ANNALS OF BOTANY

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ANNALS OF BOTANY

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Branching in the Leafy Hepaticae.

BY

ALEXANDER W. EVANS,

Professor of Botany in Yale University.

With thirty-six Figures in the Text.

THE branching of the shoot in the Hepaticae was carefully studied by Leitgeb ('71 *a*, '71 *b*, '72, '75) about forty years ago. Although most of the previous writers on this subject had confined their attention to a few thalloid genera, Leitgeb extended his observations to include a large number of leafy forms. He was able to demonstrate the fact that throughout the Jungermanniales (comprising the Metzgeriaceae and the Jungermanniaceae of many recent writers) the branches are never formed by a true dichotomy; that, in other words, the apical cell of a branching shoot never gives rise by a median longitudinal wall to the apical cells of two branches, a process which takes place in certain dichotomous Algae, such as *Dictyota*. In the Jungermanniales the branches invariably arise in segments cut off from the apical cell, and it is the persistence of the latter in the branching axis which indicates that the branching is monopodial in character.

Leitgeb showed further, however, that the apical cell of a future branch often made its appearance in an exceedingly young segment. Under these circumstances, if the branch grows vigorously and deflects the main axis to one side, the branch-system may simulate a dichotomy very closely. This is the case, for example, in *Metzgeria furcata* and in the genus *Bazzania* (Fig. 27). If, on the other hand, the branch grows less vigorously than the main axis and assumes a distinctly lateral position the branching is obviously of the monopodial type. *Riccardia multifida* and *Lepidozia reptans* (Fig. 8) may be cited as examples of this condition. When the branches arise close to the apical cell, as in the cases just considered, Leitgeb distinguishes the process as 'Endverzweigung' or 'Terminal Branching'. He contrasts with this 'Intercalary Branching', in which the branches first appear at some distance from the apical cell. In this latter type of branching the monopodial character is always apparent. It should be noted also that segments in which terminal branches begin their development are embryonic in character and are still undergoing cell-divisions; those where intercalary branches arise, on the other hand, have become more or less completely differentiated, and their cells have apparently ceased dividing.

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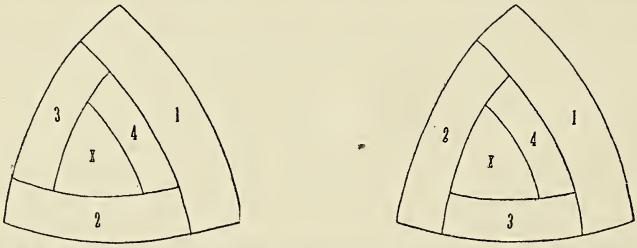
Although Leitgeb's distinctions are based on differences in development, the two types of branches can usually be recognized in mature plants on account of the fact that terminal branches arise exogenously while intercalary branches arise endogenously. The result is that the superficial tissues of a terminal branch are continuous with those of the axis, while an intercalary branch shows a distinct sheath at its base formed by ruptured cortical tissues. In the present paper the branching in the Jungermanniaceae will alone be considered. This group, which is the equivalent of the Jungermanniae akrogynae of certain writers, includes the vast majority of the leafy genera and illustrates both terminal and intercalary branching much more fully than the other groups of the Hepaticae.

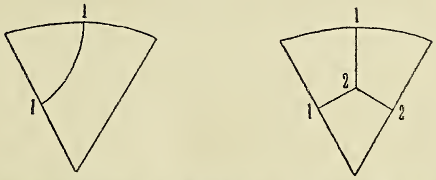
In order to give a clear idea of the way in which branches arise it will be necessary to describe briefly the normal divisions which take place in young segments. Here again our knowledge is largely based upon the work of Leitgeb. With the exception of the genus *Pleurozia,* where Goebel ('93, p. 453) has demonstrated a two-sided apical cell, the Jungermanniaceae all grow by means of a tetrahedral apical cell, which cuts off three longitudinal rows of segments in the usual way. On account of the orientation of the shoot one row of these segments will occupy a ventral position and the other two lateral positions (Fig. 1). When the segments are first cut off they are approximately in the form of flat triangular prisms, each bounded by two broad faces or walls and three narrow walls. One of the broad faces will lie in contact with the apical cell and may, as Leitgeb suggests, be designated the acroscopic wall; the other broad face, lying in contact with the next older segment of the same row, may then be distinguished as the basiscopic wall. Of the three narrow faces one, the free wall, will be exposed at the apex of the shoot, while the two others will come into contact with segments of the two other rows. In the lateral segments these two narrow faces may be distinguished as the dorsal and ventral walls respectively. They may be distinguished also according to the direction of the spiral in which the leaves are arranged. In Fig. 1, for example, which represents a dextrorse spiral, the dorsal wall in segment 4 is kathodic and the ventral wall anodic; in segment 3, on the other hand, the dorsal wall



FIGS. 1 and 2. Diagrams representing the sequence of segments cut off from apical cells. Fig. 1. Spiral dextrorse. Fig. 2. Spiral sinistrorse.

is anodic and the ventral wall kathodic. In Fig. 2, which represents a sinistrorse spiral, segment 4 agrees with segment 3 of Fig. 1 in having its dorsal wall anodic and its ventral wall kathodic, while segment 2 agrees with segment 4 of the same figure. Speaking generally, therefore, it may be stated that with a dextrorse spiral the anodic walls of the lateral segments will be ventral on the right-hand side (when a shoot is viewed from the ventral surface), while with a sinistrorse spiral they will be ventral on the left-hand side. In the ventral segments the narrow enclosed walls are both lateral, but may be distinguished as anodic and kathodic. Of course the descriptive terms which have just been applied to the walls may also be applied to portions or regions of a segment.

The early divisions in a segment follow a definite sequence, and Leitgeb was therefore able to assign the branches which he studied to definite regions. In the lateral segments the first two walls are at right angles to the two broad faces. The first wall (Fig. 3, *1*) extends from the middle of the free face to one of the enclosed narrow faces; the second wall (Fig. 4, *2*) connects the first wall with the other narrow face. The segment thus becomes divided into an internal cell and two external cells, the latter being designated by Leitgeb the 'segment-halves'.[1] These halves may be distinguished as dorsal and ventral and also as anodic and kathodic. In the ventral segments the first wall brings about a division into an inner and an outer cell, the latter usually becoming subsequently subdivided by one or more anticlinal walls. In the further development of the shoot the internal cells of the segments are destined to give rise to the axial tissues of the stem, while the external cells will form the cortical tissues of the stem and also the leaves or other appendages. Each lateral segment develops normally a single leaf, which represents the entire breadth of the segment, and Leitgeb has clearly shown that in bilobed leaves the dorsal lobe has been formed from the dorsal segment-half and the ventral lobe from the ventral half. Even in leaves which are undivided at maturity the young rudiments show evidence of being bilobed, but the shallow sinus between them soon becomes obliterated. In leaves with more than two



FIGS. 3 and 4. Diagrams representing the first two walls formed in a lateral segment and the establishment of the segment-halves.

---

[1] Leitgeb really applied the term 'segment-halves' to the two unequal cells formed by the first division wall in a lateral segment. The slight alteration in the application of the term, indicated above, makes it possible to homologize better the ventral segments with the lateral segments.

lobes one or both of the external cells of the young segment divide before the development of the leaf is clearly begun, and in this way the extra lobes are provided for. In the development of a leaf the segment-halves project and become divided by periclinal walls; these cut off the rudiment of the leaf from the cells which, by their further divisions, will form the cortical tissues of the stem. In ventral segments where underleaves are formed the development proceeds in much the same way.

Terminal Branching.

According to Leitgeb ('71 *b*) terminal branches always arise in the ventral portions of the lateral segments. He recognizes, however, two distinct kinds of terminal branching. In the first, which he describes as branching from a segment-half, the branch represents the entire ventral half of a segment, the apical cell of the branch making its appearance before the walls separating the leaf-rudiment from the cortical cells have been laid down. For the sake of convenience this may be designated the '*Frullania* type' of branching. In the second kind, which he describes as branching from the basiscopic basilar portion of a segment, the apical cell of the branch arises in a cortical cell of the axis after the leaf-rudiment has been cut off. The branch represents therefore a much smaller portion of the segment than a branch of the first kind. Branching of the second kind may be distinguished as the '*Radula* type'.

About ten years ago the writer discovered in the Hawaiian genus *Acromastigum* ('00) a third kind of terminal branching, in which the branch represents a half of one of the ventral segments. No other representatives of this kind of branching have been discovered and it may be called the '*Acromastigum* type', although other kinds of branching occur in the same genus. A fourth kind of terminal branching, in which the branch represents the dorsal half of a lateral segment, is described below. It is known only in the subgenus *Microlepidozia* of the genus *Lepidozia*, and may therefore be characterized as the '*Microlepidozia* type'.

THE FRULLANIA TYPE.

Leitgeb ('71 *b*) describes the development of branches of this type very clearly, and his diagram of a sinistrorse branching axis, which is here reproduced in a slightly modified form (Fig. 5), illustrates the sequence of the early divisions. In segments 1 and 2 the ventral halves have each undergone four divisions, which have cut off the tetrahedral apical cells of the future branches. In fact each of these segment-halves is essentially an apical cell from the beginning, the three cells first cut off acting as the first three segments of the branch. It will be noted that the walls in the segment-halves are in the following sequence: the first wall extends from the first division wall of the segment to the acroscopic wall, meeting the latter near its ventral extremity; the second wall extends from this first wall to the division wall, lying approximately parallel with the acroscopic wall; the third wall connects the first two walls. This sequence is the same in both segments, although segment 1 is situated on the right-hand side of the apical cell and segment 2 on the left-hand side, a difference which brings the branch in segment 1 in the kathodic segment-half and that in segment 2 in the anodic half. As Leitgeb points out further, this will make the spiral of the branch on the left-hand side sinistrorse, and therefore homodromous with that of the branching axis, while the branch on the right will be dextrorse and antidromous. If the branching axis were dextrorse the left-hand branch would still be sinistrorse and therefore antidromous, while the right-hand branch would be dextrorse and homodromous. In other words, the branches which arise in the anodic segment-halves are homodromous with the branching axis, those arising in the kathodic halves being antidromous.

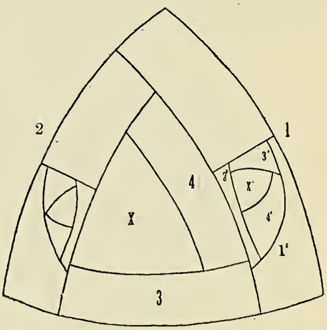


Fig. 5. Diagram representing the establishment of two branches of the *Frullania* type.

Of course the relationships which have just been brought out remain equally evident after the axis and its branches have developed further, and it is usually quite possible, from the study of an older shoot, to recognize the *Frullania* type of branching at a glance and to refer the various appendages of the branch to their appropriate segments. As might naturally be expected, the first cell cut off from the segment-half gives rise to the first underleaf of the branch, the second cell to the first lateral leaf (always situated on the side of the branch turned towards the apex of the branching axis), the third cell to the second lateral leaf (on the opposite side), the fourth cell to the second underleaf, and so on. With regard to the spirals, the branches on the left-hand side (when the shoot is viewed from the ventral surface) will all be sinistrorse and those on the right-hand side will all be dextrorse, whatever may be the direction of the spiral in the branching axis.

On account of the fact that a branch represents an entire ventral segment-half, only the dorsal half is left to form the leaf adjacent to the branch. This leaf consequently differs more or less from normal leaves, the most important differences being in form and in the number of lobes present. Sometimes, however, the leaf shows interesting peculiarities in its method of attachment. The leaves and underleaves at the base of a branch are also subject to more or less modification, both in form and in position. The differences in position are largely due to the fact that the first segments cut off from the original segment-half are less definitely orientated than those which come later. Segment 1′, for example, in Fig. 5, is not exactly ventral, but forms a considerable angle with the lower surface of the shoot, and segments 2′ and 3′ are also somewhat displaced. The differences in form are partly to be looked upon as reversions to a more juvenile condition, and partly as modifications associated with differences in environment.

The *Frullania* type of branching is more widely represented among the Jungermanniaceae than any of the other types of terminal branching. Some of the genera which show it have incubous leaves, some have succubous leaves, and some have complicate leaves. They include members of all the subfamilies recognized by Schiffner ('93–'95) with the exception of the Scapanioideae, the Stephanoideae (Raduloideae), and the Pleurozoideae. In certain genera, such as *Frullania*, *Porella*, and *Blepharostoma*, the branches all conform closely to this type; in other genera the *Frullania* type of branching occurs in connexion with other kinds. In *Bazzania*, for example, branches of the *Frullania* type and intercalary branches are both present, while in *Stictolejeunea* and a few other genera of the Lejeuneae terminal branches of both the *Frullania* type and the *Radula* type are developed. A number of characteristic examples of the *Frullania* type of branching will now be considered in detail. They are selected in order to show the modifications associated with the various methods of leaf-attachment.

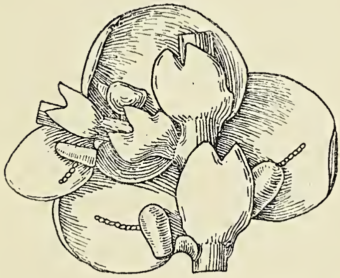


FIG. 6. *Frullania Asagrayana*. Ledyard, Connecticut (A. W. E.). × 36.

Frullania.—The normal leaves throughout this genus are deeply complicate-bilobed, the dorsal lobe being much the larger of the two. The ventral lobe, or lobule, usually develops a hollow water-sac with the opening turned towards the base of the stem or branch. The underleaves are almost always bifid. The modifications usual at the base of a branch are clearly shown in *F. Asagrayana*, a common species of eastern North America. A fragment of a branching stem is represented in Fig. 6 with the base of the branch on the left-hand side, and it will be noted that both spirals shown are sinistrorse. The incomplete leaf developed from the same segment as the branch consists of the dorsal lobe only; it spreads more obliquely than the other leaves, being almost symmetrically situated with respect to both stem and branch. The line of attachment (not shown in the figure) is partly on the stem and partly on the branch. The first underleaf of the branch is at the junction with the stem, and its line of attachment extends around the base of the branch until it almost meets the line of attachment of the incomplete leaf. Apparently, however, the leaf is never coalescent with the underleaf. The latter is not essentially different in form from other underleaves, except that it frequently bears a small tooth at about the middle of its anodic edge. The first leaf of the branch is completely covered over by the incomplete stem leaf; it shows a well-developed lobule, usually in the form of a water-sac, but the dorsal lobe is very narrow and short, scarcely exceeding the lobule in length. The following leaves and underleaves are normal in structure and exhibit a gradual increase in size, rarely, however, becoming as large and as highly differentiated as the leaves and underleaves of the stem.

The modifications noted in *F. Asagrayana* are less striking than in certain other species of *Frullania*, and the remarkable *F. aculeata*, of western South America, may be chosen to illustrate a more pronounced specialization. In this species (Fig. 7) the first underleaf of the branch is divided to the very base. The anodic division is in the form of a slender subulate lamina, but the kathodic division is a well-formed water-sac, similar in all respects to those found on normal leaves. This water-sac, from its position, looks very much as if it belonged to the incomplete leaf, which covers over the base of the branch. The first leaf of the branch has a lobule in the form of a water-sac, just as in *F. Asagrayana*, but the dorsal lobe, instead of being a lamina, is also a water-sac of the same type. The development of three water-sacs at the base of a branch, where in most species a single one is found, is apparently associated with a more xerophytic habit. The condition seems to be especially frequent in species belonging to the subgenera *Meteoriopsis* and *Diastoloba*.

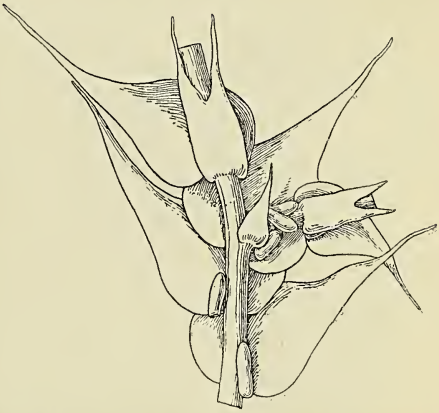


FIG. 7. *Frullania aculeata.* Galapagos Islands (A. Stewart, No. 2770). × 17.

Although in the two species just noted the first segment of a branch forms no appendicular organs except the first underleaf, Leitgeb ('75, p. 25) calls attention to the fact that in some species it often forms in addition short lobe-like appendages, which grow out between the underleaf and the incomplete leaf. These appendages, which are exceedingly variable even on an individual plant, are of considerable interest and help to explain certain peculiar structures found in other genera.

Lepidozia.—Only the subgenus *Eulepidozia* will be considered here, the discussion of *Microlepidozia* being deferred until the *Microlepidozia* type of branching is described. The common and widely distributed *L. reptans*, of

Europe, Asia, and North America, represents *Eulepidozia* adequately and is shown in Fig. 8. It will be noted that both stem and branch are dextrorse in the fragment drawn. The normal leaves in *L. reptans* are three- or four-cleft to about the middle, but the incomplete leaf at the base of a branch is only two-cleft, the apices of the lobes showing in the figure. This leaf is attached partly to the stem and partly to the branch, but is separated by a considerable distance from the first underleaf. The latter is usually bifid, although undivided or even trifid underleaves sometimes occur in this position. It is strongly concave and is inserted in such a way that the branch seems to arise in its axil. In fact, Stephani speaks of it as a subtending leaf ('91, p. 215). The sequence, however, shows clearly that it ought to be considered an underleaf, and this conclusion is supported by the fact that hyaline papillae are present at the apices of the lobes. In a leaf arising from a lateral segment there are no such papillae in the apical region. With the exception of the first underleaf the leaves of a branch show no special modifications, although a gradual increase in size is usually apparent.

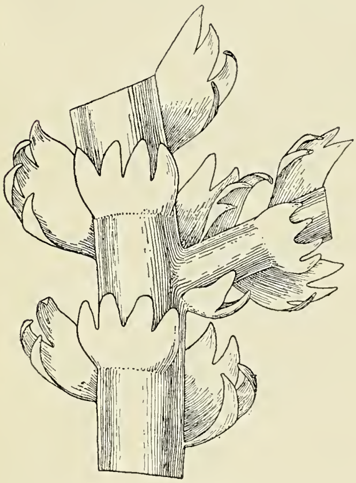


Fig. 8. *Lepidozia reptans.* Huelgoat (Finistère), France (F. Camus). × 40.

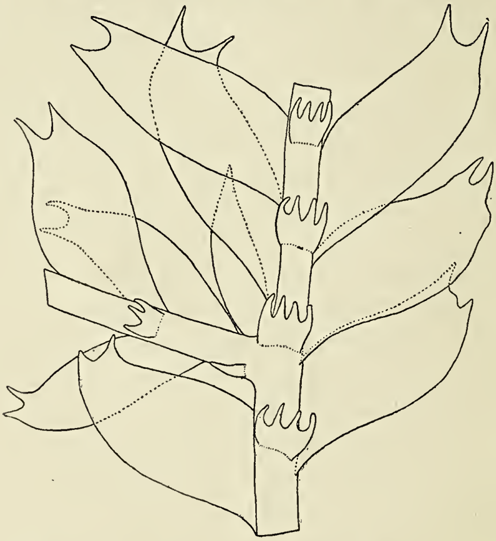


Fig. 9. *Sprucella succida.* N'dian, Kamerun (P. Dusén, Hep. Afr., No. 100). × 40.

Sprucella.—The genus *Sprucella* of Stephani ('91, p. 214), which includes the single African species *S. succida*, is closely allied to *Lepidozia*. The incubous leaves, which are almost longitudinally attached, are shortly bidentate at the apex, but are otherwise entire. The underleaves are much smaller; on robust stems they are nearly always quadrifid, but they may be only trifid or bifid. The terminal branching is essentially as in *Lepidozia*, but shows a few differences in detail. The incomplete leaf, for example, which arises from the same segment as the branch (see Fig. 9), is much smaller than normal leaves and shows no indication of teeth, being merely acuminate at the apex. The first underleaf of the branch is so nearly ventral in position that it cannot be seen when the shoot is viewed from the dorsal surface. This underleaf is very minute and is undivided; it is subulate in form and is usually only four or five cells long and three cells wide at the base. The second underleaf of the branch is normal in structure, and the same is true of the other leaves.

In comparing *Lepidozia* with *Sprucella* Stephani remarks that one of the most important differences between the genera is in the insertion of the branches. He states that in *Lepidozia* a branch springs from the inner angle of a stem-leaf, which turns its concave surface towards the branch and thereby subtends it. In *Sprucella*, on the contrary, he describes the branch as springing from the outer angle of a stem-leaf, that is, from the angle which the ventral margin of the leaf forms with the stem. As the branch develops the leaf is said to be displaced to the dorsal surface of the stem and to appear partly on the stem and partly on the branch. He states further that there is no subtending leaf as in *Lepidozia*. It will be seen from the account given above that the branches in both genera spring from the outer angles of incomplete stem-leaves, belonging to the same lateral segments, and that the subtending leaf in *Lepidozia* is nothing more than the first underleaf of the branch, and is therefore homologous with the subulate underleaf at the base of the branch in *Sprucella*. If Stephani's figure is examined ('91, Pl. XXIX, Fig. 35) it will be seen that this diminutive underleaf in *Sprucella* did not escape his attention, because he shows it clearly in the first branch on the left. He makes no allusion to it, however, in his description.

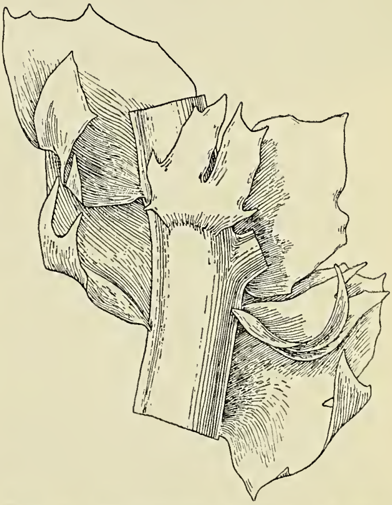


FIG. 10. *Mastigophora Woodsii*. Moidart, Scotland (S. M. Macvicar). × 40.

Mastigophora.—The rare *M. Woodsii* of western Europe is a somewhat doubtful member of this genus because its organs of reproduction are still unknown. When compared with the species already considered it shows an interesting peculiarity in its branches connected with the method of attachment of the first underleaf. The leaves in *M. Woodsii* are deeply bifid, the ventral lobe being smaller than the dorsal and usually more or less deeply subdivided (Fig. 10). The lobes are connected by a rounded keel and tend to be slightly complicate. The underleaves are large and bifid. When a branch is formed the leaf arising from the same segment is broad but undivided (Fig. 11), and its line of attachment extends across the dorsal base of the branch. The first underleaf is remarkable because it is apparently not attached to the branch at all, but to the stem at some distance below the base of the branch. It seems to be laterally inserted on the stem, and its line of attachment is almost transverse as in ordinary underleaves. This underleaf is strongly concave, but when dissected off presents no unusual features either in form or size (Fig. 12). In such a case as this it must apparently be assumed that the base of the branch is coalescent with the stem. The description just given applies most forcibly to primary branches. When these become subdivided there is little or no coalescence at the base of a secondary branch, the first underleaf occupying much the same position as in *Lepidozia.*

FIGS. 11 and 12. *Mastigophora Woodsii.* Same material as Fig. 10.
Fig. 11. Incomplete leaf at base of branch. Fig. 12. First underleaf of branch. × 40.

Pleuroclada.—Large and conspicuous underleaves are rarely associated with succubous leaves in the Jungermanniaceae. This condition, however, is found in *Pleuroclada albescens*, a widely distributed species of Europe and North America at high altitudes. The branching in this species is invariably of the *Frullania* type, and the structures at the base of a branch show a few distinctive features. Unfortunately the succubous insertion of the leaves, even here, is less distinct than in such genera as *Lophocolea* and *Chiloscyphus*, apparently on account of the fact that the bilobed leaves show a tendency to be complicate. If the line of attachment is examined it will be seen that the portion corresponding to the dorsal lobe is almost transverse, and that it is only in the portion corresponding to the ventral lobe that the obliqueness is clearly apparent. The leaves are strongly convex and appear almost hemispherical, the acute lobes being separated by a narrow sinus (Fig. 13). The underleaves approach the leaves in size, but are normally undivided and acute ; their margins are either entire or unidentate on one or both sides. Two and sometimes three papillae can be demonstrated on an underleaf, one at the apex and the others on the lateral teeth or in a corresponding position on the sides.

The leaf at the base of a branch shows its incomplete character by being undivided ; its line of attachment extends until it almost meets the first underleaf, very much as in *Frullania.* In the figure only the apex and a portion of the base are visible. The first underleaf differs from ordinary underleaves in being distinctly bilobed, thus bearing a marked resemblance to a normal leaf arising from a lateral segment. It betrays its true nature, however, by the presence of a papilla at the apex of each lobe. The second underleaf of the branch is normal in form and position, and the other leaves show no striking peculiarities.

Lophocolea.—In this genus the leaves are distinctly succubous, and the underleaves are well developed, although relatively smaller than in *Pleuroclada albescens.* The branching is largely intercalary, but terminal branches of the *Frullania* type are not unusual. The plant chosen to represent this type of branching is a small and juvenile form of *L. heterophylla*, an exceedingly common species throughout Europe, Asia, and North America. In the portion of the plant shown in Fig. 14 it will be seen that the leaves are bifid and that the underleaves are either deeply bifid or quadrifid. The leaf which belongs to the same segment as the branch does not lie over the angle between the branch and the axis as in the species previously considered. It is situated instead below the branch, although its line of attachment extends nearly or quite to the base. The leaf is nearly as broad in the basal portion as normal leaves, but is undivided, the apex being sharply acuminate. The first underleaf of the branch is normal in position, and is often larger and more complex than the second underleaf. The first leaf is small but bifid, and is completely covered over by the leaves of the

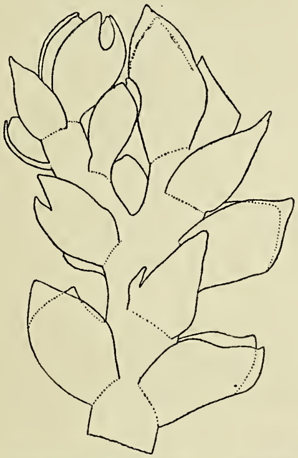


FIG. 13. *Pleuroclada albescens.* Valais Alps, Switzerland (F. Camus). × 40.

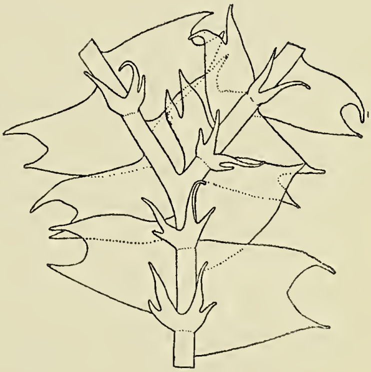


FIG. 14. *Lophocolea heterophylla.* Stafford, Connecticut (G. E. Nichols). × 27.

branching axis which come after the incomplete leaf. The second and later leaves of the branch show a rapid progression in size. The modifications just described are shown equally well by *L. bidentata* (see Leitgeb, '75, p. 24), another species with an exceedingly wide distribution, and are doubtless to be found in other members of the genus.

Harpanthus.—The present genus is composed of two species, *H. Flotowianus* and *H. scutatus*, both of which are widely distributed in the Northern Hemisphere. According to the published descriptions the branching is invariably intercalary and ventral, and this statement is apparently true of *H. scutatus.* In robust forms of *H. Flotowianus*, however, branches of the *Frullania* type occasionally occur. The leaves of this species are decurrent and spread obliquely. They are distinctly succubous and are attached by an almost longitudinal line. The apex is very shallowly indented, the apical lobes or teeth varying from rounded to pointed. The much smaller underleaves are lanceolate and acute, and are sometimes unidentate on one or both sides. When a terminal branch is developed it shows a coalescence with the branching axis very much as in *Mastigophora Woodsii*, the first underleaf being apparently situated at some little distance from the base of the branch (Fig. 15). The incomplete leaf occupies much the same position as in *Lophocolea heterophylla*; it is nearly as large as the other leaves, but differs from them in being rounded and undivided at the apex. The first underleaf of the branch is considerably larger than normal underleaves and is deeply bifid with acute divisions.

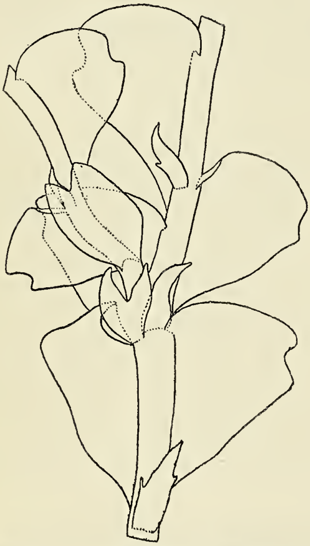


FIG. 15. *Harpanthus Flotowianus.* Riesengebirge, Bohemia (V. Schiffner, in Hep. Eur. Exsic., No. 294 *b*). × 17.

FIG. 16. *Plagiochila frondescens.* East Maui, Hawaiian Islands (D. D. Baldwin, No. 85). × 17.

The first leaf is almost transversely inserted and is much more symmetrical than the other leaves, the apical sinus being deeper and the lobes tending to be more acute. The other leaves and underleaves of the branch are not specialized.

Plagiochila.—The leaves in this genus are undivided, so that it stands in sharp contrast to the genera so far considered. Branching of the *Frullania* type is apparently restricted to species in which the secondary shoots are copiously subdivided and give rise to complex branch-systems. As an example *P. frondescens*, a widely distributed palaeotropic species, may be cited. The leaves in this plant (Fig. 16) usually show about half a dozen sharp teeth at the apex and along the ventral margin. They are attached by an oblique line which becomes abruptly arched at the ventral end. The underleaves consist of clusters of minute cilia bearing hyaline papillae. The incomplete leaf adjacent to a branch occupies the same position as in *Lophocolea* and *Harpanthus Flotowianus*; it is somewhat narrower than the other leaves, but is much like them in other respects and is attached by a line of the same type. The first underleaf of the branch is slightly displaced towards the basiscopic side (with respect to the branching axis), but shows no other modifications. The first leaf is more or less reduced in size, being sometimes very small indeed. In the example figured it appears as a lanceolate, entire lamina, but it is subject to variation and is often toothed. The succeeding branch-leaves are essentially like other leaves.

In the genus *Chiloscyphus*, as recently restricted by Schiffner ('10), branches of the *Frullania* type are not infrequent. They agree closely with those just described for *Plagiochila frondescens* and, like them, are usually associated with leaves which are undivided at maturity. Müller, however, refers these branches to the *Radula* type ('06–'11, p. 818).

Lophozia.—With the exception of subfloral innovations branches are difficult to demonstrate in most species of *Lophozia*. Terminal branches of the *Frullania* type, however, have been recognized in *L. inflata* and *L. acutiloba*, two species which Müller ('06–'11, p. 738) separates from *Lophozia*, reviving for them the old generic name *Gymnocolea*. Müller (p. 675) has also noted the exceptional occurrence of such branches in *L. longiflora*, var. *uliginosa*, and in *L. Wenzelii*, while the writer has been able to demonstrate them in several other members of the genus, some belonging to the subgenus *Dilophozia* and some to the subgenus *Barbilophozia*. As an example of *Dilophozia*, *L. Wenzelii*, an alpine and arctic species of Europe, Asia, and North America, will be described. The leaves are distinctly succubous and have a shallow apical sinus with broad and bluntly pointed lobes. The line of attachment is long and oblique and the leaves are normally plane or nearly so. Underleaves are represented by minute hair-like structures tipped with hyaline papillae and are usually short-lived and difficult to demonstrate. Fig. 17 shows a branch of the typical *Frullania* type. It will be noted that the leaf belonging to the same segment as the branch is similar to the other leaves, but is undivided, and that the line of attachment comes to an end at some little distance from the base of the branch. The first underleaf is a distinct though small lamella, and the first leaf of the branch differs from normal leaves in being somewhat complicate. The modifications are comparable with those seen in *Harpanthus Flotowianus*, although there is no marked coalescence between the branch and the axis.

In Fig. 18 an interesting variation is shown, in which the incomplete leaf is attached by a line which seems to be prolonged as far as the first underleaf of the branch. The part adjacent to the underleaf corresponds to a sharp lobe-like tooth, which is united to the leaf at its ventral base. This tooth might perhaps be considered the first underleaf of the branch; it might be considered an accessory lobe of the incomplete leaf; it might be considered an appendage of the first segment of the branch, borne in addition to the first underleaf and similar to the appendages described by Leitgeb in *Frullania*. The first interpretation is hardly permissible, because a minute underleaf very much like the one shown in Fig. 17 is visible at the base of the tooth; there are also objections to the second interpretation on account of the long line of attachment; the third interpretation, however, seems to accord well with the conditions present. An examination of Fig. 5 shows that the first segment of the branch and the dorsal segment-half are adjacent, so that a coalescence between the

FIGS. 17 and 18. *Lophozia Wenzelii.* Near Hallen (Jemtland), Sweden (H. W. Arnell and A. Grape, in Hep. Eur. Exsic., No. 172). × 17.

appendicular organs which spring from these regions would not be at all surprising. Similar unions, in fact, are frequently met with between bracts and bracteoles and are not unusual between ordinary leaves and underleaves.

As members of the subgenus *Barbilophozia*, *L. attenuata* and *L. Floerkei* may be considered. Both of these species are widely distributed in the northern parts of Europe, Asia, and North America. In *L. attenuata* the leaves are commonly trifid, although bifid leaves are not unusual. They are attached by an oblique line, but often show more or less tendency to be complicate, the dorsal lobe being connected with the rest of the leaf by a rounded keel. Sometimes the dorsal portion is attached by an almost transverse line. The underleaves are minute and inconspicuous, and were not made out in the material studied on account of the thick felt of rhizoids. When a branch is developed (Fig. 19) the leaf belonging to the same segment is undivided and acute, corresponding clearly to the dorsal lobe of an ordinary leaf. At some little distance from the incomplete leaf, on the ventral surface of the shoot, another leaf-like structure, which is also undivided and acute, can be discerned. This should apparently be interpreted as an appendage of the first segment of the branch but distinct from the first underleaf. It differs from the homologous appendage in *L. Wenzelii* in being entirely free from the incomplete leaf. The first branch-leaf in *L. attenuata* is small and often undivided; it is usually covered over by the next stem-leaf on the same side as the branch. The remaining leaves of the branch show no special modifications.

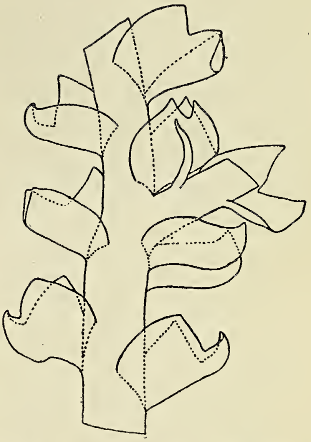


Fig. 19. *Lophozia attenuata.* Helsingfors, Finland (H. Lindberg, in Hep. Eur. Exsic., No. 95). × 40.

A study of *L. Floerkei* adds further support to the interpretation just given of *L. attenuata*. The leaves in *L. Floerkei* are normally trifid and the distinct underleaves are bifid. At the base of a branch a structure like that shown in Fig. 20 can be demonstrated. The first underleaf of the branch (at the left-hand end of the figure) is similar to the other underleaves, but is undivided; the incomplete leaf is also undivided and clearly represents a dorsal lobe; between the two and somewhat coalescent with the leaf is a broad bifid structure, which is evidently homologous with the lobe-like tooth

Fig. 20. *Lophozia Floerkei.* Bethesda, Wales (W. H. Pearson). × 27.

described in *L. Wenzelii.* The examples just considered thus form a kind of series with respect to the structures at the base of a branch. In Fig. 17 of *L. Wenzelii* the first ventral segment of the branch has formed simply the first underleaf; in Fig. 18 of the same species it has formed in addition a small appendage coalescent with the incomplete leaf; in *L. Floerkei* the appendage is much larger and the coalescence is less extensive; in *L. attenuata* the appendage is also large, but is entirely free from the leaf. In all probability an extensive study of the species here considered and allied species would show more or less variation in the structures described.

The branches in *L. inflata* and *L. acutiloba* offer a few further points of interest. *L. inflata* is one of the most widely distributed and variable species in northern Europe, Asia, and North America; the closely related *L. acutiloba*, on the other hand, is known from only a few European localities. Schiffner ('03, p. 28) states that in *L. inflata* the leaf developing from the same segment as a branch is undivided, thus differing from the normal two-lobed leaves, and he also makes similar statements about *L. acutiloba* ('09). In Fig. 21, which represents a robust aquatic form of *L. inflata*, the conditions described by Schiffner are clearly shown. The normal leaves are here somewhat more explanate than in some of the other forms of the species; they show a long oblique line of attachment and two rounded lobes separated by an obtuse sinus. The incomplete leaf is at some little distance below the branch; it is attached by a shorter line and is merely rounded at the apex. The leaves of the branch are essentially normal

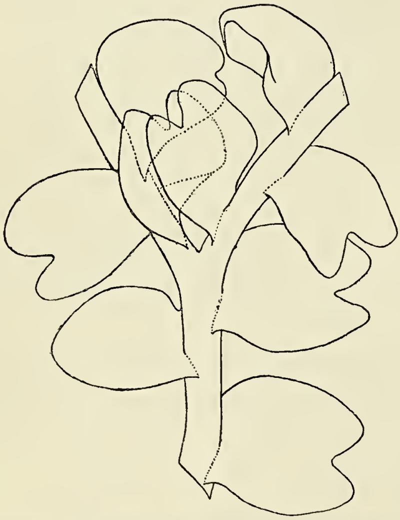


FIG. 21. *Lophozia inflata.* Riesengebirge, Bohemia (V. Schiffner and E. Bauer, Bryoth. Bohem., No. 294). × 17.

FIG. 22. *Lophozia inflata.* Same material as Fig. 21. × 17.

from the beginning. In the form figured there are no distinct underleaves, the only ventral appendages being hyaline papillae which sometimes cut off a few cells at the base. A group of these papillae can usually be distinguished at the base of a branch in the position where an underleaf would naturally appear.

In addition to the branches just discussed, Müller, in both *L. inflata* and *L. acutiloba*, describes cases in which the leaf below the branch is bilobed instead of being undivided. This condition does not seem to be infrequent and is shown in Fig. 22. It will be seen that the leaf below the branch (the lower leaf on the right-hand side) looks like the other leaves except that its line of attachment is shorter. If it were not for this slight difference and for the difference in the sequence in which the leaves are borne, it would be difficult to distinguish the branch from the branching axis. Müller refers branches like this to the *Radula* type instead of to the *Frullania* type. There are, however, two objections to this interpretation: first, the sequence in which the leaves of the branch are borne, and, second, the short line of attachment of the leaf below the branch. In the writer's opinion the branches shown in the two figures of *L. inflata* ought both to be referred to the *Frullania* type. The presence of two lobes in one of the incomplete leaves could then be ascribed to the tendency, which so many species of *Dilophozia* show, to develop more than the normal number of lobes. The terminal branches in *L. acutiloba* are in all essential respects like those of *L. inflata*.

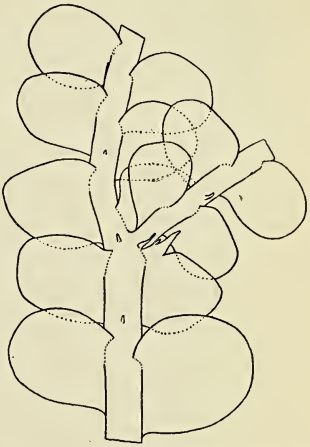


FIG. 23. *Jamesoniella autumnalis.* Cromwell Connecticut (A. W. E.). × 17.

Jamesoniella.—In *J. autumnalis*, a species which is aberrant in certain respects, branching of the *Frullania* type can occasionally be observed. In all typical members of the genus the branches are apparently always intercalary and ventral. *J. autumnalis* is widely distributed throughout North Temperate regions. The leaves (Fig. 23) are undivided and rounded, and the line of attachment is long and oblique. The minute underleaves are often short-lived and difficult to demonstrate among the rhizoids. The incomplete leaf at the base of a branch is coalescent with a lobe-like appendage at the ventral base, similar to the one figured in *Lophozia Wenzelii.* The appendage varies considerably in size and in the extent of coalescence; the apex varies also from acute (as shown in the figure) to obtuse or rounded. The first underleaf of a branch tends to be a trifle larger than normal underleaves, but the other branch-leaves show no modifications of interest.

Zoopsis.—Among the genera which Spruce ('82) included under *Cephalozia*, branching of the type now being discussed seems to be exceedingly rare. It has been reported, in fact, in scarcely a half-dozen species, two of which belong to the genus *Zoopsis* and the others to the genus *Cephalozia* in its restricted sense (the subgenus *Eucephalozia* of Spruce). In *Zoopsis* Leitgeb ('76) alluded briefly to the branching and stated that only vegetative branches ever conformed to the *Frullania* type. His observations were largely based on *Z. argentea*, a widely distributed species the range of which extends from New Zealand and Australia into tropical Asia. The leaves in this species are probably the most rudimentary found in the genus (Fig. 24). Each consists of only four cells, two of which form the base. The other two cells are in the form of papilla-like appendages attached more or less transversely to the basal cells, the one belonging to the more dorsal cell being about twice as long as the other. Although at first sight the leaves appear longitudinally inserted, they are really very slightly succubous. The underleaves are minute, but are apparently always present. In the figure the branch is on the right-hand side, and it will be seen that the leaf belonging to the same segment consists of only two cells, the long apical papilla indicating that the dorsal half is the one represented. It should also be noted that this incomplete leaf seems to be situated at the base of the branch instead of on the branching axis. Aside from this peculiar leaf there is little to show that the branch-system is not a dichotomy, the first leaf of the branch being normal in structure and position and the first underleaf (not shown in the figure) being ventral and like the other underleaves of the plant.

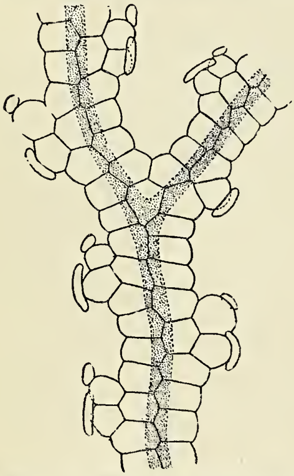


FIG. 24. *Zoopsis argentea.* New Guinea (L. Loria, No. 63). × 80.

Cephalozia.—The *Frullania* type of branching has been noted by Leitgeb ('75) in *C. bicuspidata* and *C. curvifolia*, by Spruce ('82) in *C. tubulata*, and by Stephani ('06–'09) in *C. hamatiloba* and *C. asperrima.* Only the first two of these species will be considered here. They are both widely distributed in Europe, Asia, and North America. In *C. bicuspidata* Leitgeb calls attention to the undivided leaf at the base of a branch and notes that he has observed branching of this type in but a single instance. Apparently, however, the phenomenon is less uncommon than he implies. The writer has found it to occur not infrequently in specimens from British Columbia collected by Macoun and by Brinkman, and also in a specimen from Baden collected by Jack and distributed in Gottsche and Rabenhorst's Hepaticae Europaeae (No. 353). In Fig. 25, which represents one of

Macoun's plants, it will be noted that the sharply bilobed leaves are distant and tend to be somewhat complicate. The dorsal lobe is almost transversely inserted, but the line of attachment of the ventral lobe is distinctly oblique, the conditions being much the same as in *Pleuroclada albescens*. No underleaves are present, each ventral segment bearing instead a single hyaline papilla. The incomplete leaf at the base of the branch is undivided and acuminate. The first segment of the branch bears a hyaline papilla (not visible in the figure) and, in addition, a large leaf-like appendage. Apparently there is never coalescence between the appendage and the incomplete leaf, although their lines of attachment sometimes approach each other very closely. The branches in this plant bear a strong resemblance to those described in *Lophozia attenuata*.

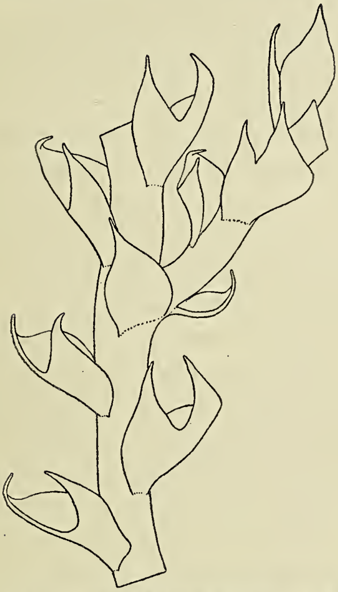


Fig. 25. *Cephalozia bicuspidata.* Ucluclet, British Columbia (J. Macoun, No. 115). × 40.

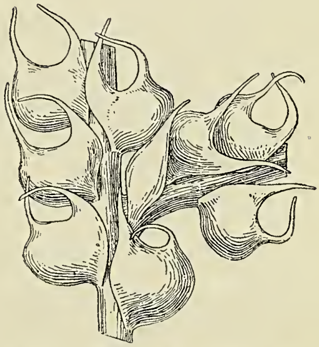


Fig. 26. *Cephalozia curvifolia.* Franconia Mountains, New Hampshire (A. W. E.). × 40.

In *C. curvifolia* (sometimes made the type of the distinct genus *Nowellia*) branching of the *Frullania* type is not unusual. The leaves in this species are attached in much the same way as in *C. bicuspidata* and are characterized by the strongly inflated base of the ventral lobe (Fig. 26). The ventral segments produce hyaline papillae, just as in *C. bicuspidata*. The leaf at the base of a branch, lacking the ventral lobe, is reduced to a narrow lanceolate lamina with a very short line of attachment. The first segment of the branch bears a hyaline papilla, but no leaf-like appendage, thus differing markedly from *C. bicuspidata* and agreeing with such species as *Lophozia inflata* and *L. acutiloba*.

Bazzania.—The terminal branching in *Bazzania*, which conforms closely to the *Frullania* type, exhibits a number of interesting peculiarities. The modifications which occur at the base of a branch are shown in Fig. 27, which represents *B. tricrenata,* a widely distributed species in the northern and mountainous regions of Europe, Asia, and North America. The spirals shown in the figure are both dextrorse, and it will be noted that the distance between a leaf on the right-hand side and the next underleaf is much less than the distance between the underleaf and the next leaf on the left-hand side. An occasional leaf in *B. tricrenata* is bluntly tridentate at the apex, but most of the leaves diverge from this typical condition. The underleaves are broader than long, and are either entire or irregularly toothed or lobed at the apex. The incomplete leaf at the base of a branch is always undivided at the apex and acute, and its line of attachment, as in similar cases, is partly on the stem and partly on the branch. The first underleaf of a branch is smaller than the other underleaves and tends to be distinctly bilobed; it is displaced in such a way that its line of attachment almost reaches that of the next underleaf in the stem-spiral. The first leaf of the branch is small, but the later leaves and underleaves show no special modifications.

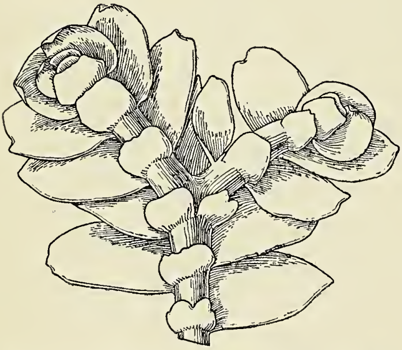


FIG. 27. *Bazzania tricrenata.* Beacon Falls, Connecticut (A. W. E.). × 27.

In the genera previously considered the branches have been partly on one side of the main axis and partly on the other. This has made the branching axis and the branch sometimes homodromous and sometimes antidromous. In *Bazzania,* however, Leitgeb ('71 *b*) brings out the fact that the spirals in a branch-system are invariably homodromous, although in some plants they may be dextrorse and in others sinistrorse. He shows that this condition is due to the restriction of the branches to anodic segment-halves. This being the case a dextrorse branching axis would develop branches on the right-hand side only (when viewed from the ventral surface) and a sinistrorse axis on the left-hand side. On account of the simulated dichotomy in *Bazzania* the shoot-system never appears one-sided, as it necessarily would if an obvious monopodium were maintained.

From the standpoint of comparative morphology Velenovský ('05, p. 112) concludes that terminal branching, especially of the *Frullania* type, represents a true dichotomy. In his opinion evidence derived from developmental study is of no value in deciding questions of this kind. Unfortunately he fails to give reasons for his opinion in this particular case. It cannot be based upon the fact that the two new axes which result from the branching are alike in all respects. Even in *Bazzania*, where the dichotomous appearance is so strongly marked, the branch can at once be distinguished from the branching axis by comparing the sequences of the leaves. It will be seen in Fig. 27, for example, that the spiral in the branch (on the right) is not continuous with that of the main axis below the branch, while the spirals in the two portions of the main axis (below and above the branch) are continuous. Of course, in such a genus as *Frullania*, the differences between a branching axis and a branch are still more striking.

According to Servít ('07), who accepts Velenovský's idea of a dichotomy, the genus *Bazzania* does form two equivalent new axes, and his Fig. 1, representing the common *B. trilobata*, seems to support his assertion. A close scrutiny of this figure, however, will show that it is incorrect and that, in the axis on the left (the main axis), the leaf on the left-hand side should precede and not follow the leaf on the right-hand side. In other words, the spirals ought to be homodromous and not antidromous, as the figure indicates and as a true dichotomy would require. Servít goes so far as to homologize the incomplete leaf in *Bazzania* (and in other genera showing the *Frullania* type of branching) with the 'Angularblatt' which Velenovský describes in the dichotomous Pteridophytes ('05, p. 249). He states that an 'Angularblatt' is distinguished from other leaves by its form, by its position in the angle formed by a dichotomy, and by its being attached equally to the two new axes. His characterization applies in all essential respects to many Jungermanniaceae with incubous or complicate leaves, but breaks down more or less in genera with succubous leaves. In *Lophocolea*, for example, the incomplete leaf is situated below the branch and shows no relation to the angle between the branch and the main axis. On the whole it seems unnecessary to consider the incomplete leaf as a special morphological modification; it would certainly be simpler to regard its peculiarities as due to the restricted portion of the segment which takes part in its formation.

THE MICROLEPIDOZIA TYPE.

The condition of homodromy which *Bazzania* secures through the suppression of branching on one side of a branching axis is secured by the subgenus *Microlepidozia* in a different way, an obvious monopodium with homodromous branches on both sides being the result. Here, as in *Bazzania*, the branches are restricted to the anodic segment-halves, but while this brings them in the ventral segment-halves on one side of a branching axis it brings them in the dorsal segment-halves on the other. This remarkable peculiarity of *Microlepidozia* is clearly illustrated by *Lepidozia setacea*, a widely distributed species in northern Europe and North America. In Fig. 28 a portion of a dextrorse axis with two branches is shown, and it will be noted that both branches are dextrorse also, that the branch on the right-hand side has arisen in a ventral segment-half, and that the branch on the left has arisen in a dorsal segment-half. In other words, the branch on the right conforms to the *Frullania* type and that on the left to the *Microlepidozia* type.

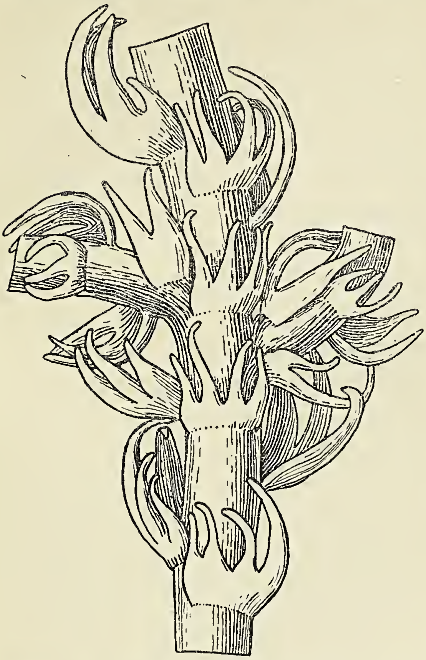


FIG. 28. *Lepidozia* (*Microlepidozia*) *setacea.* Franconia Mountains, New Hampshire (A. W. E.). × 100.

When the branching in *Microlepidozia* was first studied by the writer ('04, p. 187) it was observed that, looking at a branch-system from the dorsal surface, none of the branches on one side had incomplete leaves at the base while all the branches on the other side showed such leaves clearly. The conclusion was reached that the branches without incomplete leaves represented entire segments, thus leaving nothing for the formation of leaves. Further study, however, has brought out the fact that this interpretation is incorrect and that these branches have incomplete leaves at the base just as in the *Frullania* type, the only difference being that they are situated on the ventral surface of the shoot instead of on the dorsal. It will be seen from the figure that the first branch-leaf in the *Microlepidozia* type is not an underleaf, as in the *Frullania* type, but a lateral leaf on the side of the branch turned away from the main axis, that the second leaf also is a lateral leaf but on the other side, and that the third leaf represents the first underleaf formed. None of these branch-leaves show special modifications. Sinistrorse branching systems as well as dextrorse may be observed in *Microlepidozia* just as in *Bazzania*.

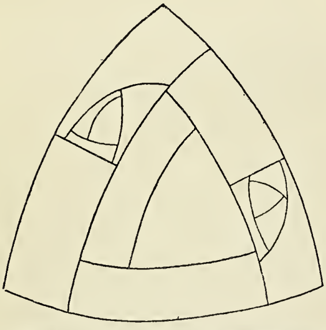


FIG. 29. Diagram representing the establishment of a branch of the *Microlepidozia* type on the left and one of the *Frullania* type on the right.

The accompanying diagram (Fig. 29) illustrates the terminal branching in *Microlepidozia*. The oldest segment on the right shows the *Frullania* type and agrees with Fig. 5; the segment on the left shows the establishment of the apical cell of a branch in the dorsal segment-half, according to the *Microlepidozia* type. It will be noted that the segments on this side follow the same sequence as on the other side and correspond with the leaves and underleaves described above. In addition to terminal branching *Microlepidozia* shows intercalary branching from ventral segments.

THE ACROMASTIGUM TYPE.

The genus *Acromastigum* contains the single Hawaiian species *A. integrifolium.* Since a detailed description of the branching in this plant has already been published by the writer ('00), only the more essential features of the *Acromastigum* type will be mentioned here. The species shows in addition terminal branching of the *Frullania* type and ventral intercalary branching. In the development of a ventral segment the first wall laid down is periclinal and gives rise to an internal and an external cell, the latter soon dividing by an anticlinal wall into two cells or segment-halves. If the development continues in the usual way one of the external cells again divides by an anticlinal wall, so that the segment shows three external cells. These three cells give rise to three hyaline papillae, which are carried forward on the apex of the underleaf developed from the segment. When a terminal branch is to be formed from a ventral segment the apical cell of the branch becomes differentiated in one of the segment-halves, leaving a single external cell for the underleaf. The result is that the underleaf is much narrower than usual and shows only one apical papilla. The branch is situated at one side of this narrow underleaf, sometimes on the right and sometimes on the left. In Fig. 30 the establishment of a branch of this character is shown diagrammatically on the right-hand side, while a branch of the *Frullania* type is shown in the lateral segment on the left. The first two segments cut off in the ventral branch give rise to lateral leaves and the third to an underleaf. It will be noted that the spiral is sinistrorse in the branch figured; if the branch had been on the left-hand side the spiral would have been dextrorse. The same thing would have been true if the spiral of the main axis had been dextrorse instead of sinistrorse. In other words, the branches arising in anodic segment-halves are homodromous with the main axis, while those arising in kathodic segment-halves are antidromous, the relation being the same as in branching of the *Frullania* type. Branches of the *Acromastigum* type are apparently always flagelliform, and their minute leaves and underleaves present no modifications of special interest.

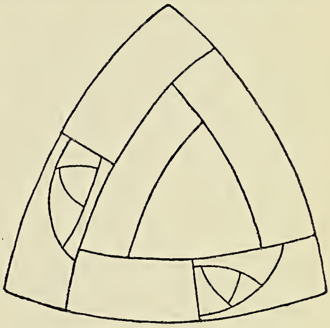


FIG. 30. Diagram representing the establishment of a branch of the *Acromastigum* type on the right and one of the *Frullania* type on the left.

THE RADULA TYPE.

The *Radula* type of terminal branching was the one first described by Leitgeb ('71 *a*) and has already been briefly characterized. It has sometimes been referred to as the '*Lejeunea* type' by the writer ('07, '08). On account of the late differentiation of the apical cell of a branch, the leaf belonging to the same segment is normally developed and shows none of the interesting peculiarities described under the previous types of terminal branching. As Leitgeb points out, a branch of the *Radula* type always arises in a cortical cell adjacent to the base of a leaf on the basiscopic side and in the ventral portion of the segment. In the genus *Radula* the branches all conform to this type, and it is also found throughout the vast group of the Lejeuneae. In most of the genera of this group the branching is entirely of the *Radula* type. In a few genera of the Lejeuneae Holostipae, however, such as *Stictolejeunea* and *Bryopteris*, branching of the *Frullania* type also occurs. These genera, together with the related genus *Jubula* (in which likewise both types of branching are found), have already been discussed by the writer in other connexions ('07, '08). Whether the *Radula* type of branching occurs in other members of the Jungermanniaceae is doubtful, although Leitgeb assigns it to *Scapania* ('75, p. 29), Goebel to *Anomoclada* ('06, p. 130), and Müller to *Lophozia inflata*, *L. acutiloba*, and *Chiloscyphus* ('06–'11). The terminal branching in *Lophozia* and *Chiloscyphus*, as interpreted by the writer, has been described under the *Frullania* type, and the branching in *Scapania* and *Anomoclada* will be considered in connexion with intercalary branching.

The development of the branches in *Radula* and the Lejeuneae is treated by Leitgeb at considerable length, but his statements with regard to the sequence of the segments in the branch-rudiments are not altogether consistent. This is probably due to the fact that the sequence conforms less rigidly to rule than in the other types of terminal branching, a condition clearly brought out by a comparison of fully developed shoots. In describing *Radula* ('71 *a*, p. 37) he states that the first segment of the branch is basiscopic (with respect to the main axis), the second acroscopic, and the third ventral. This would bring the first branch-leaf on the side of the branch turned away from the apex of the main axis, in marked contrast to what is found in the *Frullania* type. It would also make the spirals of the branches on the left-hand side of the axis dextrorse and those on the right-hand side sinistrorse. In describing the Lejeuneae, however, he states that the first branch-leaf is an underleaf ('75, p. 28). This would imply that the first segment of the branch instead of the third was ventral in position. Even under these circumstances, if the first lateral leaf retains its basiscopic situation, the branches on the left-hand side would still be dextrorse and those on the right-hand side sinistrorse. The examination of a branch in

Radula (Fig. 31) or in one of the Lejeuneae (Fig. 32) shows that his statements with regard to the lateral leaves are correct, the first leaf being constantly basiscopic. The spirals, however, show certain deviations. The determination of a spiral in *Radula* is beset with difficulties because the ventral segments bear no appendages. It is only by studying the apical cell and the young segments that the direction of the spiral can be positively demonstrated. This was done in the species figured, *R. aquilegia* of western Europe, and it was found that the branch-spirals on the left-hand side of a branching axis were sinistrorse instead of dextrorse as Leitgeb's account demands. This would, of course, make the ventral segment in the branch-rudiment second instead of third in the sequence, the basiscopic segment still being first. The conditions just described are found in both vegetative branches and subfloral innovations and are apparently constant throughout the genus.

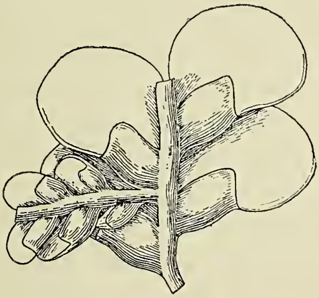


FIG. 31. *Radula aquilegia.* Moidart, Scotland (S. M. Macvicar). × 27.

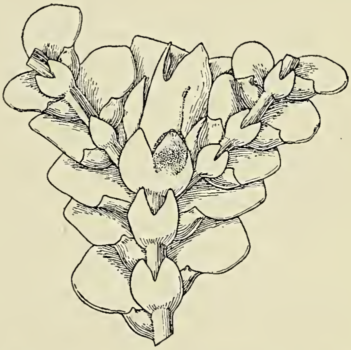


FIG. 32. *Euosmolejeunea duriuscula.* Sanford, Florida (S. Rapp). × 40.

In most of the Lejeuneae underleaves are present, so that the determination of a spiral is an easy matter. It is necessary here to distinguish between subfloral innovations and vegetative branches because they sometimes show differences which are not to be found in *Radula*. In the species figured (Fig. 32), *Euosmolejeunea duriuscula* of tropical and subtropical America, the peculiarities and variations of the innovations are well shown. It will be seen that the innovation on the right conforms closely to Leitgeb's conception, the first branch-leaf being an underleaf, the first lateral leaf being basiscopic, and the spiral being sinistrorse. The innovation on the left, however, is also sinistrorse, the first leaf being basiscopic and the underleaf being second in the sequence. A study of numerous innovations has shown that there is apparently no relation between their spirals and the spiral of the flowering axis, although the condition shown by the left-hand innovation seems to be much commoner than the other. This condition is of course identical with what has just been described for *Radula*.

In the vegetative branches the differences noted in the innovations do not seem to occur. In a large series examined the spirals were all found to agree with those in *Radula.* It may be stated, therefore, that in the *Radula* type of branching the spirals usually run in the same direction as in the *Frullania* type, dextrorse branches being on the right-hand side of a branching axis and sinistrorse branches on the left-hand side. In the *Radula* type, however, the first underleaf (or first ventral segment) comes second in the sequence instead of first.

Although the branches in *Radula* and the innovations in the Lejeuneae are clearly exogenous, the exogenous nature of the vegetative branches in the Lejeuneae is less apparent, each branch bearing at its base a sheath which seems at first sight to be composed of ruptured cortical tissue. The common *Lejeunea cavifolia,* of Europe, Asia, and North America (Fig. 33), may be chosen to illustrate the conditions present. According to Leitgeb ('75, p. 28) this sheath is really composed of the first leaf-cycle of the branch, the first underleaf and the first two lateral leaves having coalesced by their margins. The leaves are very rudimentary and form at first a covering through which the developing branch must push its way. In favourable cases he was able to demonstrate the boundaries between the leaves. He associates these foliar sheaths with the fact that the branches in the Lejeuneae, although laid down near the apex, often remain latent for a considerable period.

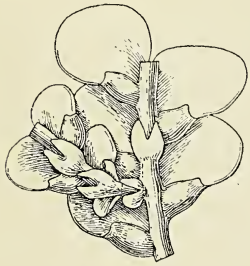


FIG. 33. *Lejeunea cavifolia.* Huelgoat (Finistère), France (F. Camus). × 40.

Leaving the foliar sheaths out of consideration, the first leaves on branches of the *Radula* type show less definite modifications than in the other types of terminal branching. They are usually smaller than normal leaves, and their lobules (ventral lobes) are often very rudimentary. The first underleaves, also, are sometimes smaller and less complex than those afterwards formed. In most cases there is a transition, sometimes very gradual, between the basal leaves and the more typical leaves which the branch later develops. The modifications present may be looked upon as reversions to a more juvenile condition.

Intercalary Branching.

Terminal branching of the *Radula* type, as Leitgeb intimates, is intermediate in certain respects between terminal branching of the *Frullania* type and intercalary branching. In the *Frullania* type (and the same thing is true of the *Microlepidozia* and *Acromastigum* types) the branches arise very close to the apex, their leaves follow a definite sequence, the branch-spirals conform to definite rules, and the exogenous origin is perfectly clear.

In the *Radula* type the formation of the branches is longer delayed, the sequence of the branch-leaves is less definite, the spirals conform less rigidly to rule, and, in the vegetative branches of the Lejeuneae at least, basal sheaths are present, whatever the morphological nature of these sheaths may be. In intercalary branching the formation of the branches is still longer delayed, the sequences of the branch-leaves are indefinite, the spirals show no conformity to rule, and the basal sheaths are constant and evidently represent ruptured cortical tissue. The peculiar features of intercalary branches are illustrated below by concrete examples.

According to Leitgeb ('72, '75) intercalary branches almost invariably arise in ventral segments. Even in cases where they appear lateral he considers that they are probably ventral in origin but displaced through subsequent inequalities of growth. The only case in which he assigns intercalary branches to lateral segments is in *Jubula Hutchinsiae*, a rare species of western Europe ('75, p. 36). In this plant the vegetative branches conform closely to the *Frullania* type and the subfloral innovations to the *Radula* type. The branches which he regards as intercalary are the short male branches, which are situated behind normal leaves, bearing the same relation to them as the subfloral innovations do to the perichaetial bracts. His opinion is based upon the fact that each branch has a distinct basal sheath, and, since he was unable to demonstrate the boundaries of coalesced leaves and saw in the sheaths evidence of active cell-division, he concluded that they represented cortical tissue. Unfortunately his explanation and figure are not convincing, and there seems to be no imperative reason why these sheaths should not be interpreted in the same way as the very similar sheaths in the Lejeuneae.

Although this doubtful case is the only one recognized by Leitgeb, the writer would refer certain intercalary branches in a number of species to lateral segments rather than to ventral. This course seems justifiable from the position which these branches occupy on adult shoots, although it has unfortunately been impossible to trace out their development from the earliest beginnings or to assign them to definite portions of the original segments. The genera where branches of this kind occur are the following: *Micropterygium*, *Diplophylleia*, *Scapania*, *Sphenolobus*, *Cephaloziella* (subgenus *Prionolobus*), *Odontoschisma*, *Anomoclada*, *Plagiochila*, and *Chiloscyphus*. In all probability this list could be considerably increased. As an illustrative example of lateral intercalary branching the genus *Micropterygium* of tropical America may be selected. In *M. Pterygophyllum*, a widely distributed species (Fig. 34), the large underleaves are very much like those of *Bazzania*, but the lateral leaves show a very peculiar structure. They are distinctly complicate, but only the dorsal lobe is attached to the axis, the ventral lobe being formed by a folding back of its lower margin. Along the keel thus formed, in well-developed leaves, a wing of variable extent may be seen. The branches are situated in the axils of the leaves and show a distinct basal sheath. In the majority of cases, as in the branch on the right, the first leaf is basiscopic with respect to the main axis, the second leaf is an underleaf, and so on. In the figure the first leaf is only partially visible and is considerably displaced from its theoretical position. In the branch on the left it will be noted that the first leaf is acroscopic, and cases may also be found where the first leaf is an underleaf. The main axis in the figure is sinistrorse, the branch on the right is dextrorse. Other cases were observed, however, in which dextrorse spirals occurred in branches on the left and sinistrorse spirals in branches on the right. So far as can be learned the spiral of the main axis exerts no influence upon the spirals of the branches. In the genera *Scapania*, *Diplophylleia*, *Sphenolobus*, and *Cephaloziella* (subgenus *Prionolobus*) the leaves are bilobed and more or less complicate, but their lobes are both attached to the axis. With the exception of certain species of *Cephaloziella* none of these genera produce underleaves. Their lateral intercalary branches are distinctly axillary and resemble in most essential respects the branches of *Micropterygium*.

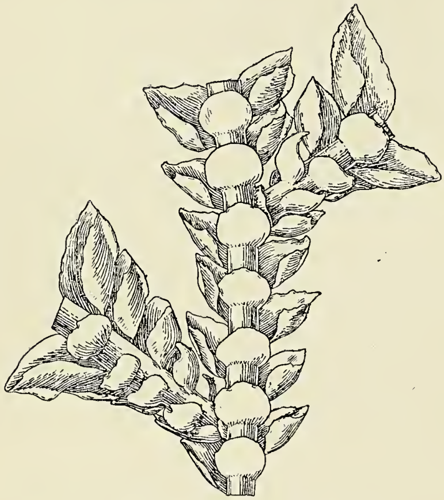


FIG. 34. *Micropterygium Pterygophyllum.* Mabess River, Jamaica (A. W. E., No. 316). × 40.

FIG. 35. *Plagiochila Sullivantii.* Quarry Run, West Virginia (A. L. Andrews). × 17.

The other genera, where lateral branches are here interpreted as intercalary, are all distinguished by succubous undivided leaves. *Plagiochila Sullivantii*, a species of the eastern United States, shows the usual conditions present (Fig. 35). The branch figured is borne in the axil of a leaf but close to the ventral margin, and an examination of *Chiloscyphus* will show that the female branches arise in much the same position. Apparently in branching of this kind there is more or less variation in the relative positions of leaf and branch, although the latter may always be described as axillary. In the common *Plagiochila asplenioides*, for example, Servít ('07, Fig. 3) figures a branch much nearer the middle of a leaf than is shown above, while in the South American *Anomoclada mucosa*, as well as in certain species of *Odontoschisma*, the lateral branches are close to the dorsal margins of the leaves. In all these cases, so far as examined, the branch-spirals and the sequences of the branch-leaves exhibit as great an irregularity as in *Micropterygium* and the other genera with complicate leaves.

The ruptured cortical sheath at the base of a lateral intercalary branch is usually conspicuous, but aside from this the basal structures show no very striking modifications. In most cases the first few cycles of leaves are rudimentary and often more or less displaced from their theoretical positions. As the branch develops the leaves gradually become more and more definite in position and in form, and the branch soon acquires the appearance characteristic of the species. Occasionally, as in *Cephaloziella Turneri*, a species of Europe, Africa, and North America, the base of a branch bears underleaves, even although these are absent from fully developed shoots. The modifications mentioned are apparently all reversionary in character.

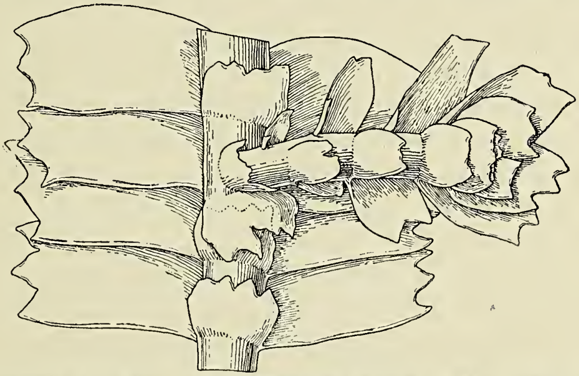


FIG. 36. *Bazzania Brighami.* Oahu, Hawaiian Islands (C. M. Cooke, Jun.). × 40.

Intercalary branches arising from ventral segments are far more common than those just described and have been less subject to discussion. Certain genera, such as *Herberta*, *Anastrophyllum*, and *Saccogyna*, seem to branch invariably in this way, and the same thing is true of many species of *Cephalozia* and *Odontoschisma*. In other cases ventral branches of this type occur in connexion with lateral intercalary branches, as in *Cephaloziella Turneri*, or with terminal branches, as in the genera *Lepidozia* and *Bazzania*. When found in connexion with lateral branches the ventral branches are usually specialized in some way. In *Lepidozia*, for example, the ventral branches are almost always sexual, while in *Bazzania* they are mostly sexual or flagelliform. Even in this latter genus, however, ventral branches with normal leaves are occasionally produced, and Fig. 36, which is drawn from the Hawaiian *B. Brighami*, shows a branch of this kind and may be considered as representative of ventral intercalary branches in general. These branches, as Leitgeb long ago pointed out, are usually situated in the axils of underleaves or in corresponding positions in species where underleaves are not formed. In the example figured it will be noted that the underleaf with the axillary branch is strongly reflexed.

There seems to be no definite sequence in the leaves of a ventral intercalary branch ; neither is there any apparent relation between the spiral of a branch and that of the branching axis. The modifications present are similar to those described under lateral branches. Although ventral branches of this type usually occur singly in the axils of underleaves, in certain genera, such as *Calypogeia* (*Kantia*), two or three of them are sometimes found side by side. In other cases, such as *Cephalozia bicuspidata*, a branch may develop from any part of a ventral segment. Sometimes branches of this character arise directly from fully differentiated cortical cells and are thus destitute of basal sheaths. Such branches are to be regarded as adventive rather than as intercalary, although the distinctions between the two are not always hard and fast. Adventive branches in this position are essentially like those which sometimes spring directly from leaf-cells. They represent a type of regenerative process and scarcely come within the scope of the present paper.

Subfloral innovations in *Radula*, *Jubula*, and the Lejeuneae have already been spoken of in connexion with the *Radula* type of branching. They apparently arise whether fertilization has taken place or not. In many plants with intercalary branching subfloral innovations are also found, but their development, in certain cases at least, is dependent upon an absence of fertilization. Such innovations, which seem to be always intercalary in origin, usually occur singly, although groups of from two to four are not infrequent in certain species. They arise both in lateral and in ventral segments, the latter position being somewhat more common. The innovations are usually more robust than other intercalary branches, but show no further distinctive characteristics.

General Considerations.

From his study of the various types of branches in the Jungermanniaceae, Leitgeb ('75, '77) reached the conclusion that they had made their appearance in a definite phylogenetic sequence. He believed that branches of the *Frullania* type came first, that these were followed by branches of the *Radula* type, and that intercalary branches came last. He obtained support for these ideas not only from the Jungermanniaceae themselves but also from the Metzgeriaceae, the branches of which he homologized with those of the Jungermanniaceae. In the more primitive Metzgeriaceae, for example, such as *Riccardia* (*Aneura*) and *Pellia*, he considered the terminal branching to be essentially the same as that of the *Frullania* type; in more advanced genera, such as *Hymenophyton* (*Umbraculum*) and *Symphyogyna*, he distinguished terminal branches of both the *Frullania* and the *Radula* types; while in the ventral sexual branches of *Metzgeria* and *Hymenophyton* he found typical intercalary branches comparable with those of *Lepidozia* and *Cephalozia*. In the Metzgeriaceae, therefore, there is at least a vague indication that the types of branches which he regarded as the more recent are associated with genera which show other advanced characteristics.

If it is assumed that the Jungermanniaceae are descended from some thallose member of the Metzgeriaceae, it would be natural to conclude that the various types of branches were among the characteristics directly inherited. The probability of a polyphyletic origin of the Jungermanniaceae must, however, be kept in mind, especially in view of the fact that the leafy genera of the Metzgeriaceae are doubtless descended from more than one thallose ancestor. There is some evidence, for example, that the Jubuloideae (of Schiffner) are descended from the Metzgerioideae, while the Epigoniantheae came from such genera as *Pellia* and its allies. If this is the case it is of course possible that some of the primitive Jungermanniaceae came from thallose ancestors showing only one or two of the types of branching characteristic of the Metzgeriaceae as a whole. The result would be that the lines of development commenced by such primitive forms would either lack the types of branches which they failed to inherit or would acquire them independently. Probably both courses were pursued by different evolutionary lines. If it is admitted, therefore, that the branches appeared in the sequence indicated by Leitgeb, it must also be admitted that this sequence may have appeared in various distinct phyla.

Aside from the unsatisfactory phylogenetic evidence just brought forward, there is also ontogenetic evidence supporting Leitgeb's ideas of sequence. In a species where several types of branching are present it will be found that terminal branching tends to appear first in the life-history of an individual plant, and that branches of the *Frullania* type tend to come before those of the *Radula* type. This is well illustrated by both the Jungermanniaceae and the Metzgeriaceae. In *Lepidozia* and *Metzgeria*, for example, the terminal vegetative branches precede the sexual intercalary branches, while in *Bryopteris* the vegetative branches of the *Frullania* type precede the sexual and flagelliform branches of the *Radula* type.

Leitgeb concluded further that the various types of branches were genetically related to one another. He considered that the *Radula* type was derived from the *Frullania* type through a delay in the process of branch formation, and that intercalary branches represented the culmination of this tendency to delay. Even if the importance of such a tendency as an evolutionary factor is recognized, it is difficult to understand how one type of branching can have been directly derived from another. Ordinarily, when two organs are genetically related, they are alike at the beginning and acquire the differences which they show at maturity during the course of their development. In other words they represent diverging lines. This is clearly seen in such organs as the scale leaves and the foliage leaves of the higher plants, the earliest rudiments of which are quite indistinguishable. In dealing with structures of this kind, Goebel ('98–'01, p. 577) has shown by experiment that it is possible to alter the course of development of a particular rudiment. He has shown, for example, that a rudiment, which from its position on a growing point would normally develop into a scale leaf, can be acted upon in such a way that it will develop into a foliage leaf instead. Nothing of the sort is conceivable with regard to the branches of the Jungermanniales. These show their distinctive differences at the beginning and tend to become more and more alike as development proceeds. Instead of representing diverging lines of development they represent converging lines (except in cases of marked specialization), and it would be quite impossible to convert a young branch of the *Frullania* type into a branch of the *Radula* type. To do so would necessitate a change in the position of the first branch-segment or else in the direction of the branch-spiral, either of which would of course be out of the question. On the whole it seems most logical to consider the differences in the branches as really due to mechanical causes, which in some way determine the position of the first branch-segment and the direction of the branch-spiral. This would obviate the necessity of looking for a genetic connexion between the various types.

The development of a branch is undoubtedly to be looked upon as a response of the shoot to some kind of a stimulus. The different kinds of branches might then be due to the same stimulus acting upon segments of different ages or to distinct stimuli. To a certain extent at least the latter hypothesis seems to correspond better with the facts, and there are even reasons for believing that intercalary branches in different positions may sometimes be induced by unlike stimuli. These ideas are best supported by the study of species in which branching is a more or less unusual phenomenon. To illustrate the conditions under which terminal branches are developed *Cephalozia bicuspidata* may be selected, while *Plagiochila Sullivantii* and *P. asplenioides* may be chosen to illustrate intercalary branching.

In *Cephalozia bicuspidata* it will be found that terminal branching is not only associated with exceedingly young segments and with young individual plants, but that the conditions necessary for a vigorous vegetative development must be present before the appearance of such branching can be induced. Under ordinary circumstances, therefore, a plant will go through life without branching at all until the characteristic intercalary branches are formed. The plants discussed in the present paper, however, grew in places where there was an abundant water supply, together with the other factors favourable for growth. Apparently, in response to these conditions, terminal branches are of frequent occurrence. It would appear as if the increased stimulus for growth had acted upon some of the young segments in the same way that it acted upon the apical cell of the shoot, the result being that new apical cells became differentiated in the segments and thus gave rise to new terminal branches.

In *Plagiochila Sullivantii* and *P. asplenioides* the leafy shoots represent branches of a prostrate caudex. These shoots continue growing for a considerable period but usually remain unbranched. It will be found, in fact, that the very conditions which induce a vigorous apical growth and terminal branching in *Cephalozia bicuspidata* tend to inhibit the formation of intercalary branches in these species of *Plagiochila*. It is only when the apical growth is diminished or brought to an end, perhaps through the enfeeblement or death of the apical region, perhaps through the production of archegonia, that intercalary branches can make their appearance. Even under these circumstances branching rarely occurs. In his interesting researches on regeneration in the Hepaticae, Kreh ('09, p. 249) has recently brought out an important fact about *P. asplenioides* (and certain other species). He finds that, when fragments of a shoot are placed under favourable conditions, the regenerative branches appear in the axils of the leaves, that is, in the position where intercalary branches would normally occur. Of course, under these circumstances, the inhibitory action of the apical region has been rendered ineffective by direct removal. From these various observations it would appear that intercalary branches are not only associated with old segments and mature plants, but that the stimulus inducing branch-formation of this type must be distinct from the stimulus which induces a vigorous growth of the shoot, and therefore distinct also from the stimulus which causes terminal branching in such a species as *Cephalozia bicuspidata.*

Terminal branching in *Lophozia* is due to stimuli which are apparently similar to those indicated in the case of *Cephalozia bicuspidata.* In *L. Wenzelii*, for example, branches of the *Frullania* type are not infrequent. The plant grows in cold bogs and is therefore under favourable conditions for vegetative growth. In the closely related *L. alpestris*, which grows in drier localities, branching is much more unusual. In this connexion *Lophocolea heterophylla* may also be mentioned. The juvenile condition in this species, in which the leaves are uniformly bifid, is often greatly prolonged. During this period the conditions favourable for growth are present, and terminal branches occur frequently. As the plant approaches maturity the shoots develop irregularly lobed or undivided leaves, and finally the sexual organs with their bracts. After these changes, associated with reproduction and the cessation of vegetative growth, have begun to make their appearance, terminal branches become very rare.

In *Cephalozia*, *Lophozia*, and *Lophocolea* the formation of terminal branches cannot be looked upon as a firmly established habit. Certain species fail to show them at all, and even in species where they may occur it is often possible for an individual plant to go through life and produce organs of reproduction without being stimulated to develop branches of this character. It is very different with such genera as *Frullania* and *Lepidozia*, where the habit of forming terminal branches is firmly fixed. Here the branches arise at fairly definite intervals as long as the conditions necessary for ordinary growth are present. Even here, however, there are a few facts which point to a slight connexion between vigorous growth and branching. In plants growing in dense shade, for example, there are often more leaves between successive branches than in plants growing under more favourable conditions. In branches, also, the growth of which seems to be more or less inhibited by the vigorous development of the main axis, branches of a higher order less frequently occur. It is only when such branches overcome the inhibitory action and assume the characteristics of the main axis that they branch with the same degree of frequency. In the first case the conditions unfavourable for growth (and for branching) are due to an unfavourable environment, in the second case to correlative influences. The second case, however, must not be confused with the one described under *Plagiochila*, where the formation of intercalary branches is inhibited by the growth of the branching axis itself, and not by the growth of an axis of higher rank.

The antagonism between apical growth and intercalary branching, which is shown so clearly by *Plagiochila Sullivantii* and *P. asplenioides*, is usually present even in cases where intercalary branching has become a characteristic habit. To illustrate this point *Cephalozia bicuspidata* may again be selected. The sexual branches in this species, which are commonly intercalary and ventral, make their appearance when a certain degree of maturity is reached. Their development, however, is longer deferred in vigorous plants, and especially in plants so vigorous that terminal branches are present. An important difference between the *Cephalozia* and the two species of *Plagiochila* in this respect is that the antagonism in the *Cephalozia* is less strong, the result being that the inhibitory influence of the apical region is more easily and more regularly overcome.

The ventral flagelliform branches in *Bazzania*, which are also intercalary, stand in marked contrast to the branches just considered, although the sexual branches in the same genus are apparently dependent upon the same conditions as those of *Cephalozia bicuspidata*. These flagelliform branches make their appearance only a short distance away from the apical region, and there is no evidence that their development is inhibited by apical growth. In fact the stimulus which induces growth and the stimulus which induces branches of this character apparently act in harmony. From the consideration of this peculiar case, therefore, it seems very probable that the stimuli inducing intercalary branching are not always identical.

Summary.

Two distinct kinds of branching, terminal and intercalary, may be distinguished in the Jungermanniaceae. In terminal branching the branches arise in very young segments; in intercalary branching they arise in segments which are more or less mature.

Terminal branching includes four distinct types, characterized by the portion of the segment which takes part in branch formation. These four types are here designated the *Frullania* type, the *Microlepidozia* type, the *Acromastigum* type, and the *Radula* type, respectively. In the *Frullania* type the branch represents the ventral half of a lateral segment; in the *Microlepidozia* type, the dorsal half of a lateral segment; in the *Acromastigum* type, one of the halves of a ventral segment; in the *Radula* type, a portion only of the ventral half of a lateral segment.

In the *Frullania*, *Microlepidozia*, and *Acromastigum* types the branch is always accompanied by an incomplete leaf, which represents the other half of the same segment; in the *Radula* type the branch is accompanied by a complete leaf, which belongs to the same segment.

In the *Frullania* type the first branch-segment is ventral, and usually gives rise to an underleaf, the second segment is acroscopic (with respect to the branching axis) and gives rise to the first lateral leaf, while the third segment gives rise to the second lateral leaf, basiscopic in position. The branch-spiral is homodromous with the axis when the branch has arisen in an anodic segment-half, and antidromous when it has arisen in a kathodic segment-half.

In the *Microlepidozia* type the third branch-segment is ventral in position, and the branch-spiral is always homodromous with the axis because the branches of this type always arise in anodic segment-halves.

In the *Acromastigum* type the third branch-segment is ventral (just as in the *Microlepidozia* type), and the branch-spiral is homodromous or antidromous with the axis, according to whether the branch is situated in the anodic or the kathodic segment-half.

In the *Radula* type the first branch-segment is sometimes ventral, but usually lateral and basiscopic. The branch-spiral is usually sinistrorse on the left-hand side of a branching axis, and dextrorse on the right-hand side (when the shoot is viewed from the ventral surface), but subfloral innovations in the Lejeuneae sometimes show variations.

The leaves at the base of a terminal branch, especially one of the *Frullania* type, are more or less modified in form, in size, and in manner of attachment, some of these modifications representing reversionary tendencies and others special adaptations.

Intercalary branches may be either lateral or ventral in position. Their spirals show little or no relation to the spiral of the branching axis, and there is nothing definite about the position of the first branch-segment. The modifications at the base of a branch are reversionary in character.

There is some evidence (derived from phylogenetic and ontogenetic considerations) that the *Frullania* type of branching was the first one to make its appearance, that this was followed by the *Radula* type, and that intercalary branches came afterwards. It is possible that this sequence appeared independently in different developmental lines. A tendency to delay in the process of branch formation was apparently an important evolutionary factor.

There is evidence also that there is a harmonious relation of some sort between vigorous vegetative development and terminal branching, and that there is usually an antagonism between vigorous growth and intercalary branching.

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On a Palaeozoic Fern, the Zygopteris Grayi of Williamson.

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With Plates I–V and one Figure in the Text.

INTRODUCTION.

THE species *Zygopteris Grayi* was founded by Williamson in 1888 (Williamson, '89) on some specimens in a nodule from Oldham, which, though imperfectly preserved, enabled him to recognize the chief points in the morphology of the plant. Williamson had previously recorded a stem of the same type under the name of *Anachoropteris Decaisnii*, Renault (Williamson, '74, p. 699).

A much better specimen than any of those described by Williamson was found later by Mr. J. Lomax, who was also the discoverer of the type-specimens. Five sections of this stem are in Williamson's collection, but his intention of figuring it was never carried out.[1] Figures have since been published by myself (Scott, '00, Figs. 97, 98[2]), Dr. P. Bertrand ('09, Pl. XI, Fig. 78), and Dr. Kidston ('10, Figs. 1–4), but the specimen has never been fully illustrated.

In 1910 I received from Mr. Lomax a series of sections of a new specimen from a fresh locality—Shore, Littleborough, so well known for other interesting fossils with their structure preserved. The present paper is based primarily on the new specimen, with comparative references to the others and especially to the best Williamson specimen mentioned above. The question whether all known specimens can really be referred to the one species, *Z. Grayi*, will be discussed in the latter part of the paper.

It will be well to preface the detailed observations with a short summary of what is already known of the structure of the *Zygopteris Grayi* type.

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[1] I know of eight sections (all transverse) of this specimen, namely 1818 A and 1919 A to 1919 D in the Williamson Collection (see p. 66), 308 in that of Dr. Kidston, R. 443 in the Manchester Museum Collection, and 184 in my own. The proper order of these sections will be given below (p. 52). The sections of the type-specimens in the Williamson Collection are: 1817–1843 (excl. 1818 A) and 1919.

[2] Figs. 115, 116 in the second edition, Scott, '08.

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The stem has a very characteristic transverse section, owing to the form of the stele, which represents a five-rayed star with the rays or arms of unequal length (Pl. II, Phots. 11–13); each arm of the stele corresponds to the insertion of a leaf-trace; the phyllotaxis is $\frac{2}{5}$, and the longest arm is that which is next going to give off a trace, while the shortest arm is that which has last done so. The most prominent arms are truncate, the broad end assuming a bicornute form. The main mass of the wood consists of a wide zone of large scalariform tracheides; this zone, the peripheral xylem, encloses a central mass of tissue, which, like the stele as a whole, is stellate, having narrow protrusions which pass out into the projecting arms. Up to 1900 this central tissue was described as a pith, with the tacit assumption that it constituted a mass of parenchyma. This, however, has been shown not to be the case (Scott, '00, p. 281, Fig. 98[1]). The central tissue contains numerous tracheides, much smaller than those of the main zone of wood. They are accompanied by parenchyma and are ranged in an irregular ring round the centre of the stele: from this central ring radial tracts of small tracheides extend outwards, up the middle of each arm of the stele; it is among the internal tracheides of the arms that the protoxylem of the stem is situated. Groups of small tracheides also occur externally at the extremities of the more prominent xylem-arms; where the arm is truncate or bicornute they form two groups, one at each angle. The nature of these external groups of small xylem-elements will be discussed below; they are connected at certain points with the internal tracheides of the xylem-arms.

The phloem surrounds and follows the outline of the xylem. It contains a single or double band of large elements, no doubt the sieve-tubes, which, however, are for the most part limited to the bays of the stele, only extending round the xylem-arms where the latter are at their shortest, having just given off a leaf-trace.

Immediately surrounding the stele is a comparatively narrow zone of inner cortex consisting of thin-walled parenchyma; beyond this comes the broad and dense outer cortex, succeeded on the outside by a few layers of delicate hypoderma and the epidermis bearing multicellular hairs.

The stem bears appendages of four kinds: (1) the large foliage-leaves, of which only the bases are shown; (2) the 'axillary shoots' of Stenzel; (3) the small scale-leaves or aphlebiae; (4) the adventitious roots.

The bases of the foliage-leaves are of large size and have a marked effect on the outline of the stem; they are given off at short intervals. The whole form of the stele is, as we have seen, dependent on the course of the bundles supplying these leaves and their axillary shoots. The latter, the nature of which has been somewhat disputed of late (Kidston, '10), are a most characteristic feature, peculiar to the *Zygopteris Grayi* type among

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[1] p. 309, Fig. 116 in the second edition.

known fossil Ferns, but finding a close analogue in recent Hymenophyllaceae. Where the trace of a foliage-leaf is to be given off the stelar arm broadens out at the end and acquires two prominent angles or horns (Pl. II, Phots. 11 and 12). At the same time the large-celled xylem becomes continuous across the arm, cutting off the peripheral part of the internal xylem, which now forms an island (Phot. 12). When the trace detaches itself from the stele the line of separation passes through the large-celled xylem, which thus forms a closed ring in the leaf-trace, while it also remains closed in the arm from which the trace has parted (Phot. 13). Nothing of the nature of a leaf-gap is formed; a real leaf-gap is of course out of the question, as there is no true pith.

The leaf-trace, immediately on leaving the stele, as shown in Williamson's later specimen, is approximately triangular in transverse section with the base of the triangle outwards (Pl. II, Phot. 13; Pl. IV, Fig. 12). At the two abaxial angles are the groups of small tracheides already mentioned, and in the middle of the strand is the island of internal xylem and parenchyma. The abaxial groups are destined for the foliar bundle, while the central island belongs to the axillary stele.

The phloem closes round the outgoing trace as soon as it becomes detached, so that the bundle assumes concentric structure from the first (Pl. IV, Fig. 12). It may be called the undivided trace, for it represents the common base of the foliar bundle and of the axillary stele. The separation between the two takes place where the trace is passing through the outer cortex, at a level where the base of the leaf is already prominent (Pl. II, Phot. 11). The abaxial part of the common trace separates, to form the foliar bundle, the line of separation passing through the large-celled xylem, so that here, as in the departure of the trace from the stele, no gap is left in the strand behind. The foliar strand, as it becomes free, begins to assume the H form, but at first the shape is rather that of a very flat W with the base of the letter outwards, for the adaxial are longer than the abaxial 'antennae' and diverge from one another towards the axis (Pl. II, Phot. 12; Pl. V, Fig. 13). The phloem at once completes itself around both foliar bundle and axillary stele; the latter at this level has an almost circular or slightly elliptical transverse section, the major axis in the latter case being tangential with reference to the parent stem (Pl. II, Phot. 10; Pl. V, Fig. 13); the island of internal xylem persists throughout. A little higher up, the stele passes into the cylindrical axillary shoot, where it becomes free. I adhere to the terminology of Stenzel, reserving all morphological questions as to the nature of the leaf-trace and branch for future discussion (p. 57).

The scale-leaves or aphlebiae, discovered by Renault in 1869 in his *Zygopteris Brongniartii*, and by Stenzel in *Zygopteris scandens* twenty years later (Stenzel, '89), are seated both on the stem itself and on the base of the leaf. They are quite distinct from the foliage-leaves and much more numerous. The small trace supplying each aphlebia traverses the cortex with a steeply ascending course. These bundles, unlike those of the true leaves, have no effect on the general morphology of the stele. On entering the aphlebia the vascular strand undergoes one or more divisions. In calling the scales 'aphlebiae' it is implied that they are of the same nature as the outgrowths borne higher up on the rachis of allied leaves. As Paul Bertrand has stated ('09, p. 109), their vascular strands in *Z. Grayi* and *Z. scandens* are given off from the leaf-traces 'avant même leur individualisation complète'.

Lastly, there are the adventitious roots; they are much less numerous than the aphlebiae and their steles pass out almost horizontally through the cortex, a fact which enables them to be easily distinguished. The roots, so far as observed, are diarch; the root-stele is inserted laterally on an arm of the main stele near its extremity (see Pl. V, Fig. 14).

The above description would apply almost word for word to the *Zygopteris scandens* of Stenzel as well as to Williamson's species. Williamson, indeed, when he first saw Stenzel's paper of 1889, was inclined to assume that the two species were identical (Williamson, '89, p. 157). We shall find that the new specimen from Shore presents some slight differences from the described specimens of both species.

The old genus *Zygopteris* of Corda, characterized by the H or double anchor form of the foliar bundle, has been broken up in Dr. P. Bertrand's memoirs, and among the smaller genera created out of it are *Ankyropteris* (founded as a subgenus by Stenzel but redefined by P. Bertrand) and *Etapteris* (P. Bertrand, '09, pp. 206, 218). The distinction is based on the petiolar structure; the well-known species *Z.* (*Ankyropteris*) *bibractensis* and *Z.* (*Etapteris*) *Lacattii* may serve as types of each. Among the most important diagnostic characters of the two genera are:

Ankyropteris. Ramifications of the frond in two series, one on each side of the rachis.

Etapteris. Ramifications of the frond in four series, two on each side.

Ankyropteris. Peripheral loops of small-celled xylem permanently present on the flanks of the foliar bundle.

Etapteris. Peripheral loops absent.[1]

Ankyropteris. Adaxial longer than abaxial antennae. Middle band of bundle often curved, with concavity outwards.

Etapteris. Adaxial and abaxial antennae of equal length. Middle band of bundle straight.

The question whether *Zygopteris Grayi* belongs to *Ankyropteris* or *Etapteris* is disputed. Dr. P. Bertrand takes the former view and suggests that the petiole may possibly be identical with *Ankyropteris bibractensis*,

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[1] Except perhaps temporarily in *E. tubicaulis* (P. Bertrand, '09, p. 206).

var. *westphaliensis* (P. Bertrand, '09, pp. 106, 109). Dr. Kidston, on the other hand, identifies the petiole of *Z. Grayi* with *Z. di-upsilon*, Williamson, which is a typical *Etapteris* (Kidston, '10). As we shall see, the new specimen is an unquestionable *Ankyropteris*; as, however, it differs somewhat from those previously described, it does not by itself suffice to settle the point in dispute, and reference to the other specimens will be necessary.

Other questions on which the new specimen throws light are the morphology of the leaf-trace and axillary stele, the structure of the internal xylem, the position of the protoxylem, and the course of the bundles supplying the aphlebiae.

THE SHORE SPECIMEN.

We will now go on to describe the Shore specimen. The specimen is from an ordinary seam-nodule and is accompanied by fragments of *Lyginodendron*, *Lepidodendron* leaves, and other familiar objects of the coal-balls. In this respect it resembles Williamson's later specimen (see above, p. 39) and the fragment originally described by him in 1874, while it differs from the specimens on which the species *Z. Grayi* was founded in 1888; the latter were contained in a roof-nodule and are accompanied by Goniatite shells.[1] This difference may raise a doubt whether all the specimens of the *Z. Grayi* type really belong to one species. The part of the Shore specimen from which my sections were cut was about two inches in length; this piece was cut into twelve transverse and ten longitudinal sections, the latter coming immediately below the former. The transverse series is very good for following the whole process of the emission of the leaf-trace,[2] but it does not show perfectly the separation between leaf-trace and axillary stele; the latter, however, is seen very well, both in transverse and longitudinal sections (Pl. IV, Figs. 9 and 10), while the form and structure of the foliar bundle are fairly exhibited, though in oblique section (Pl. I, Phot. 9).

The general structure requires no long description, as it is in all essentials of the *Zygopteris Grayi* type, as recapitulated above. The maximum diameter of the stem is about 18 mm., that of the wood about 6 mm.

The five-rayed, stellate wood is rather regular in outline, for even the shorter arms are well marked (Pl. I, Phots. 1–6). The longer arms, about to give off a leaf-trace, are more conspicuously bicornute than in some other specimens, as corresponds to the form of the leaf-trace itself. A glance at Pl. I, Phots. 4–6, shows that the leaf-trace has a very different form from that in the figured specimens of *Z. Grayi* or *Z. scandens*. In these

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[1] In my review of Dr. P. Bertrand's Études sur la Fronde des Zygoptéridées (New Phytologist, vol. viii, 1909, p. 268) I erroneously stated that '*Z. Grayi* is a roof-nodule fossil'. This is only true of the type-specimens. The correction of this slip removes one objection to Dr. Bertrand's suggested identification of *Z. Grayi* with *Ankyropteris westphaliensis*; see, however, p. 57.

[2] A selection from the transverse series is shown in Plate I, Photographs 1–6, running from below upwards.

cases, as already mentioned, the undivided leaf-trace has a somewhat triangular transverse section, as shown in Pl. IV, Fig. 12; in the Shore specimen the section may be described as crescentic (Pl. I, Phots. 4–6, 8; Pl. III, Fig. 1) with the concavity outwards and the ends obliquely truncated. The bundle here, however, is obviously of the same nature as in the *Z. Grayi* type, for in Pl. I, Phot. 1 (*l.t., a.s.*), we see it in the act of dividing to form the axillary stele and foliar bundle, though the latter is imperfectly shown. The form of the undivided trace, though a striking feature of the new specimen, is not peculiar to it; a similar crescentic leaf-trace occurs in one of Williamson's type-specimens, as shown in Pl. II, Phot. 15, *l.t.* The $\frac{2}{5}$ phyllotaxis is obvious on comparing the successive sections (Phots. 1–6) and observing the position of the stelar arms from which leaf-traces depart. The phloem and inner cortex are badly or not at all preserved; in this respect the Shore fossil is inferior to the best Williamson specimen (see Pl. IV, Fig. 12; Pl. V, Fig. 13). The wide outer cortex has the same general character as in the latter, and is traversed by numerous aphlebia-bundles. In both plants the epidermis bears great numbers of multicellular uniseriate hairs (Pl. IV, Fig. 7). The adventitious roots appear to be given off in the same way in all the specimens (see Pl. V, Fig. 14, from Williamson's later specimen).

Structure of the Stele.

The general ground-plan of the wood is not at all unlike that in the Williamson type-specimens (Williamson, '89, Pl. I, Figs. 1 and 2, and Pl. II, Phot. 15, in the present paper); the resemblance to his later specimen is not quite so exact (cf. Pl. II, Phots. 11–13). The central tissue (' mixed pith ') has a diameter of little over 1 mm., and a pentagonal outline (Pl. I, Phot. 7). The internal system of tracheides forms an interrupted zone in the outer region of the mixed pith, enclosing a considerable amount of parenchyma (Pl. I, Phot. 7).

Pl. III, Fig. 3, is from a longitudinal section of the stele, cut somewhat tangentially, so that the plane of section coincides with one side of the zone of internal xylem, and several groups of internal tracheides are shown (*x.i.*).

From the central zone extensions of the internal xylem extend outwards, up each of the xylem-arms, forming the internal rays, the length of which varies according to the level at which the arm is cut. The internal rays are narrow in this specimen and the tracheides in them much compressed. The tangential diameter of the tracheides in the outer part of the internal ray ranges from 12 to 20 μ, the radial diameter from 18 to 60 μ. Some of these compressed tracheides show a spiral thickening, and no doubt represent the protoxylem of the stele.[1]

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[1] The protoxylem elements are best shown in the axillary stele. See Pl. IV, Fig. 10, *px.*

It is to be noted that the internal tracheides are of the normal elongated form and not short, as in the stem of *Diplolabis Römeri* described by Dr. Gordon ('11). The parenchyma accompanying the tracheides in the internal rays as well as in the central tissue consists of much-elongated thin-walled cells (Pl. III, Fig. 3).

The tracheides of the internal xylem-zone range from about 45 μ to 100 μ in diameter. The smallest, in the outer part of the zone, sometimes show a spiral thickening (Pl. III, Fig. 3).

The peripheral or main zone of wood consists entirely of large scalariform tracheides from 100 μ to 250 μ in diameter, the larger sizes predominating, if we leave the ends of the arms out of consideration. In the latter region the structure varies greatly according to level, as will be seen on examining any of the Photographs 1–6, Pl. I. A xylem-arm which has just given off a leaf-trace (arm 5, Phot. 5) shows practically no differentiation; the tracheides at the distal end are scarcely smaller than the others. On the other hand, an arm which is on the point of giving off a trace already has the characteristic leaf-trace structure in its distal part, which is about to become detached (arm 5, Phot. 3, and arm 4, Phot. 6). This structure consists essentially in the presence of peripheral loops, one at each extremity of the bicornute arm, and of an island of internal xylem on the median line (Pl. I, Phot. 8; Pl. III, Fig. 1). The intermediate arms show the various stages of transition from the undifferentiated to the differentiated structure, as will be explained in describing the process of emission of the trace.

Structure of the Leaf-trace.

The structure of the undivided leaf-trace is shown in Pl. I, Phot. 8, where it has just parted from the stele, Pl. III, Fig. 1, where it has already entered the cortex, and Pl. III, Fig. 2, at a point further out on its course. The trace passes out so gradually that it is seen practically in transverse section throughout. The changes in this part of its course consist in a slight broadening of the middle part of the trace and in a gradual lengthening of the 'antennae', the name given by Dr. Paul Bertrand to the extensions of the xylem forming the lateral bars of the H-shaped foliar bundle in the old genus *Zygopteris*. At the lowest level (Pl. I, Phot. 8) the antennae are scarcely present; the peripheral loops simply occupy the truncated ends of the lunulate bundle, facing obliquely outwards (cf. Phots. 4 and 5). At the level shown in Pl. III, Fig. 1, the antennae already form recognizable protrusions, especially at one end of the bundle; at a still higher level (Fig. 2) they are quite prominent; it will be noticed that at the level shown in Fig. 2 the abaxial is longer than the adaxial antenna, contrary to the usual condition in *Ankyropteris* (cf. Pl. V, Fig. 13).

The peripheral loop consists of a double or triple band of small scalariform tracheides (forming the 'filament' of Dr. P. Bertrand) (Pl. I, Phot. 8; Pl. III, Figs. 1 and 2, *x.e.*). At the ends, this band turns round and is continuous with the xylem of the antenna. The narrow space enclosed by the loop is occupied partly by very narrow tracheides, partly by a few elongated parenchymatous cells. The narrow tracheides inside the loop are no doubt the protoxylem; I have detected ill-preserved spiral elements in this position in a longitudinal section. The protoxylem appears to form two groups, one near each end of the loop (*px.,* Figs. 1 and 2, Pl. III), probably with one or more intermediate groups as well.

On the inner side the loop is bounded by the ordinary xylem of the antenna, the tracheides in this position being only a little smaller than elsewhere. It will be seen that the structure agrees quite well with that of the peripheral loops in *Ankyropteris bibractensis*, var. *westphaliensis*, as described by Dr. P. Bertrand.[1] The agreement is especially evident where the antennae have become prominent, as shown in Fig. 2.

The external xylem-band or filament sometimes appears to be confluent with the antenna for considerable distances, the interior space of the loop then not being continuous (Fig. 2).

The stages where the leaf-trace is separating from the axillary stele are not well shown in the new specimen. A trace in the act of division is present at *l.t.*, *a.s.*, in the section represented in Pl. I, Phot. 1; the axillary stele is clear, but the foliar strand is much damaged.

In the longitudinal series a foliar bundle is shown in very oblique section (Pl. I, Phot. 9). This is seen some little way above its separation from the axillary stele, which is present in another section of the series (part of this stele is shown in Pl. IV, Fig. 10). The foliar bundle represented in Phot. 9 has, in fact, already entered the petiole. Its slender proportions and long antennae agree well with the structure of the bundle in Williamson's original specimens (cf. Pl. II, Phot. 16). The peripheral loop is quite evident, and at once identifies the bundle as that of an *Ankyropteris*. In the comparative straightness of the middle band ('apolar') and of the antennae the bundle differs widely from *A. bibractensis*, var. *westphaliensis*, P.B.; the original *A. bibractensis* of Renault is intermediate in these respects (Renault, '69, Pl. IX).

The swelling on the adaxial antenna, near its junction with the middle xylem-band, marks the presence of a lateral bundle, destined for a secondary rachis (Pl. I, Phot. 9), as is more clearly shown in an adjacent section (2529). So far as can be seen, the mode of emission of the secondary bundle agrees with that described by Dr. P. Bertrand, but the obliquity of the sections leaves the details obscure. At any rate it is clear that the lateral strands were given off singly as in *Ankyropteris*, and not in pairs as in *Etapteris*.

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[1] '09, p. 74, Pl. IX, Figs. 62, 63, &c.

Another characteristic feature of the undivided trace is the presence of a median island or strand of internal xylem, accompanied by parenchyma (Pl. I, Phot. 8; Pl. III, Figs. 1 and 2, *x.i*). The island has an irregularly elongated shape, and one or more smaller islets may be detached from it (Phot. 8). These, however, die out as the trace is followed upwards, and the median strand becomes more regular. This strand is destined to form the internal xylem of the axillary stele (Pl. I, Phot. 1, *a.s.*; Pl. IV, Fig. 9). I have found it continuous at all levels, both in the Shore specimen and in the best one of Williamson's (Pl. IV, Fig. 12; Pl. V, Fig. 13). The doubt expressed by Dr. Kidston ('10, pp. 452, 454) as to the presence of a 'pith' at the place where the axillary stele separates from the leaf-trace is evidently to be explained by defects of preservation, as he himself suspected. It must, however, be emphasized that in no case is a true pith present in *Zygopteris Grayi*, either in the main stele, the undivided leaf-trace, or the axillary stele. In all cases the central tissue contains numerous tracheides, as proved by longitudinal as well as transverse sections.

The whole form and structure of the undivided trace in the Shore specimen show that this organ is essentially a *leaf-trace* rather than the stele of a branch. It constitutes, as already stated, the common base of the foliar bundle and the axillary stele; in the Shore specimen the foliar bundle characters predominate, while in the Williamson specimens, at least those which have been figured, the stele characters are more obvious, so that the whole has been regarded as a branch (compare Pl. III, Fig. 1, with Pl. IV, Fig. 12). Such an idea would scarcely have arisen if specimens like that from Shore had first come under observation. The question will be more fully discussed later in the paper (p. 57).

Emission of the Leaf-trace.

The whole process can be followed in the Shore specimen.

If we start with that arm of the stele which has last given off a leaf-trace (e. g. arm 5, shown in Pl. I, Phots. 4 and 5) we find that it is short, with a rounded outline, and without any sign of the peripheral loops. The corresponding internal ray penetrates the arm for half its length or more. Otherwise there is little or no differentiation. At a slightly higher level the internal ray extends further outwards, and the tracheides external to its termination become markedly smaller than the rest. The extension of the ray is at first one-sided (see Phot. 1, arm 1; Phot. 2, arms 1 and 2; Phot. 6, arm 5). This is the first indication of a peripheral loop. Further up, a second branch of the internal ray appears, so that it becomes forked. The branches very nearly reach the surface, from which they are only separated by bands of small tracheides (Phots. 3 and 4, arms 1 and 2). The structure at this level is of much interest, as we here see continuity established between the protoxylem of the stem contained in the internal rays and that of the leaf-trace enclosed in the peripheral loops. It must, however, be remembered that the approach of the protoxylem-rays to the surface of the wood is not only connected with the supply of the leaf-trace; continuity with adventitious roots has also to be established. Root-steles, for example, are given off from arm 1 at a level between that of Phot. 4 and Phot. 5.

The condition at a somewhat higher level, in a case not complicated by root-insertions, is shown in detail in Fig. 4, Pl. III.[1] Here the arm already has a squarer end than lower down. The internal ray (which is still continuous) is expanded, at the point where it forks, into a little triangular body, the first indication of the central strand destined for the axillary stele. Beyond this the two branches of the ray run out nearly to the surface.

Still higher up, the arm becomes cornute; the peripheral loops are lengthened and are here more evident as loops; they now become isolated, so far as the transverse section is concerned, by the closing up of the branch rays which connected them with the triangular body. This also becomes isolated by the closing up of the internal ray behind it, a change which may take place at a lower level. (See Phots. 3–5, arm 3; for the shutting off of the main ray at a lower level see Phot. 6, arm 1.)

The rest of the process may be followed rapidly in arm 5. At the bottom of the series (Phot. 1) this arm has long lobes, each of which is obliquely truncated and tipped by a peripheral loop; the loops are quite cut off from the median triangular strand, but the latter is still in connexion with the internal ray. Above this (Phot. 2) the ray is beginning to die out behind the strand, which is lengthening tangentially. At the next higher level shown (Phot. 3) the ray has completely closed up for a long distance and the median strand appears as an island, surrounded by the large-celled wood. The arm is here distinctly constricted about midway between the island and the end of the internal ray.

Here the trace separates from the wood, as seen in Phot. 4 and Phot. 8. The outward passage of the trace and the slight changes which it undergoes within the limits of the series require no further description.

From the facts observed it is clear that the peripheral loops are constituted by the two branches of the internal ray passing out nearly but not quite to the surface of the wood, the parts which enclose their termination becoming small-celled. The internal rays contain the protoxylem, which thus comes to lie inside the peripheral loops. The point of bifurcation of the internal ray supplies the median strand for the axillary stele. All these parts become isolated as we follow them further up. In other words, the 'undivided leaf-trace' receives three branches from the internal

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[1] Arm 2, from the section next above that shown in Phot. 6.

xylem and protoxylem of the stem; the two distal branches pass into the peripheral loops; the proximal branch takes a median position and ultimately supplies the axillary shoot. Thus the whole organization of the leaf-trace is dependent on the internal xylem of the stem, for it is from this that its first-formed elements are supplied.

Structure of the Axillary Stele.

As we have seen, the axillary stele is represented in the undivided trace by the median adaxial bulge with its central strand of small tracheides and parenchyma (Pl. I, Phot. 8; Pl. III, Figs. 1 and 2). The bulge becomes more marked as the trace passes further out (Pl. I, Phot. 6; Pl. III, Fig. 2).

At the beginning of the series of transverse sections, the axillary stele seen far out in the cortex is just separating from the leaf-trace,[1] and its outline is already clearly defined, the line of separation passing through the thick abaxial xylem (Pl. I, Phot. 1, *a.s.*). It has a somewhat elliptical central strand at this level. The same stele at a rather higher level, after its complete separation, is shown in Pl. IV, Fig. 9. Allowing for slight damage, the stele is approximately circular in section; the central strand ('mixed pith') has a somewhat angular outline, possibly an indication of its ultimate stellate form. The internal xylem appears to form a solid, rather irregular mass, and has not assumed the annular arrangement characteristic of the main stele. The position of the protoxylem is not easy to determine; some of the smallest elements lie on the outside of the internal xylem, but it is not certain that they all do.

The longitudinal section (Pl. IV, Fig. 10) shows the structure of another axillary stele, no doubt that belonging to the next lower leaf-trace. The broad outer zone of scalariform tracheides encloses the strand of small elements forming the internal xylem, and in one part the spiral tracheides of the protoxylem (*px.*) are evident. They appear to be adjacent to the parenchyma of the central strand, and thus to lie on the outside of the internal xylem.

The axillary stele, in this part of its course, thus shows the stelar structure of the plant in its simplest form. The essential features—peripheral and internal xylem-systems—are present, but none of the complications of form and tissue-arrangement belonging to the fully developed stele have yet appeared.

Some further points connected with the axillary stele will be considered when we return to the Williamson specimens (see p. 54).

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[1] This is the trace corresponding to arm 1 of the stem-stele.

The Aphlebiae.

Scale-leaves or aphlebiae are borne indiscriminately on the stem and the leaf-bases, unless indeed we regard the whole surface as covered with leaf-bases, an interpretation which the form of the transverse section scarcely seems to justify. The numerous aphlebia-traces which traverse the cortex give the best idea of the number of these appendages (Pl. I, Phot. 4).

The Shore specimen shows several aphlebiae in connexion with the stem, but not many after they become free. Fig. 7, Pl. IV, represents one just detached from the cortex, with which it is connected lower down in the series. There are two gaps in the tissue, symmetrically placed, in one of which a vascular bundle is contained; no doubt the second bundle has been lost. In another case an aphlebia contained three bundles, and there was some evidence of a lateral lobe having been given off. The extreme tangential extension of the bundle in the aphlebiae still attached to the stem (cf. Pl. III, Fig. 6) is no doubt preparatory to division; in some cases the fission had begun.

From the evidence of the Shore fossil and of the second Williamson specimen we may conclude that the aphlebiae were lobed bodies containing as many as three vascular strands—possibly more.

The origin of the aphlebia-trace has been investigated, confirming Paul Bertrand's statement that they are given off from the leaf-traces, even before the complete individualization of the latter. They spring from the arms of the stele below as well as above the point where these become free as definitive leaf-traces. Thus Plate III, Fig. 5, shows an aphlebia-strand just given off from, and still connected with, one of the peripheral loops of a leaf-trace (No. 5) just detached from the stele. The level of the section figured is immediately below that of Phot. 4, Pl. I. On the other hand, in Pl. III, Fig. 4, an aphlebia-strand is shown which has separated from an arm of the stele, still far below the point where the leaf-trace will be given off, and indeed at a level where the peripheral loops are not yet fully differentiated.[1] The strand is well preserved and appears to show some remains of phloem. Other cases of stele-borne, as distinguished from trace-borne, aphlebia-strands have been observed.

The course of the strand is steeply ascending, as is well shown in the longitudinal section (Pl. IV, Fig. 8), where such a strand can be followed almost the whole way from the stele through the cortex. Numerous aphlebia-strands are given off, in succession, from each leaf-trace or stelar arm. They form four series, two to each peripheral loop.

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[1] The drawing is from the section next above that represented in Pl. I, Phot. 6, and the arm is No. 2.

The text-figure gives a rough idea of the arrangement of six aphlebia-traces, of which the course was actually followed. They are lettered (A–F) in the order in which they were observed to be given off from the leaf-trace 5, to which they all belong. Probably, however, two additional strands from the same end as C and E were missed, owing to local damage.

The aphlebia-strand first appears as a bulge of the peripheral loop. On first becoming free its xylem appears to be solid; where any differentiation can be observed, the smallest tracheides are in the middle. As the strand passes further out it becomes tangentially extended, ultimately to an extreme degree, as shown in Pl. III, Fig. 6 from the outer part of the cortex of the stem. In this condition the xylem appears to form a flattened ring, with the smallest elements in the interior; in a longitudinal section there was some indication of laxly spiral elements (protoxylem) in this position. It is often noticeable that the tracheides on the abaxial side are larger than those on the adaxial side of the ring, a point of some slight interest, as the same difference exists, in a more marked degree, in the secondary strands of the rachis of *Ankyropteris* (P. Bertrand, '09, p. 85; Pl. X, Figs. 73 and 74). So far as I have been able to make out, the aphlebia-strands are given off essentially in the same way as the secondary rachis-strands, as described by P. Bertrand, i. e. without any interruption of the external xylem ('filament'). There thus seems to be a considerable analogy between the scale-like aphlebiae and the ordinary pinnae of the leaf.

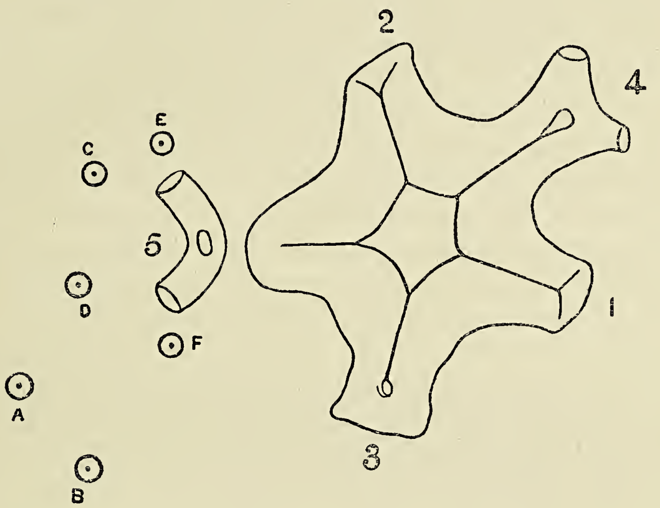

Diagram of stele, with the arms, numbered in kathodic order, the leaf-trace 5, and the aphlebia-strands (more crowded than in nature) given off from it (A–F).

The conclusions as to the aphlebiae are—

(1) that they are borne on all parts of the stem, without any special relation to the leaf-base;

(2) that their strands come off from the leaf-trace, both below and above its separation from the stele;

(3) that the protoxylem of the strand is central;

(4) that the strand divides into two or three branches when the aphlebia becomes free.

The Roots.

Adventitious roots are not very numerous in the Shore specimen; they are better shown in the second Williamson specimen; the illustration on Pl. V, Fig. 14, is from a section of this specimen from the Manchester Museum, kindly lent by Prof. F. E. Weiss. There appears to be no difference among the different specimens in the way the roots are borne. They spring laterally, as a rule, from the arms of the stele, the base of the root-stele occupying in some cases about half the length of the arm (Fig. 14). In the Shore specimen, where the peripheral loops are evident, it is seen that they contribute to the elements of the root-xylem (see above, p. 48).

The root-steles are given off on both sides of the stelar arm; sometimes two roots are opposite, in about the same plane. They arise from the arms at different levels, but I have not found them in connexion with the leaf-trace when free from the stele. They pass out horizontally.

As regards the structure of the root the evidence is still imperfect, for I have not yet seen a root in transverse section showing its connexion with the stem. In the Shore sections there are two or three roots near the stem which probably belong to it. They are about 1 mm. in diameter and diarch, with a very bulky xylem-plate, elliptical in transverse section. The cortex, if complete, is rather narrow, and sometimes has a pentagonal outline. Quite similar roots are also associated with the second Williamson specimen.

THE WILLIAMSON SPECIMENS.

The general description of the structure given in the earlier part of the paper is based essentially on the later Williamson specimen, the most perfect known before the discovery of the Shore fossil, and superior even to the latter in certain respects.

The eight sections of this specimen which I have seen are enumerated on p. 39. I have taken some pains to determine their correct order, which, from below upwards, appears to be as follows:

1. (7) Williamson 1919 D.
2. (8) „ 1919 B.
3. (1) „ 1919 C.
4. (2) „ 1919 A.

5. (3) Manchester R. 443.
6. (4) Kidston 308.
7. (5) Williamson 1818 A.
8. (6) Scott 184.

The only doubt is as to the position of sections 1919 D and B from the Williamson Collection; it is possible that they should come at the end of the series instead of the beginning. I have given the alternative order in parentheses. No question of importance is involved; the order of 6 of the sections is certain, and 1919 B clearly comes next above 1919 D (see Pl. II, Phots. 11 and 12).

The stem is considerably smaller than in the Shore specimen, having a diameter of about 12 mm. as against 18 mm., the wood measuring about 4 mm. as against 6 mm. The arms of the stele are rather shorter in proportion, and when a leaf-trace is detached only a slight protrusion is left (see Pl. II, Phots. 11–13, and compare with Pl. I, Phots. 1–6).

In the matter of preservation, the older specimen has the advantage as regards the phloem and the thin-walled inner cortex (see especially Pl. IV, Fig. 12, and Pl. V, Fig. 13). The row of dark cells in the figures cited is probably the endodermis. The outer cortex appears to have thicker walls than in the Shore specimen. On its outer border a few layers of periderm can be recognized in places. The main features of the anatomy need not be further described; they are evident from what has been said above, and from the figures.[1]

Williamson's second specimen is the typical example of that form of undivided leaf-trace which is approximately triangular in transverse section (Pl. IV, Fig. 12), thus differing widely from the Shore specimen with its crescentic trace (Pl. I, Phot. 8; Pl. III, Fig. 1). At one time I thought that this difference might be of specific value. I find, however, that among Williamson's type-specimens of 1888 both forms of leaf-trace occur. Thus, an approximately triangular trace is shown in the stem figured by Williamson ('89, Pl. I, Fig. 1), while in the stem of which a photograph is given in the present paper (Pl. II, Phot. 15) the trace (*l.t.*) is perfectly crescentic and agrees closely with those of the Shore plant. Hence, whatever the variation in form may mean, it is impossible to base a specific distinction upon it, for no one will suppose that Williamson's type-specimens, closely crowded together in the same block, belong to two different species. The form of the trace is, however, important, in so far as it has influenced the morphological interpretation of its nature. This question is discussed below (p. 57).

Returning for a moment to the later Williamson specimen, it may be worth mentioning that it differs from the Shore plant in the form of the

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[1] The anatomy of the stele is shown in my Studies in Fossil Botany, 2nd ed., Fig. 116, p. 309. See also Bertrand, '09, Pl. XI, Fig. 78.

aphlebia-strands. In the latter, these bundles, as they pass out through the cortex, become, as we have seen, much flattened and tangentially extended (Pl. III, Fig. 6). The second Williamson specimen shows nothing of the kind; the aphlebia-strands are only slightly elliptical in section, however far out in the cortex they may be. It is possible that the difference may be exaggerated by accidents of preservation, the strands in the Shore plant having to some extent collapsed, but a real distinction, though probably not an important one, certainly remains.

The Williamson type-specimens of 1888 agree in this respect, as in others, with the Shore fossil. The preservation of the cortex is very imperfect; the parts that persist are generally the external margin, the inner margin of the outer cortex, and a margin round the various outgoing strands, including those of the aphlebiae (Pl. II, Phot. 15). It is, however, evident that the aphlebia-strands are much flattened in the outer part of their course.

The type-specimens appear to be of the same variety or form as the Shore fossil, though the former came from a roof-nodule and the latter from a seam-nodule. The largest stem in the type is about 22 mm. in diameter, with the wood measuring 6 mm. The form of the stele agrees very closely (compare Pl. I, Phots. 1–6, with Pl. II, Phot. 15); as we have seen, crescentic leaf-traces occur in both and the aphlebia-strands are alike. There can be no doubt on the evidence that the new specimen belongs to the true *Zygopteris Grayi*, as founded by Williamson on the 1888 specimens. His later specimen, as we have seen, is a little different in detail, but not, as it seems to me, sufficiently so to warrant specific distinction.

Williamson's later specimen shows some points of interest as regards the axillary stele. The mode of separation between this stele and the foliar bundle is rather different from that shown in the Shore plant. In the latter, as we have seen, the undivided trace is a massive structure, and when the adaxial bulge is to be cut off as the axillary stele the line of separation simply passes through the thick band of the abaxial xylem (Pl. I, Phot. 1, *l.t.*, *a.s.*). In the other, the xylem is narrow and has to be thickened up by the addition of more tracheides, before separation can take place (Kidston, '10, p. 452; Pl. XXXIV, Figs. 3 and 4). Thus the formation of a gap in the xylem-band is avoided. Further, the axillary stele bears a much larger proportion to the foliar bundle at the point of separation than in the Shore plant. The stele and bundle a little above their severance are shown in Fig. 13, Pl. V. The structure of the stele is the same as at the corresponding level in the Shore specimen, but the phloem is well preserved, forming a ring round the xylem, and conspicuous by its large sieve-tubes. So far there is no indication of lateral appendages.

In Phot. 10, Pl. II, the stele is shown further out in its course, where the axillary branch is nearly free. Here the internal xylem is laterally extended, forming a band coincident with the major axis of the elliptical section, and tangential to the parent stem. The row of internal tracheides almost reaches the surface at either end of the band, while the main zone of xylem appears divided into two arcs; the large sieve-tubes are now limited to the adaxial and abaxial arcs, and are absent opposite the ends of the internal band. The structure strongly suggests that leaf-traces, distichously arranged, are about to be given off. The bilateral anatomy of the axillary shoot at this level presents the closest analogy with that of the stem in some recent Hymenophyllaceae, especially *Trichomanes radicans* and *T. reniforme* (Boodle, '00, Pl. XXVI, Figs. 22 and 24).

Unfortunately, in the section next above that from which Phot. 10 was taken, the axillary stele is destroyed; in the second section above, it is seen in oblique section and not quite complete;[1] it shows a relatively large 'mixed pith', with at least four prominent angles, and corresponding groups of internal tracheides; on the adaxial side, corresponding to one of the protrusions of the central tissue, there is a projecting arm or incipient leaf-trace, with an evident island of internal xylem near its end. It is thus clear that at this level the axillary shoot was already preparing to assume mature structure, with leaves in several series;[2] the distichous stage, indicated at a lower level, must have been passed through very rapidly. Intermediate conditions between that of the axillary stele (as seen at its base) and the normal stellate form have been previously figured by Williamson ('88, Pl. I, Fig. 5, see p. 157) and Stenzel ('89, Taf. VI, Fig. 53; see '96, p. 71).

ZYGOPTERIS GRAYI AN ANKYROPTERIS.

We have seen that the Shore fossil is clearly an *Ankyropteris*, as shown by the manifest presence of the peripheral loops, characteristic of that genus, on the leaf-trace, confirmed by such slight indications as we have of the arrangement of the pinnae on the rachis (see above, p. 46).

The question remains whether the *Ankyropteris* characters are common to all the specimens known. Dr. P. Bertrand includes the plant in *Ankyropteris*, and in his description, based immediately on the later Williamson specimen, he states that the leaf-trace has two poles, *interior to the wood*, on its posterior (abaxial) side, situated exactly at its extremities. He adds that, after the separation of the axillary strand, each of the poles divides into two, and that this division is the point of departure of the formation of two peripheral loops (Bertrand, '09, pp. 108, 109).

In a letter dated September 25, 1911, Dr. Bertrand informed me that

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[1] The three sections are W. 1919 A, R. 443, and Kidston 308. See list above, p. 52.

[2] It is interesting to find that in this section (Kidston 308) the cortex of the axillary shoot contains several aphlebia-bundles, which are absent from it at a lower level. Evidently, as soon as the first leaf-traces of the shoot began to be differentiated, the inevitable aphlebia-strands appeared with them.

he had strong reasons for believing that Williamson's type-specimens also belonged to *Ankyropteris*. He sent me photographs of Williamson's section 1824, showing that a petiole of the plant had evident peripheral loops I at once consulted the original section, and satisfied myself that this is the case. The section has been rephotographed by Mr. Tams, and is shown in Pl. II, Phot. 16. The petiole in question is a perfectly typical one of *Z. Grayi*, and, as Dr. Bertrand pointed out to me, is identical with the petiole of which another section is figured by Williamson ('89, Pl. I, Fig. 4). There is little doubt that this petiole belongs to one of the associated stems.

A glance at Phot. 16 will show that the band of small elements forming the external xylem (*x.e.*) of the peripheral loop is perfectly evident, especially on the left-hand side.

The proof that Williamson's type-specimens of *Zygopteris Grayi* belonged to the genus *Ankyropteris*, as now defined, is entirely due to Dr. P. Bertrand, to whom I am much indebted for calling my attention to the critical section.

The difficulty is greater in the case of the later Williamson specimen. The preservation of this specimen, good as it is for most purposes, is such as to render it difficult to make out the arrangement of the small external tracheides. When I described the structure of this stem in my 'Studies in Fossil Botany', I was inclined to regard these elements simply as protoxylem. On a careful re-examination of all the sections, I have observed cases where peripheral loops appeared to be present on the leaf-trace, but scarcely any were absolutely decisive, as it was seldom possible to distinguish with certainty between the supposed elements of the external xylem ('filament') and those of the protoxylem on the one hand or the inner phloem on the other.

The leaf-trace shown in Pl. II, Phot. 14, seems, however, fairly conclusive. The position of this bundle (*l.t.*) relative to the stele is shown in the general sketch, Fig. 15, Pl. V. One corner of the trace is obliquely truncated, recalling the leaf-traces of the Shore specimen. The truncated end is coated by a band of small elements, apparently tracheides (see Pl. II, Phot. 14, *x.e.*, and the slightly diagrammatic drawing of the same in Pl. IV, Fig. 11, *x.e.*). The appearance is remarkably like that of one of the peripheral loops of the Shore specimen, though of course less clear (cf. Pl. I, Phot. 8, and Pl. III, Fig. 1).

Taking the whole of the evidence into consideration, I feel no doubt that Dr. P. Bertrand was right in his interpretation, and that this specimen, like the others, has peripheral loops and is therefore an *Ankyropteris*. As we have seen, this conclusion is now established as regards the Shore fossil and the type-specimens of Williamson; I believe it may safely be extended to all specimens of the *Z. Grayi* type.

I think it probable, however, that there was a slight difference between the later Williamson specimen and that from Shore, the peripheral loops not becoming clearly differentiated so low down in the course of the leaf-trace in the former as in the latter. Dr. Bertrand's own description clearly points to a rather late differentiation in this respect.

The petiolar bundle of the type-specimens (Pl. II, Phot. 16) is almost identical in form with that of *Zygopteris di-upsilon* (Williamson, '80, Pl. XXI, Fig. 90), with which Dr. Kidston identified *Z. Grayi* (Kidston, '10). The resemblance is so striking that this conclusion appeared well founded at the time. Dr. Kidston, like myself, had not then recognized the presence of peripheral loops in *Z. Grayi*. *Z. di-upsilon* is a typical *Etapteris* (P. Bertrand, '09, p. 148; Pl. I, Fig. 6; Pl. XVI, Fig. 110), and, as such, has no peripheral loops and gives off four series of secondary strands from the foliar bundle. It cannot therefore belong to *Zygopteris Grayi*, which is shown to be an *Ankyropteris* by the presence of peripheral loops, and by there being (so far as the available evidence shows) only two series of secondary rachis-strands.

Dr. P. Bertrand ('09, p. 106) suggested, with much reserve, the possibility that the petiole of *Z. Grayi* might be identical with the well-known *Z. bibractensis*, var. *westphaliensis*. The differences in the form of the foliar bundle are obvious; the middle band of the xylem ('apolar') is straight in *Z. Grayi*, curved in *Z. westphaliensis*; the lateral bands (antennae) are long and but little curved in *Z. Grayi*, while in *Z. westphaliensis* they are strongly bent inwards towards the plane of symmetry; further, the antennae are of approximately equal length in *Z. Grayi*, while in *Z. westphaliensis* those on the convex (presumably adaxial) side of the median band are considerably longer than those on the concave (abaxial) side. Nothing has yet been observed to indicate that the one form passed over into the other, and there is a strong presumption that the species are distinct.

THE MORPHOLOGY OF THE LEAF-TRACE AND AXILLARY STELE.

There are two views of the nature of the organ which throughout this paper has been called the 'leaf-trace' or the 'undivided trace'. On the one view, which was that of Stenzel,[1] the first to observe the facts, this strand is essentially a leaf-trace, directly continuous with the foliar bundle, but modified, to a certain extent, by the presence of a branch, the stele of which is given off from the leaf-trace on its adaxial side and a little above its base. From its position the branch is consistently termed the axillary shoot, and its vascular strand the axillary stele.

On the alternative view, the meristele which we have called the leaf-trace is, from its base, of an axial nature, constituting the stele of a branch; the true leaf-trace is given off from it as a lateral appendage. On this

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[1] '89, p. 35; '96, p. 31.

interpretation there is no ' axillary ' organ at all ; the branching is regarded as an unequal dichotomy, of which the main stem and the smaller (so-called 'axillary') shoot are the two limbs. The leaf associated with the smaller branch is the first appendage of that branch and not its subtending organ.

This interpretation was mentioned by me in 1908 [1] as a possible one ; I then regarded the data as insufficient to settle the question. Dr. Kidston ('10, pp. 451, 454) has adopted this view ; he speaks of our ' undivided leaf-trace ' as a ' branch ', and describes the petiole trace as an independent organ arising from the periphery of the ' branch ' (i. e. branch-stele).

The interpretation of the branching of *Z. Grayi* as a dichotomy is supported by the analogy of *Z. corrugata*, in which, to all appearance, a real and equal dichotomy of the stem and its stele occurs.[2]

After the investigation of the Shore specimen of *Z. Grayi*, I can no longer regard the question of the morphology of these organs as an open one. The evidence of that specimen seems to me decisive in favour of the view that the meristele, from its base upwards, is a leaf-trace. The peripheral loops are essential parts of the leaf-trace or foliar bundle ; they have nothing to do with the stem or its branches. Now we have seen that in the Shore specimen the peripheral loops are already fully differentiated where the meristele first becomes free from the stele, and even at a considerably lower level. It is impossible any longer to contend that an organ with these characteristic foliar structures can be of the nature of a branch. The general form of the meristele further supports the obvious leaf-trace interpretation. What is true of the Shore specimen must moreover hold good for the other specimens also. In the type specimens, as we have seen, leaf-traces of the same form as those of the Shore fossil occur. In the later Williamson specimen the form of the meristele is different, and the peripheral loops perhaps differentiated later; they exist, however, where the meristele is still undivided ; the relation of the meristele to the main stele on the one hand and to the axillary stele and foliar bundle on the other is the same as in the Shore plant.

It thus seems to be established that in *Z. Grayi* the meristeles given off from the arms of the stem-stele are leaf-traces, altogether homologous with those of *Z. corrugata* or *Diplolabis Römeri*. With the former plant especially, there is a close agreement in the mode of emission of the leaf-trace, if we allow for the difference in the form of the stele from which it is given off (P. Bertrand, '09, p. 110 ; Pl. XII, Figs. 87–9).

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[1] Studies in Fossil Botany, 2nd ed., p. 318.

[2] This seems to be rare. I only know of one specimen (to be described fully in a forthcoming paper) which shows the branching in this species. Dr. Kidston's citation ('10, p. 454) of the brief reference to the fact in my Studies (p. 318) might be understood to imply that I regard the *leaf-traces* in *Z. corrugata* as of the nature of a dichotomy. This is not, of course, the case ; the forking of the stem involves an equal division of the stele and has nothing to do with the well-known process of the emission of a leaf-trace.

The conception of the meristele as a leaf-trace keeps the *Zygopteris Grayi* type in line with all the other Zygopterideae, and is in agreement with all the facts, as, for example, with the $\frac{2}{5}$ phyllotaxis.

On the branch interpretation, the arrangement of the appendages would not be a phyllotaxis at all, but a caulotaxis—a system of dichotomies in which the successive smaller limbs followed a $\frac{2}{5}$ divergence—a strange phenomenon for which we should be puzzled to find an analogy. Also, the branch theory would compel us to assume that the main stem was leafless, or restricted to scale-leaves (like a *Pinus*), while the foliage-leaves were limited to the lateral branches or smaller limbs of the dichotomy. All these are sufficiently improbable assumptions where Ferns are concerned, and, even without the evidence of the Shore specimen, would almost drive us to accept the simple and intelligible view that the leaves are borne directly on the stem.

What then are we to make of the axillary branch? Although it certainly does not give rise to the leaf-trace it must undoubtedly have some effect on the structure of the latter in the basal region. In the case of Williamson's later specimen especially, the effect is very marked, disguising to a considerable extent the leaf-trace character of the meristele; it led to the whole organ being regarded as the stele of a branch. In the Shore specimen, on the other hand, as we have seen, the leaf-trace characters predominate throughout, and the presence of an axillary shoot is only indicated in the meristele by a slight adaxial bulge and by the median island of internal xylem. It is not even certain that the latter is *necessarily* connected with the axillary stele, for a very similar central island appears in the leaf-trace of *Z. corrugata*, which has no axillary stele (P. Bertrand, '09, Pl. XII, Fig. 88); in this case, however, it soon dies out.

We may, if we like, regard the axillary stele as 'adherent' to the adaxial side of the leaf-trace, but this is a mere phrase and throws no new light on the facts.

The analogy with the axillary branching of the Hymenophyllaceae has been often insisted on (see especially Boodle, '00, p. 487), and appears to be a very close one. In the recent family the axillary shoot is sometimes abortive (Chambers, '11), and possibly, as Stenzel thought, this may also occur in *Zygopteris*, though certainly not as a rule.

Axillary branching also takes place occasionally in Ophioglossaceae (*Botrychium*, Holle, '75, p. 313, Taf. III, Fig. 4; *Helminthostachys*, Gwynne-Vaughan, '02). Judging from sections kindly shown me by my friend Prof. W. H. Lang, F.R.S., there seems no doubt that here also there is a decided analogy with the fossil forms.[1] It is interesting that the axillary branching occurring in *Zygopteris* should find its parallel in two recent

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[1] Professor Gwynne-Vaughan's suggestion that the axillary organs which he found in *Helminthostachys* were rudimentary branches appears to be fully confirmed by Professor Lang's observations.

families with which an affinity has been recognized by most investigators, from Renault onwards.

Though the branching of *Zygopteris Grayi* and its allies is not a dichotomy, it may have originated from a dichotomy, the association of the smaller branch with a subtending leaf being a derivative condition. On this view the equal dichotomy of *Z. corrugata* would be the more primitive state, as seems natural. On the other hand, it is not impossible that the case of *Z. corrugata* may be one of modified axillary branching. I hope to return to this question in a subsequent paper.

THE MORPHOLOGY OF THE APHLEBIAE.

Both Renault and Stenzel regarded the scale-leaves which they discovered in *Zygopteris Brongniartii* and *Z. scandens* respectively as abortive or reduced leaves, and therefore as comparable to the normal foliage-leaves of the plant. This view is no longer tenable. In *Z. Grayi*, as we have seen, the scale-leaves (aphlebiae) occur on the leaf-base as well as on the stem; their vascular strands are given off from the leaf-traces of the foliage-leaves and are not independent leaf-traces. Dr. P. Bertrand's view that the scales have the value of secondary petioles seems fully justified (P. Bertrand, '11, p. 53). Their vascular strands are included by him under the name 'sorties hâtives', i. e. they are secondary strands given off 'in a hurry' or below the point where the normal pinnation of the leaf begins. We have seen above that the structure of the aphlebia-strand is not unlike that of the secondary or tertiary rachis-strands in allied species, and that its mode of emission appears to be the same. The aphlebiae, then, are best regarded as modified pinnae of the leaf which have spread downwards on to the stem, but are always in definite relation, by their vascular strands, with the leaf-traces.[1] That they are modified is shown by the fact that their lamina contains two or three bundles (resulting from the division of the single supply-strand), while in all allied plants the normal pinnae are monodesmic. The function of the aphlebiae may have been a transitory one, the protection of the growing points and young leaves; in fact, they may well be analogous to bud-scales, which are often of the nature of stipules. The aphlebiae themselves might be described as multiple stipules.

Dr. Bertrand's observations appear to have cleared up the mystery which once hung about these curious organs; we need no longer feel tempted to interpret them as representing an ancestral form of foliage such as the phylloids of Lignier, which hypothetically once covered the thallus before its differentiation into stem and frond.

It is very interesting to find that aphlebiae have now been discovered

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[1] It is true that the aphlebia-strands are given off in *two* rows from each peripheral loop of the trace (unlike the pinna-strands of the *Ankyropteris* leaf); no two aphlebia-bundles, however, appear to be given off *at the same level*.

by Dr. Margaret Benson ('11) in a species of *Botryopteris*, *B. antiqua*, in which they are found to be associated with the simpler monarch form of petiole, which in this species alternates with the larger diarch petioles. These remarkable organs were evidently very widely spread among the Primofilices; how far they were truly homologous with the aphlebiae, so well known in their external form on impressions of the frond in other Palaeozoic Ferns and Pteridosperms, can only be determined when the structure of the latter has been further investigated.

AFFINITIES.

There are two other species of the same type as *Zygopteris Grayi*, namely, the *Z. scandens* of Stenzel and the *Z. Brongniartii* of Renault. The former is so closely similar to our species that Williamson thought it the same, and no one has yet found thoroughly satisfactory distinctive characters, though the horizons are so far apart (Lower Coal Measures and Permian) that specific identity is improbable. Dr. Kidston ('10, p. 455) enumerates several points of difference, but some of these are only distinctive on the assumption that *Zygopteris* (*Etapteris*) *di-upsilon* is the petiole of *Z. Grayi*, which, as we have seen, cannot be the case, the latter being an *Ankyropteris*. From sections of *Z. scandens* in my possession I have not been able to find constant distinctions; possibly a reinvestigation of the type-specimens might lead to a more definite result. It appears to be commonly admitted that *Z. scandens* is an *Ankyropteris*; this is probably the case, though the evidence is much less direct than is now the case with *Z. Grayi*; here, again, a further investigation of Stenzel's plant is desirable, though the close agreement with Williamson's species leaves no real doubt as to the genus being the same.

Z. Brongniartii, Ren., differs from the other species in the form of the stele, in which the angles are not at all prominent (Renault, '69, Pl. III, Fig. 1). This seems a well-marked distinction, though Stenzel ('96, p. 31) thought that all three species might be referable to one. There appears to be no independent evidence to show whether *Z. Brongniartii* is an *Ankyropteris*. There is a probability that it is so, but at present this only rests on the general similarity to *Z. Grayi* in structure and mode of branching.

Zygopteris corrugata, on the other hand, is certainly a typical *Ankyropteris*, as Dr. Bertrand has shown, but differs from *Z. Grayi* in its cylindrical stele, dichotomous branching, and other points.

The stem of the Lower Carboniferous *Metaclepsydropsis duplex*, of which a full description by Dr. Gordon ('11^2) has just appeared, bears a general resemblance to that of *Ankyropteris corrugata*, the tracheides of the internal xylem being here also associated with parenchyma. Another Lower Carboniferous plant, *Diplolabis Römeri*, of which Dr. Gordon has given an exhaustive and admirable account (Gordon, '11), has a solid xylem without any parenchyma, but there is a marked differentiation between the inner and the outer xylem ; the former consists of smaller elements, which are quite short, and presumably served for the storage rather than the conduction of water. The complex structure of the petiole shows beyond doubt that the plant is a Zygopterid ; Dr. Gordon regards the stem-structure as the most primitive yet known in the family. So far as the solid xylem is concerned this may well be true ; in the shortening of the internal tracheides, however, *Diplolabis* shows a differentiation not yet observed in *Ankyropteris*, where these elements are, usually at least, of the ordinary elongated form. Also, as Dr. Gordon has pointed out, *Diplolabis* and *Metaclepsydropsis* have quadriseriate pinnae, while in *Ankyropteris* they are biseriate. It therefore appears that these three genera cannot belong to the same direct line of descent. Dr. Gordon's suggestion (Gordon, '11^2) that *Diplolabis* and *Metaclepsydropsis* led on to *Zygopteris Grayi* involved the assumption that the latter species is an *Etapteris*, whereas, as we have seen, it belongs to *Ankyropteris*. Of the stem of *Etapteris* we know nothing as yet.

I am inclined to agree with Dr. Gordon's general line of argument in deriving the stem-structure of Zygopterideae from a simple protostele like that of *Botryopteris*, though, as we shall see presently, Dr. P. Bertrand takes a totally different view of the relationships.[1]

The most interesting comparison, perhaps, is that between *Ankyropteris Grayi* and *Asterochlaena*, a genus very imperfectly known until 1911, when Dr. P. Bertrand published a magnificent monograph on the Permian species *Asterochlaena laxa*, Stenzel. This fine fossil is on a much larger scale than our *Ankyropteris*, the stem (apart from the leaf-bases) reaching 50 mm. in diameter. The xylem, as is well known, is of the most extreme stellate form, with long spidery arms. The number of xylem-arms at the periphery of the star ranges from eight to thirteen ; they anastomose towards the centre. There is, however, no relation between the form of the star and the phyllotaxis, for each xylem-arm gives off two or three series of leaf-traces ; the arrangement of the bi- and tri-lobed arms is irregular ; a unilobed arm has only once been observed with certainty. The number of orthostichies ranges from twenty to twenty-seven ; in some specimens the phyllotaxis is whorled, in alternating verticils of ten or eleven members ; in the others it is described as 'subverticillate', the number of orthostichies being uneven.

In spite of these striking differences from *Ankyropteris*, many equally remarkable points of agreement exist. There is a 'mixed pith' at the centre of the star, and from this, median protoxylem-bands radiate, passing up the middle of each arm ; where the arm forks the protoxylem-bands

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[1] The discussion of the curious stem of *Zygopteris Kidstoni* (P. Bertrand, '11, p. 55), which is described as having a *stellate solid* xylem, will be best postponed till fuller information is available.

form a triangle. Dr. Bertrand believes that the median protoxylem-bands are in connexion with the protoxylem which passes out in the peripheral loops of the leaf-traces, though the connexion is often obliterated. The true protoxylem elements are accompanied by narrow scalariform tracheides, exactly as in *Ankyropteris Grayi*. There is, however, a difference in the fact that short, globular tracheides are also present in the 'mixed pith' of *Asterochlaena*; I have not found these in our plant.

Roots are inserted laterally on the leaf-trace, just where it leaves the stele, but they also occur on other parts of the xylem-arms. The structure of the roots exactly agrees with that of the roots associated with *Ankyropteris Grayi*.

The outgoing leaf-trace gives off numerous lateral strands, which pass out in advance of the trace itself. Dr. Bertrand ('11, p. 53) points out the manifest agreement with the strands in *Ankyropteris* which supply the scales (aphlebiae). In both cases the organs thus supplied have the value of secondary petioles.

Asterochlaena might be described as a multiple *Ankyropteris*, for it differs most essentially from such a plant as *Ankyropteris Grayi* in the fact that each arm of the stele supplies two or three vertical series of leaf-traces instead of one.

Dr. Bertrand, it is true, does not recognize so close an agreement between the two genera as seems to me to exist. He states (p. 50) that the Asterochlaenas are the only known Ferns which possess a system of protoxylem-bands proper to the stem. It seems clear that *Ankyropteris Grayi* and its allies are precisely in the same position. He also gives an interpretation of the *Ankyropteris* vascular system which separates it from *Asterochlaena*. In *A. Grayi* he regards the wood as constituted of five curved plates, concave outwards, the five 'réparatrices', each of which represents the left-hand 'demi-réparatrice' of one xylem-arm and the right-hand 'demi-réparatrice' of the next ('11, p. 52, Fig. 8; see also the text-fig. in this paper, p. 51). These 'réparatrices' alternate with the 'génératrices foliaires' (vertical lines of leaf-traces). On this view each leaf-trace is supposed to be supplied by the two xylem-bands lying to the right and left. In *Asterochlaena*, on the other hand, the simple 'réparatrices' are placed directly behind the 'génératrices' in the same radial plane (p. 53). Personally I can see no distinction in this respect, except that the xylem-arms are longer in *Asterochlaena*.

This whole conception of the *Ankyropteris* stele seems to me defective, for it ignores the internal xylem, which here, just as in *Asterochlaena*, 'represents, as it were, the skeleton of the xylem-star' (P. Bertrand, '11, p. 36).

The object of Dr. Bertrand's somewhat artificial interpretation of *Ankyropteris* structure appears to be to bring it into line with the vascular system of Osmundaceae; probably, however, the relation is too indirect for any such detailed comparison to be admissible.

Finally, some reference must be made to Dr. Bertrand's theory of the general course of evolution of the Zygopterideae.

Asterochlaena, though complex in the structure of the stem, has a simple form of petiolar bundle, the xylem forming a curved band, concave towards the stem, with a peripheral loop, containing the protoxylem, near each end, and slightly towards the abaxial surface. The only simpler foliar bundle of this group is that of *Clepsydropsis*, which is straight, and is thus symmetrical about two planes, while the bundle in *Asterochlaena* is only symmetrical about one plane (the radial).

Dr. Bertrand ('08) believes that *Clepsydropsis* is the petiole of *Cladoxylon*, though the two have not been found in connexion, and the proof is still incomplete. *Cladoxylon* has a very peculiar polystelic structure, the steles having, on the whole, a radiating arrangement. Dr. Bertrand holds that the xylem-star of *Asterochlaena* was probably derived by condensation from the dispersed xylem-system of *Cladoxylon*. A greater condensation, he adds, would clearly give rise to a solid or annular mass of wood with a circular contour ('11, p. 54). Both in *Diplolabis Römeri* and *Ankyropteris corrugata* he derives the circular xylem from a stellate xylem by condensation.

He appears, however, to regard the *Ankyropteris Grayi* type not as an intermediate stage of condensation, but rather as a specially high form of differentiation (see p. 56). He supposes that phenomena of condensation have occurred simultaneously in all the parallel series of the family. The more highly differentiated members may be expected to show—

(1) a solid xylem,
(2) a continuous xylem-ring, or
(3) a xylem-ring differentiated into alternate reparatory and leaf-generating regions, this being the highest type of all.

In the more primitive members of the family, on the other hand (i. e. those nearest to *Clepsydropsis*), we shall be likely to find a stellate vascular mass. Dr. Bertrand enumerates *Diplolabis*, *Tubicaulis* (which has a protostele), and *Ankyropteris* among the advanced Zygopterideae, *Asterochlaena* and *Zygopteris Kidstoni* among the primitive forms.

I do not propose to discuss this interesting theory in detail; a better opportunity may be found in a future communication. It is necessary, however, to point out that two opposite views are held: Dr. Bertrand's theory of condensation from a primitive polystelic structure, and Dr. Gordon's theory (shared, I believe, by most palaeobotanists) of the gradual elaboration of an undifferentiated protostele. Dr. Bertrand's view really depends on the truth of his attribution of the simple petiole *Clepsydropsis* to the complex stem *Cladoxylon*; it will scarcely command general assent until this identification has been placed beyond doubt.

In the meantime it seems to me to be established that a somewhat close affinity exists between the genera *Ankyropteris* and *Asterochlaena*. We cannot of course derive one from the other ; *Ankyropteris* has advanced along the line of high differentiation of the petiolar strand, while *Asterochlaena* is distinguished by the complexity of the vascular structure in the stem.

SUMMARY.

1. *Zygopteris Grayi* is a member of the genus *Ankyropteris*, as shown especially by the presence of peripheral loops on the leaf-trace. The resemblance of the petiole to *Zygopteris* (*Etapteris*) *di-upsilon* is therefore illusory.

The opinion of Dr. P. Bertrand is thus confirmed.

2. The protoxylem of the stem is situated in the internal rays of the xylem-arms, and passes out thence into the peripheral loops of the leaf-trace, which are differentiated before the trace leaves the stele.

3. The protoxylem of the axillary stele is likewise directly continuous with that of the internal rays of the main stele.

4. The undivided leaf-trace or meristele is, from its base upwards, of the nature of a foliar strand and not of a branch-stele. Its structure is, however, affected to a varying extent by the presence of the axillary stele.

5. The branching is rightly described as axillary rather than as dichotomous.

6. Internal xylem is always present, both in the main and the axillary stele. There is at present no evidence for the existence of a true pith, without internal tracheides, in any member of the Zygopterideae.

7. Aphlebiae occur abundantly both on the stem and the leaf-base. Their strands are given off from the leaf-traces both below and above their departure from the stele.

8. The aphlebiae are modified basal pinnae of the leaf, as shown by the structure and mode of origin of their vascular strand. The free aphlebia, however, contains two or three vascular strands, which are branches of the original one.

9. *Ankyropteris*, and especially *A. Grayi*, shows a close affinity with the genus *Asterochlaena*, the latter differing from the former chiefly in the presence of more than one series of leaf-traces in connexion with each arm of the stele.

10. The vascular system of *Ankyropteris* is regarded as probably a highly elaborated protostele, rather than as a condensation of a polystelic structure.

For the loan of sections or photographs I am indebted to my friends Dr. R. Kidston, F.R.S., Prof. F. E. Weiss, and Dr. P. Bertrand, to whom I wish to express my warm thanks.

The photographic illustrations are for the most part the work of Mr. W. Tams, but some were taken for me long ago by my friend Mr. L. A. Boodle.

The drawings reproduced in Plates III to V are partly by Mr. G. T. Gwilliam, partly by Miss G. C. Harrison.

To all these collaborators I am greatly obliged for their aid.

APPENDIX.

It may be of interest to reproduce Williamson's MS. description of the sections of his later, unpublished specimen. The original notes are accompanied by rough sketches to which the letters refer. I am indebted to Dr. F. A. Bather, F.R.S., for a copy of this passage, from the Williamson Catalogue, preserved in the British Museum (Natural History):

'1818 A. *R[achiopteris] Grayii.* The most perfect section yet obtained. To be figured. Oldham. Lomax.

1919 A. *Rach. Grayii.* Trans. Section of a second specimen free from the abnormal mineralization of the specimens previously described. Structure of its secondary branches distinct. Oldham. Lomax.

1919 B. *Rach. Grayii.* Trans. A second section of 1919 A, showing some marked features. The extremity of each of the five radii on the transverse section of the axial bundles is square, *a*. In another radius this square end is detached, *b*; in another ray this latter section seems to have divided into two, the "Axilspross", *c*, and the Zygopteroid petiole, *d*. It is thus evident that these two structures spring from the main vascular axis of the stem as a single bundle, *a* and *b*, but that they divide as they ascend into *c* and *d*. Stenzel says it is so with his *Zygopteris scandens*, but he thinks that the primary bundle is a Zygopteroid one from which the "Axilspross" is a secondary branch. This is *not* the case in my specimen. He also thinks that the "Axilspross" is an abortive bud. I doubt this. Oldham. Lomax.

1919 C. *Rachiopteris Grayii.* Trans. A third section of the same with one secondary branch entirely free and a second one just separating from the central axis. Oldham. Lomax.

1919 D. *Rachiopteris Grayii.* Trans. A fourth section from the above specimen. Lomax.'

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EXPLANATION OF PLATES I–V.

Illustrating Dr. Scott's paper on *Zygopteris* (*Ankyropteris*) *Grayi.*

The Photographs in Plates I and II often need to be examined with a lens.

PLATE I.

(Photographs by Mr. W. Tams.)

Shore specimen.

Phots. 1–6. Selections from the transverse series of sections, from below upwards. Phots. 1, 2, 3, 5, and 6 show the stele and surrounding tissues. Phot. 4, on a smaller scale, shows the whole stem.

Phot. 1. (Reversed, as compared with Phots. 2–6.) The arms of the stele are numbered 1–5, following the phyllotaxis in kathodic order. The leaf-trace, *l.t.* (incomplete), and axillary stele, *a.s.*, are shown in the outer cortex. × 8. Scott Coll., 2511.

Phot. 2. The arm 5 is spreading out laterally and its internal ray becoming interrupted. × 8. Scott Coll., 2512.

Phot. 3. The end of arm 5 is about to separate as a leaf-trace. × 8. Scott Coll., 2514.

Phot. 4. Complete section. The leaf-trace 5 has now separated (see Phot. 8). Numerous aphlebia-strands are seen in the cortex. × 4. Scott Coll., 2516.

Phot. 5. The leaf-trace 5 has now entered the cortex. × 8. Scott Coll., 2518.

Phot. 6. The leaf-trace 5 has passed out further into the cortex and is assuming a stouter form. The arm 4 is preparing to detach its leaf-trace. × 8. Scott Coll., 2520.

Phot. 7. Middle part of stele, showing irregular ring of internal xylem, *x.i.*, with the five internal protoxylem rays. × about 30. Scott Coll., 2516.

Phot. 8. Leaf-trace 5 (see Phot. 4). *x.e.*, peripheral loops formed by the small-celled external xylem; *x.i.*, median island of internal xylem, destined for the axillary stele. × about 30. Scott Coll., 2516.

Phot. 9. Foliar bundle, in oblique section, from the base of a petiole in connexion with the stem. *x.e.*, peripheral loop; *s.v.b.*, base of a secondary vascular bundle belonging to a pinna. × 8. Scott Coll., 2530.

PLATE II.

(Photographs 10–13 by Mr. L. A. Boodle, F.L.S.; 14–16 by Mr. W. Tams.) Photographs 10–14 from Williamson's later specimen.

Phot. 10. Axillary stele, from the transverse section figured as a whole in Scott, '08, Fig. 115. *x.i.*, band of internal xylem, tangential to the parent stem; *ph.*, phloem; *i.c.*, inner cortex of the axillary shoot. × 34. Williamson Coll., 1919 A.

Phots. 11–13. General transverse sections.

Phot. 11. Leaf-base prominent. *st.*, stele of stem; *l.t.*, leaf-trace in the act of separating from the axillary stele; *aph.*, aphlebiae, two in connexion with the leaf-base, and the third in connexion with the stem. The strands of other aphlebiae are seen in the cortex. × about 4. Williamson Coll., 1919 D.

Phot. 12. Here the foliar bundle, *l.t.*, and the axillary stele, *a.s.*, have already separated. For details see Pl. V, Fig. 13. × about 4. Williamson Coll., 1919 B.

Phot. 13. (Reversed, as compared with Phots. 11 and 12.) *Pet.*, petiole, in oblique section, with the axilllary shoot, not yet quite free, between petiole and stem; *a.s.*, axillary stele; *l.t.'*, another leaf-trace, diverging from the former by $\frac{2}{5}$, just leaving the stele. × about 4. Williamson Coll., 1919 C.

Phot. 14. Undivided leaf-trace in the outer cortex. *x.e.*, small tracheides of a peripheral loop. The position of this trace is shown in the diagram, Pl. V, Fig. 15, *l.t.* × 35. Scott Coll., 184.

Phots. 15 and 16 from Williamson's type-specimens.

Phot. 15. Transverse section of a stem, showing the stele and surrounding tissues (imperfectly preserved). *l.t.*, crescentic leaf-trace, as in the Shore specimen; *aph.b.*, aphlebia-bundle (several shown); *r.*, root passing out through cortex. × 7. Williamson Coll., 1824.

Phot. 16. Transverse section of the vascular bundle of the petiole and adjacent tissues. *x.e.*, small-celled external xylem of the peripheral loops. × 26. Williamson Coll., 1824.

PLATE III.

Figs. 1, 3, and 5 by Miss G. C. Harrison. Figs. 2, 4, and 6 by Mr. G. T. Gwilliam.

Shore specimen.

Fig. 1. Undivided leaf-trace, after entering the cortex (level of section between that of Phots. 5 and 6). *x.*, large-celled xylem; *x.e.*, small-celled external xylem of the peripheral loops; *px.*, probable positions of protoxylem; *x.i.*, island of internal xylem, destined for the axillary stele; *ph.*, remains of phloem; *i.c.*, band of inner cortex between leaf-trace and stele. × about 40. Scott Coll., 2519.

Fig. 2. Leaf-trace further out in cortex. Rather more than half is shown (level two sections above Phot. 6). Note the increased width of the middle part of the trace, in preparation for the detachment of the axillary stele. Lettering explained under Fig. 1. × 50. Scott Coll., 2522.

Fig. 3. Longitudinal section of the stele of the stem passing tangentially through the 'mixed pith', and showing the internal xylem, *x.i.*, at several places. *p.p.*, parenchymatous cells; *x.x.*, inner edges of the outer, large-celled xylem-zone. × 100. Scott Coll., 2525.

Fig. 4. Transverse section of arm 2 of stele (level immediately above Phot. 6). *x.i.*, internal protoxylem ray, the forks nearly reaching the surface of the xylem; *aph.*, aphlebia-strand, given off from the arm of the stele. × 65. Scott Coll., 2521.

Fig. 5. Part of leaf-trace, just detached from arm 5 of the stele (level between Phot. 3 and Phot. 4), showing one of the peripheral loops and an aphlebia-strand just given off and still in connexion. *x.e.*, external xylem of peripheral loop; *ph.*, remains of phloem; *aph.*, the aphlebia-strand. × 120. Scott Coll., 2515.

Fig. 6. Aphlebia-strand, *v.b.*, in the outer cortex. The strand is much extended tangentially. × 40. Scott Coll., 2516.

PLATE IV.

Fig. 9 by Miss G. C. Harrison; the others by Mr. G. T. Gwilliam. Figs. 7–10, Shore specimen.

Fig. 7. Aphlebia detached from cortex of stem, *c.* *v.b.*, one of the vascular bundles of the aphlebia; the other is only indicated by a gap in the tissue; *h.*, multicellular hairs of the stem. × 30. Scott Coll., 2516.

Fig. 8. Somewhat diagrammatic longitudinal section of stem. The cross-hatched area is the outer cortex, *st.*, stele, cut rather obliquely so that the 'mixed pith' is seen below, and two of the internal rays, passing out from it, above. *aph.*, aphlebia-strand, passing out through the cortex. × 5. Scott Coll., 2525.

Fig. 9. Transverse section of an axillary stele (the same as shown in Phot. 1, but two sections higher up). *x.i.*, internal xylem; *p.*, parenchyma of the 'mixed pith'; *x.*, large-celled xylem-zone; *c.*, part of cortex; → *S.*, direction of main stele of stem. × 44. Scott Coll., 2513.

Fig. 10. Longitudinal section of another axillary stele, passing through the 'mixed pith'. *x.i.*, internal xylem; *px.*, protoxylem, consisting of spiral tracheides; the parenchyma of the 'mixed pith' is shown above; *x*, large-celled xylem-zone. × 70. Scott Coll., 2527.

Figs. 11, 12. Williamson's later specimen.

Fig. 11. Undivided leaf-trace in the outer cortex, the same as shown in Pl. II, Phot. 14, and in the diagram Pl. V, Fig. 15, *l.t.* *x.e.*, small-celled external xylem of a peripheral loop. × about 50. Scott Coll., 184.

Fig. 12. Undivided leaf-trace on the border of the inner and outer cortex. From section figured in Scott, '08, Fig. 115. A zone of inner cortex surrounds the trace. *x.i.*, internal xylem; *x.*, outer xylem-zone; *ph.*, phloem with large sieve-tubes. × 28. Williamson Coll., 1919 A.

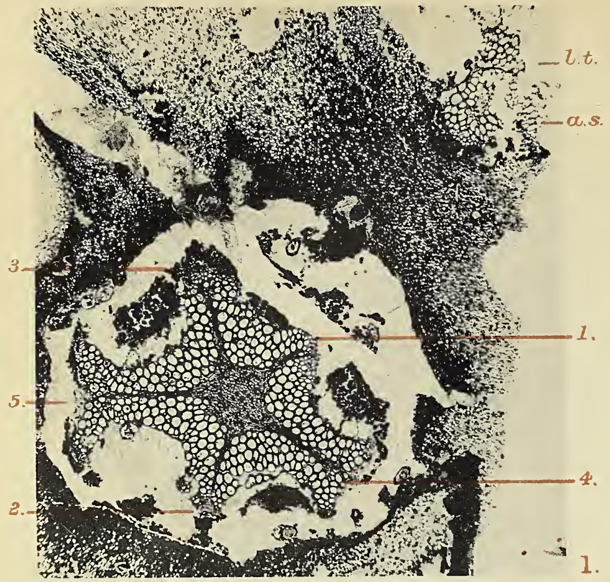
PLATE V.

Figures by Mr. G. T. Gwilliam, from Williamson's later specimen.

Fig. 13. Foliar bundle, *L.T.*, and axillary stele, *A.S.*, just separated, with inner and outer cortex between them; whole section shown in Pl. II, Phot. 12. *x.*, *x.*, large-celled xylem of bundle and axillary stele; *ph.*, *ph.*, phloem of bundle and stele; *x.i.*, internal xylem of stele. × 28. Williamson Coll., 1919 B.

Fig. 14. Arm of stele, *st.*, in transverse section, giving off laterally the stele of a root, *rt.*; *c.*, inner cortex. × about 40. Manchester Coll., R. 443.

Fig. 15. Diagram of transverse section, showing relative positions of the stele, *st.*; an undivided leaf-trace, *l.t.* (shown in Pl. II, Phot. 14, and Plate IV, Fig. 11); a foliar bundle, *f.b.*, and its axillary stele, *a.s.* The gap in the cortex was filled by a Stigmarian rootlet. × 6. Scott Coll., 184.



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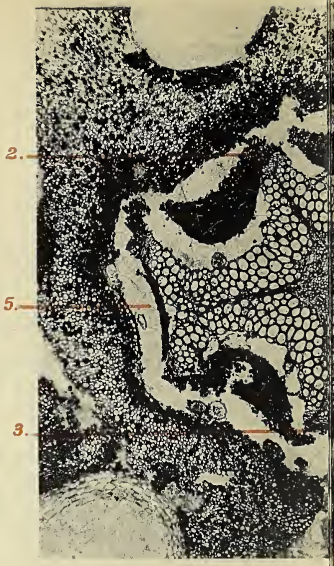

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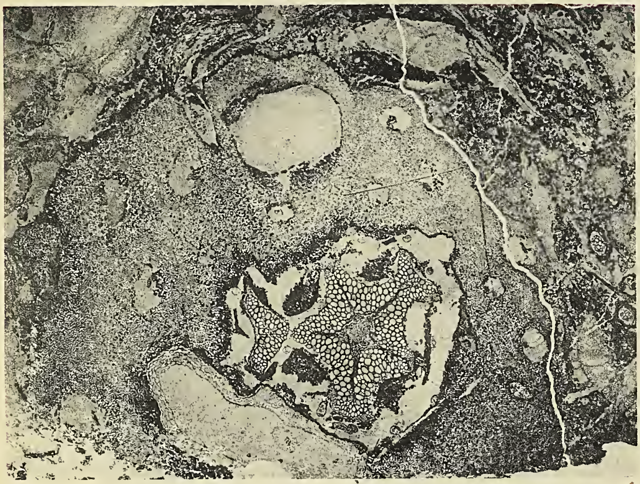

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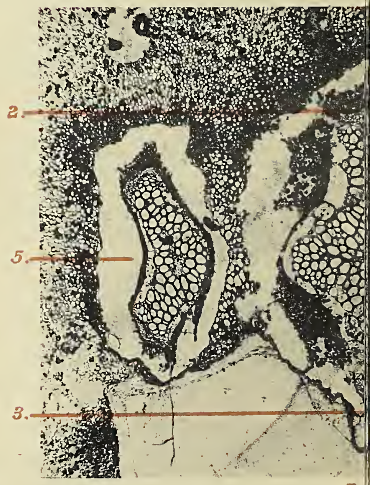

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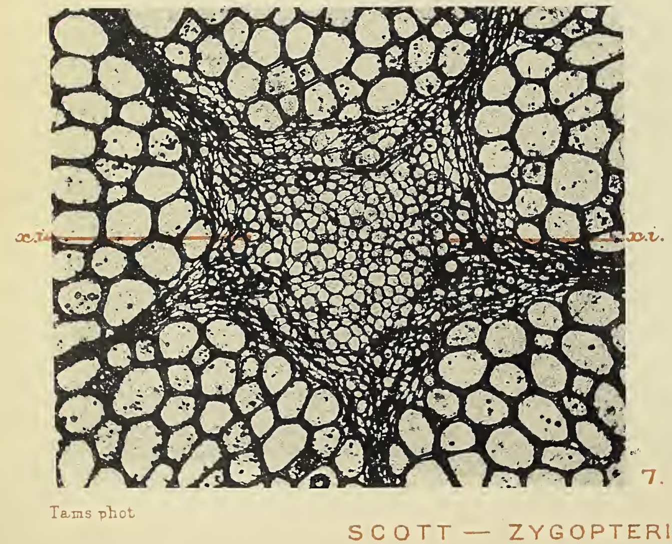

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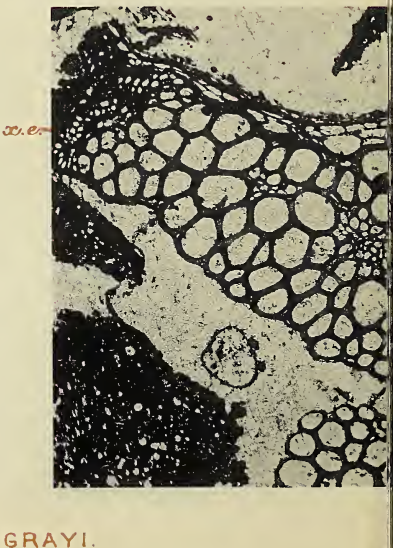

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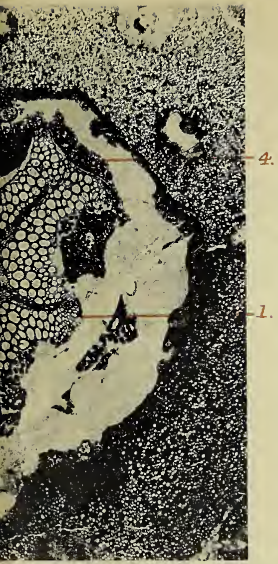

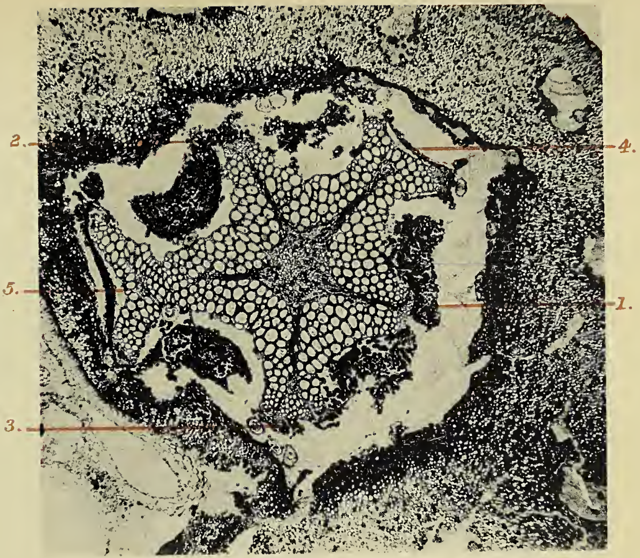

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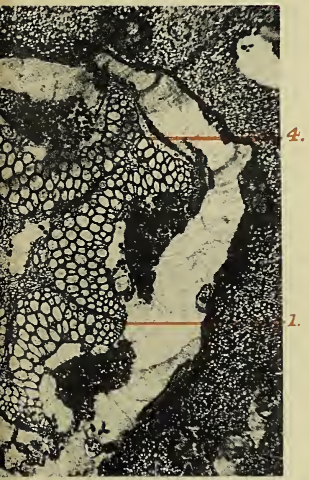

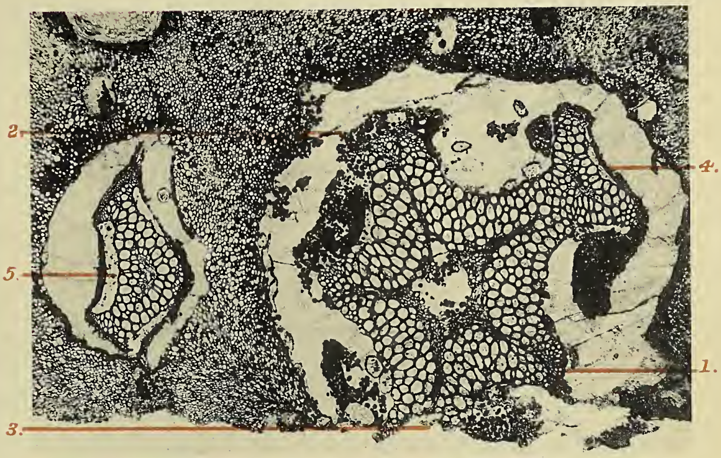

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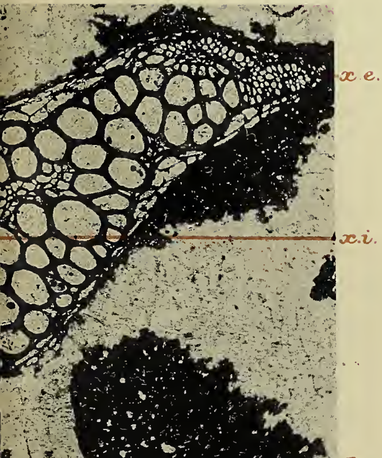

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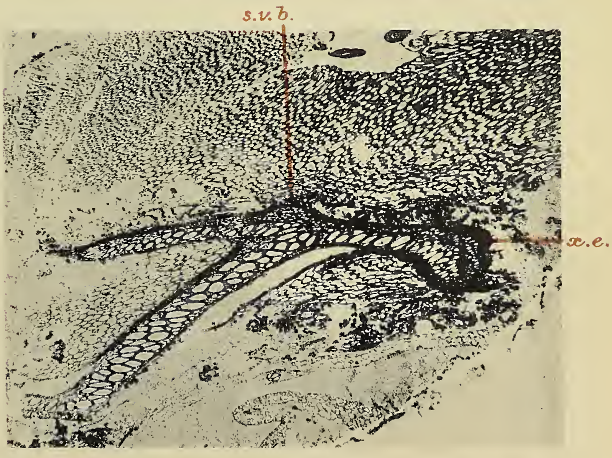

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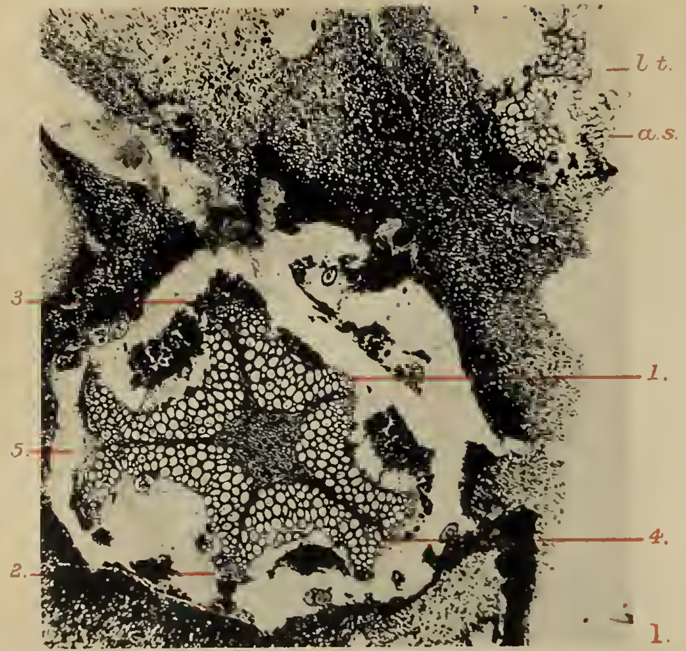

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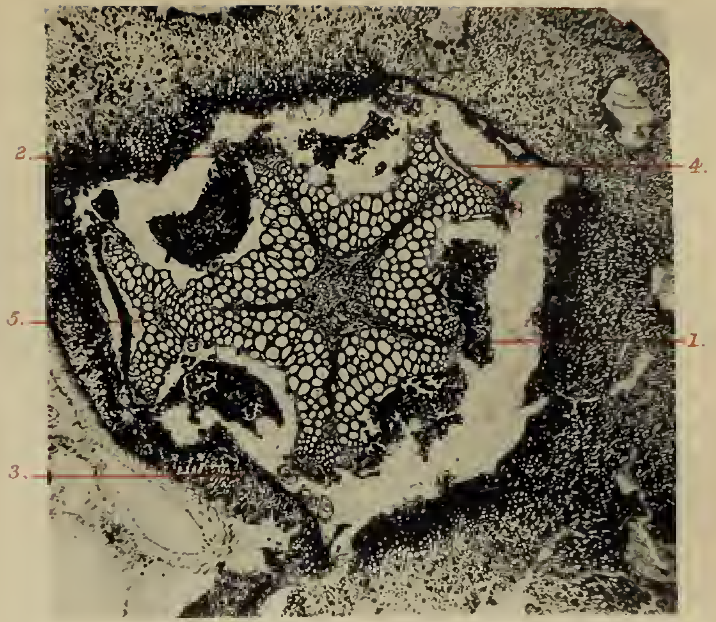

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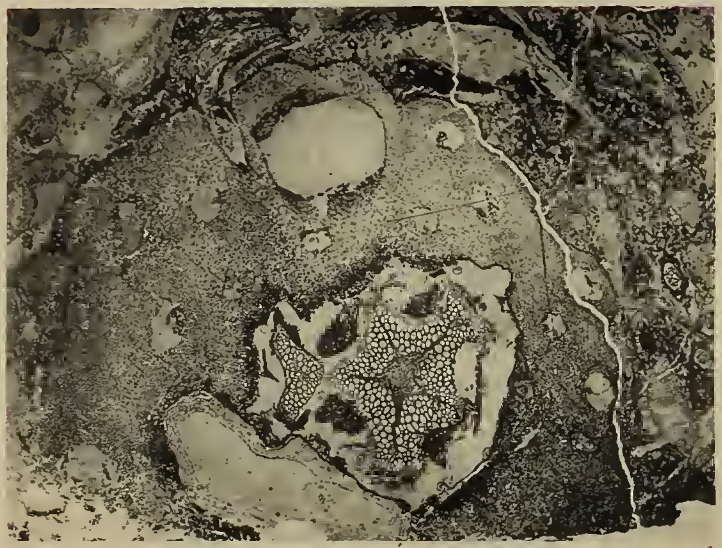

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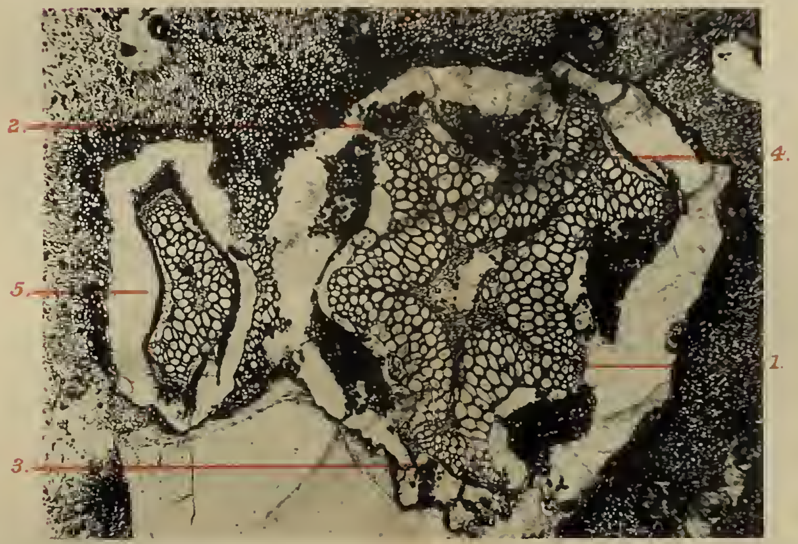

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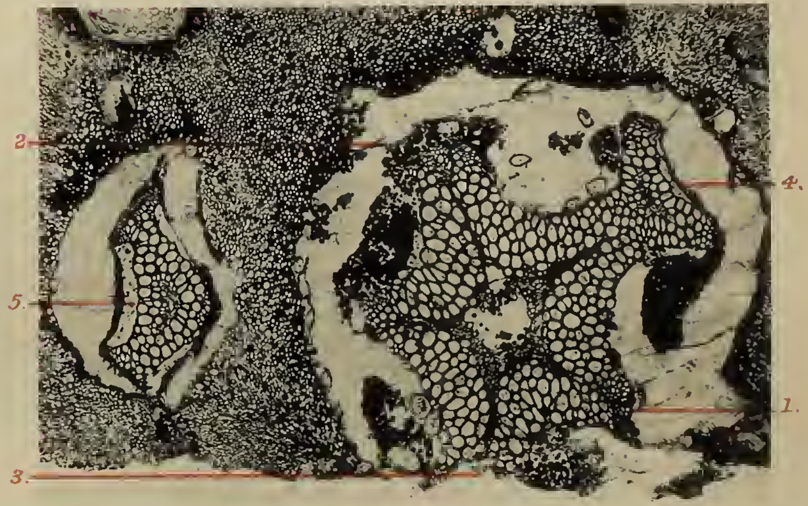

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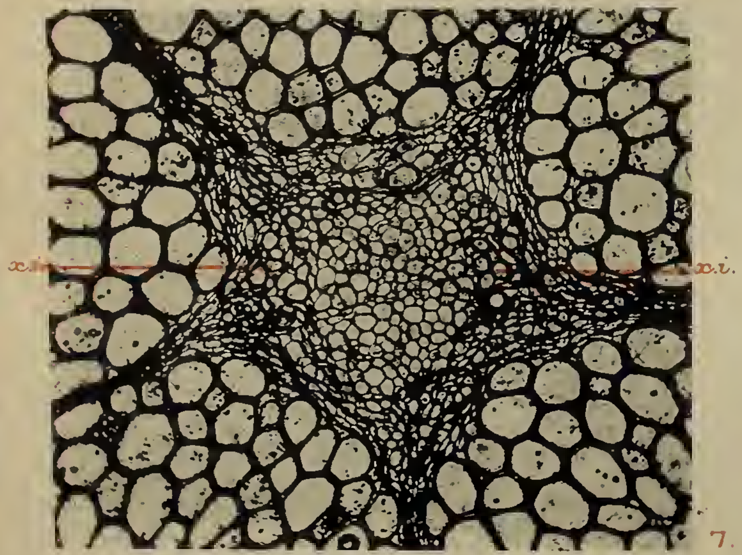

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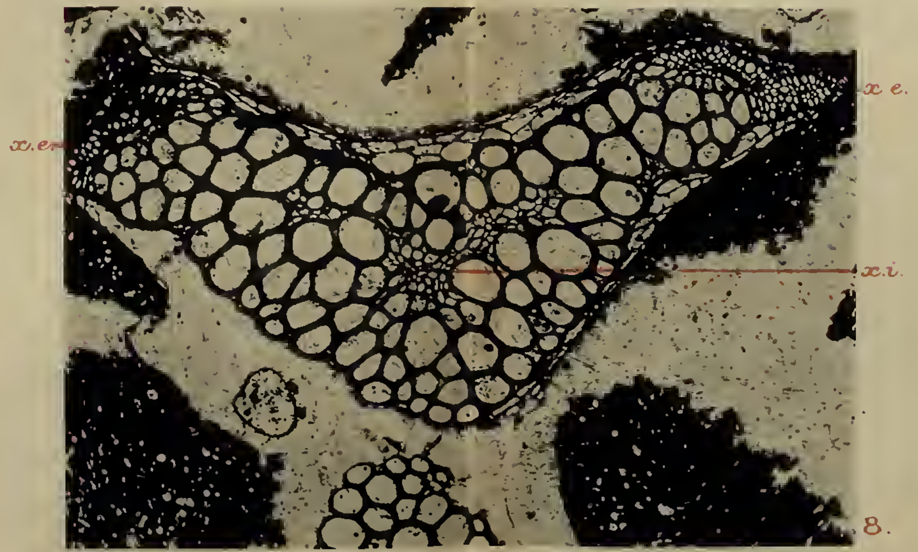

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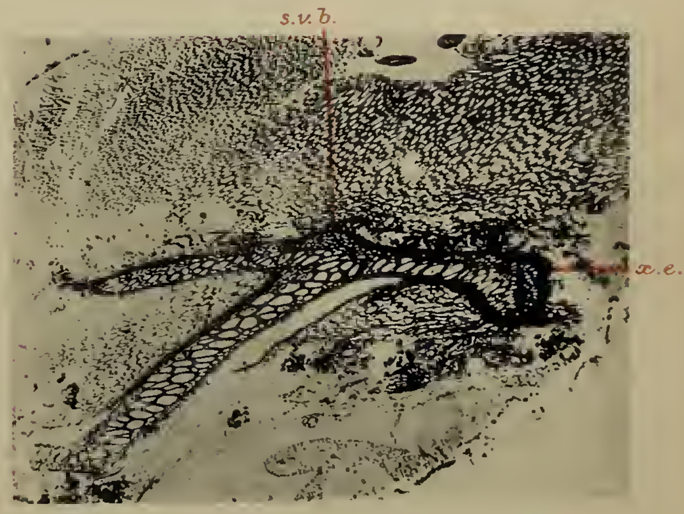

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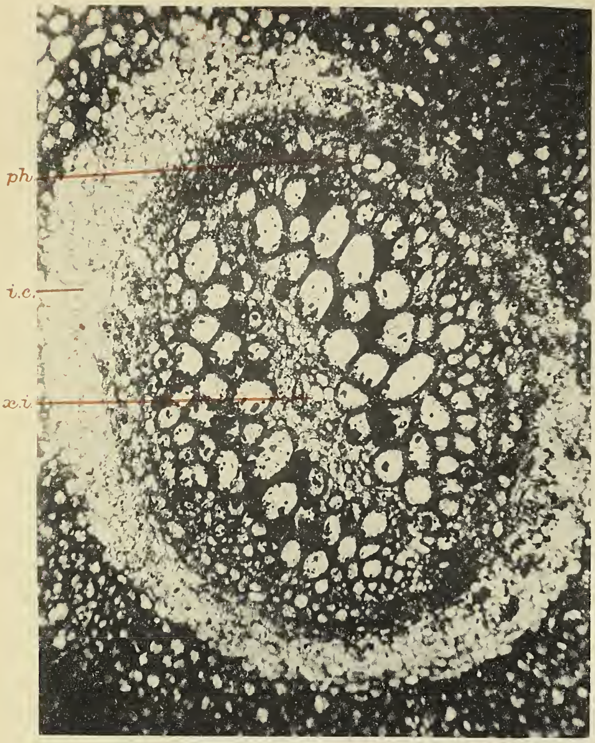

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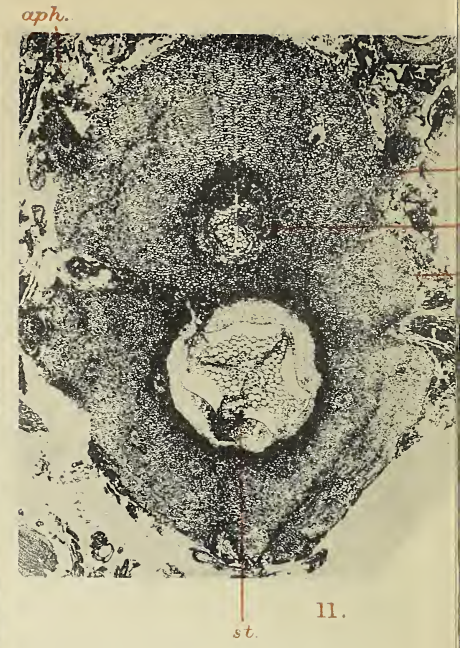

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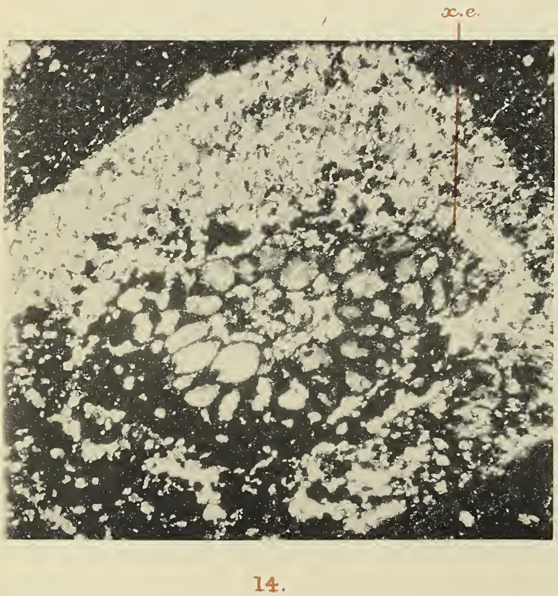

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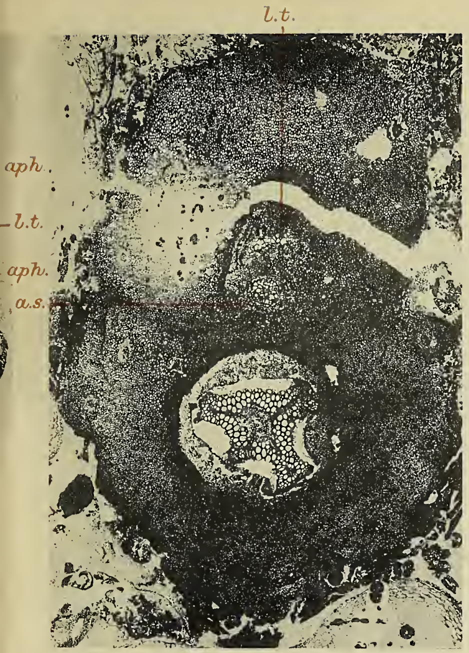

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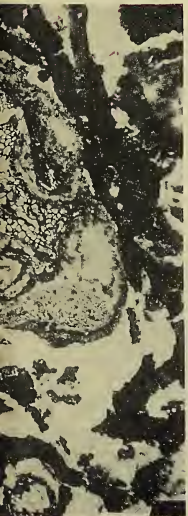

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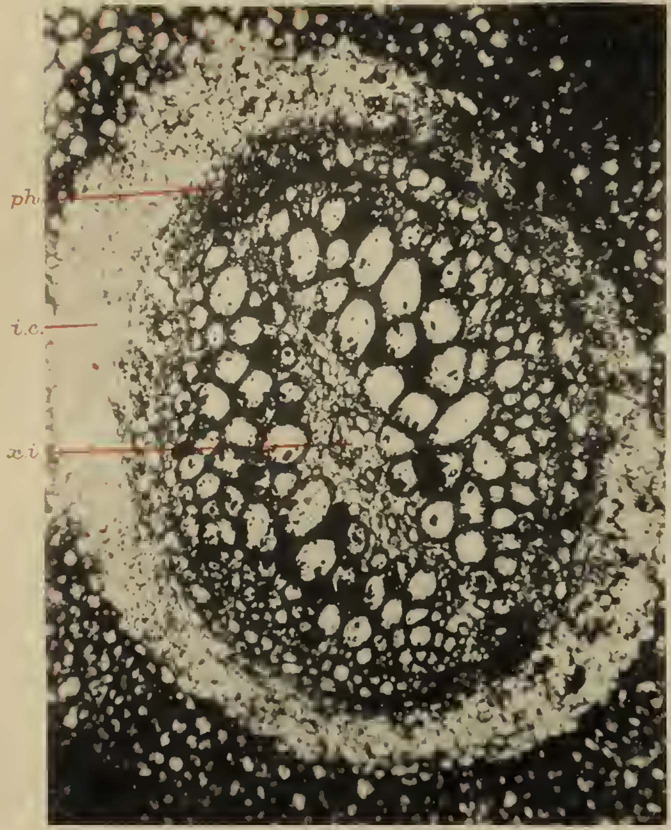

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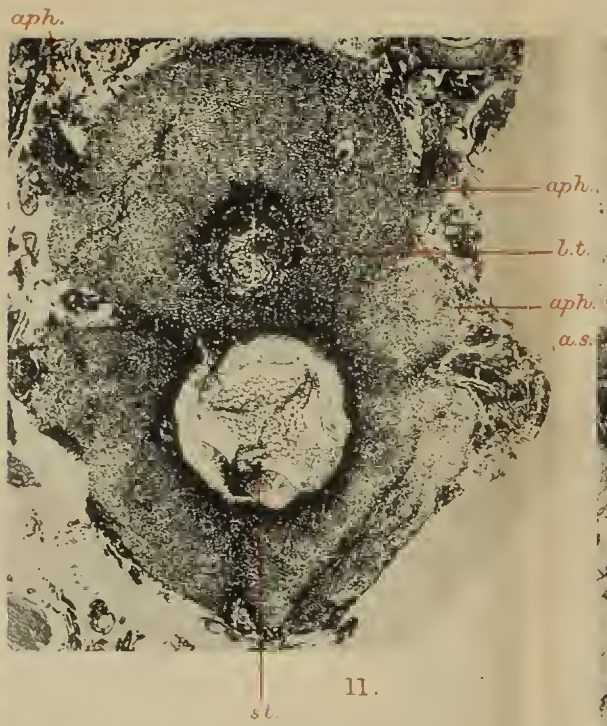

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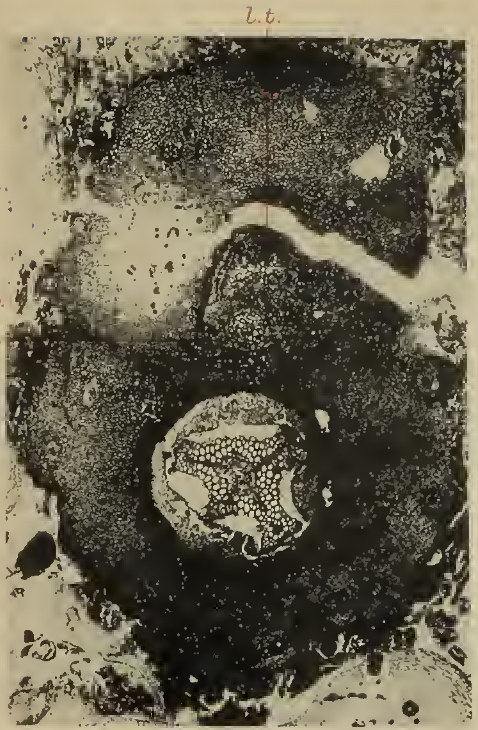

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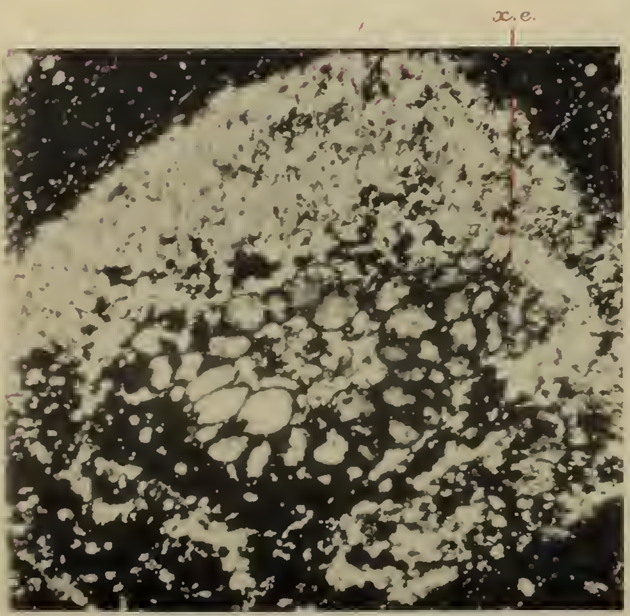

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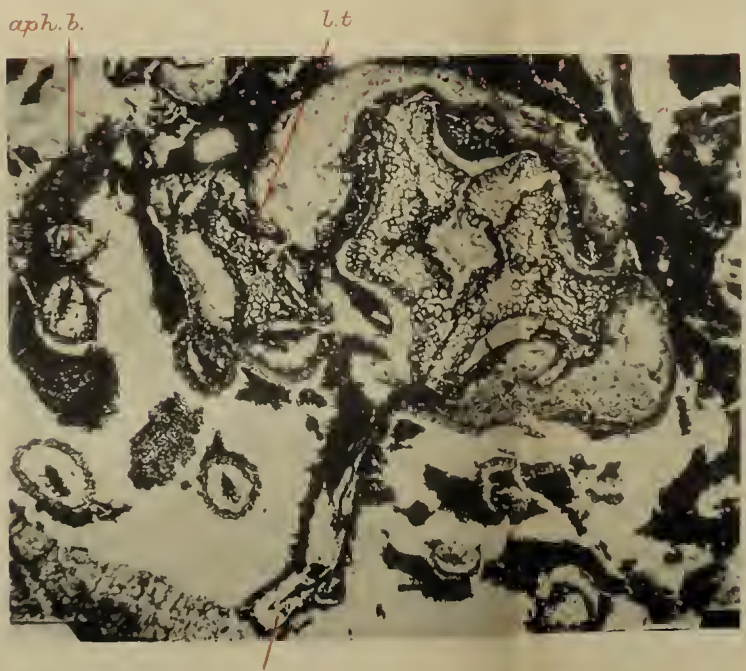

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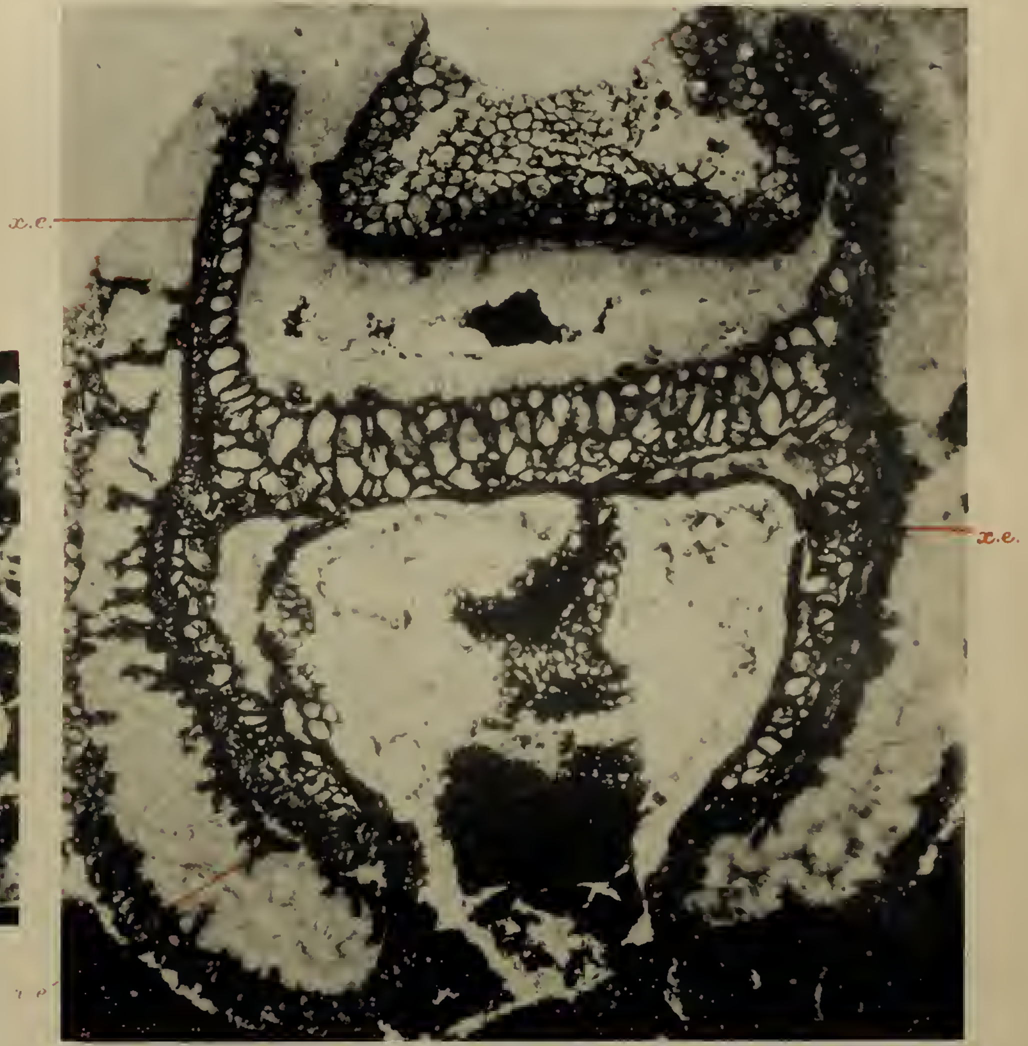

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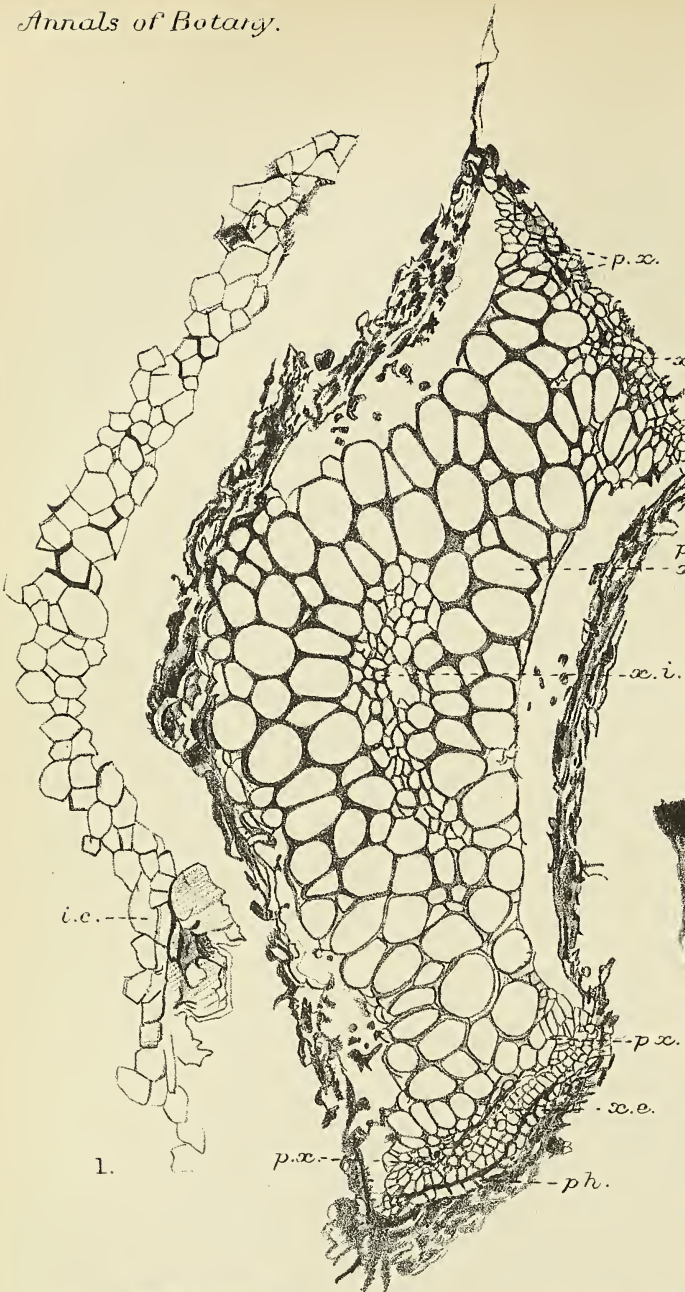
SCOTT — ZYGOPTERIS GRAYI.



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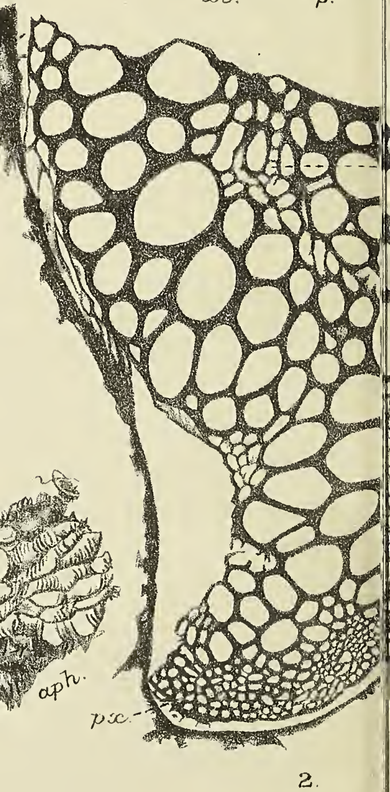

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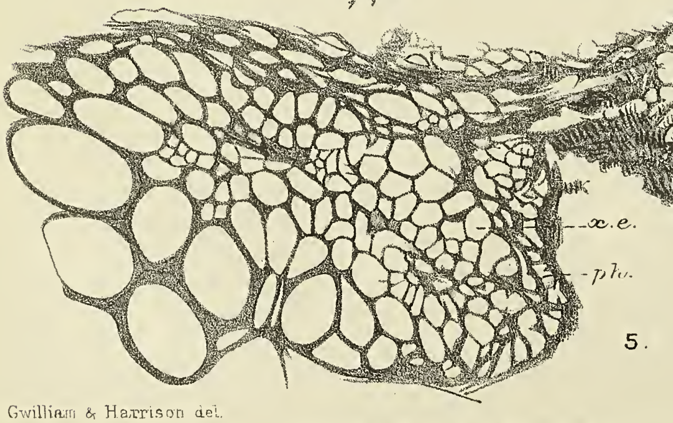

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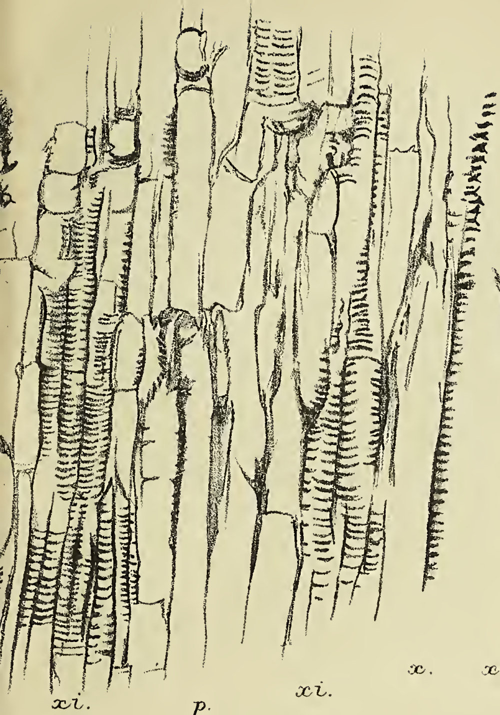
SCOTT—ZYGOPTERIS GRAYI.



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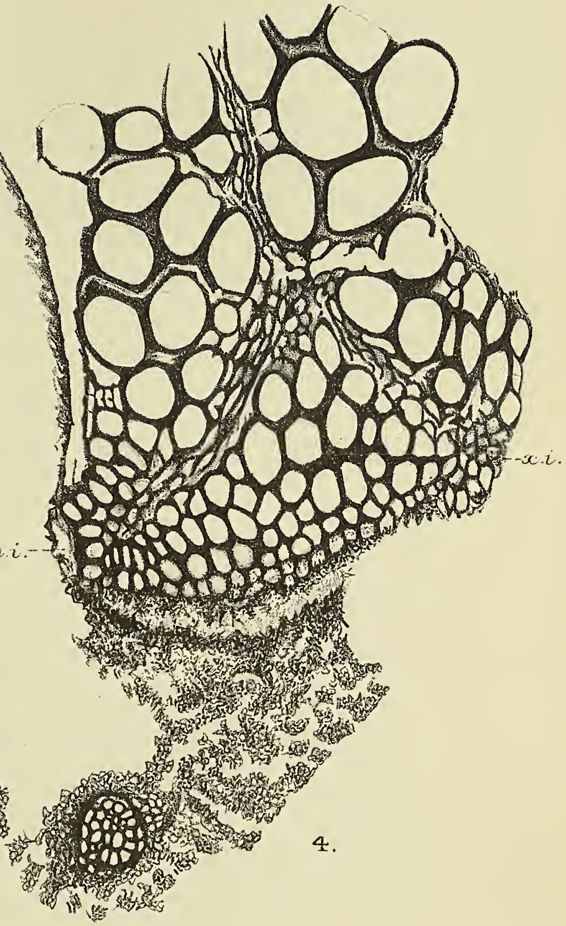

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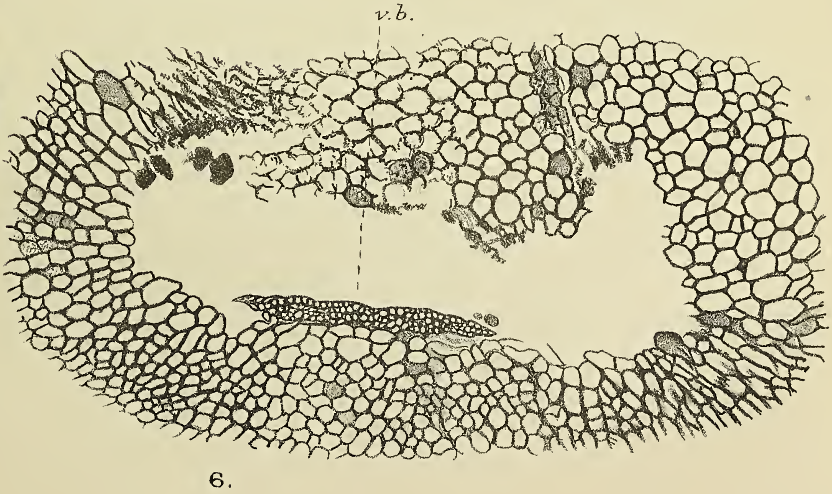

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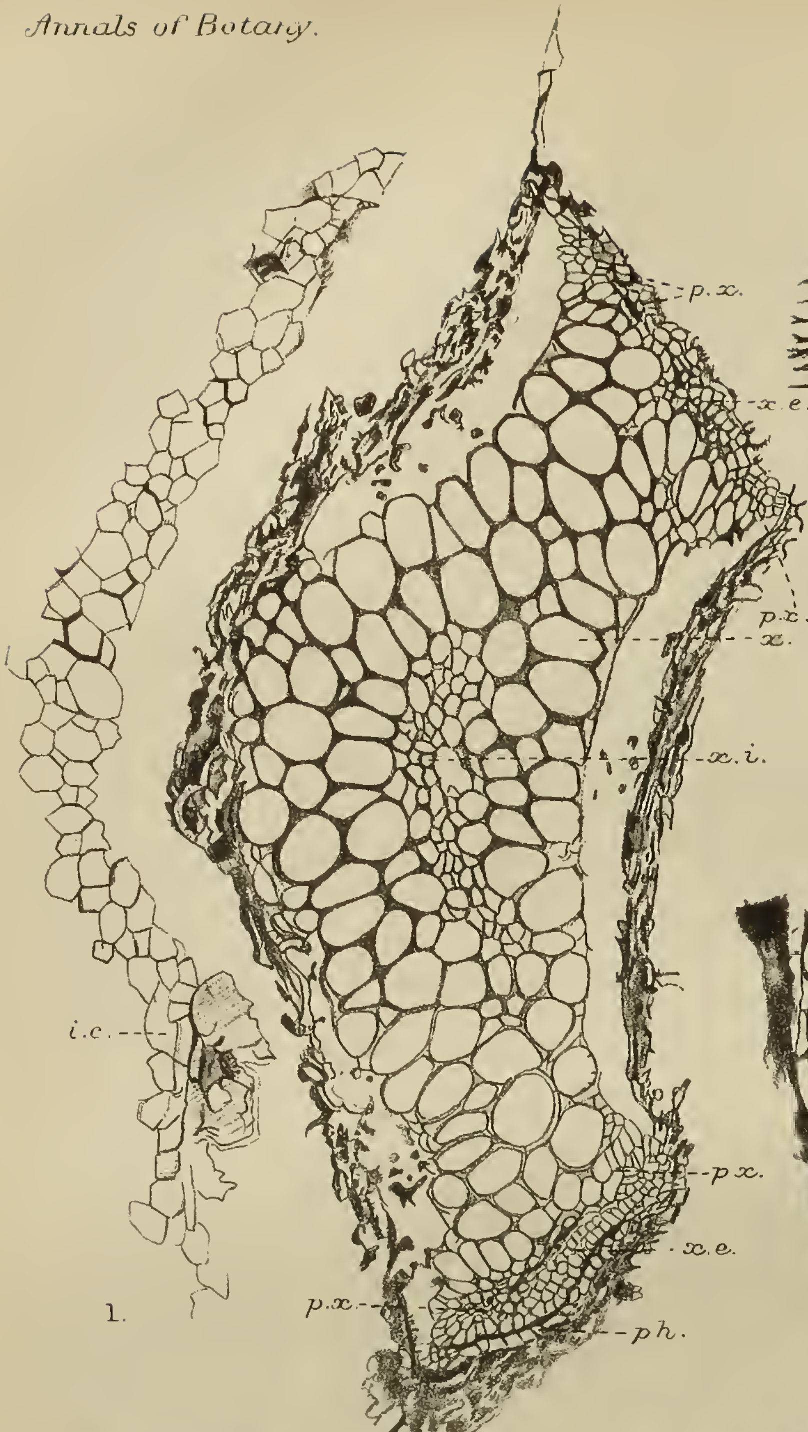

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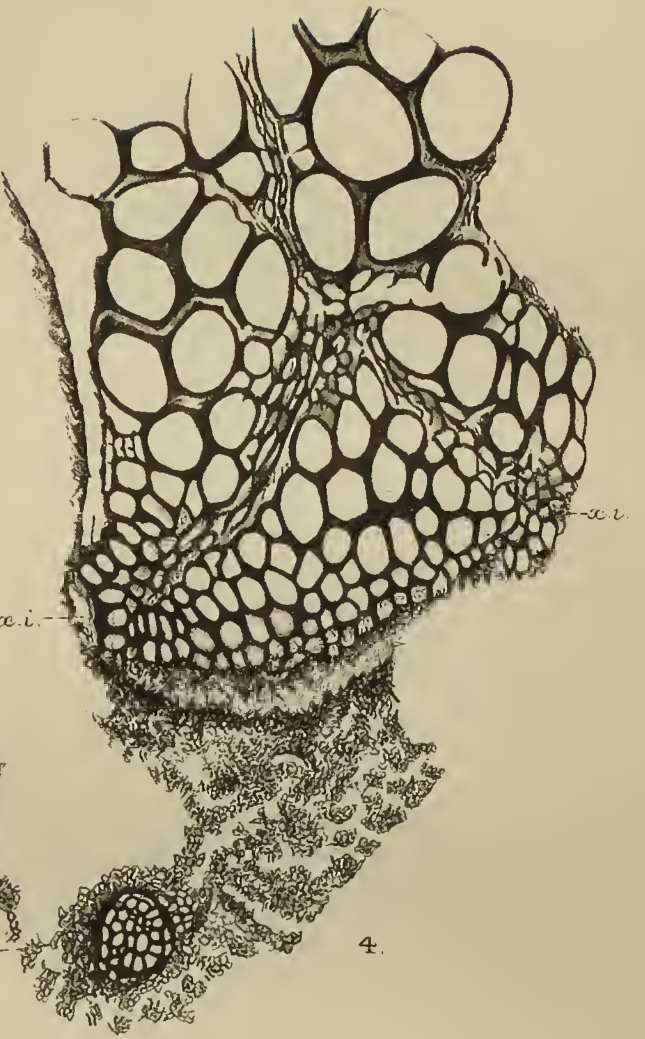

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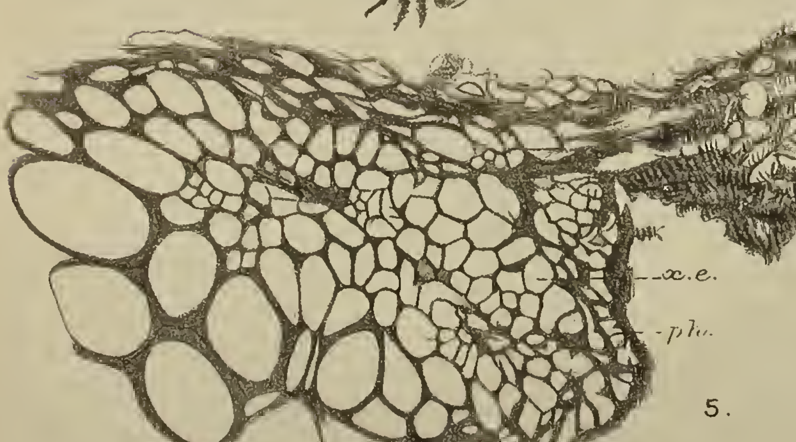

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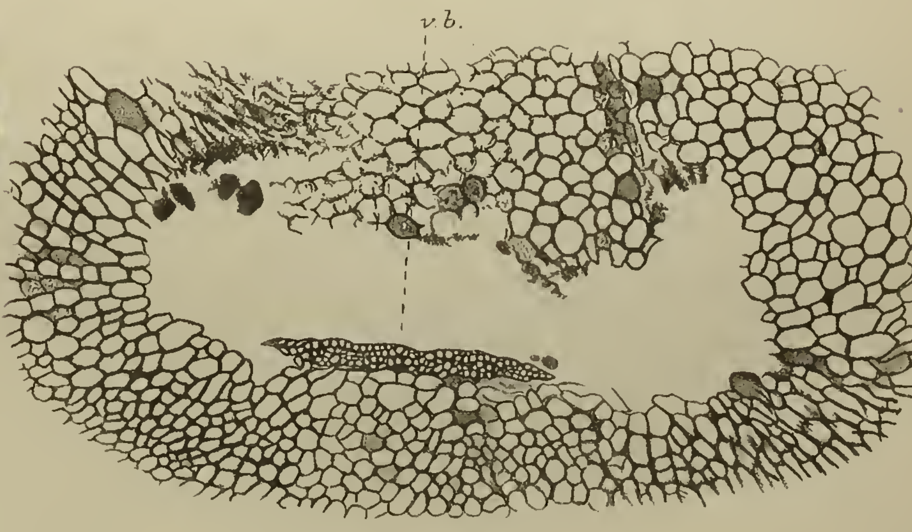

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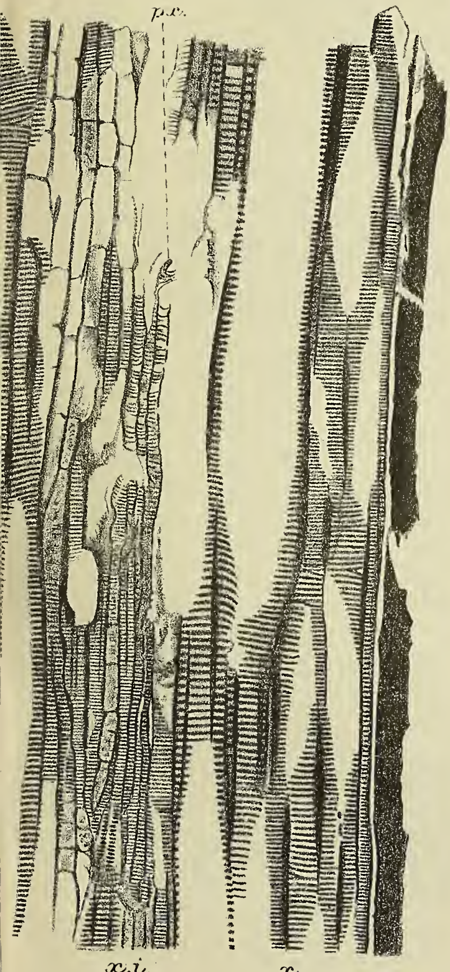

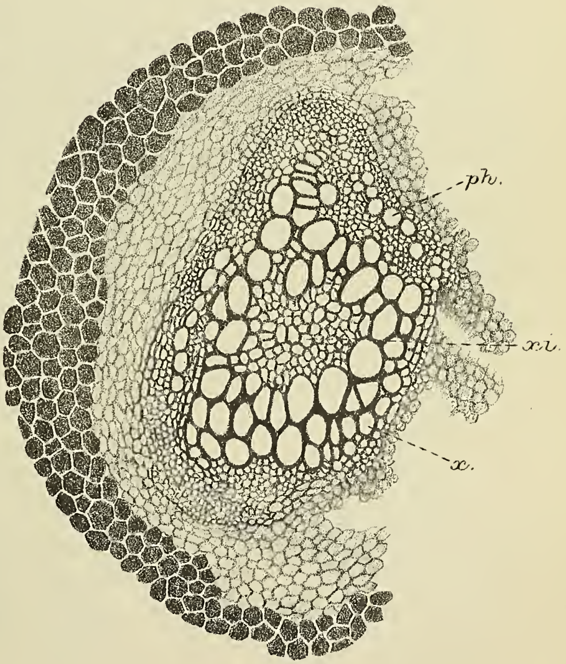

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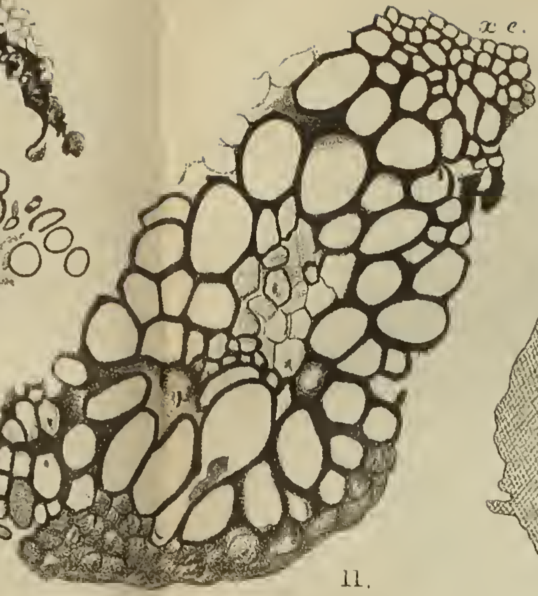

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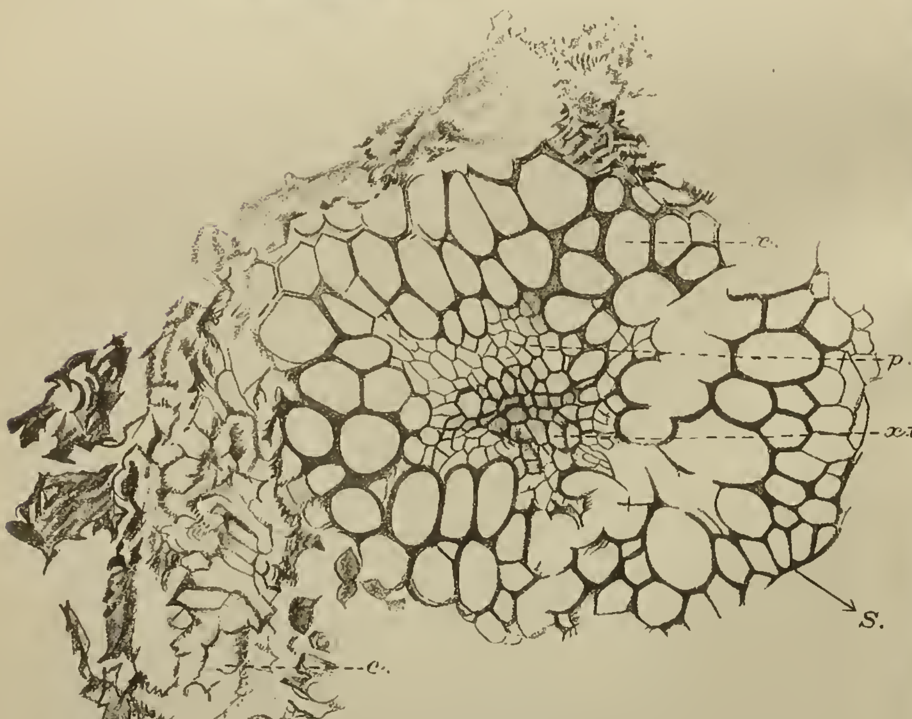

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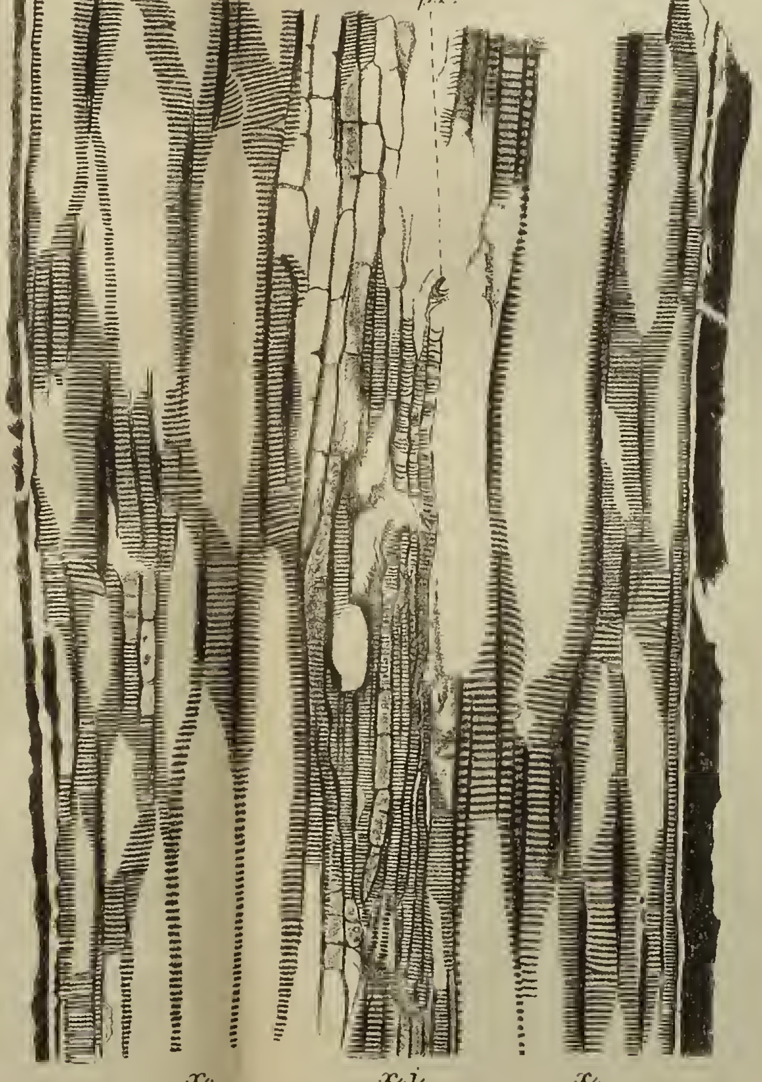

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SCOTT—ZYGOPTERIS GRAYI.

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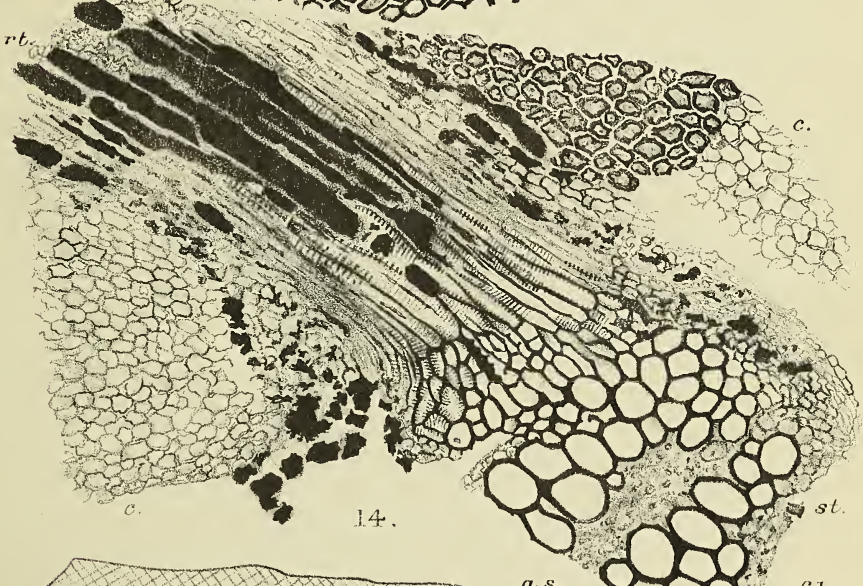

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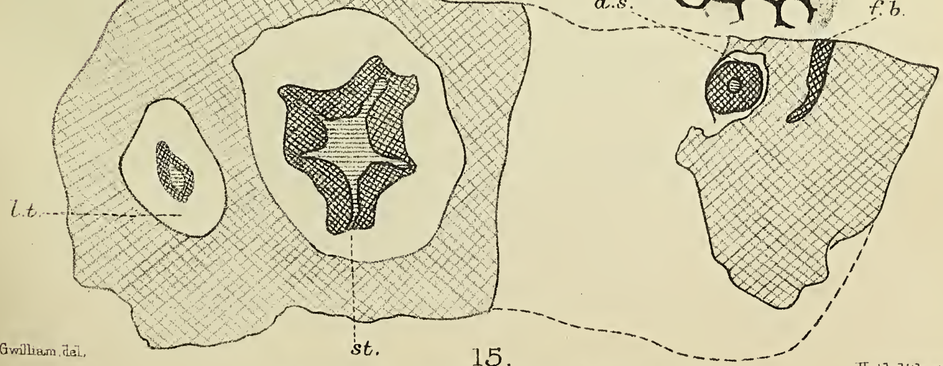

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Gwilliam del.

Huth, lith et imp.

SCOTT — ZYGOPTERIS GRAYI.

The Water-balance of Desert Plants.

BY

D. T. MACDOUGAL, PH.D., LL.D.

Director, Department of Botanical Research, Carnegie Institution of Washington.

With Plates VI–X.

THE conducting tissues of seed-plants are closely connected with morphologically distensible tracts of medullary and cortical tissue which have an appreciable capacity for the retention of water in plants of even the strictest habit and stature. As the ascending current passes from the absorbent elements to the transpiratory surfaces, some of it may go into such masses of cells constituting reservoirs, in the roots, stems, or leaves. This accumulated supply may be drawn out to the transpiring cells when the pressure of the solution in the cell-sap is overcome.

All plants with massive stems may thus carry a large balance of water, and this stored solution may play a very important part in the life of the individual. The relatively largest balances are carried by some of the species characteristic of the arid regions of the south-western and southern parts of North America, some parts of South America, and the southern part of Africa, while Northern Africa, Asia, Australia, and arid regions in high latitudes everywhere have but few plants with a large water-balance.

The author began some work on the water-balance of desert plants in 1908, and the earlier results obtained have already been published.[1] A few of the plants survived the original tests, and these with other living specimens have yielded evidence upon phases of the subject not discussed in full in the earlier paper.

The Tucson region, in which the observations were made, has a winter rainy season and a wet midsummer, with a hot dry fore-summer and arid after-summer. The total average annual precipitation is about 12 inches. The extremest arid effects are seen in June and early July, when the humidity falls as low as 6 per cent. with midday temperatures of 110° and 112° F. Some of the material was observed in the open, and other specimens were kept in a shaded laboratory room in which the extremes of temperature were not reached by ten or fifteen degrees.

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[1] MacDougal, D. T., and Spalding, E. S.: The Water-balance of Succulents. Publication No. 141, Carnegie Institution of Washington, 1910.

[Annals of Botany, Vol. XXVI. No. CI. January, 1912.]

ECHINOCACTUS WISLIZENI.

This species is native to the Tucson region, being found on rocky slopes between 500 and 900 metres elevation. The globose or cylindrical stems attain a length of 1·5 metres and a diameter of half or even two-thirds the length in some instances. The root-system is horizontally disposed.[1] The stems may have a weight of 80 to 100 kg., and the long axes are generally parallel to the axis of maximum illumination, which accounts for a slight leaning to the southward in a perfectly open country. The southern side has the folds more closely drawn together, while the cortex of the shaded side will be more heavily developed with the external ridges of the stem widely separated. The extra weight on the shaded sectors of the plant counterbalances the tendency to fall towards the southward. (See Pl. VI.)

About twenty individuals of various ages were examined during a period of four years. It will be necessary to summarize briefly some of the data already published in order to interpret the newer facts presented.

Echinocactus No. 1. A large plant weighing 42·743 kg. was taken up March 4, 1908, and placed on a suitable support on a platform in a shaded room. The rate of loss at the beginning was 17 g. daily or one part in 2,500, which rose to 29 g. daily in the arid fore-summer, dropped to 14 g. during the summer rains, rose to 18 g. in the dry after-summer in September, and then fell irregularly to 1 g. daily in December. This rate of one part in 40,000 is the lowest recorded for any plant that came under observation. The highest daily rate, one part in 1,100, was observed in May, 1908. A year later, when the plant had lost about 4 kg. or 10 per cent. of its original balance out of a total weight of nearly 43 kg., the daily rate was but one in 2,500 of the total weight. With reference to the amount of the water-balance, the daily rate was one in 1,300 in May, 1908, and one in 2,000 in May and June, 1909, at which time the weight was about 38 kg.

Echinocactus No. 2 was taken up November 5, 1908, and placed in the room with No. 1, weighing 5·136 kg. The midwinter rate of loss was as great as that of No. 1, but it soon fell to 0·6 g. daily. This was equivalent to one part in 8,056. The plant was placed in an equable temperature room in mid-February in which the thermometer showed a rise from 56° F. to 71° F. in midsummer, and the humidity ranged from 80 to 90 per cent. The rate under such circumstances rose to 0·7 g., seemingly independent of humidity, with a direct relation to the temperature at first, but the rate continued to increase despite the falling temperature until it reached 1·3 g. daily in October. A decrease now ensued parallel to the falling temperature which carried the rate down to 0·27 g. daily, or one part in 18,000.

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[1] See Cannon, W. A.: The Root-habits of Desert Plants. Publication No. 131, Carnegie Institution of Washington, 1911.

Late in February, 1910, the plant was removed to the open and the rate immediately rose to 6 g. daily, and with the coming of the arid fore-summer rose to 21 g. daily, or one part in 150 of the weight at the beginning of the period taken into account. The rate now fell to 7 g. in the rainy season, 3 g. in October, 1910, 1 g. in November, and 0·5 g. in December to February, 1911, or one part in 5,000 of the total weight. The loss in December to February, 1908–9, was one part in 3,000 of the water-balance and about one part in 4,000 of the water-balance two years later (1910–11). The weight on March 20, 1911, was 2·220 kg., showing a loss of 2·916 kg. or 56·5 per cent. of the original weight in the total period of desiccation extending over thirty months.

A chemical analysis of the plant made in April, 1911, showed total solids of 8·097 g. per 100 c.c. of sap, and 3·149 g. of ash, with an average osmotic activity of 2·3 atmospheres at 25° C. This plant, as well as No. 3, may be supposed to have reached the limit of endurance, and disintegration had set in, although a high concentration of the sap contents had not been reached.

The record of this plant includes some data of importance as bearing on the capacity of such plants to absorb moisture through their aerial surfaces. The weight in the open on February 28, 1911, after a drizzling rain lasting through the night, was 2·430 kg., which was 8 g. more than on February 20, 1911. The loss during the eight days would have been about 4 g., so that an estimated absorption of 12 g. by the spines and external structures must have taken place. The plant showed no adhering drops or films of water, and the weighing was done at noon on the 28th.

Echinocactus No. 3 was taken from the soil on March 6, 1908, and placed on a base of loosely-piled black volcanic rocks in the open, its estimated weight being 5·480 kg. It weighed 3·893 kg. when brought into the laboratory on November 5, 1908, and during the next month gained 14 g., which may be attributed to absorption of water vapour by the dead and dried spines. The daily rate of loss was 0·8 g., or one part in about 5,000 in the period from November to February.

The plant had now been deprived of a water-supply for a year, and when placed in the soil exhibited the usual phenomena of growth and flower formation during the summer of 1909. The roots were embedded in soil in a suitable box and the preparation set under a glass shelter without sides, an arrangement which would allow desiccation to proceed as usual. The plant was freed from the soil on November 5, 1910, cleaned, and the roots cut away to simulate the conditions in November, 1908, and the weight was found to be but 4 kg., showing that practically all accretion of dry material had been used in the construction of roots, although some expansion of the head had taken place in 1909. Still supported under the shelter, but separated from its roots and soil, the rate of loss was 1·6 g. daily from

November, 1910, to February 3, 1911, or one part in 2,500, the glass shade having been removed. Its weight was 3·855 kg. on February 28, 1911, and after a rain of 0·19 inch in the form of a slow drizzle throughout the night, the same weight was found. Estimating the total loss at the rate in the preceding period, it must have amounted to 40 kg. in twenty-five days, and this amount appears to have been balanced by the absorption on the part of the dried spines and other external parts. It may not be asserted that any of the above absorption was carried on by living tissue, nor does it appear that the water taken up might be of use to the plant.

The chemical analysis of the sap in April, 1911, showed 9·512 g. solid matter per 100 c.c. of sap and 3·864 g. of ash, the average of over a dozen determinations giving an osmotic pressure of 2·2 atmospheres at 25° C. The total solid and ash were thus about five times the minimum shown by a turgid plant (see p. 76). The concentration of the sap was greater than that of any plant previously examined, yet it is notable that the osmotic activity was scarcely more than half that of any other specimen examined. This would seem to indicate that actual disintegration had begun, and that this individual might not have been able to recuperate if supplied with soil moisture.[1] The total loss was 2·075 kg. or 36·2 per cent. of the original weight in three years.

Echinocactus No. 4 was taken from the soil, the root-system cut away, and placed on a base of loosely piled volcanic rock in the open on March 5, 1908. The estimated weight was 1·192 kg., which was reduced to 795 g. on November 5, 1908. A slight gain in weight was noted, as in No. 3, when this plant was brought into the shaded laboratory. The rate of loss was 0·5 g. daily in the 164 days ending May 21, 1909. This was equivalent to one part in 1,500. The rate was 0·6 g. in August, then fell to 0·4 g. and to 0·2 g. in November, and then during the midwinter period fell to 0·13 g., or one part in 5,300. A gain of 2 g. in weight ensued when the plant was placed in the humid dark room for seven days. The humidity was 62 per cent. with a temperature of 58° F. Upon being taken out the rate rose to 0·4 g. in March and 0·6 g. in May, which was practically equivalent to the rate at the corresponding period of the previous year. A rate of 0·4 g. was maintained from July to November, when it fell during the period of November to February to 0·11 g., or one part in 5,000, which, with corrections for variation in periods of observation, is not materially different from the rate of the year before.

The room in which this plant was placed was heated during February so that the rate rose to 0·24 g. daily and the weight fell to 549 g. on February 28. It was now placed outside for four days, its final weight on March 5, 1911, being 549 g. It was then set on the parapet wall of

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[1] See MacDougal and Cannon: The Conditions of Parasitism. Pub. 129, Carnegie Institution of Washington, p. 33, 1910.

the laboratory. The high temperatures and intense sunlight soon killed it, however. (See Pl. VII.)

Echinocactus No. 6 was taken from the soil November 7, 1908, and after being freed from roots was mounted on a stand in the laboratory. The initial net weight was 28·573 kg., and the rate of loss during the first month was 13 g. daily, which was about one part in 2,200 of the total weight. The rate during the winter fell to 0·6 g. daily, or one part in 5,000, and rose in the hot fore-summer to 10 g. daily. By October, 1909, the rate had fallen to 8 g. daily, and to 2·5 g. daily in November, which was one-fifth of the rate of the previous year. The rate in January was 1·7 g. daily, or one part in 16,000. Observations now showed 2·2 g. in March, 4·6 g. in April, 6 g. in May, 7·2 g. in June, 7·7 g. in July, 5·8 g. in August, 5 g. in November, and 2 g. daily in the period extending into February, 1911, a rate of one part in over 12,000, which was greater than that of the previous year, but was still less than the rate of loss during the first year of observation. The rate increased to 2·6 g. daily during February as the room was heated daily during that time. The rate of loss during the first seventeen days of March was nearly 4 g. daily, and 2·3 g. daily during the twenty-four days ending April 10. The entire loss during twenty-nine months was 3·713 kg., or nearly 13 per cent. of the original weight. No noticeable growth had taken place, but sixteen greenish-yellow fruits were retained. It is to be seen that the changes in the form of this plant would be such as to maintain fairly constant transpiratory conditions, and the variations from year to year are to be ascribed to other causes than variations in temperature, air currents, &c.

Echinocactus No. 7 was taken from the soil November 7, 1908, freed from its roots, and set on a metal support in the laboratory. The net weight was 35·818 kg., and the rate of loss during the first month was 18 g. daily, or about one part in 2,000, which was slightly more than that of No. 6. The rate during the winter was 10·5 g. daily, or one part in about 3,500, which was higher than in No. 6. The rate ran very irregularly, falling to 8 g. daily in June, 9 g. in August, and decreasing to 4 g. in November, which, like that of No. 6, was much less than in the previous year. The winter rate did not fall below this, however, and amounted to one part in over 8,000, which was double the rate of No. 6. The loss rose to 9·8 g. daily in June, fell to 6 g. in August to October, to 4 g. daily in October, while the midwinter rate, 1910–11, was 2·1 g. daily, or one part in 15,000, less than that of No. 6 and slightly less than its own rate of the previous year. The rate from February 3 to February 28 was 1·6 g. daily, a notable decrease and less than that of No. 6 during the same period. The erratic record from February to April was found to be due to errors caused by a mouse's nest built among the arms of the balance. During the next seventeen days the rate rose to 2 g. daily, and to 3·3 g. daily during the twenty-four days ending April 10, 1911.

The weight was now 31·570 kg., the total loss during the entire period of the experiment extending over twenty-nine months being but 42·48 kg., or less than 12 per cent. of the original. During this time some apical growth had ensued and eighteen fruits were retained. This plant, with No. 6, was set up for further observation.

Echinocactus No. 10 was taken from the soil November 7, 1908, freed from its roots, and set on a tin box near the south side of the laboratory in the open. Its net weight was 14·588 kg. and it lost 61·6 g. daily during the first month, or one part in 220, about ten times the rate of plants in a shaded room. The rate during the next six months was 30 g., the total being 4·860 kg., out of the original noted above. This rate was one part in 430, which is about five times that of Nos. 6 and 7 during the same period in the shade.

The analysis of the expressed juice of the sap of the cortex gave the following data:

Specific gravity	1·035
Acidity calculated as H_2SO_4 per 100 c.c. . . .	0·1064
Total solids per 100 c.c. of sap	7·060
Ash content of sap per 100 c.c.	3·009

A similar analysis of a turgid plant taken up on September 9, 1909, gave the following:

Specific gravity of sap	1·0095
Acidity calculated as H_2SO_4 per 100 c.c. of sap . .	0·0887
Total solids per 100 c.c. of sap	2·092
Ash content of sap per 100 c.c.	0·792

Echinocactus No. 13 was taken from the soil and freed from its roots on February 18, 1910, after which its net weight was found to be 49·390 kg. It was now mounted on a base of loosely piled black volcanic rock near a *Carnegiea* taken up at the same time. Definite points were located for calibration, and the greatest length was 585 mm. and the greatest diameter 413 mm. The rate of loss was 150·5 g. daily from February to May, this being one part in over 300 total weight. The total loss of 12·490 kg. up to May 12, 1910, was accompanied by a decrease in length of 25 mm. and of diameter amounting to 43 mm. The rate of loss fell to 102 g. daily in July, some new spines being formed and flower buds developed at this time. The rate fell to 33 g. daily by October 3, 1910, at which time the total loss was 12·845 kg. This rate amounted to one part in 873. Although the plant had lost over one-fourth of its total water-balance, eighteen flowers had been formed and eight small fruits matured. The length had decreased to 500 mm., a further shrinkage of 60 mm. in length and of the diameter to 325 mm., a loss of 45 mm. The lowest rate, 9·3 g. daily or one part in nearly 2,900, was found in the period of thirty-five days ending November 9,

1910. The rate rose to 14 g. daily during the period ending February 6, 1911, and the external dimensions remained unchanged from the measurement of October 5, 1910. During this period of eighty-nine days the external measurements did not follow the transpiratory loss.

The final weight, taken on March 20, 1911, was 25·540 kg., indicating a total loss of 455 g. in forty-two days at the rate of nearly 11 g. daily. The total loss in thirteen months amounted to 23·850 kg., or 48·3 per cent. of the total weight. The total solids in the sap amounted to an average of 3·7 g. per 100 c.c., the ash being 1·4 g. per 100 c.c. The average of a large number of freezing-point determinations gave an osmotic activity of about 3 atmospheres at 25° C. These results show a different aspect from anything previously examined. The plant seemed alive as indicated by its osmotic pressure, but the soluble matter in the sap had been reduced to a minimum.

Some interesting relations appear when the amount of loss is contrasted with the degree of succulency. Turgid specimens of *Echinocactus* contain 90 to 95 per cent. of their total weight in water, and this plant may be estimated to have contained about 45 kg. of water at the beginning of the experiment. The cylindrical body, with its fluted surface, offers about twice the transpiratory area of a plain cylinder of the same measurement, which would amount to about 15,000 sq. cm. The degree of succulence would, therefore, be about 3 or 3 g. of water present to each sq. cm. of surface. This is to be compared with 10 g. in leaves of *Suaeda* as determined by Miss Delf.[1] In this condition the plant lost in the open one part in 300 on the average during a period when daily temperature ranged from 32° to 90° F., and in the full blaze of the desert sunlight.

The total loss in weight up to May 12, 1910, was 12·490 kg., which may be taken as being principally water, the total amount remaining now being about 32·5 litres. The surface had shrunken to 14,000 sq. cm., but the succulence had also decreased to about 2·3. These conditions, together with the higher relative humidity prevalent in July, were seen to result in a lessened rate of loss during the summer. At the end of the dry after-summer, the surface showed a further estimated decrease to about 3,000 sq. cm., and as but small loss had ensued since the last observation, the total now being 12·485, the remaining water may be estimated to have been about 32,000 litres, which indicates a succulency of 2·4 g. per 100 sq. cm. of surface, which is slightly greater than that shown in midsummer, although the rate of loss was much lower. Other factors may be taken as contributing to this result. The approximated position of the folds and the cooler night temperatures would both tend to lessen the rate of total loss. Next it is to be seen that as soon as the transpiratory loss was not taken up by the changes in external dimensions, the rate increased even under the retarding

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[1] See Transpiration and Behaviour of Stomata in Halophytes, Ann. of Bot., vol. xxv, 1911, pp. 485–505.

influence of seasonal factors least favourable to transpiration. Nothing may be safely said as to the cause of this rise. It is to be noted in this connexion, however, that the amount of dissolved solids in this plant, instead of being about seven or eight parts per hundred, as shown by other desiccated specimens, was about half this amount, not very much more than the proportion shown by a turgid specimen. The disintegration of certain substances in the walls of the cells or membranes may have altered their permeability and permitted a more rapid water loss.[1]

The Tree Cactus *Carnegiea gigantea*, Britton and Rose, or Sahuaro forms a trunk 25 to 50 cm. in diameter with a heavy cylinder of woody tissue, enclosing a medulla of a diameter amounting to 8–10 cm. in some instances. The cortical layer of colourless cells is also very thick, the outer layers of this tissue being chlorophyllose. Externally the trunk presents a series of longitudinal folds or ridges, separated by furrows which vary in depth much more widely than similar structures in the various species of *Echinocactus*. The older portions of the trunk may become so distended as to eliminate the folding or plaiting. The trunk may reach a height of 12–15 metres and bear many heavy branches. The total weight of the larger plants might be estimated at 2–4,000 kilos, 85–90 per cent. of which may be taken as water. The water-content may, however, undergo a wide variation, as is well illustrated by the following paragraphs.

The root-system penetrates the soil more deeply than those of the *Echinocactus*, and even these organs are thick, soft, and contain a great amount of fluid.

Carnegiea No. 1 was taken up May, 1908, and freed from soil and roots, after which it was set in a perpendicular position on a base of loosely piled rocks in the open. Its estimated weight was about 40 kg. November 5, 1908, the weight was 32·518 kg. It was now brought into the laboratory with the Echinocacti already described. Water was lost at a rate of 62 g. daily during October, and at the rate of 23 g. daily during the next six months, which amounted to one part in 1,700 of the weight at the beginning of the period. The analysis of the expressed juice obtained from the terminal portion of the trunk gave the following results :—

Specific gravity	1·0355
Acidity calculated as H_2SO_4 per 100 c.c. of sap	0·103
Total solids per 100 c.c. of sap	9·622
Ash content of sap per 100 c.c.	2·754

Carnegiea No. 1 *a* was taken up, freed from soil and roots, October 22, 1909, when it weighed 45·325 kg. It was suitably mounted in the laboratory, and lost weight at the rate of 114 g. daily during November. This

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[1] The transpiratory mechanism of *Echinocactus* has been described by Cannon. See Biological Relations of Certain Cacti, American Naturalist, vol. xl, Jan. 1906, p. 27.

rate fell to 8 g. in January, 5·5 g. in February, and rose to 67 g. daily when removed to the open in May, June, and July. The rate fell to 6 g. daily in October, 1910, and to 3·8 g. in the period of 89 days ending February 6, 1911. The loss during a part of February, 1910, was one part in over 8,700, while in the corresponding period of a year later it was but one part in over 9,300. The last calculation was made for a longer period, and the actual minimum may have been much lower.

The total weight of the plant was reduced from 45·325 kg. to 34·810 kg., a total loss of 10·535 kg., or 23·3 per cent. of the original weight. The chemical analysis made in April, after some further loss had been undergone, showed the following :—

Total solids in juice per 100 c.c. . . .	10·44 g.
Ash content of sap per 100 c.c. . . .	1·83 g.

Three determinations of the osmotic activity by the freezing-point method gave 10·15, 9·64, and 9·72 atmospheres, the average being 9·84 atmospheres at 25° C.

Three analyses of growing plants previously reported[1] show total solids in the juice ranging from 3·4 g. to 5·9 g. with an ash content of 1 to 1·7 g. per 100 c.c. of sap.

The desiccation of this plant, which had progressed without any repletion of the water-balance for over 16 months, may be taken as representing about the limit of endurance of the species without water, both as to time and amount of loss. It is of interest to note also that determinations of the osmotic activity of the sap of individuals rooted in moist soil by the freezing-point method gave pressures of 6·78 atmospheres, although some as low as about 4 atmospheres by plasmolytic methods are on record.[2] It is to be seen from this that the range of variation with regard to this condition is as 1 to 2.

The desiccation of *Carnegiea* was not found to cause any noticeable reduction in length, a fact that may be readily understood when the heavy woody cylinder is seen to be well formed to within a few centimetres of the upper end of the trunk. The shrinkage in the cortex would result in some decrease in diameter, but this would be accomplished by contraction of the folds without any great decrease of the outer surface. Turgid trunks consist of over 90 per cent. water, but their slender cylindrical form would operate to give them a relatively lower succulence than *Echinocactus*. Furthermore, the degree of succulence would be much lowered during desiccation by the maintenance of the surface area as suggested. Transpiratory loss seemed to be more or less closely correspondent to the succulence, and to be

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[1] In Publication No. 141, Carnegie Institution of Washington, 1910, p. 47, 1911.

[2] See MacDougal and Cannon : Conditions of Parasitism in Plants. Pub. 129, Carnegie Institution of Washington, 1910, p. 25.

affected by the seasonal changes in an obvious manner. No changes of mode or discontinuity in the rates of loss, such as were seen in *Echinocactus*, were recorded. The extremest stages of desiccation were accompanied by highly increased total solids and ash content of the sap.

Carnegiea seedlings. Twenty young plants, seven months old, were taken from the flat in which the germinations had taken place, and put in a dish in the laboratory, November 4, 1910. The net weight of the lot was 27·244 g., and the loss during the first three days was 6·865 g. at the rate of 2·286 g. daily, this being one part in 12 of the total. Much of this may be attributed to the desiccation of the exposed root-surfaces, although all of the finer branches had been removed. The rate of loss was 0·5 g. daily, or one part in 40 during the next nine days, while in the next 79 days ending February 3, 1911, the rate was 0·85 g. or one part in 30, an increase for which no adequate cause may be given. February 24, 1911, the net weight was 12·499 g., the loss in the preceding 21 days having been 0·055 g. indicating a rate of one part in 46. The total loss was 14·745 g. or 54 per cent. of the original weight. Four of the seedlings were wilted beyond revival, and as many more were in a doubtful condition, the above may be taken as roughly representing the average resistance of the seedlings. From this it may be seen that the seedling loses water at such a rapid rate during even the cooler season as to exhaust its effective balance in about 100–120 days. The death of some of the individuals ensues when something less than 54 per cent. of the total water-balance is lost. This is, however, a greater degree of endurance than that exhibited by adult plants, as may be seen by reference to page 79. The older individuals are not able to withstand a loss greater than about 23 per cent. of their original turgid weight.

Thirty-six resting corms of a native *Brodiaea* were taken from the soil on October 30, 1910, and brought into the laboratory to obtain data which might be of interest in comparison with those furnished by the seedlings of *Carnegiea*. The net weight of the lot was 46·7 g., and the loss during the first 24 hours was 4·9 g., which was one-eleventh of the total. This doubtless represents the desiccation of the outer dried coating of the bulbs. The loss during the following day was 0·78 g. or one part in 53. During the next week, ending November 9, 1910, the loss fell to 0·31 g. daily or one part in 130. The loss during the week ending November 16, 1910, was 1·680 g., 0·24 g. daily, or one part in 200. The total loss was 18·425 g. or 39·5 per cent. of the original weight during 96 days. During the latter part of this period young corms were developed, and the contents were slowly withdrawn from the older ones. The desiccation of the other structures described in this paper resulted in depletions in water-balance followed by shrivelling and contraction of the affected members. The reaction of *Brodiaea*, however, is seen to consist in the formation of new smaller corms which are plump and turgid, although each one contains but the fraction of the water of the larger member from which it originated. The repetition of this action might result in the survival of the species through several arid seasons by the seasonal development of a series of diminishing size (Pl. IX, D).

IBERVILLEA SONORAE.

The indurated tubers of this plant are irregularly globose or flattened, and lie on the surface of the ground. A network of small fibrous roots is formed during the summer rainy season and serves to take in a supply of solution which is added to the enormous balance already present. The growing points which are distributed irregularly over the surface of the tuber awaken at the same time, and produce vines which may reach a length of two to three metres, quickly maturing flowers and fruits (Pls. VIII and X). The rhythm of the plant is such that this activity ensues for many seasons after the plant has been separated from the substratum, as has already been described.[1]

A tuber of a plant which had been established at the Desert Laboratory in 1906 was taken up October 22, 1909, and when cleaned and freed from roots and dead vines, weighed 530 g. The loss was 0·4 g. daily during the first 15 days, or one part in 132, 0·16 g. daily in the next 12 days, and in the 51 days ending January 8, 1910, the loss was but 0·08 g. daily or one part in 6,500. The rate fell to 0·04 g. daily in the latter part of January and February, which was but one part in nearly 13,000, but rose to 0·11 g. daily during the 55 days ending April 21, 1910.

The rising temperature stimulated the formation of green stems in May, the rate of loss rising to 0·3 g. daily, or one part in 1,500. The rise continued until the rate was 0·08 g. daily in July, which was one part in 573, this being the greatest transpiratory activity exhibited at any time. A decrease now began which brought the rate to 0·05 g. in the period ending October 3, 1910, and to 0·3 g. in November, after the vines had died. The rate during the following 91 days was 0·09 g. daily, or one part in nearly 4,500, which it may be seen is in excess of the rate during the corresponding period of the previous year. The room containing this plant was warmed in February so that the rate increased to 0·16 g. daily, the weight being 389 g. on the last day of the month. The rate during the first 20 days of March was less than 0·1 g. daily, decreasing slightly during the next 21 days, but rising again to exactly 0·1 g. daily during the 20 days ending May 1, 1911, slightly less than the rate of loss during the corresponding period in 1910.

May 26, 1911, the weight was 387 g., indicating a daily loss of 0·12 g. during May, an increase commensurate with the higher temperatures prevailing.

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[1] MacDougal, D. T.: Botanical Features of North American Deserts. Pub. No. 99, Carnegie Institution of Washington, 1908, p. 20.

The total loss from this plant in 19 months was 143 g. or 27 per cent. of the total weight. This included the material used in the construction of stems and leaves in the summer of 1910. In addition to the increased transpiration from these stems, some marked loss would be attributable to respiration and to the falling away of dead stems and branches. (See upper right-hand figure in Pl. X.)

Ibervillea Nos. 4 and 5 were received as freshly collected tubers from Dr. J. N. Rose, who was in the field near Guaymas, Sonora, in March, 1910. The tubers were cleaned and freed from roots, then placed on suitable supports in a shaded laboratory. The weight of No. 4 was 1·452 kg. on March 17, 1910. The loss during the first five days was 25·6 g. daily, which was about one part in 520. Much of this accelerated loss was due to the transpiration from cut surfaces and bruised tissues. With the closure of such places the rate fell to 1·3 g. daily in April, and 0·85 g. in May, although the rising temperature would have tended to increase the rate, which continued to fall until it was but 0·36 g. in June, which was one part in nearly 3,700. The development of a number of branching stems caused the rate to rise to 0·66 g. daily in July, which fell to 0·5 g. in the period ending in early October, when the stems were dead. A decrease continued which brought the rate to less than 0·2 g. daily in late October, and to 0·04 g. daily in the period ending February 3, 1911, which was but one part in 30,000. The room was warmed to 18° C. for a few hours daily during February, and the rate of No. 4 rose to 0·3 g. daily in March, but fell to 0·05 g. daily in the 20 days ending April 10, but rose to 0·35 g. in the 21 days ending May 1.

May 26, 1911, the weight was 1·260 kg., indicating a daily loss of 0·16 g. in May, which was less than that in April, although green stems were being formed. A similar decrease in the rate of loss was recorded for the corresponding period of 1910 as noted above. No explanation may be offered for the erratic behaviour of this plant, which was kept within a few centimetres of the other tubers used.

The total loss in 14 months amounted to 192 g. or but 13 per cent. of the original weight, although many branches and small leaves were produced in the summer of 1910. (See left-hand figure in Pl. X.)

Ibervillea No. 5 weighed 1·006 kg. on March 17, 1910. The rate of loss during the following days was but 3·6 g., which was probably less than that of No. 4, largely by reason of the small amount of damaged surface. The rate fell to 0·9 g. in April, and to 0·5 g. in early May. The rising temperatures failed to accelerate the loss, but the rate rose only when green stems were formed. These brought the loss to 0·6 g. later in May, 0·9 g. in June, and to 1·5 g. with the fullest development of the leafy stems in July. The gradual death of the stems brought the rate down to 1 g. daily in the 68 days ending October 3, 1910, 0·9 g. in November, and to 0·15 g. daily in the 91 days ending February 3, 1911. This minimum was equivalent to one part in over 5,000, which was much in excess of the loss displayed by No. 4. The excessive loss is to be attributed to the greater development of green stems, five of which remained alive. The room was warmed to 18° C. for a few hours every day in February, but the rate of loss rose to only 0·16 g. daily. The rate was 0·2 g. daily during the first 20 days of March, 0·2 g. daily during the next 60 days, and 0·32 g. daily in the period of 25 days ending May 1, when a number of rapidly elongating stems were being formed. (See lowermost figure in Pl. X.)

TUMAMOCA.

Tumamoca is a relative of *Ibervillea*, native to the region about the Desert Laboratory, and having similar habits. The tuberous formations which retain the balance of food-material and water, however, are formed underneath the surface of the soil, and the outer layers are not so impervious to water. One of these structures taken from the soil and cleaned, November 2, 1910, weighed 125 g. Upon being placed on a suitable support in the laboratory it lost 1·3 g. daily during the first two days, or one part in 93, although care was taken not to damage the outer layers. This high rate continued, being 1·37 g. during the next five days, although it fell to 0·3 g. daily during the 86 days ending February 3, 1911. Its net weight at this time was but 90 g. and the minimum loss was one part in 400, which is greatly in excess of that of its relative *Ibervillea*, under the same conditions. (See uppermost figure in Pl. X.)

The total loss from this plant amounted to 258 g. or about 25·5 per cent. of the original weight, which was greater than that shown by any other plant of this species. The excess is probably attributable to the greater number of green stems formed.

GROWTH ACTIVITIES OF DESICCATING PLANTS.

In addition to the observations made on the growth or quiescence of plants with a water-balance during a period of depletion recorded in the preceding pages, the unpublished results of a study of the formation of stems from desiccating tubers of *Dioscorea alata*, which were made in the New York Botanical Garden, by the author in 1902, are of interest in connexion with the subject under discussion.

Mature tubers produced by vines in the conservatories were placed in shallow glass dishes on tables within two or three metres from large windows in January of that year. The illumination did not differ greatly in intensity from that of the glass houses. Growth soon began and two or three buds awakened on each tuber, and the stems produced extended at an extremely slow rate during the following 20 months, with the result that an extension of 10 to 15 cm. was reached (Pl. IX, C). A comparative examination was made of this material with stems of vines in the conservatories and of the young stems sent out by tubers in the propagating houses. The work of Duchartre upon *D. Batatas*, Dcsne., was found of great interest in connexion.[1] Duchartre placed tubercles in a dry chamber where they would be compelled to sprout without a supply of soil moisture, in a manner similar to that noted above. He noted that the stems developed were 50 to 80 cm. long, composed of elongated internodes, and that the leaves remained extremely small with diminutive laminae, entirely lacking from lower internodes, and showed a lack of mechanical tissues, from which he points out the resemblances to etiolation as exhibited by some plants. The bases of the stems remained green, but the remainder of the epidermal structures were of a reddish brown colour. He also noted that the base of the stem became the seat of the formation of a tuberous swelling which must have been the beginning of the large tuber usually formed by these plants underground. No stomata were found on the reduced leaves, the starch was not all converted, and the unequal thickenings were not seen in the layer of cells usually becoming collenchymatous. (See Pl. IX, A and B.)

Miss Dale performed similar cultural experiments with *Dioscorea sativa*, although no reference is made by her to the previous experiments of Duchartre.[2]

In this case also elongated stems were formed which measured as much as six or eight feet. The formation of buds at the bases of the stems was noted, but no comparisons of the lengths of internodes with those of the normal were made. Tuberous structures were seen to develop in the axils of the leaves, and the branches remained very short. The formation of adventitious roots around the bases of the stems, with a scaly epidermis, was noted.

The stems in my cultures of *D. alata* did not seem capable of reaching the lengths described by Duchartre and by Dale in the two species named, but this deficiency may be accounted for in part by the smallness of the tubers and correspondingly small supply of food-material available. The stems consisted of two to five internodes, and were angular, reddish brown, and bore numbers of trichomes and glandular structures. The internodes were of a length less than that of the normal in every instance. The bases of the stems underwent a tuberous swelling from which arose numbers of short thick adventitious roots (Pl. IX, C).

The leaves were extremely small, and were in the form of narrow reddish brown scales, with no differentiation into lamina and petiole. The axillary buds showed an activity similar to that of the main buds of the

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[1] Duchartre, P.: Influence de la sécheresse sur la végétation et la structure de l'igname de Chine *Dioscorea Batatas*, Dcsne. Bull. Soc. Bot. de France, vol. xxxii, 1885, p. 156.

[2] Dale, E.: On the Origin, Development, and Morphological Nature of the Aerial Tubers in *Dioscorea sativa*, Linn. Ann. of Bot., vol. xv, 1901, p. 491.

tubers, and the bases of the primary branches were expanded into tuberous formations in the same manner as the primary stems, but the adventitious roots formed did not progress beyond the form of papillate outgrowths. In no instance did the branches attain a length beyond a centimetre.

An examination of my material showed that the xerophilous stems had attained a degree of differentiation of the tissues much less than the normal, which may be attributed directly to the lack of formative material, including water. The pericycle might not be distinguished, and the cortical tissue was composed of cells of greater angularity of outline. Despite the lack of development in the stelar elements, the sub-epidermal parenchymatous elements showed marked collenchymatous thickenings, not seen in normal stems, while the outer walls of the epidermis were much heavier and were distinctly cutinized. (See Pl. IX, A and B.) It is thus to be seen that, despite the lack of nutritive material and the capacity for photosynthesis, the small resources of the shoot were directed towards the formation of elements which would operate to husband the minute supply of water and diminish transpiration. The differences in structural reaction in *D. Batatas* and *D. alata* may be accounted for chiefly by the fact that the tuberous formations of the first are thickened roots, while those of *D. alata* are undoubtedly stem structures.

GENERAL DISCUSSION.

The vegetation of almost all desert regions usually includes a number of rapidly maturing forms, indigenous or of ancient or recent introduction, which carry out their entire cycle of existence during regular or irregular periods of rainfall, and are physiologically mesophytes. Other forms that require much moisture are to be found along streamways.

The specialized forms not affected by the streamways and more or less active during the dry seasons comprise two types, viz. the sclerophyllous and the succulent. The first includes a large number of woody and spinose herbs and shrubs with reduced branches, restricted spread of leaves, and indurated surfaces. These xerophytes have a very small water-balance, and the cell-sap may show extreme concentration. Determinations of the osmotic pressure of the sap of a number of such types at Biskra,[1] in Africa, by Fitting, gave pressures of 100 atmospheres and over. The roots of such plants are usually in continuous absorptive contact with soil particles from which some moisture may be withdrawn, and sclerophyllous forms are notably difficult to transplant, since they wilt so quickly when removed from the soil. Mesophytic plants, when grown under arid conditions, simulate sclerophylls to some extent, although the extremes of osmotic pressure of the sap are probably not approximated.

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[1] See Fitting, H.: Die Wasserversorgung und die osmotischen Druckverhältnisse der Wüstenpflanzen. Zeitschr. f. Botan., vol. iii, 1911, p. 209.

Morphogenic reactions of a xerophytic character are well illustrated by the structural features of *Dioscorea* described in this paper.

The specializations exhibited by sclerophyllous forms are therefore of a direct physiological character and entail the least morphological change. This group is represented in American deserts by a large number of leguminous trees and shrubs, such as *Prosopis*, *Acacia*, *Calliandra*, *Parkinsonia*, *Cercidium*, *Olneya*, &c., and by *Covillea* (*Larrea*), *Fouquieria*, *Lycium*, *Koehberlinia*, *Condalia*, *Zizyphys*, *Manzanita*, *Quercus*, *Aster*, *Encelia*, *Franseria*, *Jatropha*, *Sapindus*, *Vauquelinia*, &c.

Succulents display most of the external features of the spinose xerophytes, which may be carried to their extremest limits. This is well exemplified by the Cacti, in which the entire shoot may be reduced to a short cylindrical or globose form. In addition to these reductions secondary morphological changes have ensued, which have resulted in exaggeration of the medullary or cortical tissues in roots, stems, or leaves, which may contain large balances of water. The sap of succulents generally shows a comparatively low osmotic pressure. Three to five atmospheres are usually found in *Echinocactus Wislizeni* when turgid. *Carnegiea* shows 6–8 atmospheres, and *Opuntia* 10–12; *Agave* slightly more; and the pressure in all of these may increase greatly with desiccation. The greater number of succulents in South-Western America are to be found in the regions in which the rainfall occurs regularly within well-defined seasons. This is true of the plants used in the experimental work described in the preceding pages. The root-systems of such plants are generally horizontally disposed within a few centimetres of the surface, and are in a position where the moisture is most available during the rainy seasons. The cessation of the rains is soon followed by a low water-content of the surface layers of the soil, and the passage of water from the soil to the plant is reduced to a minimum. The finer rootlets perish, and the plant stands self-contained until the next rainy season, when new absorbing branches are formed.

The actual physiological value of large water-balances varies widely, as may be seen by an examination of the experimental data given in this paper. The great Tree Cactus, or Sahuaro (*Carnegiea gigantea*), may survive a year or perhaps two, under certain circumstances, without receiving additional water from the soil. The formation of flowers in the arid fore-summer, however, will not take place unless the plant has received its supply during the previous winter rainy season. Neither would apical growth ensue in midsummer unless the summer rains were available. The death of a plant from the base upwards may sometimes result in the development of flowers on isolated branches which have received no water for a year, but this must be taken as a special case in accord with many experiences that approaching death stimulates reproduction.

Echinocactus was seen to exhibit both root development and apical growth of the stem after one or two years of depletion of the water-balance, and showed some capacity also for flower formation. Individuals exposed to the full intensity of the Arizona sunlight might not survive for more than a year, although even the slightest amount of shade would greatly enhance the value of the enormous water-balance. Plants in an ordinary room were in good condition after three years of deprivation of water.

Repeated observations show that the flattened Opuntias may exist for extended periods, perhaps two or three years, without a water-supply, and may carry out seed-formation during this period. New joints may be formed, but generally at the expense of the older ones, which are destroyed during the process.

Ibervillea has a great balance of food material as well as water, which is accumulated in woody tubers with heavily indurated outer tissues. These plants have been seen to be capable of developing the thin shoots for many years with a small rate of depletion. One individual has been under continuous observation for ten consecutive seasons. The intensity of the illumination and temperature, of course, figure largely in the matter.

The corms of *Brodiaea* respond to seasonal stimuli by the development of smaller new and turgid corms in prolonged periods of desiccation. The older corms are emptied and destroyed in the process, which may be repeated a few times.

No notable morphogenic departures were seen in the phenomena of growth and reproduction as exhibited by *Echinocactus*, *Opuntia*, and *Ibervillea* with a depleted water-balance, except that the new structures were generally of a minimum size. The stems of *Dioscorea* developed under such conditions, however, showed some notable departures towards a sclerophyllous habit. (See pp. 83–5.)

All of the plants examined showed a high rate of water loss immediately upon removal from the soil, due to evaporation from moist outer surfaces and from abraded tissues, regardless of the season. Next it was found that the curve of transpiration was lowest in the cool months of November, December, and January. At this time the daily rate of *Echinocactus* was between one in 40,000 of the total weight and one in 8,000 in a well lighted room, while individuals in the open showed a rate of one part in 5,000 to one part in 2,500, and even one part in 300 in a single instance. The maximum rate during the hot, dry fore-summer with relative humidities between 6 and 20 per cent. and midday temperatures as high as 90° F. indoors and 112° F. in the open, varied between one in 800 and one in 4,000 in a well lighted room, while it rose to one part in 300 for the entire period of February to May, and must have been double this rate in May.

The minimum daily rate of loss in *Carnegiea* (adults) was about one part in 9,000 of the total weight in a shaded room in winter, and the maximum in the open was one part in 640 after the plant had suffered several months' depletion. Seedlings of this plant lifted from the soil in November lost weight at the rate of one part in 12 daily for three days, the average during the next nine days being one in 40, which then rose to one part in 30 in midwinter, and fell later to one part in 227 in the period when some of the individuals were dying. This expected high rate of loss by desiccation would contribute to the enormous mortality of seedlings in the open. Germination takes place in the summer rains in July, and the tender plantlets must then endure a period of four months of extreme aridity, but with falling temperatures.

The resting corms of *Brodiaea* lost one part in 11 by evaporation from the dead outer coats the first day after removal from the soil in November, and this rate fell at once to one in 53, then to one part in 164, and finally to one part in 200. The latter rate was calculated for the period including the formation of new corms. This rate of loss is probably greater than that which would be exhibited by corms *in situ* in the soil.

The minimum rate of loss of *Ibervillea* during the relatively humid cool winter season was one part in 4,500 to one part in 32,000. The maximum in a well lighted room during the arid fore-summer was one part in 573 to one part in 2,000. This applies to the period in which new stems were being formed.

The behaviour of a plant in successive seasons with especial relation to the state of depletion of its water-balance of succulency was a matter which received some attention. *Echinocactus* No. 1 showed a maximum rate of 29 g. daily in 1908, and 15·6 g. a year later, after it had lost but 3 kg. or 7 per cent. of its weight. The minimum rate in March, 1908, was 10 g. daily, while a year later, in the corresponding season, it was less than 4 g. after a loss of but 3 kg. or about 7 per cent. of its weight.

Echinocactus No. 6 lost at the rate of 0·6 g. daily during 155 days including the winter of 1908–9, and 3·7 g. daily during a corresponding period of 173 days a year later, after it had decreased 1·370 kg. or 3·8 per cent. of its earlier weight. A year later the rate was 2·8 g. for a period of 188 days after a loss of but 1·600 kg. more. The maximum rate in June, 1909, was 10 g. daily, and in June and July, 1910, 7 and 7·7 g. daily, after a loss of 1·865 kg. or less than 7 per cent. of its total weight. *Echinocactus* No. 7 lost 9 g. daily in 56 days in midwinter of 1908–9, while the rate was 2·3 g. daily for 109 days in the corresponding period of 1909 and 1910, after a loss of 2·653 kg. or 7·5 per cent. of the earlier weight.

The above data were taken from plants kept in the laboratory, in which corresponding seasons would be fairly equalized.

Echinocactus No. 13 lost at the rate of 150 g. daily during 83 days ending May 12, 1910, and 10 g. daily in the 42 days ending March 20, 1911, after its weight had decreased nearly 50 per cent. This plant was exposed in the open.

An *Ibervillea* tuber lost at the rate of 0·8 g. in May, 1910, and at the rate of but 0·12 g. daily in May, 1911, after the weight had decreased 15 per cent. Green stems were present, and the conditions fairly equivalent in the two periods.

Ibervillea No. 4 lost at the rate of 0·8 g. daily in 15 days ending May 26, 1910, during which time new stems were starting, and at the rate of 0·16 g. daily under equivalent conditions in 1911, after the weight had decreased 15 per cent.

Ibervillea No. 5 lost at the rate of nearly 0·7 g. daily in 15 days ending May 26, 1911, under equivalent conditions, after the weight had decreased 22 per cent.

The green stems were especially vigorous in No. 5, and to their activity must be attributed the excessive total and high rate shown in May, 1911. An inspection of these data shows that a total loss of 15 per cent. in weight was followed by a decrease of 85 per cent. in the rate, a loss of 8 per cent. in weight by a decrease of 80 per cent. in the rate, a loss of 22 per cent. in weight by a decrease of 56 per cent. in the rate, in tubers of *Ibervillea*. The significance of these measurements is partly obscured by the activity of newly developing green stems, but it is evident that the major variation in the transpiratory loss with progressive depletion of the water-balance (which in turgid individuals would be 60–80 per cent. of the total weight) is not to be attributed to climatic variation, or the activities of stems formed, although the influence of the last-named feature is obvious.

The evidence furnished by the individuals of *Echinocactus* free from the influence of factors which would prevent a fair comparison affords some further illustration of the matter.

Echinocactus No. 1 lost water at the rate of 109 g. daily in March, 1908, and at the rate of 3–5 g. daily in March, 1909, a decrease of 50–66 per cent. in rate with a lessening of the water-balance of about 7·7 per cent. The maximum rate in May, 1908, was 29 g. daily, in June, 1909, 15·6 g. daily, a decrease of nearly 47 per cent. in rate with a loss of 7·2 per cent. in weight.

Echinocactus No. 4, which had already been exposed in the open for eight months, lost at the rate of nearly 0·45 g. daily in 182 days ending May 21, 1909. The loss during the 209 days ending May 13, 1910, was at the rate of about 0·24 g. daily, a decrease of nearly 50 per cent. in rate after a total decrease of about 7 per cent. of the earlier weight.

Echinocactus No. 6 lost at the rate of 6 g. daily for 155 days ending May 12, 1909. In 177 days ending May 13, 1910, the rate was 3 g. daily, a decrease of 30 per cent., although the weight had fallen but 4·7 per cent.

The rate was but 2·3 g. in 157 days ending April 10, 1911, a decrease of 23 per cent. in the rate although the weight had fallen but 3·5 per cent.

Echinocactus No. 13 lost at the rate of 150·5 g. daily in 83 days ending May 12, 1910, and after a decrease of 28·6 per cent. of the remaining weight, the rate was less than 11 g. daily, a decrease of 92·6 per cent.

Five possible causes which might have influenced the rate of transpiration of a desiccating succulent present themselves. These are as follows: 1st, the increased concentration of the cell-sap, which was of such degree in the experiments as to increase osmotic pressures from 4 or 5 to 10 or 12 atmospheres, might retard evaporation from the cell-membranes; 2nd, a diminution of the degree of succulence, or proportion of water per unit area of surface present might lessen evaporation; 3rd, desiccation may result in alterations in the character of the outer membranes, or of any of the transpiring walls of the plant; 4th, desiccation may stimulate the formation of new tissues or the alteration of existing cells in such manner as to close openings through which water vapour might pass; and 5th, the positions of the surfaces might be shifted in such manner as to vary the exposure and lessen transpiration.

Livingston has recently pointed out that a concentration of the sap, even if carried to a point where an osmotic pressure of 100 atmospheres was exhibited, would not give a retardation of more than 10 per cent. from the rate afforded by a pure water surface.[1] It is evident, therefore, that this factor is negligible in the present discussion, as the increases found were not more than 5 or 6 atmospheres.

The records of the observations are not sufficiently complete to permit any accurate integration of the influence of succulence upon the rate of transpiration. The course of events in the behaviour of *Echinocactus* No. 13 shows a fair correspondence between the two in the course of rapid desiccation in the open air. The rate of loss diminishes so much more rapidly than the estimated degree of succulence in slowly desiccating individuals that it is impossible to escape the conclusion that other agencies are operative. Thus, *Echinocactus* No. 1, weighing over 42 kg. in March, 1908, may be safely estimated to have contained at least 92 per cent. or 39 kg. of water, and by comparison with No. 13 would have a relative succulence of 3 and a surface of 13,000 sq. cm. The rate of transpiration was 10 g. daily. A year later the degree of succulence after a loss of 3 kg. may be estimated at 2·76, with no allowance for diminished external area. The rate of loss, however, was 5 g. daily, a decrease of 50 per cent., although the succulence had fallen but 8 per cent. If an allowance were made for the shrinkage of external area, the disproportion would be greater, as the

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[1] Livingston, B. E.: The Relation of Osmotic Pressure in Plants of Arid Habitats. Plant World, vol. xiv, 1911, p. 153.

actual decrease in succulence was probably not more than 5 per cent. Data from *Echinocactus* No. 6 for longer periods are equally interesting. This plant contained about 27 kg. of water December 8, 1908, and with a succulence of 3 would offer a surface of 9,000 sq. cm. The rate of loss for the 155 days ending May 12, 1909, was 6 g. daily. November 18, 1909, a further loss, 2·335 kg., had ensued and the succulence was then 2·7 daily. In the 178 days to May 13, 1910, the rate of loss was but 2·8 g. daily. The succulence had decreased 10 per cent. and the transpiration rate 53 per cent. The annual growth and consequent accelerated loss in *Ibervillea* makes the data from these plants ineligible in the present connexion.

The approximation of the ridges or folds in *Echinocactus* would operate to diminish the angle of exposure to the sun and the consequent transpiratory loss. A detailed examination of *Echinocactus* has not been made, but the corky outer layer of the tubers of *Ibervillea* is being added to at intervals, and changes of this character would lessen the water loss.

The total losses from various plants in which the course of desiccation was not disturbed is given below :—

Name.	*Original weight.*	*Total loss.*	*Proportion.*	*Period.*	*Remarks.*
Echinocactus.					
„ No. 1.	42·743 kg.	4·583 kg.	11%	15 mo.	In shaded room. Died.
„ No. 2.	5·136 kg.	1·724 kg.	33%	18 mo.	Shade and open air. Living.
„ No. 4.	1·192 kg.	0.643 kg.	54%	36 mo.	Died.
„ No. 6.	28·573 kg.	3·713 kg.	13%	30 mo.	Shade. Living.
„ No. 7.	35·818 kg.	4·248 kg.	11%	30 mo.	Shade. Living.
„ No. 13.	49·390 kg.	23·850 kg.	48%	13 mo.	Open. Living.
Carnegiea.					
„ No. 1.	40·000 kg.	11·250 kg.	28%	12 mo.	Open air and shade. Living.
„ No. 1*a*.	45·325 kg.	10·535 kg.	23%	16 mo.	Open air. Living.
20 seedlings.	27·244 g.	17·745 g.	54·7%	102 da.	Shade. 4 dead.
Brodiaea.					
36 resting corms.	46·700 g.	18·425 g.	39·5%	96 da.	Shade. Young corms formed.
Ibervillea.					
„ No. 0.	530· g.	143· g.	27%	19 mo.	Shade. Sending up shoots annually.
„ No. 4.	1·452 kg.	164· g.	11·5%	14 mo.	Shade. Annual shoots.
„ No. 5.	1·006 kg.	278· g.	27%	14 mo.	Shade. Annual shoots.
Tumamoca.					
Tuber	125· g.	34·4 g.	17·5%	4 mo.	Shade. Died.

No special attention was given to the matter of absorption of water or water vapour by the plants which were used in the experimental work. The results of the weighings, however, disclose the fact that small individuals of *Echinocactus* made a net increase in weight when placed in a dark room with a relative humidity of 80–90 per cent., after removal from a room with a humidity of 15–30 per cent. Other specimens made similar gains when brought into a shaded room from the open air. Still another specimen showed a distinct gain in the open during a night of drizzling rain. As these plants are provided with a dense armature of long curved spines (see Pl. VII) which are hygroscopic, their dead tissues dry out under arid conditions and appear to take up water or aqueous vapour, as any bit of dry wood might do it. The bases of these spines are made up of heavy-walled tissues through which water might pass with difficulty. It is probable, therefore, that the changes in weight of *Echinocactus* due directly to humidity do not affect the succulence of the living tissues, and concern the dead spines only.

The actual behaviour of the spines with regard to atmospheric moisture was tested in July, 1911. The spines from two ridges of a plant 50 cm. in height were detached and sent to the acclimatization laboratory at Carmel, California. The lot was placed upon a suitable sheet of paraffin paper and found to weigh exactly 20 g., in a sunny place, at noon on July 8. After exposure to the humid air in the open throughout the night, the weight was found to be 20·400 g. at 9.15 a.m. on the 9th, after some drying out had taken place. Desiccation occurred as before and the spines were again placed in the open and sheltered from precipitation, at 7 p.m. on the 10th. The night was foggy and misty and the spines absorbed so much that the weight had increased to 20·745 g. at 7 a.m. on the 11th. The lot of spines was practically equivalent to those clothing *Echinocactus* No. 4, and was about one-tenth of the whole number on the plant from which they were taken. The total weight variation on such a plant due to imbibition and desiccation of the spines might, therefore, be about 7 or 8 g. daily, and on the largest individuals two or three times this amount. The proportion of weight variation due to such causes would be large in small plants.

The conditions presented are different from those studied by Spalding,[1] Schönland,[2] and Marloth. Marloth [3] detected positive absorption of water and water vapour by the hairs of *Mesembryanthemum* and *Crassula*, and the structure of one type of trichome in the younger stage is such as to permit the passage of water into the living cells. Lloyd found that water absorbed by the loose bark of *Fouquieria* passed into the living tissues, and that the consequent increase of the water-balance was followed by the unfolding of new leaves in stems, quiescent at the beginning of the experiment.[4] Miss Delf demonstrated that water in liquid form as well as a vapour might pass the epidermis of the green parts of *Suaeda maritima* and *Sali-*

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[1] Absorption of Atmospheric Moisture by Desert Shrubs. Bull. Torr. Bot. Club, vol. xxvi. 1906, p. 367.

[2] On the Absorption of Water by the Aerial Organs of some Succulents. Trans. Roy. Soc. South Africa, vol. i, part II, 1910, p. 395.

[3] Notes on the Absorption of Water by Aerial Organs of Plants. Trans. Roy. Soc. South Africa, vol. i, part II, 1910, p. 28.

[4] The Artificial Induction of Leaf-formation in the *Ocotillo*. Torreya, vol. v, 1905, p. 175.

cornia annua, when the water-balance was low, and that absorption through immersed surfaces was exhibited by many plants.[1]

Purpose and adaptation may be easily read into such action. This kind of an interpretation seems forced, however, when it is considered that a capacity for absorption depends upon physical qualities, the primary significance of which relates wholly to other features of the activity of the plant.

EXPLANATION OF PLATES VI–X.

Illustrating Dr. MacDougal's paper on 'Water-balance'.

Plate VI. *Echinocactus Wislizeni*, normal and cristate specimen below. Two plants of *Carnegiea gigantea*, the taller of which is about 150 years old. The smaller plant has been girdled by rabbits, and may survive many years in this condition.

Plate VII. *Echinocactus* No. 4, with water-balance depleted by desiccation for 30 months.

Plate VIII. *Ibervillea sonorae*, with old stem climbing on branches of *Parkinsonia microphylla*.

Plate IX. A, Partial transverse section of stem of *Dioscorea alata* formed by desiccating tuber; B, from normal stem of same; C, tuber of *Dioscorea alata* with stems formed during desiccation. D, *Brodiaea*, with young corms formed during the desiccation.

Plate X. Tubers of *Ibervillea* and *Tumamoca* after desiccation for one year. Living and dead stems formed during this period are still attached.

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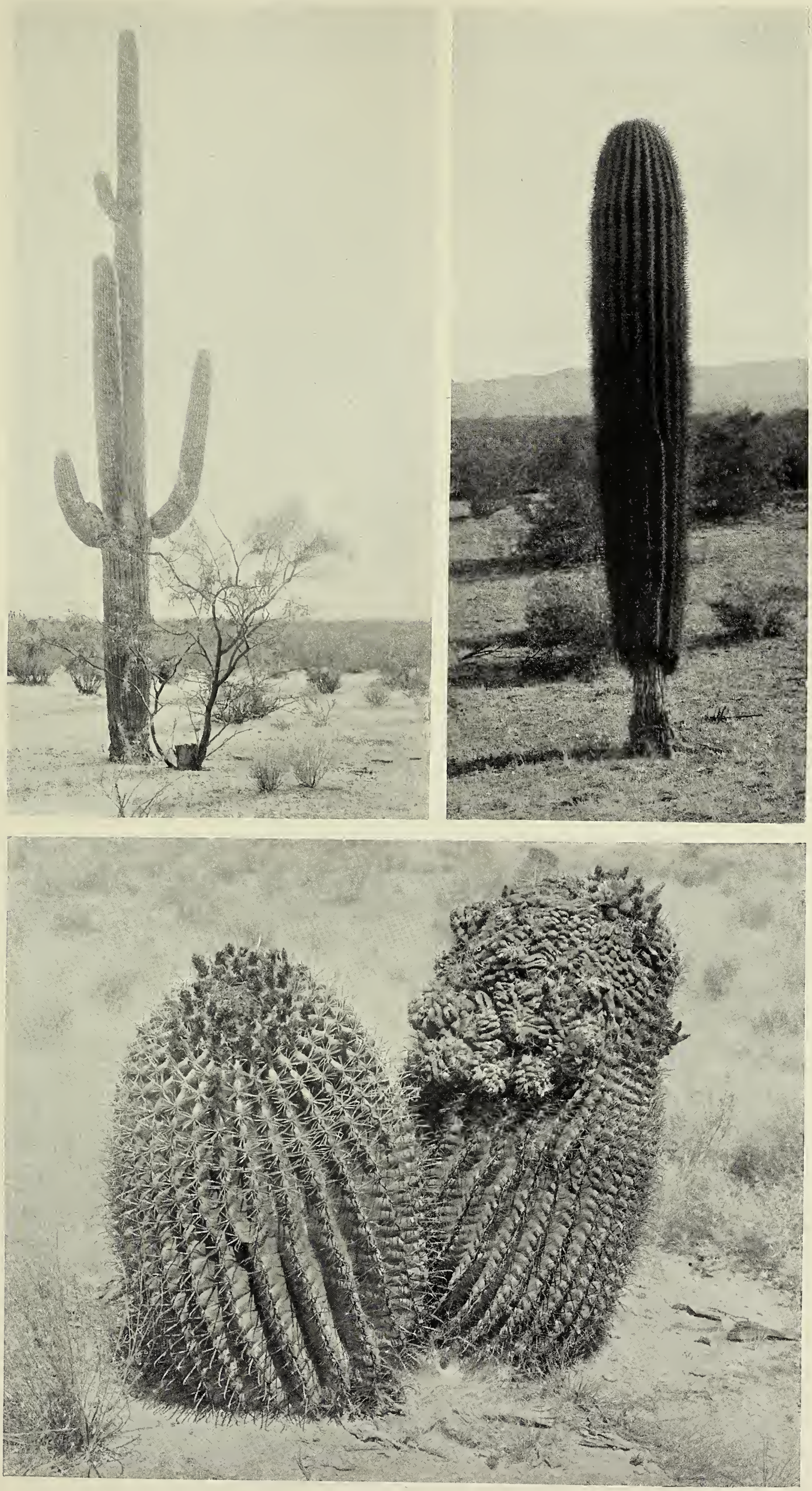
[1] Transpiration and Behaviour of Stomata in Halophytes. Annals of Botany, vol. xxv, April, 1911, p. 485.



MACDOUGAL—DESERT PLANTS

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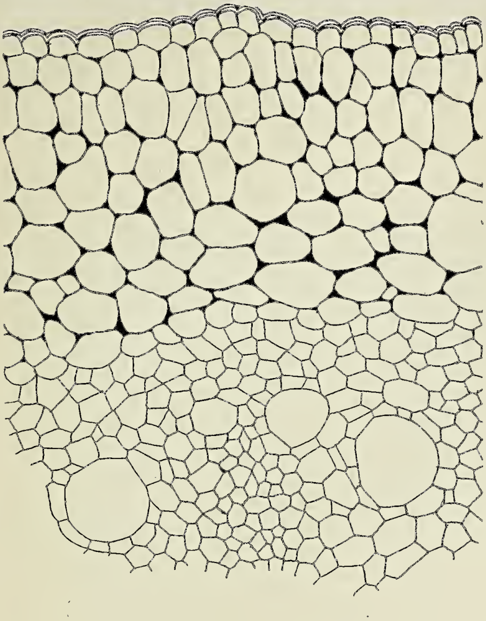

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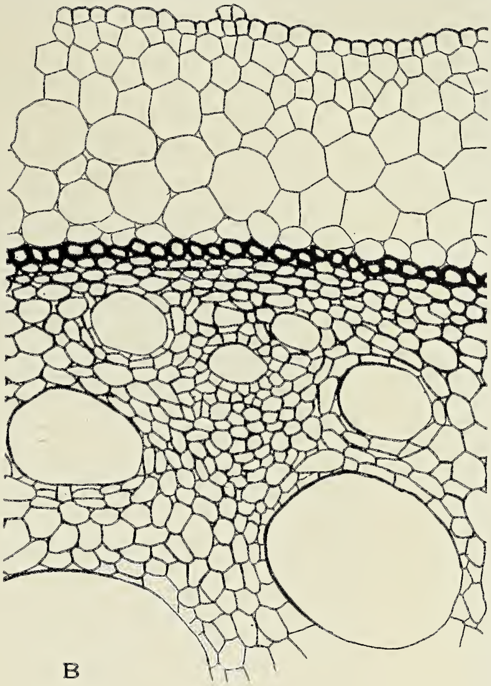

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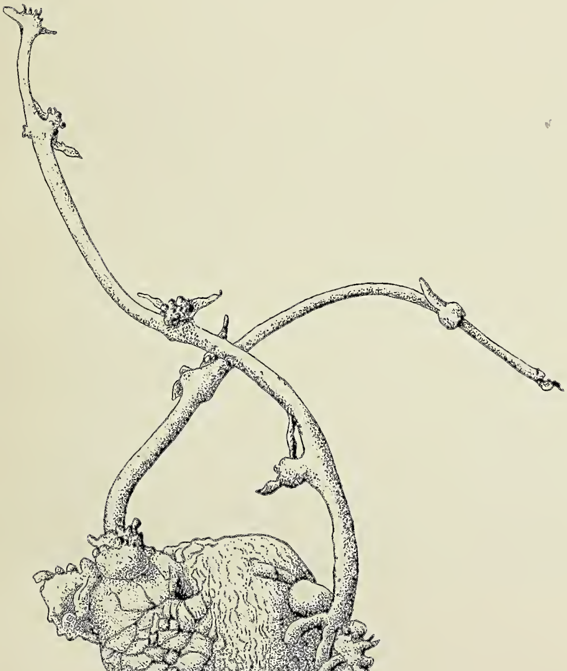

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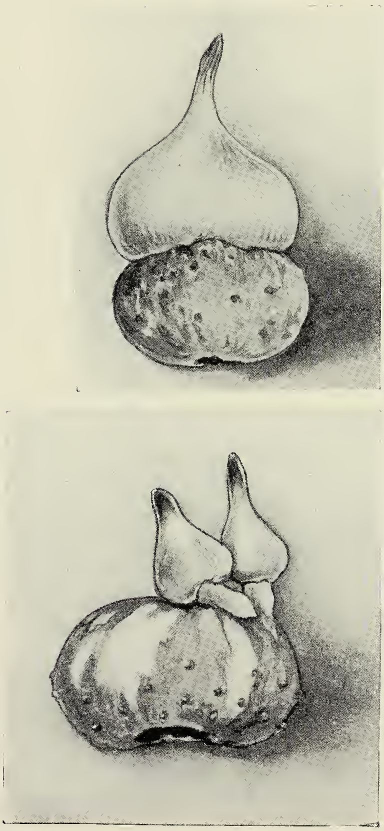

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MACDOUGAL—DESERT PLANTS

MACDOUGAL—DESERT PLANTS

The Weeds of Arable Land in relation to the Soils on which they grow. II.

BY

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DURING the season of 1910 an investigation was carried on in certain parts of Bedfordshire to determine the relation between the weeds of arable land, the crop grown, and the soils upon which they grow.

It was recognized at the outset that the results must be regarded as tentative in nature until confirmatory evidence was obtained from similar work carried out in other parts of the country, on soils both of the same and different geological derivation. Consequently in 1911 the field of investigation was transferred to certain parts of Wiltshire and Somerset, to districts round Warminster (Upper Greensand), Shrewton (Chalk), and Bath (Clay). In that part of the country a large number of geological formations crop out within a comparatively short distance, but attention was confined to two or three main types of soil.

In the area investigated no sand was available to compare with the Lower Greensand of Bedfordshire, the soils of the Upper Greensand being generally rather heavy. In place of the Oxford and Gault clays (here only represented by pasture), which are non-calcareous in nature, the calcareous clays of the Oolite and Lias series came under consideration.

The working plan adopted was the same as in 1910; the areas were characterized by the comparative absence of hedges, which greatly facilitated the work.

The relative prevalence of the weeds was again noted as:—

(1) Dominant.
(2) Sub-dominant.
(3) Distributed.
(4) Occasional.
(5) Scarce or rare.

During the season's survey 106 species of weeds were met with, representing 74 genera. Of these 29 species, representative of 26 genera, were

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each seen once or twice only. As in the previous investigation, attention was confined to those weeds growing in among the crop, the weeds of the surrounding hedgerows being left out of consideration. The classification of the soils did not present so much difficulty as in the case of the Bedfordshire work. The areas dealt with were much larger, and adjoining fields did not show the sudden change in the nature of the soil that occurred so frequently in the eastern county. In the chalk areas rather more subdivision had to be made this year. The presence or absence of lime in the various soils was frequently tested for with dilute hydrochloric acid.

The seasons in the two years of investigation were of totally different character, that of 1910 being wet and cold, with a deficiency of sunshine, while that of 1911 was characterized by an excessive amount of sunshine and by high temperatures, coupled with a low rainfall, so that conditions of drought prevailed. This radical difference may have influenced the relative occurrence of certain species, so that it is quite probable that some of the diversity in results may be due to seasonal variation rather than to the actual differences in the nature of the soil.

The chief species of weeds, with their habitats and dominance, were as follows :—

Ranunculaceae. *Ranunculus acris.* Found on clay and sand. Absent from chalk. Never dominant.

Ranunculus arvensis. Chiefly found on clay, once on chalk. Never dominant. (Rarely seen in this district; far less frequent than in Bedfordshire.)

Ranunculus repens. Most frequent on chalky soils; also seen on clay containing a fair proportion of lime. (N.B.—It is possible that in the Bedfordshire districts *R. repens* occurred in some instances, but was not separated from *R. acris.*) Never dominant.

Papaveraceae. *Papaver hybridum.* Only occasionally seen, then on chalky soil. Never dominant.

Papaver Rhoeas. Characteristic of chalky soils; hardly seen elsewhere. Occasionally dominant. (Many observations of poppies were made when the plants were too small to permit the species to be identified. These usually occurred on chalky soils, with one record (dominant) from sand.)

Fumariaceae. *Fumaria officinalis.* Chiefly on chalky soils, but fairly frequent on loam. Several records from clay, but then it was usually occasional or scarce in distribution. Occasionally dominant on chalk or loam.

Cruciferae. *Brassica alba.* Practically confined to chalk and brashy loam (limestone). Frequently dominant; one instance of dominance on clay, which proved to contain much limestone.

Brassica Sinapis. Chiefly on chalk and red land; also much on clay and loam. No record from ordinary sand. Frequently dominant on all types of soil, including 'bake'. (This plant is perhaps more widely distributed in area than in Bedfordshire, but as it is not so abundant in quantity in the West Country, it is consequently less in evidence.)

Capsella Bursa-pastoris. Practically confined to clay and loam. Never dominant.

Resedaceae. *Reseda lutea.* Characteristic of chalk, but only occurring in very small quantity.

Violaceae. *Viola tricolor.* Practically confined to chalky soils. Very rare on clay. Never dominant.

Caryophyllaceae. *Arenaria serpyllifolia.* Occasionally found, usually on chalky soils. Never dominant.

Cerastium vulgatum. Distributed on various types of soil, but seldom seen and never very prevalent.

Lychnis Githago. Confined to chalk. Once dominant.

Lychnis vespertina. Chiefly on chalky soils, sometimes on clay and loam. Of frequent occurrence as an occasional or scarce weed.

Silene Cucubalus. Chiefly on chalky soils ; occasionally on clay, where it was once dominant.

Stellaria media. Universally distributed. Occasionally dominant on any soil.

Rosaceae. *Potentilla anserina.* Occasionally seen on chalky soils and clay. Once dominant on chalk.

Umbelliferae. *Daucus Carota.* Generally found on pure chalk, sometimes on chalky loam. Never dominant, usually only occasional.

Heracleum Sphondylium. Characteristic of chalky soils. Never dominant.

Scandix Pecten. Characteristic of chalky soils. Very occasional elsewhere. Once dominant on red land.

Rubiaceae. *Galium Aparine.* Generally distributed. Rarely dominant or sub-dominant, though of very frequent occurrence.

Sherardia arvensis. Rarely seen, but then chiefly on chalk in very small quantities.

Valerianeae. *Valerianella olitoria.* Confined to chalk. Rarely seen.

Dipsaceae. *Scabiosa arvensis.* Characteristic of chalk soils. Never dominant.

Compositae. *Anthemis sp.* Chiefly on clay or loam, but distributed over other soils. Once dominant on clay.

Carduus arvensis. Universally distributed and very common. Dominant on any soil.

Centaurea nigra. Chiefly found on chalk and red land; only occasionally recorded from other soils. Never dominant.

Senecio vulgaris. Universally distributed, but by no means so frequent in occurrence as in Bedfordshire. Never dominant.

Sonchus arvensis. Generally distributed, but chiefly on chalk and clay. Frequently dominant on chalk, also dominant on clay and sand.

Taraxacum dens-leonis. Recorded most frequently from chalky soils, but also occurs on all other types. Never dominant.

Tussilago Farfara. Chiefly on clay, but frequent on other soils. Dominant on clay and chalk.

Primulaceae. *Anagallis arvensis.* Distributed over various types of soil but rarely in any quantity. It does not seem to appreciate chalky soils. Never dominant; once sub-dominant on 'bake'.

Convolvulaceae. *Convolvulus arvensis.* Universally distributed as to soil and dominant anywhere. One of the most commonly occurring weeds.

Boraginaceae. *Lithospermum arvense.* Characteristic of chalky soils. Never dominant.

Myosotis arvensis. Confined to chalky soils. Never dominant.

Orobanchaceae. *Orobanche minor.* Only recorded from one district on chalky soils. Never dominant.

Scrophulariaceae. *Bartsia Odontites.* Practically confined to chalky soils; very occasional on clay. Never dominant.

Linaria spuria. Occasionally recorded from the lighter soils—loam and red land. Never dominant.

Linaria vulgaris. Characteristic of chalky soils. Never dominant.

Veronica agrestis. Found on all types of soil. Never dominant.

Veronica hederaefolia. Recorded from all types of soil, but only dominant in one place, on chalk.

Labiatae. *Mentha arvensis.* Chiefly found on clay soils; probably characteristic of heavy land. Never dominant, once sub-dominant on clay.

Plantagineae. *Plantago lanceolata.* Practically confined to chalky soils. Never dominant.

Plantago major. Recorded from all types of land, but chiefly found on clay and chalky soils. Never dominant.

Plantago media. Confined to clay; never seen on chalk. Never dominant.

Illecebraceae. *Sceleranthus annuus.* Only seen on 'bake'.

Chenopodiaceae. *Chenopodium album.* Universally distributed both as to soil and dominance.

Polygonaceae. *Polygonum aviculare.* Chiefly found on chalk and clay. Often dominant on both types.

Polygonum Convolvulus. Chiefly on clay and chalky soils. Never dominant.

Rumex Acetosella. Only seen on 'bake'. (Characteristic of acid sandy soils.)

Rumex crispus. Most frequent on chalky soils, but often seen on clay and other types of land. Occasionally dominant on sand and red land.

Euphorbiaceae. *Euphorbia exigua.* Chiefly on clay, but frequent on other soils. Never dominant, but occasionally sub-dominant on clay.

Euphorbia Helioscopia. Fairly well distributed, but most frequent on chalky soils and loam. Never dominant, usually only occasional or scarce.

Liliaceae. *Ornithogalum pyrenaicum.* Characteristic of heavy clay soils in the Bath district. Rare.

Gramineae. *Alopecurus agrestis.* Occurred on various types of soil. Occasionally dominant on chalk.

Arrhenatherum avenaceum var. *tuberosum.* Distributed as to soil. Once dominant on 'black gravel'.

Bromus arvensis and *B. sterilis.* Very seldom seen, then only on chalk.

Lolium perenne. Only on chalk and clay. Never dominant.

Poa annua. Chiefly on clay, but also found on other soils. Never dominant.

Poa pratensis. Only recorded from chalk. Once dominant.

Poa trivialis. Universal in distribution. Once dominant on clay.

Triticum repens. Universal in distribution. Dominant on heavy land.

Equisetaceae. *Equisetum arvense.* Universally distributed as to soil, but not very frequent in occurrence. Once dominant on clay.

Analyses of the data have been made showing the distribution of the various weeds on the different soils. It must be remembered that a weed that is 'absent' from any particular soil may occur in isolated instances in that very habitat, in which case it is usually only occasional or very rare in distribution.

A. Clay and Heavy Brashy Soils.

In the districts round Inglescombe and Inglesbatch, near Bath, the land is of an exceptionally heavy nature, being a very sticky clay, which needs special care and methods to farm it properly. During the hot weather it bakes very badly and opens out into deep cracks. This soil is derived from three geological formations which crop out in succession up the hillsides, and it is characteristically calcareous on account of the limestone which occurs with it, in contradistinction to the non-calcareous clays worked in Bedfordshire.

1. Inferior Oolite; soil very reddish in colour and generally brashy in nature. Contains much limestone.

2. Fuller's earth; an argillaceous deposit between 1 and 3. Below the surface this appears as a very sticky yellow clay.
3. Bath or Great Oolite; clay and limestone.

In the Wilmington district, on the further side of the valley from the above, the soil is derived from—

1. Lower Lias; clay and limestone.
2. Inferior Oolite.

[In both these districts there is an outcrop of the Midford Sands, but this is generally either wooded or under pasture, and so does not affect the investigation in hand.]

The derivation of the soils does not appear to affect the varieties of plants occurring, as the different species of clay weeds were found indiscriminately on land derived from all the four geological formations, all being calcareous in nature.

The number of species occurring was relatively less than those found on chalky soils, and the majority of the plants were such as occurred on any type of soil, hardly one appearing to be *symptomatic* of clay. A very few species, however, were chiefly associated with clay, though occurring on other types of soil as well, and one, *Plantago media*, appears to be confined to clay in this district.

Plantago media—practically confined to clay.

Capsella Bursa-pastoris—also recorded from loam.

Mentha arvensis	chiefly found on clay, but also seen on other soils.
Poa annua	
Ranunculus arvensis	
Tussilago Farfara	

Ranunculus acris—also on sand.

Brassica alba	also on chalk.
Euphorbia exigua	
Plantago major	
Polygonum aviculare	
„ Convolvulus	
Potentilla anserina	

B. Chalk.

The chalk districts investigated were chiefly on the Wiltshire Downs in the neighbourhood of Shrewton and Stonehenge, a radius of some four miles being worked. Data were also obtained from the chalk at Heytesbury and Sutton Veny, not far from Warminster. The whole of the soils were derived from the chalk formation, though for convenience of working and classification they were subdivided into—

(*a*) *Chalk*, where the soil was white or very light in colour.

(*b*) *Red Land*, lying above (*a*), rather darker in colour and perhaps a little heavier in texture.

(*c*) *Chalky loam*, very light calcareous soil, but slightly heavier than the above.

The noticeable feature of the weed flora is the large number of species occurring, many of which are essentially characteristic or even symptomatic of chalk. In both these respects the flora is in marked contrast to that of the clay soils.

Bromus arvensis	confined to chalky soil.
„ sterilis	
Campanula hybrida	
Daucus Carota	
Linaria spuria	
„ vulgaris	
Lychnis Githago	
Myosotis arvensis	
Poa pratensis	
Reseda lutea	
Centaurea nigra	very characteristic of chalk, but occasionally seen on other soils.
Heracleum Sphondylium	
Lithospermum arvense	
Papaver Rhoeas	
Plantago lanceolata	
Scabiosa arvensis	
Scandix Pecten	
Sherardia arvensis	
Viola tricolor	
Arenaria serpyllifolia	chiefly on chalk, sometimes on clay.
Bartsia Odontites	
Brassica Sinapis	
Fumaria officinalis	
Lychnis vespertina	
Papaver sp.	
Ranunculus repens	
Silene Cucubalus	
Brassica alba	equally abundant on clay.
Euphorbia exigua	
Plantago major	
Polygonum aviculare	
„ Convolvulus	
Potentilla anserina	

C. 'Bake.'

This is a thin layer of soil, often only a few inches thick, overlying the chalk on the top of some of the Downs. Instead of being calcareous, this layer is destitute of lime, and is indeed sandy and acid in nature, of a red colour. The local theory is that for some reason or other the lime has been entirely washed out from the soil and carried down, leaving a non-calcareous residue. The peculiar nature of the soil is reflected in the flora, the abrupt change from the typical chalk flora being quite startling in its suddenness. Unfortunately very little of the land was accessible. The number of species was relatively few, and the quantity of weed was comparatively scanty.

The actual weed flora may prove of interest:

Brassica Sinapis Geranium sp.[1] Scleranthus annuus	dominant in places.
Anagallis arvensis	sub-dominant or distributed.
Carduus arvensis Rumex Acetosella	occasional.
Poa trivialis Scandix Pecten Spergula arvensis	scarce.

The presence of *Rumex Acetosella* and *Spergula arvensis* is most significant, as these are unfailing indicators of an acid soil. The prevalence of *Anagallis arvensis* is also noteworthy, as on the chalky soil very few plants of this species are to be seen.

While so many species are more or less definitely associated with particular soils, others are universally distributed, and occur on various types of soil, on any of which they may be dominant. A few plants, while universal, are more particularly associated with chalk.

Weeds of universal occurrence.

Alopecurus agrestis
Anthemis arvensis
„ cotula
Arrhenatherum avenaceum var. tuberosum
Carduus arvensis
Cerastium vulgatum
Chenopodium album
Convolvulus arvensis
Equisetum arvense
Galium aparine
Lolium perenne
Poa trivialis
Senecio vulgaris
Sonchus arvensis
Stellaria media
Triticum repens
Veronica agrestis
„ hederaefolia

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[1] The species was unfortunately not identified, but was probably either *G. molle* or *G. dissectum.*

Anagallis arvensis—(only in very small quantities on chalk).

Euphorbia Helioscopia Rumex crispus Taraxacum dens-leonis	especially on chalk.

Geranium columbinum „ dissectum „ molle	(Probably some species are specially associated with chalk or clay, but there were not sufficient records to justify such association.)

Any plants not mentioned in the foregoing lists will be found in the following table. Most of these were seen very seldom or only once during the investigation.

Ranunculaceae	Ranunculus bulbosus	sandy loam
„	„ hirsutus	clay
Cruciferae	Brassica oleracea	chalk
„	Cochlearia Armoracia	sandy loam
„	Senebiera Coronopus	clay
Caryophyllaceae	Lychnis diurna	„
Papilionaceae	Trifolium repens	chalk
„	Vicia hirsuta	sand
Rosaceae	Alchemilla arvensis	clay
„	Potentilla reptans	various
Umbelliferae	Caucalis nodosa	clay
Valerianeae	Valerianella olitoria	chalk
Compositae	Bellis perennis	chalk and clay
„	Carduus lanceolatus	clay
„	„ nutans	red land
„	Centaurea Scabiosa	„ „
„	Chrysanthemum Leucanthemum	various
„	Lapsana communis	chalk and clay
„	Matricaria inodora	sand
Boragineae	Borago officinalis	chalk
„	Symphytum officinale	sandy loam
Solanaceae	Solanum nigrum	sand
Orobanchaceae	Orobanche minor	chalk
Scrophularineae	Linaria minor	chalk and clay
„	Veronica arvensis	chalk
Labiatae	Glechoma hederacea	chalky soil
„	Lamium amplexicaule	chalk
„	„ purpureum	sandy loam
„	Prunella vulgaris	clay
„	Stachys arvensis	„
Polygonaceae	Rumex obtusifolius	„

Urticaceae	Urtica dioica	clay and sandy loam
Liliaceae	Ornithogalum pyrenaicum	clay
Gramineae	Avena fatua	black gravel
,,	Phleum pratense	clay

COMPARISON OF THE WEED FLORA OF THE WILTSHIRE AND SOMERSET DISTRICTS WITH THAT OF EAST BEDFORDSHIRE.

A. Weeds of clay soils.

The general aspects of the weed floras are fairly comparable, both as regards the distribution and variety of the species. Certain differences are however evident, of which the following are the most striking:

	Bedfordshire.	*West Country Districts.*
Bartsia Odontites	Typical and practically confined to clay. Never seen on chalk	Chiefly on chalk; scarce on clay
Matricaria inodora	Very plentiful	Totally absent
Plantago media	Scarce; distributed on various soils	Fairly frequent; practically confined to clay
Capsella Bursa-pastoris	Scarce on clay	Chiefly on clay
Tussilago Farfara	Universal in distribution	Chiefly on clay
Euphorbia exigua	Scarce; only one record on clay	Frequent; chiefly on clay

B. Weeds of chalk soils.

The West Country chalk soils exhibit many species of plants, of which a large proportion are definitely characteristic of the chalk. The Bedfordshire soils, on the contrary, have relatively few distinct chalk weeds, of which still less can be called definitely characteristic.

	Bedfordshire.	*West Country Districts.*
Lychnis Githago	No record	Confined to chalk
Silene Cucubalus	Symptomatic of chalk	Also occurred on clay
Campanula hybrida	One record; sand and clay	Symptomatic
Linaria spuria	,, ,, heavy clay	,,
,, vulgaris	,, ,, chalk and clay	,,
Myosotis arvensis	Universal in distribution	,,

	Bedfordshire.	*West Country Districts.*
Poa pratensis	One record; gravelly loam	Symptomatic
Daucus Carota	„ „ on chalk	„
Reseda lutea	„ „ „	„
Chenopodium album	No record from chalk	Frequent on chalk
Heracleum Sphondylium	Not recorded	Very characteristic of chalk
Lithospermum arvense	„ „	„ „ „
Papaver Rhoeas	Seldom recorded from chalk	„ „ and frequent
Plantago lanceolata	Universally distributed	Characteristic of chalk
„ major	Scarce	Frequent on chalk
Scandix Pecten	Universally distributed	Characteristic of chalk
Euphorbia exigua	Designated as 'Calcifuge'	Frequent on chalk
„ Helioscopia	„ „ „	„ „ „
Potentilla anserina	No record on chalk	Occasionally seen on chalk

C. 'Calcifuges.'

Of the various so-called 'Calcifuges' of the Bedfordshire districts *Poa annua* is the only species which retains that character in the West Country. All the others are more or less associated with chalk.

Bedfordshire Calcifuges.	*Occurrence in West Country.*
Alopecurus agrestis	Recorded several times on chalk; dominant in one field.
Anagallis arvensis	Frequently recorded from chalk, but usually very scanty.
Euphorbia exigua	Frequent on chalk.[1]
„ Helioscopia	„ „ „
„ peplus	„ „ „
Veronica arvensis	Only three records, but all from chalk.
„ hederaefolia	Several records from chalk, with one of dominance.

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[1] In Bedfordshire all species of *Euphorbia* are apparently absent from chalk, while certain of the species are frequent in that habitat in the West Country. It has been criticized that 'it is therefore a mistake to call them "calcifuge", as it can only mean that there is some other factor, commonly present with chalk in Bedfordshire, which determines absence'. Strictly, this criticism is quite just, but for the purpose of this investigation the word 'calcifuge' is used in a restricted *local* sense, in default of a better term, to indicate the absence of any species from chalk soils in a particular locality. Future work will probably sort out the true calcifuges from the local calcifuges.

An examination of the above analyses and of the available data shows:—

1. Many of the differences between the two floras are simply differences in quantity, which change the balance of the distribution of the weeds on the different types of soil.

2. Several plants which occur on various soils in Bedfordshire are definitely associated with chalk soils alone in the West Country.

3. Other plants which are calcifuges in Bedfordshire are more or less frequently observed on chalk in the West.

4. A few weeds are recorded from each district which are totally absent from the other. The number of such species, though, is far less than might have been expected, considering the distance between the two localities, involving some difference in climate and in soil.

Points of Interest.

1. While the association of particular weeds with certain soils is so well marked, the correlation between the weeds and the crops with which they occur is much less definite. The great majority of species occur indiscriminately with any kind of crop, whether cereals, roots, or 'seeds', the nature of the soil being the determining factor of their occurrence. Still, a few plants do show some correlation with the crop, and to a large extent the West Country results tally with those of Bedfordshire in this respect. The 'seed' crops were chiefly clover, vetches, and a mixture of Italian rye-grass and clover. A very few species were almost entirely confined to such crops, but a larger variety of plants were noticeable on account of their rarity or absence among seeds. Also a very few plants were exclusively associated with cereals.

Weeds only associated with cereal crops.

Campanula hybrida
Lamium amplexicaule
Lapsana communis
Poa trivialis
Potentilla reptans
Ranunculus arvensis

Weeds only associated with seed crops.

Arenaria serpyllifolia
Cerastium vulgatum
Geranium columbinum
Geranium dissectum
„ molle
Sherardia arvensis

Weeds absent or very rare in seed crops.

Arrhenatherum avenaceum var. tuberosum
Heracleum Sphondylium
Lolium perenne
Mentha arvensis
Plantago media
Poa annua (though once dominant among seeds)
Triticum repens
Veronica hederaefolia

A number of species which were absent from seed crops in Bedfordshire frequently occurred in association with them in the West Country. This may partly be due to the fact that a large proportion of the clover and rye-grass crops had only recently been seeded, and had made little growth owing to the long drought. In consequence the weeds had not suffered so much from the overshadowing effect of the leafy crop. Under the circumstances the occurrence of such weeds in conjunction with seed crops lends support to the hypothesis that their absence, when it occurs, is due to the starvation of light or air by the crop rather than to any inherent antagonism between the two plants, weed and crop. It is noticeable that the number of absentee species in the West Country records is only nine, as against twenty-one in the Bedfordshire lists.

2. *Couch or Twitch* was frequently noted as a troublesome weed, but as a general rule only two species were designated as such, instead of the half-dozen met with in other places.

(*a*) Triticum repens—the true Couch.

(*b*) Arrhenatherum avenaceum var. tuberosum—the 'Onion Couch', or 'Knotty Couch'. This is one of the worst of weeds when it occurs in any quantity, as it easily spreads and is most difficult to eradicate on account of its swollen tuberous stems, each joint of which will develop into an independent plant if detached.

3. In one instance the most pestilent weed was *Avena fatua* or Wild Oat, which existed side by side with the 'Onion Couch'. This weed is particularly troublesome, as

(*a*) it spoils the crop;

(*b*) it spoils the sample of corn;

(*c*) it gets into the thrashing-machine parts and clings together by its hairs until it blocks every outlet.

4. *Equisetum and Coltsfoot* did not occur in company so frequently as they did in Bedfordshire. The Coltsfoot was more often associated with clay soils, but as before the distribution of both weeds was general. It has been suggested that the presence of Coltsfoot is an indication of the nearness of underground water, and that the plants can only thrive if they can send down their long roots to tap it. This idea, however, needs confirmation.

5. Two plants are totally different in their habitat in the two districts:

(*a*) *Bartsia Odontites*, which is a clay weed and calcifuge in Bedfordshire, is practically absent from clay and is very frequent on chalk in the West.

(*b*) *Chenopodium album*, which is never recorded from chalk in Bedfordshire, is noted more frequently from that habitat than from any other in the West.

6. *Rhinanthus Crista-galli*, Yellow Rattle, was very prevalent in some places in the West. Generally this is a weed of pasture land, but in one case observed the barley crop was full of it and was utterly ruined by it. Some seasons are far more favourable to the growth of this weed than others, though in both 1910 and 1911 large quantities have occurred in this particular district.

7. The total absence of Mayweed, *Matricaria inodora*, was very noticeable. A small amount of *Anthemis* sp. occurred in places, but the plants rarely attained any size, and so a very conspicuous feature in the weed flora of Bedfordshire was missing in that of the West Country, on similar soils.

8. Where two or more species of the same genus occur as weeds, it frequently happens that the species differ among themselves in their distribution, being associated with different types of soil ; e. g.—

Euphorbia exigua	chiefly on clay
„ Helioscopia	„ „ chalk
Plantago lanceolata	chalk only
„ major	chalk and clay
„ media	clay only
Poa annua	chiefly on clay
„ pratensis	chalk
„ trivialis	distributed
Ranunculus acris	clay and sand
„ arvensis	clay
„ repens	chiefly on chalk
Rumex Acetosella	acid sandy soil
„ crispus	chiefly on chalk

SUMMARY.

1. In each district investigated a definite association exists between the species of weed plants and the soil on which they grow. This association may be either—

(*a*) *Local.* When a weed is symptomatic of a certain soil in one district but is not so exclusively associated with it in another.

(*b*) *General.* When a certain species is symptomatic or characteristic of the same type of soil in different districts.

2. In most cases there is no association between the weed and the crop, though a few species do show a relationship. The weeds usually found in ‘ seed ’ crops appear to be constant, and certain other plants exhibit

the same association with particular types of crop in Bedfordshire and in the West Country.

3. The relative prevalence of the weeds varies somewhat in the different districts, certain species which are more or less common in one place being practically absent or very scarce in another, on similar soils.

In conclusion I wish to tender my thanks to Mr. E. S. Beaven, who has given me every possible assistance, both by putting me into touch with various farmers, and by the personal interest that he has taken in the work, together with much friendly advice and criticism. Also I must express my indebtedness to all those whose land came under investigation, for their unfailing kindness and readiness to facilitate the work.

The Root-nodules of Myrica Gale.

BY

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With Plates XI and XII.

THE discovery by Hellriegel and Wilfarth in 1886, that the root-nodules of leguminous plants are able to assimilate atmospheric nitrogen by means of the Bacteria present in the cortical cells, directed attention to the possibility of certain non-leguminous root-nodules having a similar function. The earlier observers failing to demonstrate the presence of Bacteria in the nodules they investigated, but finding the nodules, especially the older ones, filled with hypha-like threads, considered them instances of 'Wurzelsymbiosen' caused by mycorrhizal filaments.

In 1896 Hiltner demonstrated the presence of Bacteria in the root-nodules of Alder and *Elaeagnus*, and showed that young Alder plants without nodules would not thrive in a nitrogen-free soil. When, however, these starved plants were inoculated with organisms from Alder tubercles, nodules were produced and growth was normal. He also demonstrated that the poorer the soil in available nitrogen, the greater is the number of root-nodules developed on the Alder roots, provided the necessary organisms are in the soil. Hence under conditions of nitrogen deficiency the number of nodules on the roots becomes a measure of the nitrogen assimilating activities of the plant.

In 1899 Nobbe and Hiltner reported that the root-nodules of *Podocarpus* were active agents in the assimilation of nitrogen. A *Podocarpus* plant possessing root-nodules was grown for five years in nitrogen-free sand, thriving well during the whole period.

In 1907 the author demonstrated the presence of nitrogen-fixing organisms in the root-nodules of *Cycas*.

Hitherto, these four groups of non-leguminous plants—Alder, Elaeagnaceae, *Podocarpus*, and Cycadaceae—have been the only ones recognized as possessing root-nodules concerned with the assimilation of atmospheric nitrogen.

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MYRICA GALE.

The peculiar nodule formations on the roots of *Myrica Gale* were first described and figured by Brunchorst in 1886. He states that the nodules are caused by an endotrophic fungus with septate hyphae and terminal spores, and considered them simply disease formations.

Möller in 1889 confirmed Brunchorst's observations, and, without adequate reasons, placed the fungus in the group *Frankia*, naming it *Frankia Brunchorstii*, considering it to be closely related to a similar fungus said to occur in Alder nodules.

Marshall Ward in 1889 mentions *Myrica* nodules as possible instances of symbiosis as yet unexplained.

Tubeuf in 1896 speaks of the Mycodomatia of *Myrica*. He says the nodules 'may increase to very large tubers with surfaces resembling a bunch of grapes. In the large cells of the middle layers of the primary root-cortex of these growths, coils of very fine fungus-threads are sheltered; these extend year after year into the younger parts of the enlarging tubercles, and gradually disappear in the older parts. What may be the significance of these structures for plants possessing chlorophyll and furnished with normal roots is as yet unknown.'

In 1902 Shibata stated that the inhabiting fungus is found exclusively in a peripheral sub-cork layer of parenchymatous tissue one to three cells thick, whilst the remaining parenchymatous cells of the rind are filled with starch bodies. Because of the peculiar ray branching of the fungus and its club-shaped spores he considered it to belong to the group *Actinomyces*.

Peklo in 1910, working on material from plants grown in a greenhouse, supported Shibata's views.

External Structure of the Nodules.

For the purpose of this investigation root-nodules of *Myrica Gale* were obtained from plants growing wild in Wales, Ireland, and the North of England, and from cultivated plants in the Chelsea Physic Gardens. The roots of all the plants were found to possess nodules of varying sizes. The young nodules (Pl. XI, Fig. 1) are visible first as tiny swellings on the sides of the roots. These grow until they are from 2–3 mm. long and 0·8–1 mm. broad. In this stage they resemble the single nodules found on *Vicia sativa*. This primary nodule then ceases to elongate, but from the distal end a thin hair-like rootlet grows out, and around this rootlet, from the body of the primary nodule, three secondary nodules arise. These secondary nodules grow to the size of the primary nodule and then each again gives rise to a rootlet and three tertiary nodules. These again repeat the process until by repeated branching the characteristic 'cluster' nodules (Fig. 2), with their fringe of radiating rootlets, are formed. When the branches become closely crowded together sometimes only two branches arise from the end of the nodule, instead of the normal three, owing to lack of space for development.

The branching is apparently associated with the outgrowth of lateral roots, and is not due to dichotomy of the apical meristem of the nodule as in the case of the branched nodules of Alder, *Elaeagnus*, and *Cycas*.

Internal Structure of the Nodule.

A transverse section of a single nodule (Fig. 3) shows a central tetrarch stele like that of the normal root, and indicates that the nodule itself is a modified root. The stele is surrounded by an endodermis characterized by neither radial dot nor thickened walls, but by the cells being filled with oil drops. Outside the endodermis are several layers of cortical parenchymatous cells covered on the outside by a definite small-celled cork layer. In mature nodules this cortical tissue is characterized by the presence of (1) somewhat enlarged cells filled with Bacteria, (2) cells filled with oil drops. In sections cut from material fixed in alcohol and stained with Kiskalt's amyl-gram stain the Bacteria can be seen *in situ* in the enlarged cells as small rods. In transverse sections cut near the apex of the nodules zoogloea threads of Bacteria are seen passing from cell to cell. These are comparable to the 'infection threads' seen in leguminous nodules.

In a median longitudinal section of a nodule (Pl. XII, Fig. 6) four fairly well defined areas can be distinguished:

1. *Meristem zone.* An apical mass of meristematic cells beneath the outer protective cork layer. This crowns the end of the stele and extends some distance down the sides (Figs. 4, 5, 6, *m.*).

2. *Infection zone.* A zone of cells in which the infection threads are seen passing from cell to cell. In a young nodule some of the cells contain starch grains (Fig. 8).

3. *Bacterial zone.* This area includes the bulk of the cortical tissue of the nodule and consists of parenchymatous cells. The majority of the cells are enlarged and crowded with Bacteria. Amongst these are scattered cells containing reserve food material in the form of oil drops (Fig. 3, 4, 5, 6, *b.* and *o.*).

4. *The basal zone.* In this region the bacterial cells are few in number, and in fully matured nodules are quite absent, but the oil-containing cells are numerous.

As the individual nodule matures and branches, the seat of bacterial activity is transferred to the branch nodules, and the Bacteria gradually disappear from the bacterial zone.

True fungal hyphae are often found ramifying through the cells of the basal zone, and in the old nodules they may sometimes be seen filling the majority of the cortical cells. These fungal hyphae were considered by the earlier investigators of *Myrica* to be the cause of the formation of the nodules. This view, though now seen to be erroneous, was quite natural before the bacterial nature of the nodule was demonstrated, and it is possible that these hyphae, whilst not the cause of nodule formation, may be of a mycorrhizal nature and benefit the *Myrica* plant.

Origin of the Nodules.

The nodules evidently arise as modifications of lateral roots. The method of infection has not yet been observed, but it is presumably similar to that of leguminous plants. The Bacteria enter a root-hair and pass into the cortical cells as an 'infection thread'. It is possible that the presence of these Bacteria in the cortical cells stimulates the stele to produce a lateral branch at this point, for every nodule possesses a well-defined tetrarch stele. The cortical cells become enlarged by the growth of the infecting Bacteria, and a short swollen nodule is formed instead of a typical lateral root.

The tip of the nodule is rounded and does not possess a root-cap, for the stele of the lateral root does not break through the surrounding cortex until later.

When the nodule has reached its mature size further expansion ceases, but the stele, which up to this stage has remained completely within the nodule, ending a short distance below the apex, now grows on and out through the apex, and forms a thin hair-like rootlet (Fig. 6, *r.*) with a typical tetrarch stele (Fig. 7) surrounded by a few layers of cortical cells, but possessing no definite root-cap.

Around the rootlet three secondary nodules or branches arise from the primary nodule in a similar manner to the formation of the primary nodule from the root. These secondary nodules repeat the growth and branching of the primary nodule, a rootlet and three tertiary nodules arising from each. By repeated branching in this manner the peculiar 'cluster' nodules are produced.

Bacteria—Isolation and Cultivation.

Small nodules were removed from the roots and sterilized by being placed for two minutes in the sterilizing fluid used by Harrison and Barlow in their investigations on leguminous nodules. This fluid consists of hydrochloric acid 2·5 c.c., mercuric chloride crystals 1 grm., distilled water 500 c.c. After removal and washing in distilled water one of the nodules was crushed on a slide. The exuded matter adhering to the slide was then air-dried under sterile conditions and stained with Ziel's carbol fuchsin. On microscopic examination numerous small rod-shaped Bacteria were seen. These were evidently similar to *Pseudomonas radicicola*, the organism found in the root-nodules of the Leguminosae. They further showed the characteristic staining reaction with Kiskalt's amyl-gram stain, the aniline gentian violet being removed by ethyl alcohol, but retained when treated with amyl alcohol. Harrison and Barlow consider this a special differentiating stain for *Pseudomonas radicicola.*

Pure cultures were also obtained by plating out some of the expressed matter from a sterilized nodule on to nutrient agar plates.

The nutrient material consisted of maltose 1 grm., potassium phosphate 0·5 grm., magnesium sulphate 0·02 grm., ammonium phosphate 0·5 grm., agar 1 grm., and 100 c.c. distilled water.

The inoculated plates, after incubation for two days at 28° C., showed numerous colonies which were round to lenticular in shape, raised, entire, viscous, and 0·75 to 1 mm. in diameter. These colonies corresponded in all respects to the characteristic colonies of *Pseudomonas radicicola.* Microscopic examination of the colonies showed the typical rod-shaped Bacteria.

Estimation of Nitrogen Fixation by the Bacteria.

Cultures of the Bacteria were made in Erlenmeyer flasks of 300 c.c. capacity containing a nutrient solution consisting of 100 c.c. distilled water, 1 grm. maltose, 0·5 grm. potassium phosphate, and 0·02 grm. magnesium sulphate. Some of the flasks were inoculated with a loop from the colonies on a nutrient agar plate; others were left uninoculated and formed controls. All the flasks were incubated at 25° C. for seven days. During this time the contents of the uninoculated flasks remained clear, but the inoculated flasks became cloudy.

Kjeldahl nitrogen determinations of the contents of the flasks gave the following average results:

Control flasks . . .	0·53 mg. N per 100 c.c.
Inoculated flasks . .	2·58 „ „

showing a fixation of nitrogen of 2·05 mg. per 100 c.c. of culture.

Effect of Nodules on Growth of Myrica Plants.

To ascertain the effect of the presence of nodules on the growth of *Myrica* plants attempts were first made to strike *Myrica* cuttings in sand watered with nutrient solution, but all ended in failure.

Excellent results were, however, obtained with young *Myrica* plants procured from Heysham Moss last spring. Some of these plants possessed small nodules on their roots; others appeared to be quite devoid of nodules. Six of each were planted out in a greenhouse, in pots containing sterilized soil deficient in nitrogen. All the plants with nodules flourished well. Of the six plants without nodules, two made no growth for some time, but afterwards began to shoot. On examining their roots a few nodules were found. Evidently these were already infected with Bacteria, but the nodules had been too small to see, when they were planted out. The remaining four made no fresh growth, and after a time showed evident signs of starvation. At this stage two of these starved plants were watered twice, at an interval of seven days, with a liquid culture of *Myrica* nodule organisms. Soon these plants commenced active growth, and caught up and surpassed the two plants possessing root-nodules. By the end of the summer they were larger than any of the *Myrica* plants in the greenhouse. The two starved plants, which were not inoculated, lingered on for a time and were quite dead by the end of June.

It is evident from these results that the root-nodules of *Myrica* are definitely concerned with nitrogen assimilation, and the Bacteria in the bacterial zone of the nodule are the active agents in nitrogen fixation.

SUMMARY.

1. The root-nodules of *Myrica Gale* are modified lateral roots.

2. The young primary nodules give rise by branching to the characteristic 'cluster' nodules, surrounded by rootlets which grow out through the end of each branch.

3. Three branches or secondary nodules arise from the end of each primary nodule, and, like it, are modified lateral roots. After the formation of these branches the stele of the primary nodule elongates and grows through the apex of the nodule, giving rise to the hair-like rootlet.

4. In each mature nodule four zones can be distinguished:

(*a*) The apical meristem;

(*b*) The 'infection thread' area;

(*c*) The 'bacterial' zone, which includes most of the cortical tissue of the nodule, and consists chiefly of the enlarged cells containing Bacteria;

(*d*) The basal zone. The lower end of the nodule, devoid of bacterial cells, but containing numerous cells filled with oil drops.

5. After the nodules have branched and reached their full size the Bacteria disappear from the cells of the bacterial zone, and the basal zone gradually encroaches upon and finally replaces all the other zones.

6. In old nodules, and sometimes in the basal zone of younger nodules, mycorrhiza filaments are found.

7. Pure cultures of the Bacteria from the 'bacterial' cells were made, and on examination were found to be identical in structure and growth with *Pseudomonas radicicola*, the organism of the root-nodules of the Leguminosae.

8. Nitrogen determinations of liquid cultures, incubated for 7 days at 25° C., showed a fixation of 2·05 mg. of nitrogen per 100 c.c.

9. Young *Myrica* plants grown in sterilized nitrogen-poor soil did not flourish unless they possessed root-nodules. Plants devoid of nodules, after inoculation with a culture of *Myrica* nodule Bacteria, developed root-nodules and grew well.

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EXPLANATION OF FIGURES IN PLATES XI AND XII.

Illustrating Prof. Bottomley's paper on the Root-nodules of *Myrica Gale.*

Fig. 1. Photograph of root and nodules of *Myrica*. Natural size.

Fig. 2. Photograph of 'cluster' nodule. × 5.

In the following figures *c.* denotes cork; *b.*, bacterial cells; *o.*, oil-drops; *e.*, endodermis; *p.*, pericycle; *ph.*, phloem; *x.*, xylem; *m.*, meristematic zone.

Fig. 3. Transverse section of nodule, showing the tetrarch main stele. × 70.

Fig. 4. Transverse section of nodule, showing the stele producing three branches. *p.x.* denotes protoxylem; *br.*, branch of stele. × 70.

Fig. 5. Transverse section of nodule near the apex, showing the main vascular bundle (*m.b.*), which is continued into the emerging root, and the three branches of the bundle (*br.b.*) which supplies the branches of the nodule. × 70.

Fig. 6. Longitudinal section of the root-nodule, showing the branched vascular tissue and the emerging rootlet (*r.*) passing through the broken tip of the nodule (*t.*). × 70.

Fig. 7. Transverse section of the rootlet which has emerged from the tubercle. × 140.

Fig. 8. Section of the cortical cells of the nodule, showing the Bacteria (*b.r.*) and the infection threads (*i.t.*). *n.* denotes nucleus of host cell. × 325.

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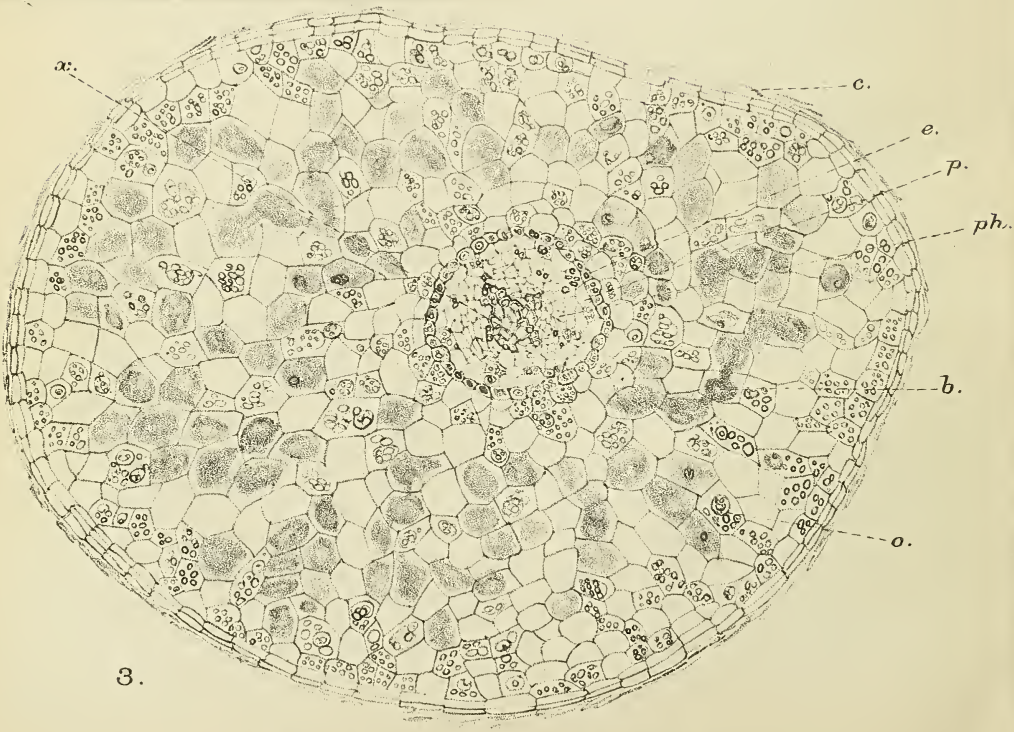

3.

BOTTOMLEY — MYRICA GALE.

4.

5.

Huth, lith et imp.

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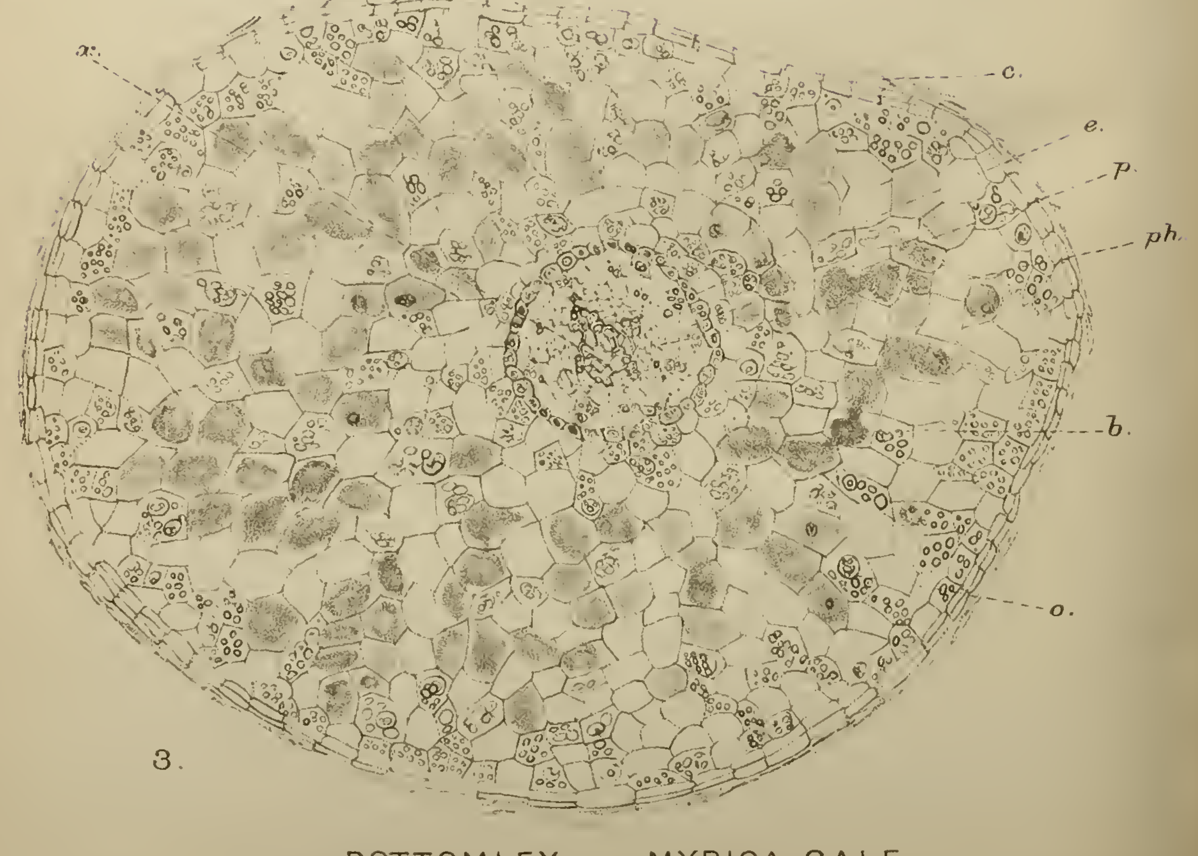

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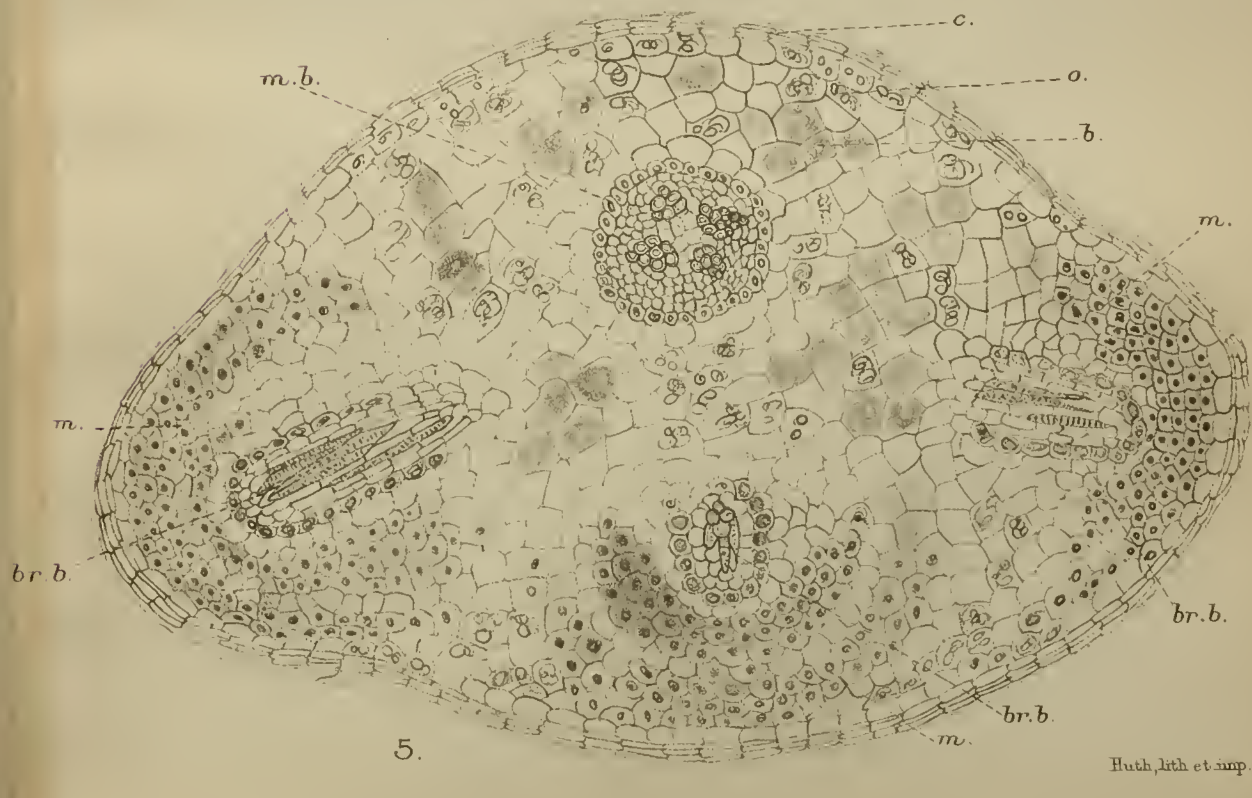

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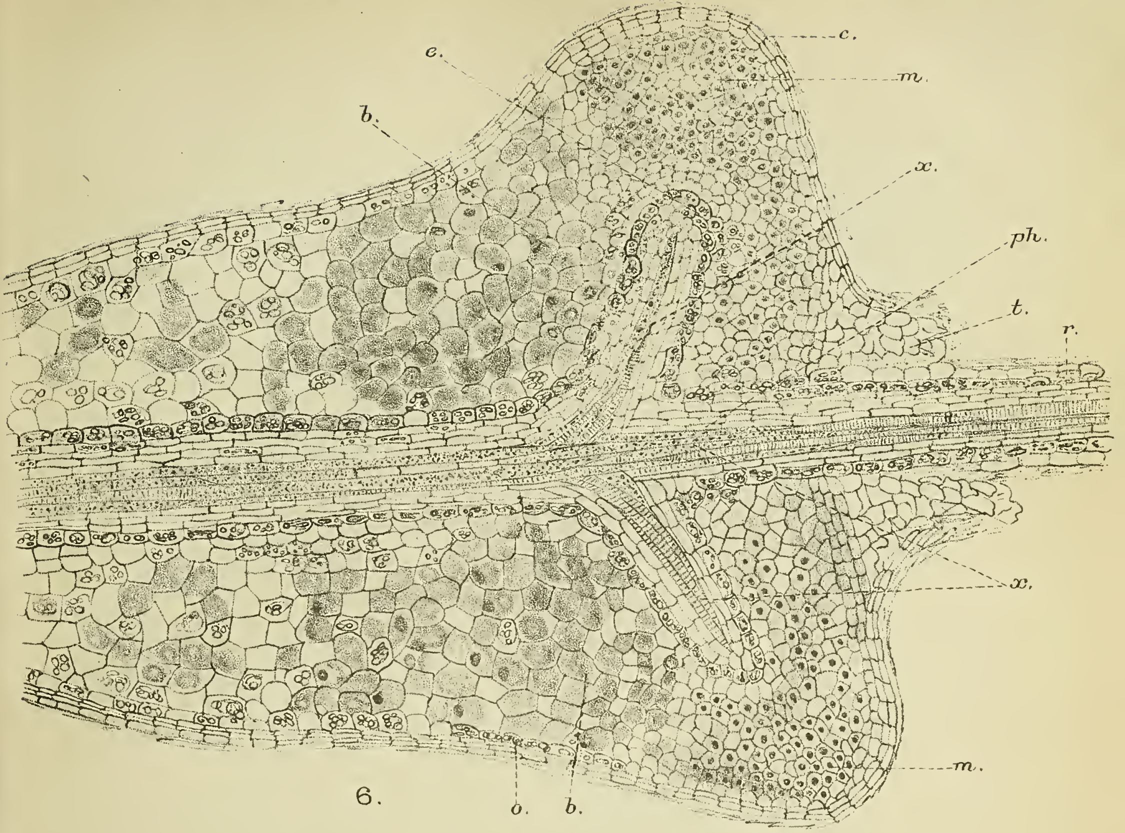
BOTTOMLEY — MYRICA GALE.

Huth, lith et imp.



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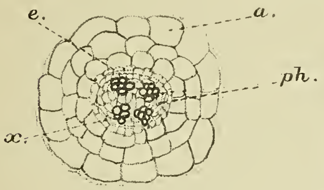

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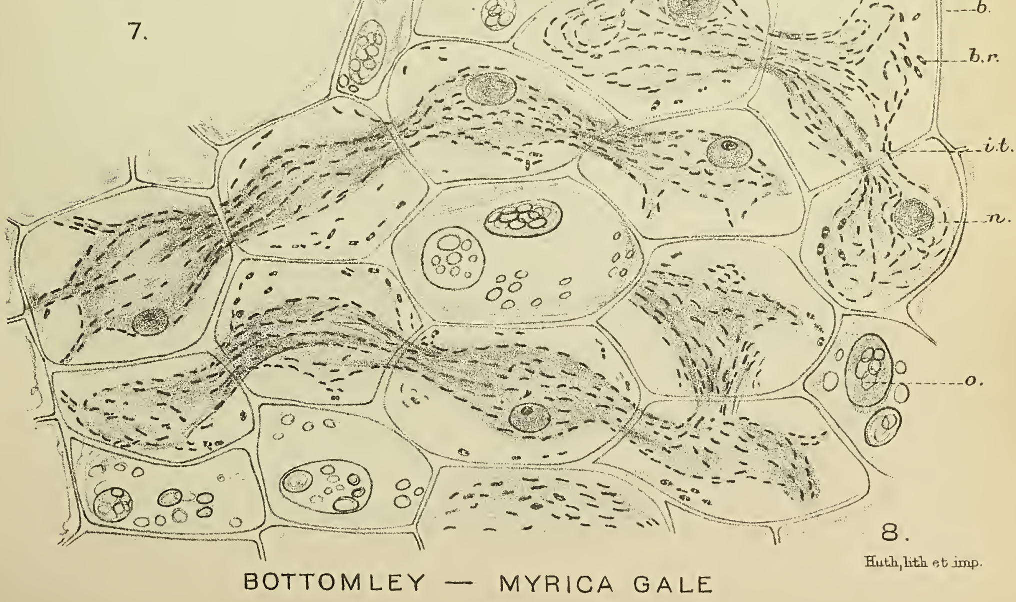

8.

BOTTOMLEY — MYRICA GALE

Huth, lith et imp.

The Morphology of the Root Tubercles of Alnus and Elaeagnus, and the Polymorphism of the Organism causing their Formation.

BY

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With Plates XIII and XIV.

AMONGST the earlier investigators in the domain of the utilization of atmospheric nitrogen by plants, Hiltner stands out prominently, and in his work of 1899 the roots of *Alnus* and *Elaeagnus*, two non-leguminous plants, were shown to possess root tubercles which were associated with the assimilation of atmospheric nitrogen. These observations have been amplified more recently by Bottomley, who isolated a bacillus from the internal tissue of these nodules, and with a pure culture of this organism inoculated some *Vicia sativa* seedlings, upon the roots of which characteristic tubercles containing *Pseudomonas radicicola* were subsequently produced. From this experiment it was inferred that the tubercles of *Alnus* and *Elaeagnus* contained *Pseudomonas radicicola*, and infection of the roots by this organism was regarded as the probable cause of their formation. The literature concerning the tubercles of both these plants has greatly increased during recent years, and the various authors all find within the nodules a spore-producing hyphal-fungus, which has been placed in the group Hyphomycetes, but they do not appear to agree with one another in the detailed structure of this parasite, and its effect upon the host plant. The present communication is an account of some investigations which entirely support the original view, put forward by Hiltner and upheld by Bottomley, that there is a symbiotic relationship between these two plants and the organisms causing the tubercle formation.

Nodules have been obtained, at various seasons of the year, from plants of *Alnus incana*, *Elaeagnus edulis*, and *Elaeagnus rhamnoides*, growing at Chelsea, Sevenoaks, and North Wales. The material used for sectioning was fixed in either Flemming's or Bouin's fixative or absolute alcohol, the latter having the advantage that only in this case is the oil which is present in large quantities in the tubercles of *Elaeagnus* removed by

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subsequent treatment with xylol. Microtomed sections were almost exclusively employed, as the details could not be satisfactorily determined with certainty from the freehand sections which have been recommended by some writers. A large variety of stains were used, the principal being Flemming's triple, Heidenhain's iron haematoxylin, Ehrlich's haematoxylin, Löffler's blue, methyl green and fuchsin, methyl violet and fuchsin, carbol fuchsin, and Kiskalt's amyl gram. The last named proved the most satisfactory, since it is a good nuclear stain and also one by means of which *Pseudomonas radicicola* can be differentiated.

In order to ascertain whether these root tubercles were connected with the presence of organisms possessing the power of assimilating atmospheric nitrogen, an attempt was made to isolate any such Bacteria from their internal tissues. For this purpose a medium of the following composition was utilized:

1 grm. saccharose,
0·5 grm. acid potassium phosphate,
0·02 grm. magnesium sulphate,
100 c.c. distilled water.

Into each of three Erlenmeyer flasks, 300 c.c. capacity, 50 c.c. of such a medium was placed, and then submitted to a temperature of 140° C. and a pressure of two and a half atmospheres for ten minutes in an autoclave. They were then neutralized with sodium hydrate and allowed to cool. Some of the tubercles were removed from the roots of both *Alnus* and *Elaeagnus*, thoroughly washed, and then placed in a sterilizing fluid composed of:

2·5 c.c. concentrated hydrochloric acid,
1 grm. mercuric chloride,
500 c.c. distilled water.

They were allowed to remain in this solution for two minutes, when they were removed with sterile forceps and washed in distilled water contained in a sterile vessel. These nodules were then crushed with previously sterilized instruments, and the *Alnus* nodules were put into one of the above flasks, the *Elaeagnus* into another, and the third was left untouched. All were then incubated at a temperature of 25° C.

After two days the solutions into which the nodules had been placed were quite cloudy, whilst the other one had remained unchanged. A drop of the cloudy liquid was placed on a slide, air-dried, then stained with carbol fuchsin, and examined microscopically. In both cases it was a pure culture of rod-shaped organisms, many of which contained two or three small round densely staining bodies, and were apparently very characteristic *Pseudomonas radicicola* (Pl. XIV, Fig. 9). Another slide was prepared, and aniline gentian violet employed as a staining reagent, and this was found to be immediately withdrawn from the organisms by absolute ethyl alcohol, but was retained by them when amyl alcohol was used as a dehydrating agent; which reactions clearly indicated that they were *Pseudomonas radicicola*, according to the investigations of Harrison and Barlow.

Exactly the same results were obtained when maltose or glucose replaced saccharose in the above culture medium. Solid media were also prepared by adding 2 per cent. agar-agar to the above media, boiling, and then pouring into sterile Petri dishes and allowing to cool. These media were inoculated with very dilute crushed nodular tissue, prepared as above, by means of a sterile platinum loop, and incubated at 25° C. Very characteristic colonies were produced on the agar media, which were ovoid to circular in shape, with an entire margin, slightly raised above the surface, translucent, shining, and mucilaginous. They increased in size from 0·5 mm. diameter in one day to 1·5 mm. diameter in three days.

Streak and stab methods of cultivation produced equally typical growths. The streak produced an abundant, translucent, shining, mucilaginous growth, along the line of infection, with an entire margin, increasing in opacity, size, and viscosity from day to day. The stab induced the formation of a large typical colony on the surface of the agar, and a filiform growth along the stab in the medium.

The colonies embedded in the agar medium were very small, translucent, and disc-shaped or lenticular.

All the above cultures, after a period of incubation lasting four days or upwards, revealed on microscopic examination the presence of some larger organisms, tending to become ovoid in shape, amongst the characteristic rod-shaped *Pseudomonas radicicola* (Fig. 10). These larger organisms also contain the densely stained round bodies which were present in the rods, their larger size being caused by an increase in the enveloping capsule. Division takes place so that one organism contains only one of the small bodies, which then grows until it fills the surrounding capsule, and the whole structure becomes spherical in shape and appears to have a very definite cell-wall (Fig. 11).

When the larger bodies are present in the colonies on the solid media, a white opaque appearance is assumed. The agar media were very favourable to the production of the larger spherical form, whilst in liquid cultures their appearance seems to be correlated with depletion of the medium of its carbohydrate, their source of energy, and probably also with the accumulation of the products of their own metabolic activities. That they are better able to resist the influence of the external environment than are the actively motile bacilli was demonstrated by boiling two cultures for ten minutes, one of which contained only the small rods, whilst the other contained only the larger spherical bodies, and subsequently inoculating freshly prepared culture media with them, and incubating for two days, at the end of which period a normal healthy culture had been produced only in the medium inoculated from the culture containing the larger spherical organisms. These bodies appear to be analogous with the so-called 'bacteroid' forms produced by other species of *Pseudomonas radicicola*, since by inoculating freshly prepared media with them the small typical bacilli are formed, which subsequently give rise to them again.

The above data indicate that the organisms living inside the root tubercles of these two non-leguminous plants are morphologically identical with *Pseudomonas radicicola*, which inhabits the tubercles of leguminous plants. An attempt was made to determine also their physiological capabilities with regard to the assimilation of atmospheric nitrogen in the following experiments:

Into each of two Erlenmeyer flasks, 400 c.c. capacity, was placed 100 c.c. distilled water, 1 grm. saccharose, 0·5 grm. potassium hydrogen phosphate, 0·02 grm. magnesium sulphate. To one of these flasks, the control, was added 2 c.c. of a culture obtained from *Alnus* as described above. Both were then autoclaved and subsequently neutralized with sodium hydrate. The other flask, after cooling, was inoculated with 2 c.c. of the same culture as the control, and both were incubated at 25° C. for ten days. During this period the control remained apparently unaltered, but in the one containing the living organisms there was a visible change as described above.

The nitrogen content of both flasks was determined by the Kjeldahl method of analysis.

The following results have been obtained with cultures from *Alnus* and *Elaeagnus* respectively:

	Nitrogen founa in control.	*Nitrogen found in culture.*	*Gain in Nitrogen due to organisms.*
Alnus	0·47 mg.	3·96 mg.	3·49 mg.
	0·62 ,,	3·85 ,,	3·23 ,,
Elaeagnus	0·45 „	3·07 ,,	2·62 ,,
	0·82 ,,	3·32 „	2·5 ,,

The increased nitrogen content can only have been produced by the growth of the living Bacteria introduced, and must have been derived by them from the atmosphere. Every precaution having been taken to exclude all other organisms except those derived from the tubercles, these experiments demonstrate that the root tubercles of *Alnus* and *Elaeagnus*, like those of leguminous plants, assist in the assimilation of atmospheric nitrogen, and this explains how, as Hiltner observed, the plant benefits from the presence of these tubercles on its roots.

The root tubercles of *Alnus* and *Elaeagnus* are usually present in clusters (Pl. XIII, Figs. 1 and 2), which may in *Alnus* attain a diameter of three inches or even more, but in *Elaeagnus* they are rarely more than an inch and a half across. The tubercles are enabled to grow in clusters of such dimensions by their perennial habit and the repeated bifurcation which occurs when they are from a quarter to half an inch in length and about an eighth of an inch in diameter. In some cases, particularly when the tubercles occur in isolated positions on the roots, trifurcation occurs.

Transverse sections of the tubercles of both plants (Figs. 3 and 13) immediately reveal the fact that they are modified lateral roots, each possessing a well-defined central stele, and the increased diameter is caused by the enlargement of the cortical cells owing to their infection with the Bacteria. The organisms penetrated the root-hair, and entering the cortical cells caused them to hypertrophy, and this arrested the growth in length of the stele, so that a short swollen structure is produced instead of a typical lateral root. The tip of the nodule is occupied by a meristematic zone (Figs. 7 and 16), by means of which it grows, not only during one season, but also from year to year, growth being renewed each spring, when both meristematic apex and central stele branch (Figs. 7 and 16). The vascular tissue in the tubercles of *Elaeagnus* is in the form of a triarch stele (Fig. 13), possessing a relatively large amount of phloem, two or three layers of pericycle, and a distinct endodermis which is always closely packed with reserve food material, usually in the form of oil, but in some of the tubercles gathered in the spring there was a large proportion of starch. The xylem is composed of annular and spiral vessels and parenchyma. Towards the base of the older nodules a considerable amount of secondary vascular tissue is produced, and amongst this xylem there are scalariform vessels. In the tubercles of *Alnus* the central stele varies from triarch to hexarch (Figs. 3, 4, and 5). In the older regions there is a solid central mass of xylem (Fig. 5), whilst at the tip the centre of the stele is occupied by parenchyma, and the protoxylems are arranged round this (Fig. 4). Here also secondary thickening occurs, a considerable quantity of phloem is present, surrounded by two or three layers of pericycle and an endodermis, the cells of which are filled with reserve food material in the form of definite structures usually somewhat spherical in shape and giving very definite proteid reactions.

In the roots the phellogen has its origin in the pericycle, and forms a few layers of periderm externally, and several layers of secondary cortex on the inside. The tubercles are always surrounded by a few layers of periderm (Figs. 3, 7, 13, 16), which are formed by the continued growth of the original phellogen of the root to keep pace with the growth of the tubercle. In the spring the periderm near the tips of the tubercles in *Elaeagnus* frequently becomes very irregular, large masses being split off at various places, giving rise to small excrescences on the surface which are sometimes visible to the naked eye. This is caused by the inability of the phellogen to divide sufficiently rapidly to keep pace with the rapid expansion and growth of the nodular tissue when reawakened to activity.

In the tubercles of *Elaeagnus* the cortical cells situated immediately behind the meristematic zone appear to contain a protoplasmic network, while those a little further back are often somewhat abnormally enlarged and are filled with densely staining spherical bodies. This appearance suggests the idea which has been held by Woronin, Brunchorst, Shröter, and others, that the tubercles are produced by one of the Plasmodiophoraceae. When, however, the sections are stained with Kiskalt's amyl gram it becomes evident that the cortical cells contain the nitrogen-fixing organism *Pseudomonas radicicola* (Fig. 8).

The apparent network is then seen to be a zoogloea thread in which are embedded the small rod-shaped Bacteria (Fig. 8, *b*, *z*). This is further demonstrated by applying a little ethyl alcohol, when the stain is instantly removed from the network, remaining only in the nuclei of the host cells. This condition always prevails in the youngest cells inhabited by the Bacteria, and is undoubtedly the form in which the organism passes from cell to cell, being exactly comparable with the so-called 'infection threads' produced in leguminous tubercles, and, as in them, seems to be attracted by the host nucleus around which it twines. The contents of the slightly older, enlarged cells are also very clearly defined by Kiskalt's amyl-gram stain, as distinct spherical bodies, having a definite wall and a strong resemblance to the organisms obtained in the old cultures and on the plates (Fig. 8, *c*). Detailed examination of a large number of sections of nodules of various sizes and ages, at different seasons of the year, showed that these spherical organisms are produced from the zoogloea after it has attained certain dimensions with regard to the host cell, and consequently has reached a particular stage in its development. In addition to the cells containing only the small rods in the zoogloea, others were examined in which there were a number of larger structures in the slime thread, and amongst these some very definite spherical bodies, the number of which evidently increases in proportion, until the host cell contains only these, the network being at first masked, but afterwards entirely used up as the bacilli assume the coccoid form (Fig. 8). The nuclei of the host cells under the influence of the zoogloea frequently assume a somewhat amoeboid form, become very vacuolate, may have several nucleoli, and in some cases appear to disintegrate (Fig. 8).

In order to ascertain the connexion between the two structures described above, the following methods of cultivation were tried. Part of the internal nodular tissue was crushed and put into a hanging drop so that it could be watched under the microscope, or incubated as desired. Some of these preparations were subsequently dried and stained. Thin hand sections were treated in the same way. The most satisfactory method of cultivation, however, was to take a nodule, the outside of which had been sterilized, and cut it into three or four pieces, in order to expose some of the cortical cells directly to the influence of external agencies, and then place them in liquid culture media and incubate them at 25° C. The media used were those described above for the cultivation of the isolated organisms. The influence of adding a trace of ammonium phosphate or asparagin was also investigated. The pieces of nodule were incubated for periods varying from 12 hours to 6 days, when they were removed from the culture medium, fixed in Bouin's fixative, embedded and microtomed, and the sections stained with amyl gram or carbol fuchsin. In every case there was a steady increase from day to day in the proportion of bacilli present, and a corresponding decrease in the spherical bodies until after the fourth day, when all the latter forms had disappeared. The coccus forms were seen to divide, leaving a clear area across the centre, which always remained unstained (Fig. 14). Each half then divided again (Fig. 14), and the resulting bodies each produced a typical bacillus. In some cases there appeared to be more than four rod-shaped organisms produced from the one coccus, but the spherical body very soon loses its identity, especially when they are not too crowded together. All these stages are very evident in freshly gathered nodules, especially in the spring. These observations, like those in connexion with the isolated organisms, point to the conclusion that the amount of available carbohydrate present is one of the factors determining the production of the coccus form. It is extremely probable that the twining of the zoogloea round the nucleus of the host cell, as well as the activities of the Bacteria in these cells, impairs to a large extent the metabolic processes of the latter, and consequently renders it more difficult for the Bacteria to obtain the carbohydrate, their source of energy, and they consequently undergo a morphological change which renders them more resistant to the influence of their environment, and in this condition they rest. In the tubercles themselves, they become much more abundant in the autumn, in the winter appear to be the only form of organism present, and in the spring, when food material is once more available and fresh cortical cells are being formed in the nodule, they divide and once more assume the active bacillus form. The greater resistant power of the cocci was illustrated by moving some of the pieces of tubercle which contained none of this form from one culture medium to another, when it was found that in 12 hours the formation of a large number had been induced by this sudden change in the constitution of the environment. The two forms are clearly polymorphic of the same organism, which is a species of *Pseudomonas radicicola.*

The tissue at the base of the tubercles contains an abundant supply of reserve food material, which consists mostly of oil, but in the spring a good deal of starch is present. In the older nodules this tissue becomes somewhat compressed laterally, and here and there are little colonies of Bacteria (Fig. 15) which have not migrated to the younger tissue nearer the tip of the tubercle, although they assume the rod-shaped form in the spring.

The cortical cells of the tubercles of *Alnus* also clearly contain organisms of two forms—small bacilli, undoubted *Pseudomonas radicicola*, and larger coccus forms (Fig. 6). These forms are evidently analogous to the same forms in *Elaeagnus*. Cells are frequently met with also, which contain a mixture of these two forms in varying proportions (Fig. 6), and in some tubercles the spherical bodies are seen to be in a state of division, so that they clearly produce rods again (Fig. 6, *d*). *Alnus* tubercles were subjected to the cultural methods described above for *Elaeagnus*, which revealed the same polymorphic nature of the organism inhabiting it, the only difference being that the *Alnus* bacillus does not form such an evident zoogloea in the cells of its host. Probably correlated with this, the nuclei of the cortical cells do not appear to undergo any definite changes. In *Alnus*, too, the bacteroidal cells traverse the complete length of the nodule, and between them, in zones more or less concentric with the endodermis, are cells containing chiefly reserve food material in the form of oil and proteid globules.

The entire absence in all the tubercles examined of any filaments or hyphae in *Alnus*, and only the very narrow zoogloea threads being present in *Elaeagnus*, together with the inability of the spherical bodies, which appear to have been called 'spores' by some authors, to germinate after the manner of a spore, seems to preclude the possibility of the tubercles being produced by the parasitism of a spore-producing hyphal-fungus. The present series of investigations entirely support the idea that the root tubercles of *Alnus* and *Elaeagnus*, although morphologically different, are physiologically analogous to the root tubercles of the Leguminoseae, which have been shown to be actively concerned in the fixation of atmospheric nitrogen, thus rendering this vast store available to the leguminous plant. This is done for *Alnus* and *Elaeagnus* by a species of *Pseudomonas radicicola*, which, in one of its forms, is morphologically identical with the organism found in leguminous nodules, but like the latter it is polymorphic and assumes a comparatively large spherical form, which does not usually occur in other species.

In conclusion, my heartiest thanks and gratitude are due to Professor W. B. Bottomley, in whose laboratory the investigations have been pursued, for his kindness, sympathy, and advice.

SUMMARY.

1. The root tubercles of *Alnus* and *Elaeagnus* are modified lateral roots. They are perennial, dichotomously or trichotomously branched structures.

2. The tubercles are produced by the infection of the root with a species of the nitrogen-fixing organism *Pseudomonas radicicola.*

3. The bacillus enters the root and afterwards propagates itself in the cortex of the nodule as a rod-shaped organism. In *Elaeagnus* it produces a very definite zoogloea.

4. The further development of the organism, in both cases, gives rise to relatively large spherical bodies, which increase in numerical proportion until they fill the entire cell.

5. Under certain conditions the larger bodies divide into two, and then each divides again, and possibly even further, until they lose their identity and a group of bacilli remain in their place.

6. *Pseudomonas radicicola* is a polymorphic organism, the bacillus and coccus being different forms of one and the same organism.

7. In *Elaeagnus* the nuclei of the host cells appear to undergo some change under the influence of the zoogloea.

8. In *Elaeagnus* the Bacteria are found mainly in the region immediately behind the growing point, whilst in *Alnus* the bacteroidal tissue traverses the entire length of the nodule.

9. In *Elaeagnus* the food storage cells are found towards the base of the tubercle, in *Alnus* there are zones of tissue concentric with the endodermis, and in both the endodermis performs this function.

10. In *Elaeagnus* isolated groups of bacilli occur in the basal region.

11. The coccus form appears to be correlated with scarcity of available carbohydrate and change of environment. It is much more resistant to the influence of external agencies than the rod-shaped form.

12. The organism is capable of fixing free atmospheric nitrogen when isolated from the tubercles, and its presence is undoubtedly beneficial to the plant.

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DESCRIPTION OF PLATES XIII AND XIV.

Illustrating Miss Spratt's paper on the Root Tubercles of *Alnus* and *Elaeagnus*.

In Figs. 3, 4, 5, 7, 13, and 16, *x* = xylem; *ph* = phloem; *p* = pericycle; *e* = endodermis; *c* = cork; *b* = bacteroidal cells; *f* = reserve food material; *m* = meristematic zone.

In Figs. 6, 8, 14, 15, *b* = bacillus form; *c* = coccus form; *d* = coccus form dividing; *z* = zoogloea; *n* = nucleus.

Fig. 1. Root tubercles of *Alnus incana*. Natural size.

Fig. 2. Root tubercles of *Elaeagnus edulis*. Natural size.

Fig. 3. Transverse section of root tubercle of *Alnus*. × 70.

Fig. 4. Transverse section of stele at tip of tubercle of *Alnus*. × 70. *s* = parenchyma.

Fig. 5. Transverse section of stele near base of tubercle of *Alnus*. × 70.

Fig. 6. Cortical cells of *Alnus incana*. × 325. *g* = proteid globules.

Fig. 7. Longitudinal section of tubercle of *Alnus*. × 37.

Fig. 8. Cortical cells of *Elaeagnus*, showing bacillus and coccus forms, zoogloea, and host nucleii. × 325.

Fig. 9. *Pseudomonas radicicola* isolated, rod-shaped form. × 1,180.

Fig. 10. *Pseudomonas radicicola* isolated, rod-shaped form changing to coccus. × 1,180.

Fig. 11. *Pseudomonas radicicola* isolated, coccus form. × 1,180.

Fig. 12. *Pseudomonas radicicola* isolated, coccus form changing to bacillus. × 1,180.

Fig. 13. Transverse section of tubercle of *Elaeagnus*. × 70. *s.x.* = secondary xylem.

Fig. 14. Cortical cells of *Elaeagnus*, showing coccus forms changing to bacillus. × 325.

Fig. 15. Cortical cells from basal region of *Elaeagnus* tubercle showing Bacteria, and reserve food material as oil at *O*. × 325.

Fig. 16. Longitudinal section of root tubercle of *Elaeagnus*. × 37.

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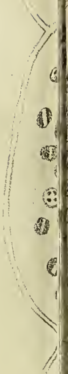

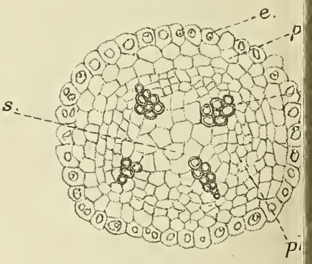

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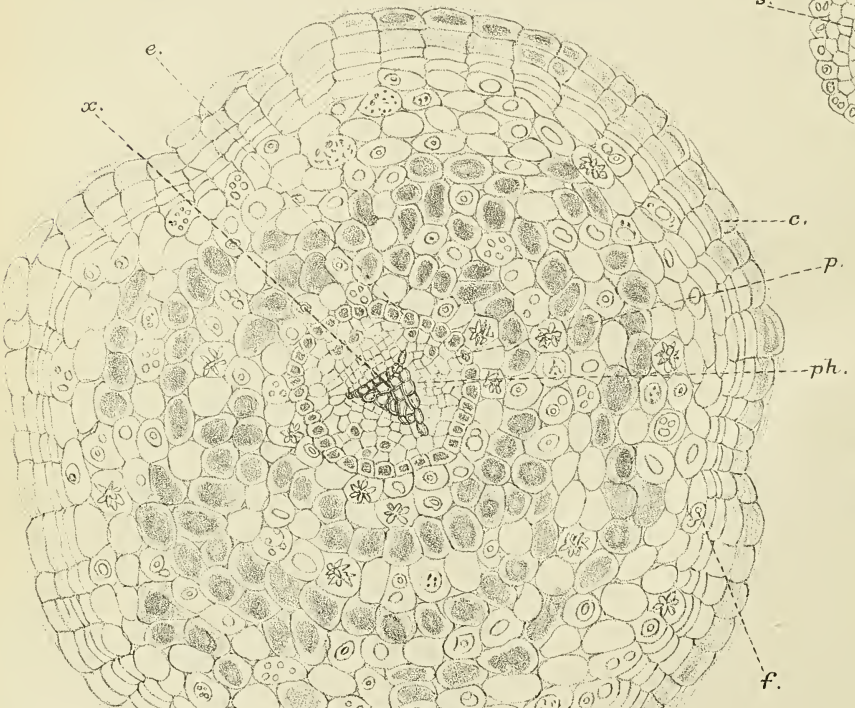

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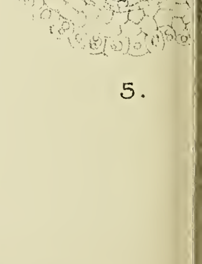

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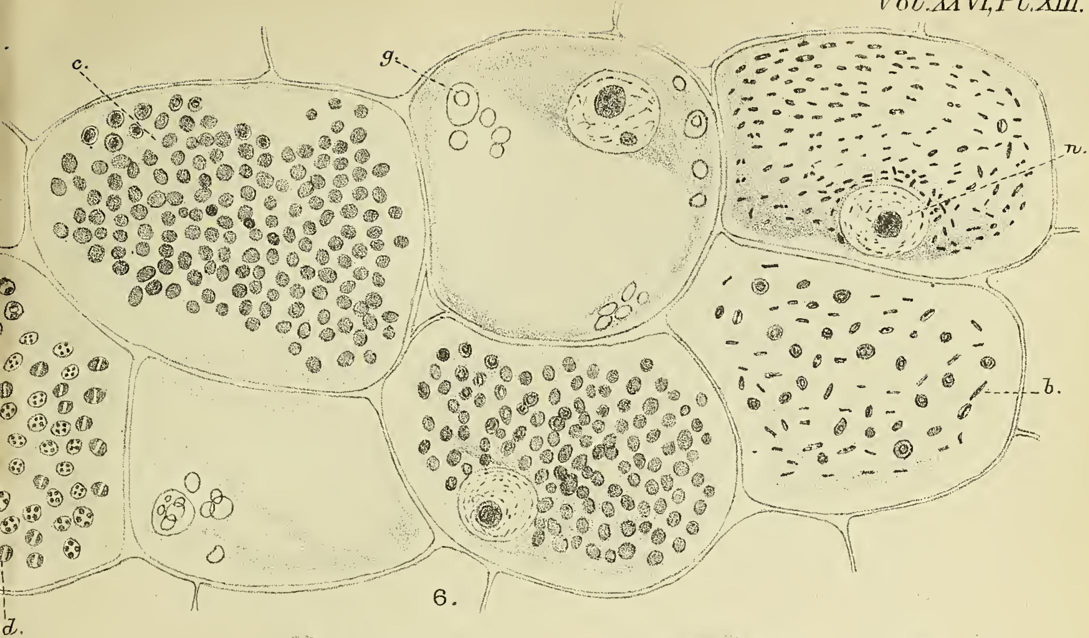
SPRATT — ROOT-TUBERCLES OF ALNUS AND ELÆAGNUS.



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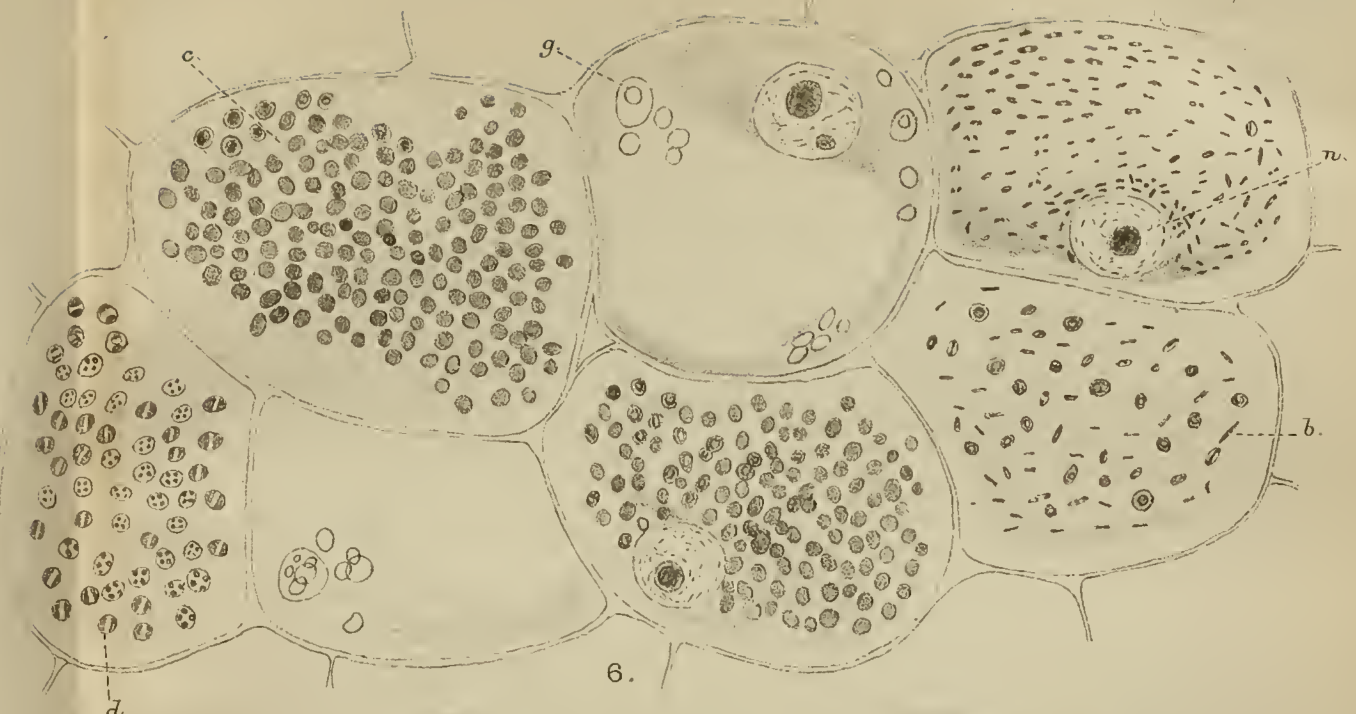

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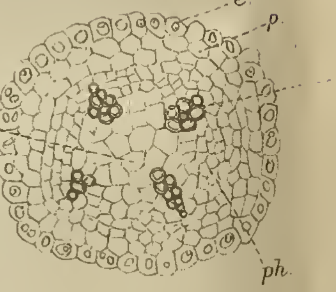

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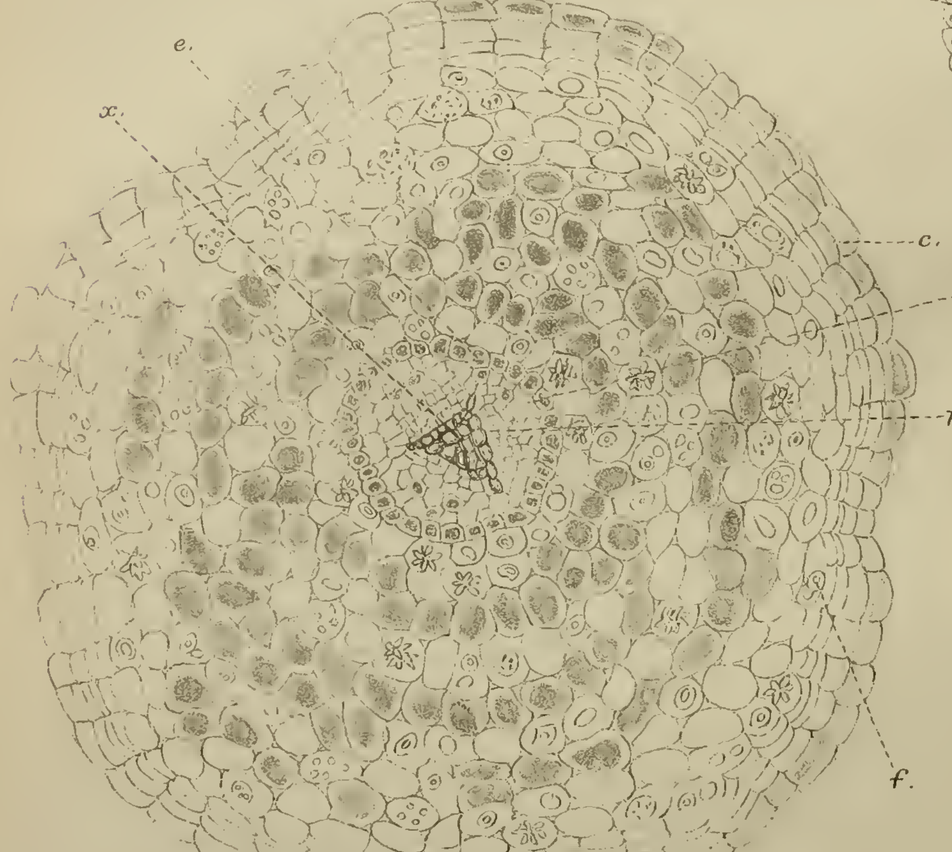

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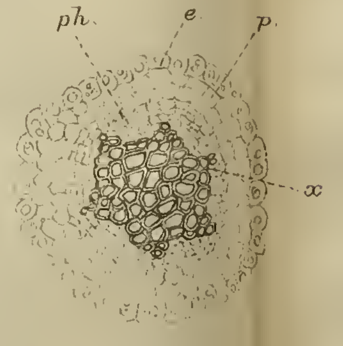

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SPRATT — ROOT-TUBERCLES OF ALNUS AND ELÆAGNUS.

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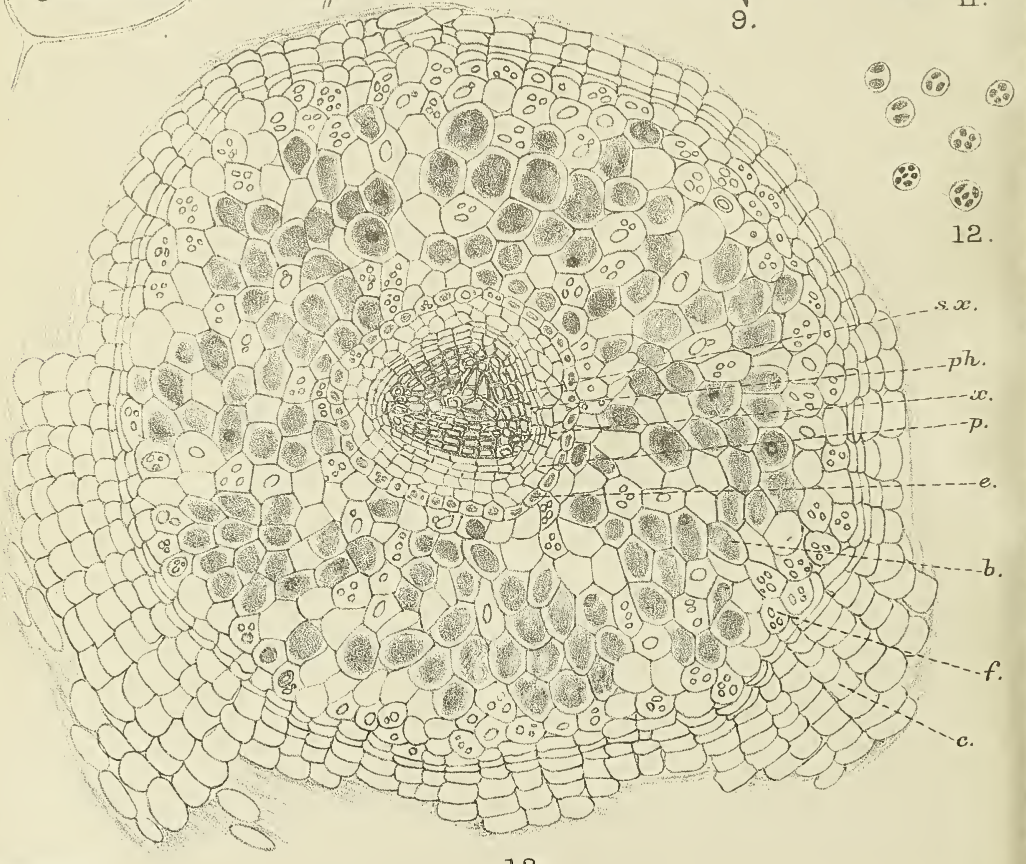

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SPRATT—ROOT-TUBERCLES OF ALNUS AND ELÆAGNUS.

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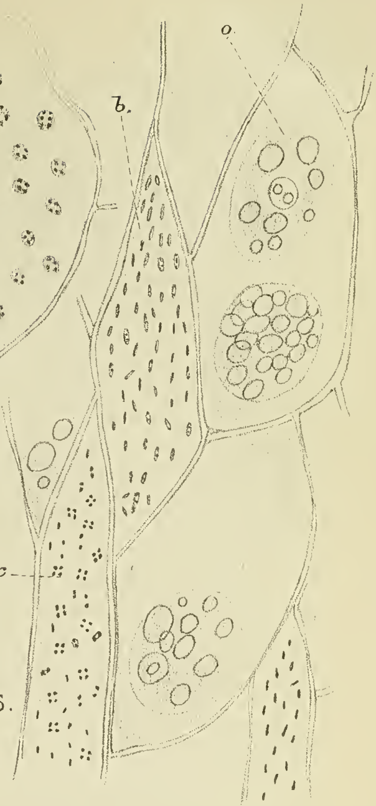

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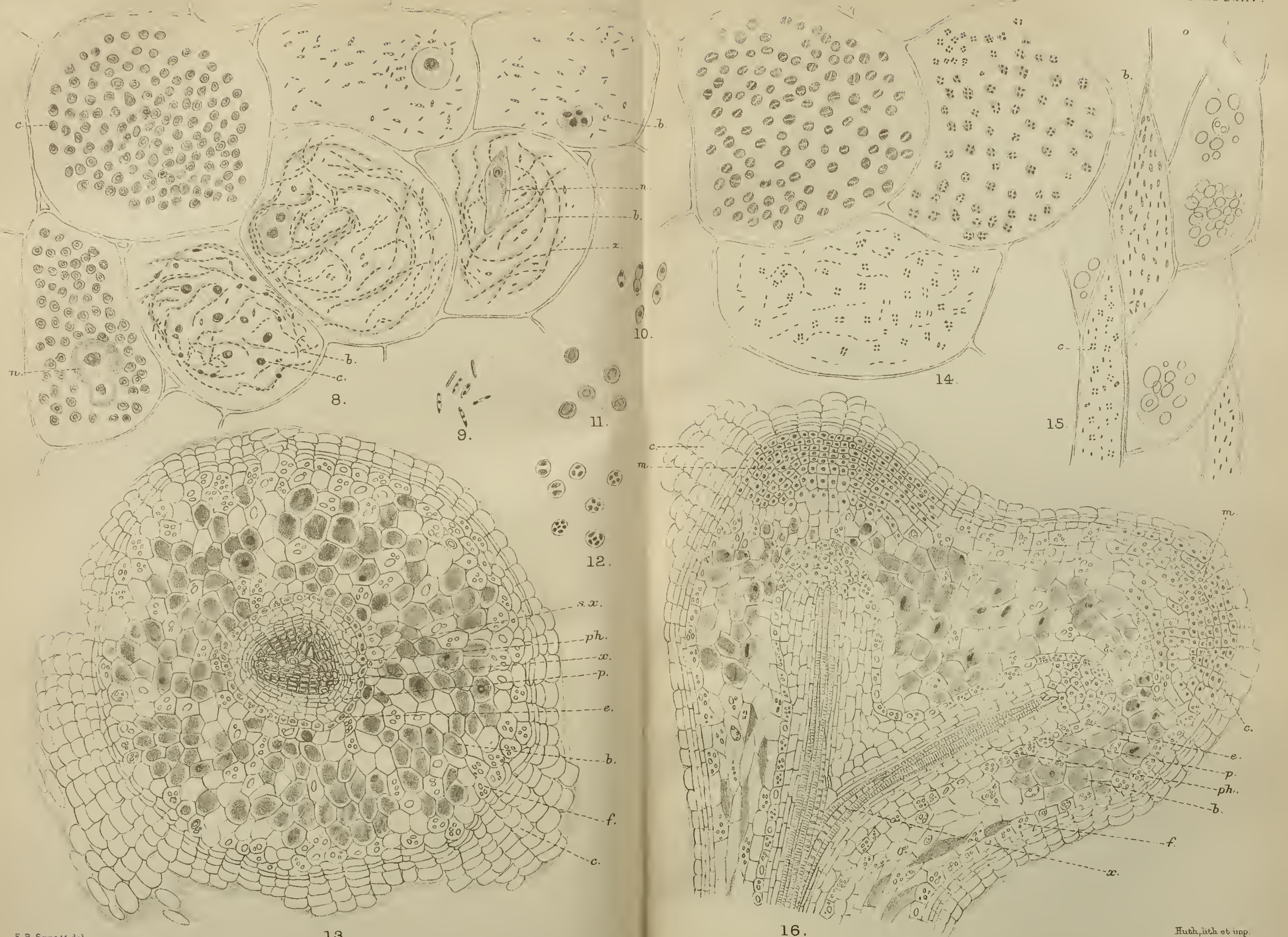

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SPRATT—ROOT-TUBERCLES OF ALNUS AND ELÆAGNUS.

On the Cause of ‘Blindness’ in Potato Tubers.

BY

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THE disease known as ‘blindness’ in potato tubers is so called because it more or less completely destroys the ‘eyes’, so that tubers thus affected are worthless for use as ‘seed’. In addition to the symptom of blindness the surface of the tubers becomes rough and dark brown on account of a large formation of cork. When such a tuber is placed in damp air it becomes covered with a fine white mycelium, belonging to *Verticillium albo-atrum*, which produces the characteristic small conidia. If a blind tuber is cut across, the disease is seen to be confined exclusively to the eyes and to a narrow zone immediately below the cortex, which is thickened by the cork formation mentioned above. In this zone there is the fine mycelium of *Verticillium albo-atrum*. The whole of the interior of the tuber remains sound.

Amongst a number of tubers which had been stored for seed during the winter, by far the greater number were blind. Some of these were planted in pots in a cool greenhouse, and others in the open. A few rotted away, some remained sound but produced no shoots, others formed fresh eyes which developed into shoots. These at first appeared healthy, but later most of them showed signs of leaf-curl and leaf-roll, becoming yellow in colour, and gradually withering and drying from below upwards. In the leaves of the plants grown in the greenhouse a bacterial disease appeared which is considered in a separate paper.[1] No mycelium was in any case found in the subaerial parts of the plant, though one shoot was cut down and every internode and leaf microscopically examined by means of sections. The subterranean stems, especially those nearest to the old tuber, were covered with elongated brown patches due to *Verticillium albo-atrum* and exactly resembling the figures given by Reinke and Berthold.[2] These brown patches spread from the old tuber up the new shoots and along the underground stems which produce the new tubers, and so into these new

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[1] Dale: A Bacterial Disease of Potato Leaves. Annals of Botany, vol. xxvi, No. ci, Jan. 1912, p. 134.

[2] Reinke and Berthold: Zersetzung der Kartoffel, 1879.

[Annals of Botany, Vol. XXVI. No. CI. January, 1912.]

tubers themselves, through the heel. The track of the fungus may easily be traced with the naked eye by means of the brown coloration. On some of the new tubers were many long, straight, brown hyphae, especially near the eyes. These hyphae apparently attack the tubers by means of the eyes and spread through the superficial tissues of the tubers. Possibly the hyphae may also obtain an entrance through the lenticels.

Microscopic sections show that the mycelium on the underground shoots is partly external and partly in the cortical tissues, but that it never penetrates as far as the vascular tissues. Some of the external hyphae are long, straight, and relatively thick-walled, of a light brown colour, and branching at long intervals almost at right angles to the main hyphae. If kept in damp air these hyphae produce the slender colourless mycelium of *Verticillium albo-atrum.* The old mycelium forms, both on the living plant and also on cultures of sterilized potato tuber, a dark brown resting mycelium with small rounded segments containing numerous drops of oil. This mycelium, when kept damp, also puts out colourless hyphae.

According to Reinke and Berthold[1] the disease known as leaf-curl is also due to *Verticillium albo-atrum.* These observers found a mycelium in the *vascular* tissues of the *subaerial* stems, and also in the *cortical* tissues of the *subterranean* stems. In both cases the mycelium was that of *Verticillium albo-atrum.* The fungus was not found in the tubers except near the heel. Reinke and Berthold distinguish three types of fungus affecting two generations of potato plants, and state that some races of potato are more liable to infection than others.

The late Professor Marshall Ward[2] found what seems to be the subaerial mycelium mentioned above, and also noticed some of the other symptoms described by Reinke and Berthold, but apparently not leaf-curl. He succeeded in tracing the fungus down the stem into the heel of the new tuber.

None of these observers seem to have noticed the fungus in tubers stored through the winter, nor to have observed the destruction of the 'eyes'.

Both the disease known as 'blindness' and that causing 'leaf-curl' seem to be due to *Verticillium albo-atrum*,[3] and tubers from crops affected with these diseases should not be used for 'seed', though they are quite fit for culinary purposes and generally keep well on account of the corky layer round the cortex.

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[1] Reinke and Berthold : l. c.

[2] Marshall Ward : A Potato Disease. British Association Report, Bristol, 1898.

[3] *Note.* Leaf-curl and leaf-roll almost certainly occur as symptoms in other diseases.

SUMMARY.

The mycelium of *Verticillium albo-atrum* is present in ‘blind’ potato tubers, where it causes the destruction of most of the ‘eyes’. It grows up into the new shoots, when any are formed, and in some cases it may pass into the subaerial shoots. In other cases it never goes beyond the subterranean stems and creeps along them into the newly-formed tubers, *internally* as a colourless mycelium in the cortical tissues, and *externally* as a scanty thin brown mycelium. Thus the tubers may be infected by means of the vegetative mycelium only, without the formation of any kind of spore. The course of the fungus from the old to the new tuber may be traced by means of the brown coloration of the affected tissues. Tubers have been grown in three successive years from the original diseased crop, and in each year some have been blind and have had a warty and corky outer surface.

SCHOOL OF AGRICULTURE,
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A Bacterial Disease of Potato Leaves.

BY

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With Plates XV and XVI.

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1. SYMPTOMS AND MODE OF OCCURRENCE.

IN the course of some experiments made in 1909 with potato plants grown from tubers affected with 'blindness', i. e. in which the 'eyes' had been more or less completely destroyed by *Verticillium albo-atrum*, certain pathological phenomena were observed which were at first thought to be due to the disease then under investigation, namely 'blindness'.[1]

Further observations, however, showed that two distinct diseases were in question. One of these, 'blindness', affects the tubers and underground stems exclusively, while the other, which is due to Bacteria, is almost as exclusively confined to the leaves.

The plants in which the latter disease was first observed were three, grown from tubers affected with 'blindness', and containing the mycelium of *Verticillium albo-atrum*. Two of these tubers had been kept in damp air for nine days, and had begun to form new and apparently healthy 'eyes'. The plants grown from these tubers were labelled respectively '1 *a*' and '1 *b*'. A third tuber, which was dry and resting, had only traces of 'eyes'. It was marked '3'. Each tuber was planted in a flower-pot on

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[1] Dale: On the Cause of 'Blindness' in Potato Tubers. Annals of Botany, vol. xxvi, No. ci, 1912, p. 129.

May 12, 1909. Besides these tubers, cuttings of shoots from apparently healthy tubers were planted. It is noteworthy that the plants grown from these cuttings were not attacked by the disease, which appeared on the plants grown from the 'blind' tubers.

Each of the three tubers produced shoots which at first were apparently healthy. But by June 10, i. e. in about a month after the tubers had been planted, the shoot of 'plant 3' began to show symptoms of disease. The leaves were somewhat wrinkled, becoming slightly yellow and covered with small brownish spots.

One of the leaves was cut. In the fresh leaf the brown spots were seen to be due to isolated cells, or rows of cells, densely filled with granules showing active movement. These cells are to be distinguished from other cells containing *colourless crystalline* granules.

In the former cells the granules are stained by dyes, while in the latter the granules are not stained and are refractive, so that if the granules are examined in reflected light they appear white, whereas the stainable granules are black, though both usually exhibit active movements in sections of fresh leaves. The stainable 'granules' were found later to be Bacteria.

By June 15 'plant 3' showed clear symptoms of 'leaf-curl'. The leaves were rolling up at the edges and were going yellow in large patches, and *the veins of the leaves were turning brown from the edge towards the centre of the leaves.* The leaves gradually became more yellow and dropped off, from below upwards. Sections of the leaves with brown veins showed that, in addition to the isolated cells with stainable granular contents, referred to above, the vascular tissues were turning a bright brown. At this stage were first observed, in the larger veins, certain *colourless tubes, with highly refractive walls*, of very irregular size and thickness. These tubes were obviously not fungal, and their significance and nature were not at first apparent.

In 'plants 1 *a* and 1 *b*' there were similar signs of disease, and sections showed both cells with granules and also large numbers of tubes.

By June 21 'plant 1 *a*' (which had been covered with a bell-glass for about a week) was almost dead. It had five large shoots (one had been cut off and fixed earlier) and three small shoots. Three out of the five had no green leaves; the stems were yellow and covered with elongated brown patches. The leaves were brown, shrivelled, and very slightly attached to the stem. The other two shoots had green stems with a few yellowish green leaves at the top. The three short shoots had yellowish green or brown leaves.

'Plant 1 *b*', which had not been covered, had green leaves on all its three shoots, but the veins were going a purple-brown colour on the under sides of the leaves, and the leaves were shrivelling from the tips downwards, while some were dropping off. 'Plant 3' was almost as badly affected as 'plant 1 *a*'.

A leaf of 'plant 1 *a*' was cut to see if any change had taken place in the tubes in the veins. They were still quite distinct, only slightly branched, and they nearly all ended in cells filled with stainable granules.

Plants with this disease almost invariably show marked symptoms of 'leaf-curl' and 'leaf-roll', but 'leaf-curl' may occur in plants which have not, as far as could be determined, yet been attacked by the organism causing the disease. A plant showing marked 'leaf-curl', but no traces of browning or wilting, was cut down, and each internode and its adjoining leaf put into separate bottles in absolute alcohol. A microscopic examination of these leaves and stems showed no traces of tubes, granules, Fungi, or any other sign of disease beyond the actual 'curl'. On the other hand, the brown veins and tubes may occur in leaves which are flat and remain so.

In the spring of 1910, and again in 1911, the disease appeared spontaneously in plants grown in pots in a cool greenhouse, some from the original 'blind' stock and some from tubers which were apparently healthy. Attempts were also made to grow seedlings for infection in sterile soil, but, though the seedlings readily germinated, further growth would not take place in sterile soil, so that plants grown in ordinary soil had to be used. The tubes obtained were in all cases similar. As before, some of the plants, but not all, showed symptoms of 'curl'.

2. HISTOLOGICAL CHARACTERS OF THE AFFECTED POTATO PLANTS.

Leaves from the three plants described above were fixed in chrome-acetic acid. Various stains were used, but by far the best method was found to be double staining with diamond fuchsin and light green. The sections were placed for about two minutes in fuchsin, washed in water, then *very rapidly* stained with light green by pouring the stain on the slide and washing it off again *at once* with absolute alcohol, which must not be left on the slide more than a minute before the sections are cleared in oil of cloves. Hand sections, however, require a longer time in absolute alcohol after the light green, in order to differentiate the stains and wash out the excess of fuchsin.

In the sections thus treated the tubes and Bacteria were stained bright red, while all the tissues of the host plant, with the exception of those which were lignified and of the nuclei, became green. It is therefore quite easy to trace the distribution of the parasite in the host plant by means of the coloration.

The tubes also stained well with alkaline methylene blue. In some cases the sections were treated with alcoholic caustic potash, before staining, to swell the tubes.

In stained microtome sections the tubes are to be found in all parts of the leaf, though they are largest and most numerous in the large veins. In sections of fresh leaves the tubes are easily overlooked in the mesophyll on account of the presence of chlorophyll. They are also inconspicuous in unstained hardened material because of their small size compared with those in the veins.

It was at first uncertain whether the tubes were formed inside the leaf and grew outwards, or whether they originated at the periphery. Various considerations led to the conclusion that the latter is the case.

In early stages of the disease, when there are few or no cells containing Bacteria, the tubes are seen to occur at the periphery and to extend inwards for varying distances. They were never observed in the deeper tissues only.

In many cases the tubes are narrow at the surface of the leaf and widen irregularly towards the centre, but not infrequently they show a large swelling at the surface. In the case of branching tubes the branching usually occurs in the neighbourhood of the vascular tissues and not near the surface. It is much more probable that the tubes branch than that they anastomose, though this does seem to occur occasionally.

It may therefore be concluded that the tubes begin at the surface and pass inwards. How is the entrance effected? *In no single case*, amongst the hundreds of sections examined, *was a tube seen to enter the leaf through a stoma.* This point has been studied carefully both in surface view and in transverse sections. *In every case the tube pierces the epidermis*, almost always where two adjacent cells are in contact. This may be seen by looking at any of the diagrams representing tubes at the surface, either in surface view or in section.

A horizontal section of a vein is shown in Pl. XV, Fig. 1, where the epidermal cells are seen in surface view. Two stomata are represented surrounded by epidermal cells, on the walls of which, in almost every case where two adjacent cells are in contact, are numerous tubes in early stages of formation. The unaltered tissues of the host are stained green, the parts attacked by the disease take up the red colour.

Figs. 2 and 3 show two later stages. In surface views there is usually a deeply staining, approximately circular mass, often with an irregular lumen in the centre. Comparison with a section (Fig. 4) shows that this deep red area is a more or less hemispherical mass at the mouth of the tube (cf. Figs. 2 and 3 with Fig. 4).

In other cases the tube is extremely narrow at the surface (Figs. 5 and 8, &c.). Whether narrow or wide, it frequently shows one or more points projecting above the surface (Figs. 4, 5, 10, &c.).

After piercing the epidermis the tube grows *at right angles to the surface* along the middle lamella of two adjacent cells. Rarely it seems to cross the centre of a cell. At the point where the tube pierces a wall there is often a swelling (Pl. XV, Fig. 6, and Pl. XVI, Fig. 20). On arriving at an intercellular space the tube crosses the space (Fig. 7), often with a very tortuous course, and passes between two cells on the other side. Occasionally tubes branch (Fig. 9) and, more rarely, anastomose (Fig. 8). Sooner or later the tube enters a mesophyll cell (Figs. 8, 9, and 12), in some cases near the epidermis (Fig. 8), or even into an epidermal cell, but more usually into a cell near the vascular tissue (Figs. 8 and 9). Though frequently occurring in the vascular tissues, tubes are scarcely ever seen in the vessels, though often in the phloem. The cells in which the tubes end are filled with Bacteria which usually stain red.

The tubes themselves are very irregular in outline and vary considerably in thickness. Irregular constrictions are common (Pl. XV, Figs. 5, 7; Pl. XVI, Fig. 11), and these are independent of the cell-walls of the host plant, as they are clearly visible in tubes crossing intercellular spaces (Fig. 7). The walls often present a corrugated appearance (Figs. 5 and 11). On the other hand, some tubes may be very straight and cylindrical (Figs. 6, 10, and 20). There is usually a lumen which is relatively narrow, though it varies in diameter (Figs. 6 and 7). The two sides of a tube are in many cases by no means parallel (Figs. 7, 11, 13).

The growing end of a tube is generally swollen and pointed (Fig. 5). Such swollen ends may be seen in intercellular spaces (Fig. 7) and also in the cells containing Bacteria. In some cases the tubes appear to pass right through the Bacteria-containing cells, or several tubes may end in one cell (Figs. 10, 12).

In the later stages of the disease the tissues of the host become extremely disorganized, especially in the neighbourhood of the veins. In these stages the affected tissues of the host plant, as well as the parasite, take up the fuchsin, so that the tubes cannot be so clearly followed.

It is noteworthy that sections of *stems* show no tubes or Bacteria, or at most very slight traces. The disease is confined, as far as could be determined, to the leaves. The explanation of this fact seems to be that the cuticle of the stem is too thick for the tubes to penetrate. In this connexion it may also be noted that in pure cultures of the organism grown on sterilized potato there was little or no growth on the 'skin' of the tuber, whereas the cut surface was rapidly covered with a thick mass of Bacteria.

3. THE PARASITIC ORGANISM AND ITS CULTURE.

The appearance of the tubes and of the granule-containing cells strikingly recalls, in many respects, those occurring in the nodules of leguminous plants, and shown by Miss Dawson[1] to contain, and to be formed by, organisms resembling Bacteria. Sections of nodules of *Pisum sativum*, fixed and prepared in the same manner as those of the potato leaves, showed similar reactions towards dyes. Morphologically there is also

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[1] Dawson: 'Nitragin' and the Nodules of Leguminous Plants. Phil. Trans., Series B., vol. cxcii, 1899, p. 1.

much resemblance, but the tubes in the potato leaf are the bigger, and in many cases the less branched of the two. The mode of entry in each case is similar, as the infection tubes of leguminous plants penetrate the cell-wall of the root-hair, while those of the potato plant pierce the thin cuticle of the leaf. In both cases there is frequently a swelling at the point of entry and a dilatation where the tube pierces the cell-walls of the host. This may be seen by comparing the diagrams of the different tubes (cf. figures in this paper with those in Miss Dawson's).

In both cases the tubes finally pass into cells which are filled with granules that stain like the tubes. It seemed probable, therefore, that in each case a similar organism was concerned. Attempts were accordingly made to isolate an organism from leaves in which tubes and granules had been found by previous microscopical investigation. These attempts were not made until 1910, as in 1909 the nature of the tubes was still undetermined.

Leaves showing marked 'curl' were obtained from plants grown in a greenhouse from small but apparently healthy tubers which were planted on February 3. By March 11—i. e., as in the preceding year, after about a month—tubes and stainable granules were found in the leaves. From pieces of these leaves Bacteria were obtained by means of plate cultures of gelatine containing extract of potato tubers. The leaves were remarkably free from Fungi or Bacteria, other than the one obtained, so that pure cultures were easily and quickly made.

It may here be noted that various kinds of Bacteria were obtained from potato *tubers*, and compared with those from potato *leaves*, but in no case were they the same in their behaviour on culture media.

In the spring of 1911, from a badly diseased plant, in which tubes and Bacteria were abundant, three kinds of organisms were obtained. One of these was identical with that isolated in 1910. Of the other two, one was an organism with a most remarkable habit of growth: on potato tubers it formed large *Tremella*-like gelatinous masses, a centimetre or more above the surface of the tuber.[1] These masses were corrugated at the edges, and resembled a gelatinous fungus far more than Bacteria. The culture was not slimy but exceedingly tenacious, so that strong pressure with a cover-slip did not break it up. Microscopic examination showed that the gelatinous mass consisted of roundish, highly refractive Bacteria, embedded in masses of gelatinous substance, and relatively widely separated from one another.

The third form was somewhat like the second, but so feebly growing that the culture of it was soon abandoned.

The original Bacteria obtained from leaves in 1910 grew well on a number of media, but most successfully on pieces of sterilized potato

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[1] This growth was confined to the cut surfaces of the tuber and never spread to the 'skin' of the potato.

tuber, at a temperature of 25° C. Under such conditions the Bacteria form a dirty white film which quickly grows in thickness, but which is almost exclusively confined to the cut surface. The cultures are very slimy and pull out into strings. As they grow, they give off a quantity of gas which raises bubbles on the surface of the cultures. The cultures, when old and becoming dry, sometimes turn a purplish or reddish-brown colour, recalling that of the veins of potato leaves in which this organism is parasitic.

The organism is very dependent on an abundant supply of water, and growth soon ceases, even on potato tuber, when the substratum becomes in the least dry, even though the food supply is by no means exhausted. Pieces of sterile (boiled) potato tuber, with cultures of the organism, were fixed in chrom-acetic acid, cut with a microtome, and stained with diamond fuchsin and light green (Fig. 14). Sections showed that the Bacteria were present in large numbers *between* the cells, filling up the intercellular spaces, whereas only a few, even in a culture a month old, were seen *inside* the cells and attached to their contents. The starch was apparently not attacked at all, as the contents of the cells gave a dark purple colour with iodine. These contents were chiefly the starch grains, boiled to a paste in the process of sterilization. The distribution of the Bacteria in the tuber was very clearly shown, as they took the usual bright red stain, while the walls and contents of the cell became a uniform green, with the exception of the starch grains and nuclei. It is noteworthy that the abundant proteid crystals resisted all the processes of sterilization, fixing, washing, and staining. They still retained their crystalline form, and stained red with fuchsin.

The organism was also grown on *living* potato tuber, which was sterilized as far as possible by cutting pieces from the centre of a sound tuber with a sterilized knife. The pieces of tuber were put in a sterile Petri dish, and sterile distilled water containing the organism was poured over them. A control section was placed in a dish containing sterile distilled water only. In about a week the control piece of tuber was unchanged, and the infected slice was brown and rotten and smelt strongly ammoniacal. Sections of the living material showed Bacteria *between* the cells, and few or no Bacteria *inside* the cells, which contained unchanged starch grains, stained red with fuchsin. One of the pieces of rotting tuber was fixed in chrom-acetic acid, cut with a microtome, and then stained with fuchsin and light green. In the parts where rotting was only beginning and the tissues were still firm (Fig. 15), the Bacteria were seen to be abundant between the cells, which were widely separated so that they easily fell apart. Apparently the middle lamella is first attacked and dissolved, and then the cellulose walls, which stain red as in the leaves. In some sections spores were abundant, as well as ordinary Bacteria, in the intercellular spaces. Some of the spores are shown in Fig. 15, in the angles between the cells. The spores stain a deep red; many of the Bacteria stain green, perhaps because the red colour was washed out in the course of preparation of the slide.

In comparing sections of sterilized (boiled) tuber with those of living tuber, it is suggestive that in the former the walls of the host do not stain red in the parts attacked by Bacteria, as they do in the latter. The explanation seems to be that in the living tuber the middle lamella is first attacked and dissolved by the organism, and then the cell-wall itself, whereas in the sterile tuber the isolation of the cells is brought about, before infection, by the process of steaming. Thus the organism finds a path already opened.

On potato extract in gelatine the organism grows well and liquefies the gelatine slowly. In a streak culture it first liquefies the part below the streak, forming a deep channel down which the culture slips into the liquefied medium at the base of the tube, where it proceeds to liquefy the remaining gelatine. The completion of the process takes days or even weeks in a low room-temperature.

On potato extract in agar, especially in an incubator at about 25° C. (the ordinary temperature of the laboratory in winter being low), the organism grows well. The cultures were slimy, as in gelatine, and pulled out into strings. As this medium dried up rather soon the growth ceased.

On 'Lemco' beef bouillon in gelatine at a somewhat low ordinary temperature (about 13°–15° C.) the organism grew well. The gelatine was slowly liquefied, at first along a groove as in potato gelatine cultures. The liquid part of the culture was turbid and contained flecks of pellicle.

In a *stab* culture a large circular mass of Bacteria was formed, thick in the centre and thin at the edges. Growth also occurred, but not very abundantly along the line of stab, the separate colonies each appearing as isolated round dots.

In the incubator, at 25° C., a good growth was obtained in liquid gelatine, the organism falling to the base of the tube as a whitish precipitate. A thin pellicle was formed on the surface. This film broke up and sank when the tube was shaken.

Wood ashes in agar, at 12°–15° C.

Streak culture. There was a considerable growth in all the cultures made.

Stab culture. A fair growth was formed along the line of stab, but there was no special development on the surface. The organism spread along the line of stab in wavy films or cloudy masses, which are very characteristic, and quite different from the isolated colonies formed in Lemco, and in cane-sugar and peptone gelatine. The difference seems to be due to the medium, isolated colonies being formed in gelatine, and wavy, cloudy growths in agar.

At 25° C. a fair growth developed on a streak, and a stronger development in a stab culture, as in the cultures at lower temperatures.

Urea in agar. At ordinary temperatures and in the incubator good growths were obtained on both streak and stab. The streak culture forms a broad, straight, and rather wide band. The stab culture formed a film over the surface, and also grew down the medium in a filmy manner as in wood ashes in agar.

On cane-sugar and peptone in gelatine[1] growth was very abundant. Large bubbles of gas were given off in such numbers as to form a frothy mass on the surface of the solid medium. Bubbles were also given off along the line of stab cultures, in spite of the solidity of the medium. As in other gelatine cultures, the colonies in a stab were clearly distinguishable with a hand lens as isolated round dots. Liquefaction here also was very slow and was not complete for some weeks. There is a distinct formation of acid, during which the neutral litmus becomes first red and then colourless.

On sterile milk. Several tubes, prepared according to the method given by Percival,[2] were infected with the organism, and one uninfected tube was placed with them as a control. In the incubator, at 25° C., by the next day the infected milk was going yellow at the top. At about 13° C. no change was visible. On the second day the infected milk in the incubator was coagulated and the violet colour had disappeared. There was a yellow liquid at the top and an almost pure white coagulum below. The milk in the control tube remained unchanged. At the lower temperature of the room the infected milk was changing colour but was not yet coagulated. After some time the coagulum disappeared.

On beer-wort gelatine there was very little growth, which is somewhat surprising as this organism grows so freely on so many media, and also because most organisms grow well on this particular medium.

Aerobism. A test-tube containing a stab culture of the organism in cane-sugar, peptone, and gelatine was placed in a larger tube containing a mixture of caustic soda and pyrogallin. Growth occurred abundantly, as was shown by the evolution of gas in the culture tube.

A second experiment was made, using the method given by Pethybridge and Murphy.[3] A streak culture of the organism was made in agar (urea was used in this case) and immediately plugged with melted agar to exclude the air. In this tube also growth was vigorous. The organism can therefore live under anaerobic conditions.

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[1] This medium was made up as follows: cane-sugar 1·5%; peptone 0·5%; gelatine 10·0%; distilled water 30 c.c.; neutral litmus.

[2] The milk was prepared as follows: Milk was passed through a separator. If not neutral it was rendered so, and a little neutral litmus added, which gave the milk a purple colour. The separated milk was sterilized in plugged tubes. Percival, Agricultural Bacteriology, 1910.

[3] Pethybridge and Murphy: A Bacterial Disease of the Potato Plant in Ireland and the Organism causing it. Proceedings of the Royal Irish Academy, vol. xxix, section B, no. 1, 1911.

The size and shape of colonies. Plate cultures were made in gelatine and agar. The colonies obtained were round or oval, and in some cases showed pseudopodial-like prolongations, but this may be due to the pressure of the cover-slip on the gelatine. In the colonies the Bacteria are quiescent, but when liberated they are actively motile.

The organism grows well at 25° C. and also at 35° C. There is no growth between 35° and 45° C., and the thermal death point is about 50° C.

The cultures on the various media were examined microscopically at different ages, fresh, stained alive with water methylene blue, and in permanent preparations made on cover-slips and stained with various aniline dyes. The organism differed considerably according to different conditions, so much so that doubt was felt as to whether the cultures were the same. But the point was settled by cross cultures from one medium to another, the results being constant.

The organism is much more motile in liquid than on solid media. It is rod shaped, but the rods vary greatly in size, and especially in length, and sometimes grow into filaments which may be as much as $5\,\mu$ long. The average size is $3{\cdot}7\,\mu \times 1{\cdot}23\,\mu$, the minimum $2{\cdot}4\,\mu \times 0{\cdot}8\,\mu$, the maximum $5\,\mu \times 1{\cdot}7\,\mu$.

In cultures on potato tuber, which are extraordinarily vigorous, rods of all lengths were found, some very small and scarcely motile in the slimy masses of zoogloea, others longer and wider in the more liquid parts of the culture. In this same culture, after only three days' growth in an incubator at 25° C., there were quantities of ripe spores as well as spores in process of development.

Formation of spores. The spores and spore-bearing individuals are so much larger than the average vegetative bacillus that at first sight they do not seem to belong to the same organism. But similar preparations have been made from different cultures, and in one and the same preparation all stages may be traced from the ordinary small vegetative bacillus to the ripe spore. The development is shown in Fig. 16. The ordinary bacilli are shown at a^1 and a long filament at a^2. Larger and somewhat more deeply staining individuals are seen at *b*. Still larger, and in some cases longer, individuals are represented at *c*, where the contents may be seen to be contracting and leaving the wall. In the next stage, *d*, the protoplasm is breaking up into segments, which vary in number according to the length of the spore-bearing rods. At this stage or earlier the rods may break up into smaller pieces. The spore-bearing rods are often seen to be surrounded by a faintly staining halo (*c* and *d*) which shades off gradually, separating the rods more or less widely from one another. This is no doubt the slimy matrix in which the rods are embedded if they are in a zoogloea, as is frequently the case in subaerial cultures. Inside the rods the spores are formed by the rounding off of the protoplasm, which becomes surrounded by a thick wall. The contents of the ripe spores show marked apical staining (Fig. 16, *e*). Most frequently two spores are formed in a short rod, or segment of a rod. In some cases a long rod breaks up into many spores, some of which do not attain to maturity. In the case of a short rod with two spores, only one may develop fully (*g*).

A portion of zoogloea with the vegetative rods embedded in a gelatinous matrix is seen in Fig. 16, *h*.

4. INFECTION EXPERIMENTS.

Attempts were next made to infect potato leaves, both of seedlings and of mature plants, with the organisms described above. Some of these experiments were made in 1910 and the others in 1911, some in a greenhouse, others in the open. In both years the climatic conditions were unfavourable for experiments in the field. In 1910 the weather was so damp that the plants soon succumbed to *Phytophthora*. In 1911, on the other hand, the heat and drought were so great that, except early in the year, the Bacteria all dried up before they were able to enter the tissues of the host.

1. In 1910, owing to an unavoidable interruption in the work, the infections could not be made until August. Cultures of Bacteria from both leaf and tuber were used. The latter gave negative results, however, and they were consequently abandoned.

Bacteria from the same culture tube were used to infect both the seedlings and the mature plants. In some cases the Bacteria were laid on the leaf and left to find their own way in; in other cases a wound was made with a platinum needle which had been first dipped into the culture.

The infections were made on August 13. The seedlings used had been grown in a greenhouse and were infected *in situ*. By August 29, i. e. in sixteen days, the infected leaves showed symptoms of leaf-curl, and were also turning brown in places. Some of these leaves were fixed in chrom-acetic acid and examined later.

The mature plants, grown from tubers, were some of an unusually late crop which appeared to be healthy, although most of the potatoes in the neighbourhood had been badly attacked by *Phytophthora*. The infections were also made on August 13. As in the case of the seedlings the organism obtained from tubers did not infect the leaf. The Bacteria were either laid on the leaves, which were wet with rain, or were stabbed into the tissues with a platinum needle. At first the infected plants were covered with bell-glasses and shaded with cloths. The glasses were removed after three days, but the cloths were retained as a screen from the sun and also as a possible protection from the attacks of *Phytophthora*. By August 29 several infected shoots showed signs of leaf-curl and of browning. These were fixed in methylated spirit.

In November of the same year (1910) some potato and tomato seedlings were infected with Bacteria obtained by carrying on the original cultures from the potato leaf. In these later experiments the Bacteria were all laid on the surface (either upper or lower or both) of the leaf, which had previously been wetted with boiled distilled water. *No mechanical injury whatever was done to the tissues of the host by stabbing.*

The plants were covered with bell-glasses and placed in a cool greenhouse. The infections were made on November 16. In two days brown spots began to appear on the infected leaves of those plants which had been kept most *damp*. Some which were *drier* were at first unaffected until sterile distilled water was put upon the leaves. Both the tomato and potato plants excreted large drops of water round the edges of their leaves, and it was observed that some of these became cloudy with Bacteria. This fact suggests the reason why the leaves begin to wither first at the apex and round the edges. Most probably infection takes place where there is the greatest amount of water, though, as stated above, it is quite independent of the stomata. Several leaves were fixed three days after infection. The infected leaves later became brown in patches and along the veins, and some appeared glassy-looking in patches. By no means all the leaves showed signs of 'curl' even when badly infected.

2. In 1911, infection experiments were begun earlier in the open at the University Farm, in a field of potatoes which was kindly put at my disposal by Professor Biffen.

This year the original organism was used, and also those obtained from the diseased plant cut down and examined in February, 1911.

The first experiments were made on June 8, when the weather was already very hot and dry, but there was some rain at the end of the month. With this exception there was hardly any rain at all until the end of the season, and the temperature not infrequently rose to over 90° F. in the shade[1] (35° C.), and was of course much higher in the sun.

The method used was slightly altered, and was as follows: The lower leaves were cut off to prevent their being confused with those which had been infected. The young upper leaves were wetted with distilled water. In the case of very hairy leaves it was found necessary to rub the water gently on the leaf, especially on the under surface, to ensure its being thoroughly moistened. The Bacteria were laid on the wet leaves and no punctures were made until quite late in the summer, when, owing to the drought, the Bacteria could not enter the host.[2] Each of the infected shoots was covered with a bag made of thin typing paper, waterproofed with paraffin and petrol, and then sewn up. By this means the plant was pro-

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[1] It is important in this connexion to remember that the organism will not grow above 35° C.

[2] The drought would tend to make the epidermal walls of the host thicker and firmer, and therefore more difficult for the Bacteria to pierce.

tected to some extent from dryness and excessive light. Some leaves were infected on the upper surface only, some on the lower surface only, and others on both sides. Several varieties of potato were infected, including 'White City', 'Up-to-Date', 'British Queen', and 'Maresland Queen'.

The results of these experiments will not be given in detail, partly because later in the season so many gave no results, and also because the plants were attacked by a fungus, apparently *Cladosporium*. The symptoms of the disease caused by this fungus are externally so like those due to Bacteria that only a microscopic examination shows which is present. Both diseases may occur in the same leaf.

The positive results, though few in number, are indisputable, as well-developed infection tubes were found in the leaves. These will be described in the next section of the paper.

With regard to *Cladosporium* it may be mentioned in passing that this fungus attacks the potato leaves chiefly after the plants have been earthed up. It is one of the common Fungi of the soil, and, when the soil is brought into contact with the leaves in the process of earthing up, the spores attack the lower leaves and the fungus gradually passes upwards, until the whole plant may be involved. Each spore and the hyphae formed from it kill a small area of the leaf, causing a yellow or brown spot. The veins are also very frequently attacked and become brown. The diseased areas gradually spread over the leaf, which becomes yellow, then dries up, and finally falls off.

Some experiments, both with seedlings and with plants from tubers, were also made during the summer in a greenhouse, but gave no results, without any doubt because it was not possible to prevent the temperature from rising far too high. On one occasion, with open windows and white-washed roof, the temperature was 104° F. Since the maximum growing point is 35° C. (95° F.), the optimum temperature for growth is of course lower. This fact, as well as the excessive drought, accounts for the fewness of the positive results obtained in the summer of 1911 after artificial infection.

5. EXAMINATION OF THE ARTIFICIALLY INFECTED PLANTS.

1. 1910 *Material.* Few sections were cut through the material infected by puncture, because one of the chief objects in the experiments was to determine whether the infection took place through the uninjured epidermis, and this of course could not be seen when the leaf had been pierced artificially.

As in the case of naturally infected plants, no infection was seen to take place through either the water stomata or the ordinary stomata, though diseased leaf edges were specially examined. The fact that the withering of the leaves often begins at the apex or round the edges, and that the veins are the parts chiefly affected, seems to be explained by the fact (already pointed out, p. 144) that the water excreted at night round the edges of the leaves at the ends of the veins serves as a medium for the Bacteria, which thus obtain the large amount of moisture necessary for their growth. On the lower surface of the leaves the angle between the projecting vein and the lamina is also a common point of infection, doubtless because it affords shelter for the parasite (cf. Fig. 11).

The Seedling Plants. Only one case of really clear and unmistakable tubes was found in cutting sections of seedling potato leaves. This was in a hand section of a comparatively large vein (Fig. 17). But other sections showed Bacteria in the cells of the affected parts of the leaves.

The Mature Potato Plants. These gave better results, though the experiments had to be stopped sooner than was desirable on account of the attack of *Phytophthora.* But definite tubes were obtained, and also early stages of infections were observed with less well defined tubes. In all cases, as in the spontaneously infected plants, the entrance of the Bacteria was through the epidermis and never by means of the stomata. Fig. 18 is an example of a tube which shows clearly the contained Bacteria, whereas in the naturally infected tubes distinct Bacteria were rarely if ever seen, perhaps because the tubes were older and emptied of their contents. This section also shows many Bacteria between the cells and outside the tube from which they may have escaped. It will be noticed that the tube is lying on the surface of the wall, on which are also Bacteria. Intercellular spaces are also to be seen with bacterial contents. Apparently the tubes are in an early stage of development, and the Bacteria have not yet penetrated into the interior of the cells, nor far into the tissues.

Fig. 19 represents a case of infection without definite tubes, in which the bacteria make their way between the epidermal cells and the palisade cells and cross the intercellular spaces. As in the last figure the epidermal cells are injured, apparently by the entry of the Bacteria. In both cases Bacteria occur outside the section on the surface of the leaf. The Bacteria, both in spontaneously and in artificially infected leaves, cling so tenaciously to the outer surface of the leaf that they are not washed off by the various processes involved in making permanent microtome sections. It is therefore often possible to determine the exact spot where the infection was made.

In the case of the *tomato seedlings* no trace of tubes was found, but Bacteria were abundant inside the leaves, especially in the intercellular spaces and in the thin-walled cells of the larger veins. In both potato and tomato plants the epidermal cells were attacked very seriously. This is not usually the case in naturally infected plants. Perhaps the contact of the larger mass of material, often including some of the potato tuber underlying the Bacteria, may account for the greater injury.

Several series of sections through the edges of leaves showed Bacteria entering between the cells and not by the stomata.

2. *Material infected in* 1911. The open-air experiments in 1911, in the earlier part of the season, gave more positive results, though on account of the hot, dry weather, these were fewer than had been hoped and expected. Of the plants infected on June 8 some leaves were cut and examined fresh on June 23, i. e. fifteen days after infection. As before stated, it is difficult, except in the larger veins, to see tubes in fresh material on account of the presence of chlorophyll. It is quite possible, therefore, that tubes in the lamina may have been overlooked.

Three leaflets from a plant of 'White City' infected with the original organism were cut. The signs of disease were not far advanced, and the leaflets were only slightly brown. *A very definite tube was seen in the first leaflet cut.* From these leaflets a fresh culture of the organism was made, which resembled the original culture. On June 30, that is, twenty-two days after infection, some more leaves were cut. A leaf of 'Up-to-Date' infected with the original organism contained good tubes in a vein which was cut. One large tube and some smaller ones were also seen in a leaf from a plant of 'White City' infected with the original organism (Fig. 20). A leaf of 'White City' infected with the thick gelatinous form also contained well-marked infection tubes and also many fungal hyphae. There is, however, no danger of confusing the two, as the fungal hyphae are thin-walled, have granular contents, branch, and are septate, whereas the bacterial tubes have thick refractive walls, and a narrow lumen with no contents, and seldom branch. There is, however, a doubt as to whether these tubes were really formed by the gelatinous organism or by the original Bacteria, as the infections were made on the *upper* side only, while the tubes were found only on the *lower* side. It is possible that some of the original Bacteria, known to form tubes, may have been transferred from one plant to another by the hands of the operator. To avoid this danger, subsequently only one kind of organism was used for infection on a given day, as there were no means of sterilizing one's hands properly in the field.

After this time, however, a severe drought set in, and the plants also became attacked by *Cladosporium*. No definite and unmistakable tubes were found in the leaves which were subsequently cut, except on July 8, on another leaf of 'White City' infected on June 8 with the original organism. It is noteworthy that the tubes were confined to the upper side of the leaf, and that the upper side only had been infected. The leaf was so very slightly diseased that to the naked eye it seemed almost healthy.

No tubes were found in plants infected with the organism which was obtained from the plant in 1911, and which seemed identical with that obtained in 1910. Bacteria were, however, found inside infected leaves. Leaves infected with the gelatinous organism, though showing no tubes, except in the doubtful case recorded above, not infrequently showed masses of Bacteria, in the condition of zoogloea, inside the tissues of the host and between the cells. It therefore seems as if this organism were also pathogenic to the potato.

In the case of plants infected with the original organism, Bacteria were sometimes found inside the host plant when no tubes were seen. This would seem to be a case of infection without definite tubes like those noticed the year before.

6. DISCUSSION OF RESULTS.

The experiments and observations recorded above lead to the conclusion that the disease under consideration is bacterial in nature. Various bacterial diseases of the potato and allied plants have already been described by different authors. A history and comparison of these diseases has lately been published by Pethybridge and Murphy [1] in a paper describing a bacterial disease of potato shoots known as '*Black stalk-rot*' or 'Blackleg', occurring in Ireland.

The bacterial diseases of the potato which have been most fully described are the one just referred to, and another worked out by Erwin Smith [2] and called by him '*Potato Bacteriosis*', which attacks not only the potato but other species of *Solanum*, especially the egg-plant (*S.* [*Melongena*] *ovigerum*) and tomato (*Lycopersicum esculentum*).

In potato 'Black stalk-rot', or 'Black-leg', as described by Massee, the symptoms are as follows: [3] The leaves wilt and turn yellow, the lowest first. When the leaves droop the surface of the underground parts of the stem bearing such leaves is more or less covered with brown stains. Discoloration *goes up the stem*, which becomes black and decays. This disease was regarded by Carruthers,[4] who does not, however, describe it in detail, to be due to *Bacillus phytophthorus*, which was first isolated and named by Appel.[5] An organism was also isolated by Pethybridge and Murphy from potatoes affected with black stalk-rot, but it was not considered by them to be the same as (though closely allied to) that obtained by Appel. They therefore regard it as a new species, to which they give the name *B. melanogenes*.

The symptoms of 'Black stalk-rot' differ considerably from those of the disease at present under consideration, especially in the fact that the disease begins from *below* and spreads *upwards*, attacking chiefly the stems

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[1] Pethybridge and Murphy: v. p. 141, *supra*.

[2] Erwin Smith: A Bacterial Disease of the Tomato, Egg-plant, and Irish Potato (*Bacillus solanacearum*, n. sp.). U. S. Department of Agriculture, Bull. No. 12, 1896.

[3] Massee: Diseases of Cultivated Plants and Trees, 1910, p. 514.

[4] Carruthers: Journal of the Royal Agricultural Society of England, vol. lxviii, 1907, p. 226.

[5] Appel: see bibliography in the paper by Pethybridge and Murphy quoted above.

and tubers, whereas in the disease here described the mischief not only *begins* in the leaves but is exclusively confined to them, so that, as far as can be determined at present, the stems and tubers are only indirectly affected by means of the destruction of the foliage. As is to be expected, the organisms isolated in each case also differ from one another. In the potato *bacteriosis*, described by Erwin Smith (l. c.), the parts chiefly attacked are also the stems and tubers, though the organs *first* attacked are the leaves, as in the disease now being described. But the two diseases differ essentially in the fact that, while in *bacteriosis* the *vascular* tissue is first attacked and the vessels blocked with the organism, the parenchyma being only affected subsequently, in the present disease the injury begins in the *parenchyma*, and spreads to the non-vascular tissues of the veins, while not apparently affecting directly the stems and tubers. For convenience in description the latter disease may shortly be called *bacterial leaf disease* to distinguish it from bacterial diseases chiefly affecting other organs, and also to distinguish it from leaf diseases, such as 'blight', due to *Phytophthora* or other Fungi. The *mode of infection* in the two diseases is also different. *B. Solanacearum* enters the vessels by means of the water stomata or through wounds, whereas in bacterial leaf disease the organism pierces the uninjured epidermis and passes first between the cell-walls.

Again, as might be expected, there are marked differences between the *organisms* causing the two diseases. The differences between the three organisms, viz. *B. Solanacearum*, causing bacteriosis, *B. melanogenes*, causing black stalk-rot, and the bacillus causing bacterial leaf disease, may most readily be seen and compared by means of the accompanying table[1] (p. 151).

There seems to be no doubt, as a study of the table shows, that the organism causing bacterial leaf disease differs markedly from those already described, and that it must therefore be regarded as a new species, reluctant though one is to add another to the already long list. Since its most distinguishing characteristic is the power of forming tubes in the tissues of the host, like those already known in the Leguminosae, the most obvious specific name is one indicating this character. I propose, therefore, to call it *B. Tubifex*, n. sp.

The species, though parasitic on potato leaves, is also a saprophyte, as it grows strongly on various culture media as well as on the living leaf and tuber.

With regard to the mode of formation of the tubes a few words may here be said.

The organism evidently pierces the epidermis and passes between the cells by a process of fermentation in which the middle lamella is dissolved. That Bacteria possess this power has already been demonstrated by previous

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[1] Reference may also be made to the table given by Erwin Smith, in the paper above referred to, showing the differences between *B. Solanacearum*, *B. tracheiphilus* (the organism causing cucumber rot), and 'Kramer's Bacillus', causing a rot in potato tubers.

workers. That *B. Tubifex* is able to cause fermentation is shown by its behaviour on culture media.

The tube itself is doubtless formed, as in the Leguminosae, by the union of the individual organisms into a zoogloea by means of the gelatinous envelope surrounding them. This envelope is only developed in subaerial cultures and not in liquid media. That the tube is not formed from the dissolved middle lamella of the cells of the host plant seems to be indicated by the fact that the tubes can be formed in, and cross over, large intercellular spaces in the mesophyll of the lamina.

The tube is therefore a kind of zoogloea, which penetrates the host by means of a ferment action which dissolves the middle lamella of the cells of the potato leaf. Apparently the Bacteria pass out of the tube into the cells of the host plant, leaving the matrix as a resistant, deeply staining tube, since all attempts to differentiate Bacteria by means of staining failed in the old tubes, though the same methods were used which were successfully employed by Miss Dawson in the case of the nodules of the Leguminosae.

Whether there is any relation between bacterial leaf-disease and leaf-curl is uncertain. The constant symptoms of the former disease are a yellowing of the leaves accompanied by the formation of brown patches in the lamina and of a brown colour in the veins. These symptoms may or may not be accompanied by leaf-curl or leaf-roll. On the other hand, as stated above, leaf-curl may occur when no organism can be found in the subaerial shoots. Reinke and Berthold[1] observed this fact, and came to the conclusion that curl is due to the presence of *Verticillium albo-atrum*, which may, however, be confined to the subterranean parts of the host and to the lower parts of the subaerial stem, though it may eventually grow upwards along the whole length of the shoot, and kill it by blocking up the vessels.

7. CONCLUSIONS AND SUMMARY.

Bacterial leaf-disease is due to *Bacillus Tubifex*, n. sp., which attacks the leaves of the potato plant by piercing the cuticle where this is thin enough for it to penetrate. The bacilli form a kind of zoogloea and dissolve the middle lamella of the host plant by means of a ferment, and so pass between, or in some cases across, the cells of the host plant by means of infection tubes like those present in the nodules of certain leguminous plants. Infection generally takes place near the edges of the leaves or along the veins either on the upper or under surface. The infection tubes grow at right angles to the surface of the lamina and sooner or later end in cells of the host plant, which become filled with Bacteria.

The disease appears to be more of theoretical than of practical importance, as in a fairly dry, hot summer the cuticle of the potato leaves would

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[1] Reinke and Berthold: Zersetzung der Kartoffel. Die Kräuselkrankheit der Kartoffel, 1879, p. 67 et seq.

generally be too thick, and the temperature too high, to allow of the penetration of the organism. Any considerable attack would, however, be serious, as the leaves and shoots are completely destroyed and the formation of tubers consequently stopped. The disease has been most marked in plants grown in a *cool greenhouse*. The organism causing the disease is a bacillus differing in so many respects from those previously described as the cause of other potato diseases that it must be considered as a new species. On account of its power of forming infection tubes in the host plant it has been named *Bacillus Tubifex*, n. sp.

In culture it forms slimy masses of zoogloea on steamed potato tuber. These masses string out and stretch in films over large bubbles of gas, which are given off as the organism grows. The Bacteria have the power of causing fermentation. They are aerobic and anaerobic, and can live either as parasites or as saprophytes. Spore formation readily occurs.

My grateful thanks are due to Professor Biffen for allowing me to work in the School of Agriculture and at the University Farm, and for helping me with his advice during the course of my investigations.

TABLE SHOWING THE CHARACTERISTICS OF DIFFERENT BACTERIA PATHOGENIC TO SOLANUM TUBEROSUM.

	Bacillus Solanacearum (Smith).	*Bacillus melanogenes* (Pethybridge).	*Bacillus Tubifex*, n. sp.
1.	Not sticky in the plant or on culture media, except slightly on agar.	Exceedingly sticky and slimy in cultures, especially on potato tuber. It strings out from the needle.	Like *B. melanogenes*.
2.	Dirty white or yellowish white, then brownish and finally *smoke-black*. Browns vascular bundles.	White, except on sterile potato.	White, then dirty white.
3.	Strictly aerobic. Will not grow in closed fermentation tubes with any sugar.	Slight growth occurs in stab cultures covered with agar.	Not strictly aerobic. Will grow in presence of caustic soda and pyrogallin, and also in agar stab covered with agar.
4.	No acids with any sugar.	Forms acid in glucose broth and in cane-sugar broth.	Forms acid in cane-sugar and peptone in gelatine.
5.	Intense alkali producer.		
6.	Slowly saponifies milk.	Coagulates milk within 48 hours. Gas bubbles formed. Colour of litmus goes.	Coagulates milk in 1 or 2 days with formation of acid. Gas bubbles formed. Decolorizes neutral litmus.
7.	Decided but not particularly obnoxious smell—like some, but not all, rotting potatoes.	—	Slight odour. On living potato smells ammoniacal.
8.	Grows readily at 37° C.	—	Grows readily at 35° C.
9.	Thermal death-point 52° C.	Thermal death-point between 45° and 50° C.	Thermal death-point between 45° and 50° C.
10.	Zoogloea in top layers of beef broth or peptone water as innumerable tiny whitish flecks, which are sometimes slightly inclined to unite into pellicle but diffuse through the liquid on shaking.	Zoogloea not recorded.	Like *B. Solanacearum*, but also forms films on the surface which break up and sink when the fluid is shaken.

	Bacillus Solanacearum (Smith).	*Bacillus melanogenes* (Pethybridge).	*Bacillus Tubifex*, n. sp.
11.	Growth on gelatine not leaf-like.	—	Forms circular colonies on gelatine plates.
12.	Does not liquefy gelatine.	Liquefies gelatine fairly rapidly. Begins the second day in gelatine stab.	Liquefies gelatine rather slowly.
13.	No gas in potato or in media with various sugars.	Slight formation of gas with sugar.	Forms large bubbles of gas on potato and in cane-sugar and peptone in gelatine.
14.	No acid formed in potato or glucose solutions containing nitrogenous substances.	Forms acid.	Forms acid in cane-sugar and peptone in gelatine.
15.	Goes through bundles into tuber, where cavities are formed.	In subaerial stems.	Not found in tuber nor in stems, but only in leaves, especially in the veins but not in the vessels.
16.	No spores.	No spores.	Spores formed readily and plentifully in a few days on potato tuber.
17.	Sluggishly motile, active in young cultures.	Actively motile. Peritrichous flagellae.	Actively motile in liquid media.
18.	Bacilli in short rods.	Bacilli often in pairs or in threes or in long chains. Vary little in size. 1·3 to 1·8 × 0·9 μ. Chains up to 20 or 70 μ. 5 to 10 peritrichous flagella.	Bacilli in short rods and long filaments of all lengths according to conditions of growth, nature of medium, age of culture, &c. Flagellate. Average size 3·7 × 1·23 μ, minimum 2·4 × 0·8 μ, maximum 5 × 1·7 μ.
19.	Bacilli blocking up *vessels* of host plant. At first *only* in the vascular tissues, but later in parenchyma.	In vascular tissue of the stem of the potato plant.	Bacilli in and between the cells of the parenchyma and phloem. Seldom in the vessels and only in later stages.
20.	Infection through wounds caused by insects, &c.	Infection chiefly from the tuber, the organism growing up the stem through the vascular tissues.	Infection through uninjured epidermis by means of infection tubes, as in the nodules of certain leguminous plants.
21.	Injury caused chiefly by the blocking up of the vessels. Almost entirely confined to *stems* and *tubers*.	Injury chiefly as in *B. Solanacearum*.	Injury caused by disorganization of tissues due to passage of infection tube, and by lesions in cells filled with Bacteria. Also indirectly by the using up of the products of metabolism. Almost confined to *leaves*.
22.	Causes wilting and browning of foliage and stems.	Causes blackening of stems, withering of leaves, and rotting of tubers.	Causes withering and drying of leaves from outside inwards. Often accompanied by 'curl', but not always.
23.	—	On *gelatine* plates forms minute white dots. Liquefaction after the second day.	White dots on second day on gelatine plates and along line of stab. Liquefies gelatine.
24.	—	On *agar* white dots are formed in 48 hours as in gelatine. Surface covered with greyish white growth, somewhat crenated along the margin.	On *agar* plates dots as on gelatine. In stab cultures forms wavy films along the line of stab.
25.	—	Not vigorous in beef extract peptone.	Rapid growth in 'Lemco'.
26.	—	In cane-sugar broth gas and acid produced.	In cane-sugar-peptone gelatine gas and acid produced.
27.	—	Active growth in potato juice.	Active growth in liquid extract of potato tuber.

	Bacillus Solanacearum (Smith).	*Bacillus melanogenes* (Pethybridge).	*Bacillus Tubifex*, n. sp.
28.	—	Produces enzyme capable of dissolving the middle lamella of cells.	Produces enzyme capable of dissolving the middle lamella of cells.
29.	—	On *sterile* potato at 25° C. yellow slimy growth is formed in 2 days. After 6 days becomes brown.	On sterile potato at 20° C. a dirty white slimy growth is formed, raised up into bubbles.
30.	—	On *living* potato. Slight brown colour, later black. Middle lamella dissolved. Moisture needed.	As *B. melanogenes.* Smells strongly ammoniacal on living potato. Goes brown.

SCHOOL OF AGRICULTURE,
CAMBRIDGE.
October, 1911.

EXPLANATION OF FIGURES IN PLATES XV AND XVI.

Illustrating Miss Dale's paper on a Bacterial Disease of Potato Leaves.

PLATE XV.

Fig. 1. Surface view of the epidermis of a potato leaf, showing the bacterial infection tubes entering the tissues of the host through the cuticle and not through the stomata. The tubes are coloured red in all the figures. 4 *oc.* 2 *mm. obj.* (oil immersion).

Fig. 2. Surface view of older stage to show the lumen. Note that the cell-walls on each side of both tubes are stained pink because they have been chemically altered by the parasite. 4 *oc.* 2 *mm. obj.*

Fig. 3. Another surface view of older stage with the end of the tube more thickened. 4 *oc.* 2 *mm. obj.*

Fig. 4. Transverse section of an epidermal cell and of adjacent cells, showing two tubes which are markedly thickened at the surface of the leaf. Note the prolongations above the surface in the left-hand tube and the lumen, and the dilatation where the right-hand tube pierces the cell-walls of the host. 4 *oc.* 2 *mm. obj.*

Fig. 5. A young complete tube with a single narrow projection beyond the epidermis. The tube widens gradually with very irregular contours and shows several constrictions. Towards its inner end it becomes narrower, and there are traces of granular contents in the lumen. 4 *oc.* 2 *mm. obj.*

Fig. 6. A short piece of a tube, shown where it crosses three cell-walls. The swellings at the points where the walls of the host cells are pierced is very marked. 4 *oc.* 2 *mm. obj.*

Fig. 7. A portion of a tube to show the behaviour in an intercellular space. The surrounding cells are more or less diseased and contain some Bacteria. 4 *oc.* 2 *mm. obj.*

Fig. 8. Two tubes in a later stage of the disease. These tubes anastomose and branch, ending in the phloem of a small vein. A few Bacteria are to be seen on the surface of the leaf near the outer ends of the tubes. One of the cells of the host, immediately below the epidermis, is filled with Bacteria and its chloroplasts are degenerating. The intercellular course of the tubes is very clearly shown in this section. 4 *oc. D obj.*

Fig. 9. One tube is here seen branching and passing between the cells of the host into a small vein where some of the cells and vessels contain Bacteria. 4 *oc. D obj.*

Fig. 10. This shows an unusually large number of tubes, in close proximity, in the neighbourhood of a large vein. The tubes are seen at the surface, where each ends in several points. They have a more or less nearly straight course and are unbranched. Numerous cells containing Bacteria are also represented. Other examples, showing equally numerous tubes, were met with in examining series of microtome sections. 4 *oc. D obj.*

PLATE XVI.

Fig. 11. A long tube is here seen from the point of entrance in the angle between the vein and the lamina of a leaf to its ending in the phloem of the vascular bundle. On each side of the tube, along its tortuous course, are diseased cells containing Bacteria, and a few Bacteria may be seen lying outside the leaf round the mouth of the tube. Incidentally this section shows how the tubes, so to speak, avoid the stomata, one of which lies close to the outer end of the tube. 2 *oc. D obj.*

Fig. 12. The section is of a considerably diseased leaf with several branching and anastomosing tubes passing from the exterior into a small vein. The Bacteria have only attacked the cells and not the vessels. Many cells of the parenchyma are filled with Bacteria. 4 *oc. D obj.*

Fig. 13. This shows a few cells filled with Bacteria and containing the endings of the tubes. One tube is seen in transverse section in an intercellular space. 4 *oc.* 3 *mm. obj.*

Fig. 14. Section of potato tuber, *sterilized by steaming*, and infected with Bacteria. The Bacteria are very numerous between the cells, which, after being steamed, are only loosely attached to one another. There are only a few Bacteria inside the cells, whose starchy contents are apparently only slightly attacked. 2 *oc.* $\frac{1}{2}$ *inch obj.*

Fig. 15. Section of *living* potato tuber infected with Bacteria. Rotting was only beginning and the tissues were still firm. As in the dead tuber, the Bacteria are most abundant *between* the cells, whose walls have here been separated by the organism, which has destroyed the middle lamella and chemically changed the cell-walls so that they stain red. The organism has formed spores in the intercellular spaces. The large red spheres are starch grains, which have not been attacked by the Bacteria. 2 *oc.* $\frac{1}{12}$ *obj.*

Fig. 16. Formation of spores in the bacilli. *a* 1. Ordinary vegetative bacilli. *a* 2. Very long rod. *b.* Rods enlarging to form spores. *c.* Contents contracting from the wall. A faintly staining halo of zoogloea surrounds these rods. *d.* The contents breaking up into short lengths. *e.* Ripe spores showing deep apical staining. *f.* Two spores in a rod. This is very frequent. *g.* Rod with two spores, only one of which has matured. *h.* Portion of a zoogloea. 4 *oc.* 2 *mm.*

Fig. 17. Section of a leaf of a *seedling potato plant*, artificially infected with Bacteria from a pure culture. Two tubes are seen entering at the lower surface and growing towards the vascular tissues. The plant was grown and infected in a cool greenhouse. 2 *oc. D obj.*

Fig. 18. An artificially infected leaf of a *mature plant* from a tuber, grown and infected in the open. Many of the Bacteria used for infection are still clinging to the upper epidermis. A single tube is seen passing along a palisade cell. Bacteria are also seen in the intercellular spaces and inside the epidermal cells. 4 *oc.* 2 *mm.*

Fig. 19. Represents a case of infection without definite tubes. Numerous Bacteria may be seen passing between the cells and also inside an epidermal cell. 4 *oc.* 2 *mm.*

Fig. 20. Several tubes from a leaf of 'White City' variety of potato, artificially infected on the University Farm. The tubes are typical and exactly like those found in naturally infected plants. The swellings where the cell-walls of the host plant are pierced are very marked. 4 *oc.* 2 *mm.*

N.B.—It should be noted that in all cases the tubes run *across* the veins and lamina, and never in any case parallel to the surface of the leaf.

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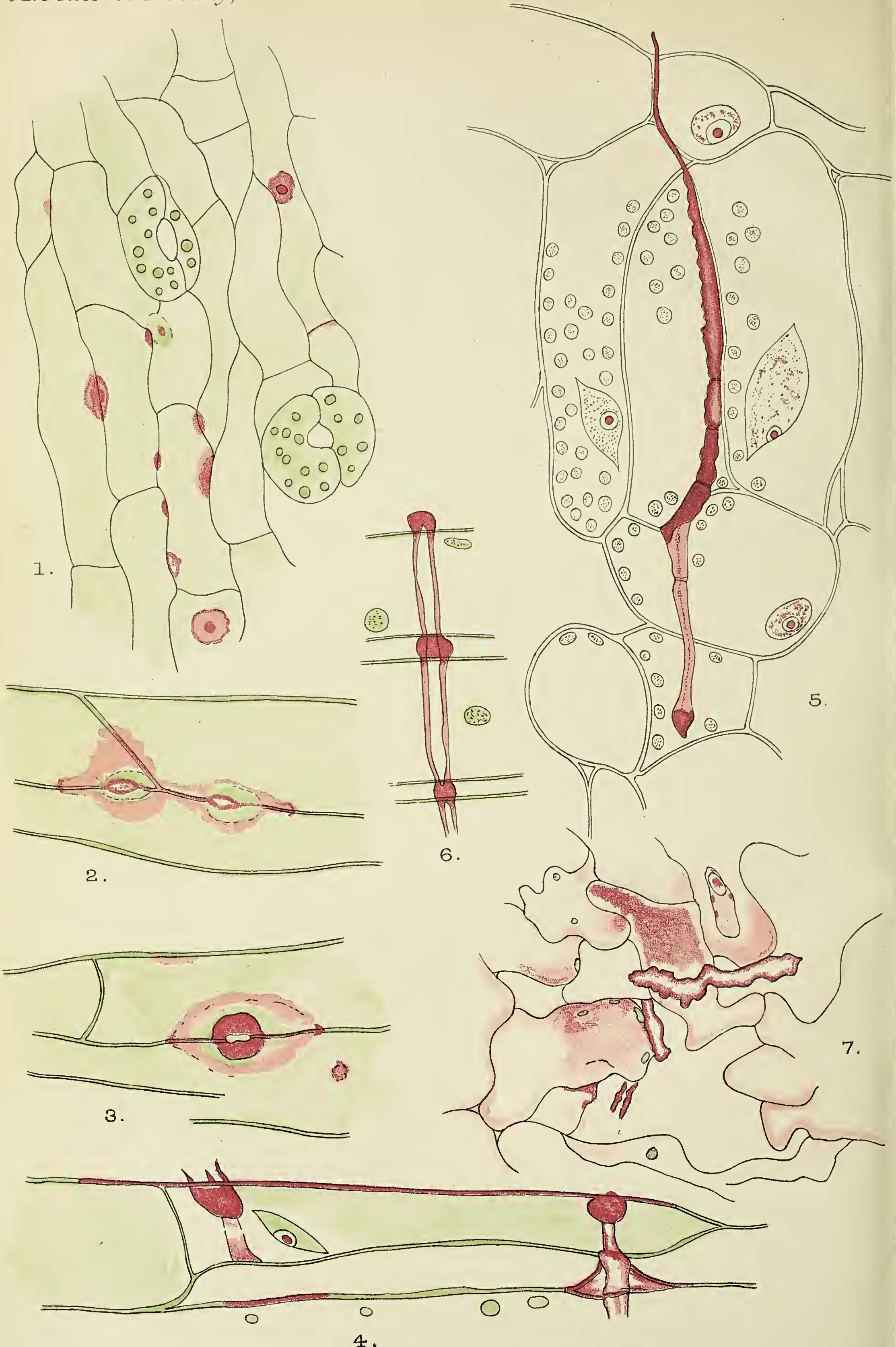

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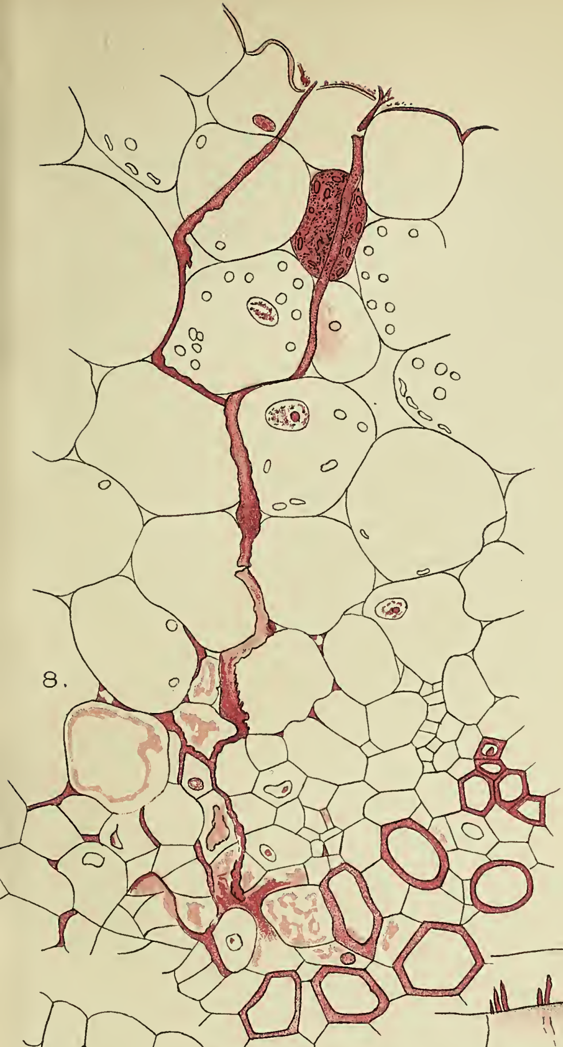

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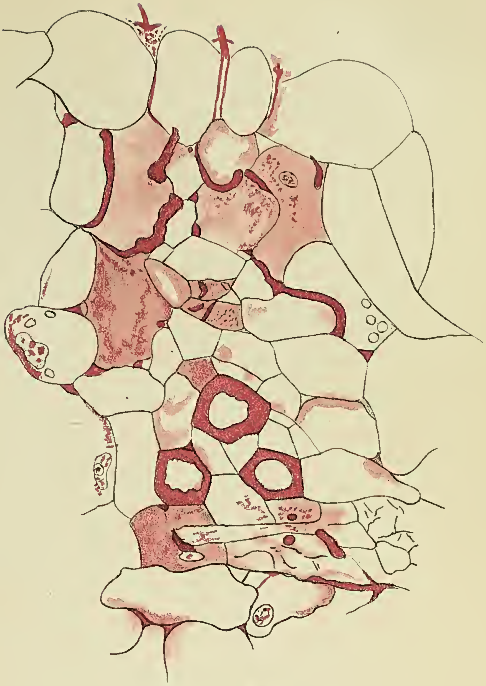

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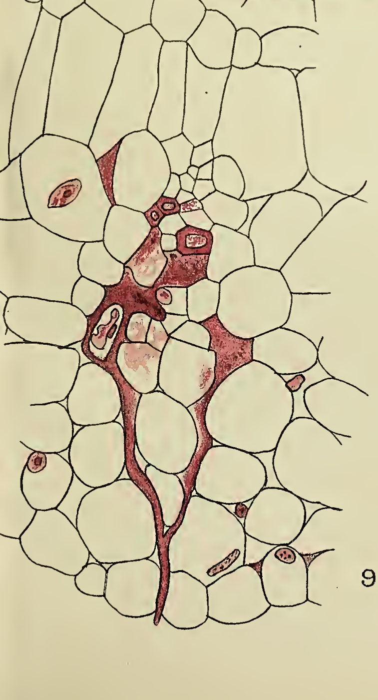

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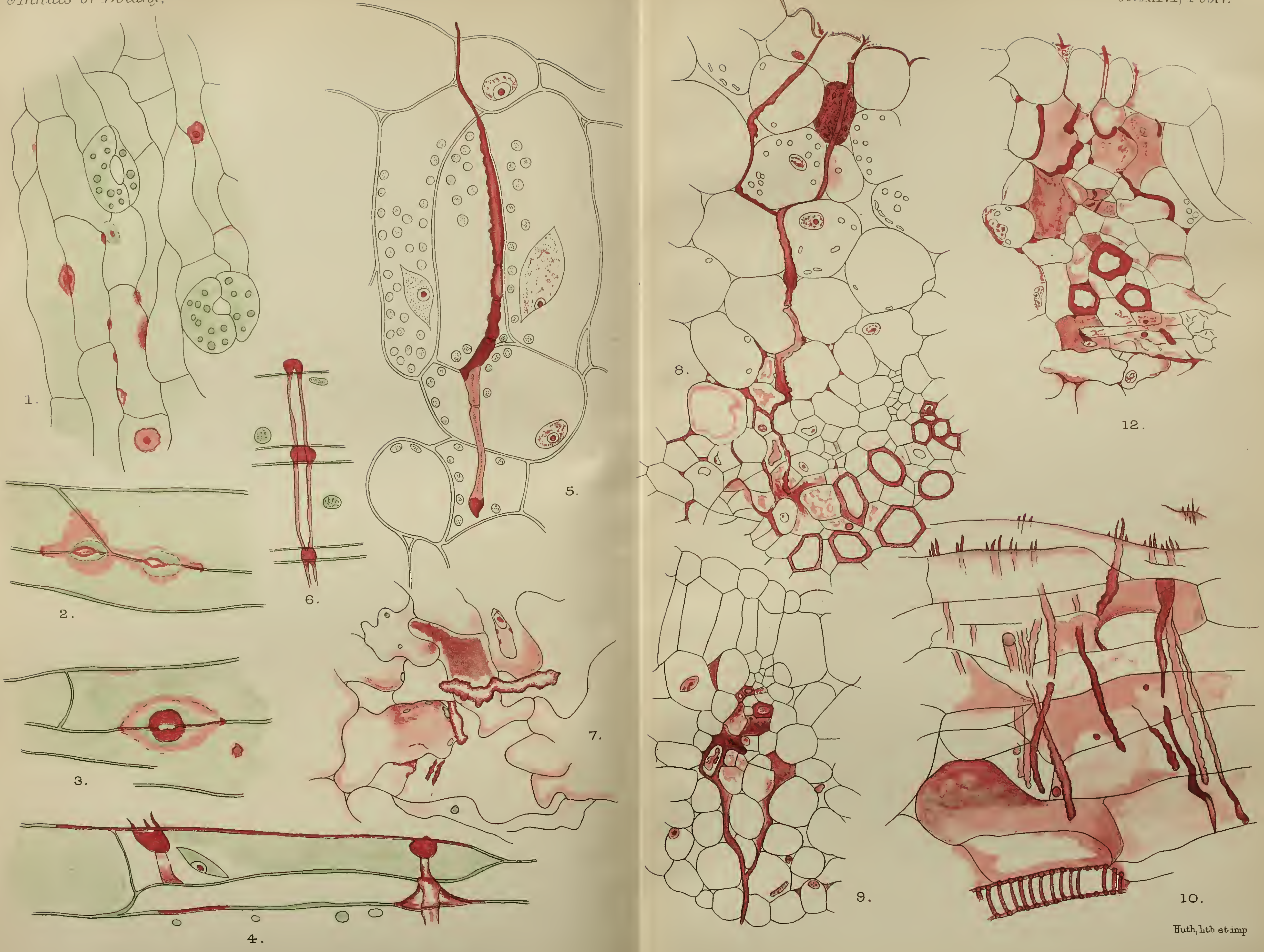

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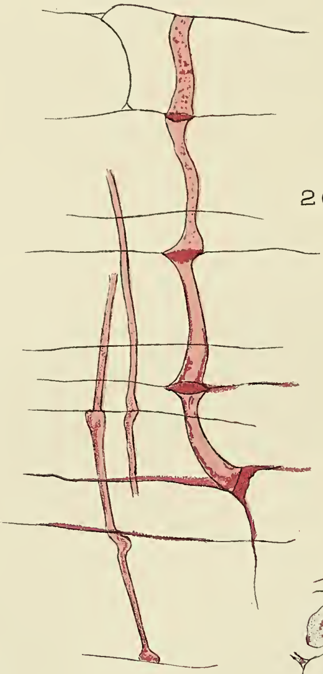

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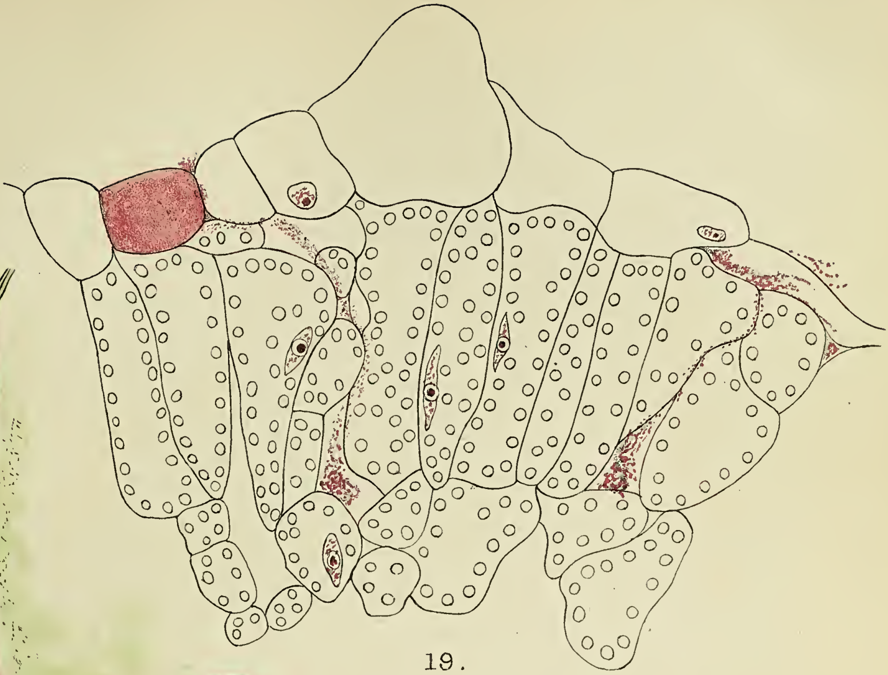

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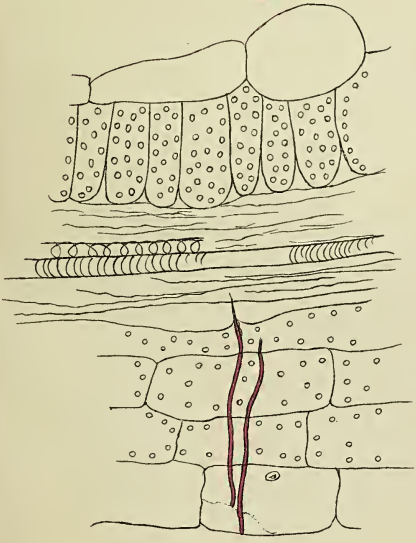

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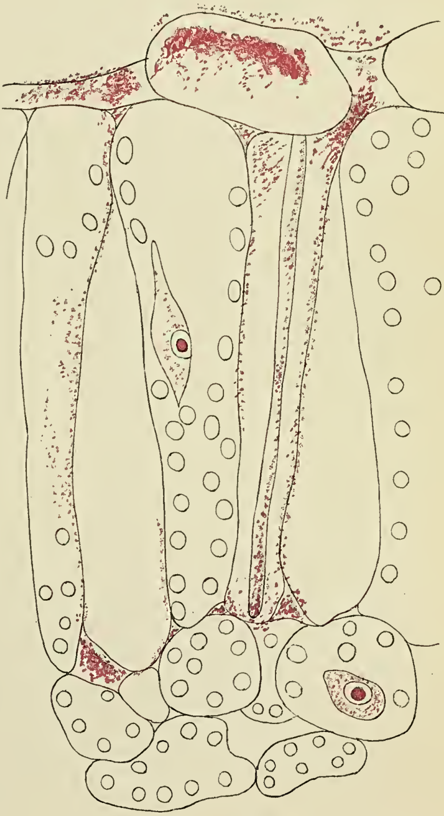

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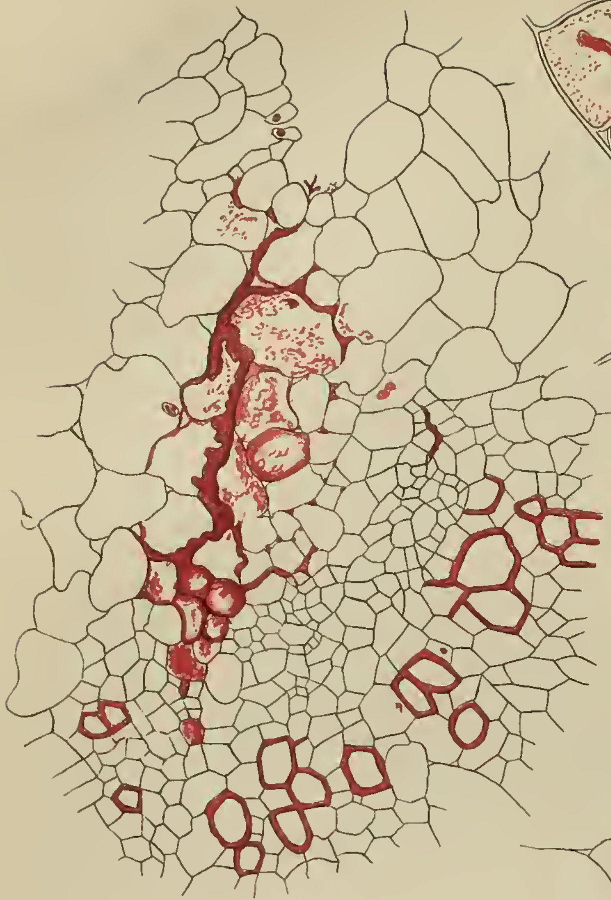

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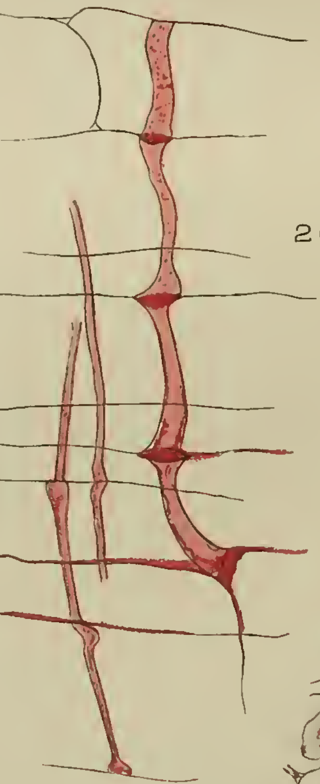

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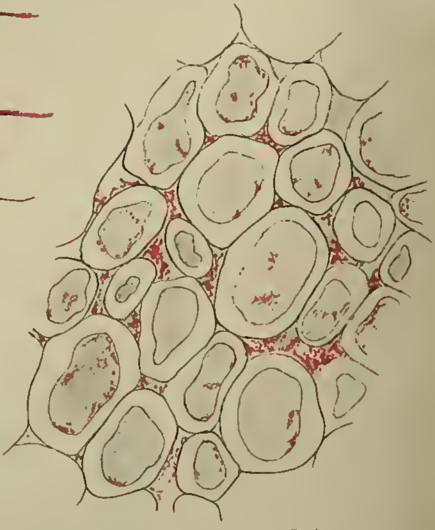

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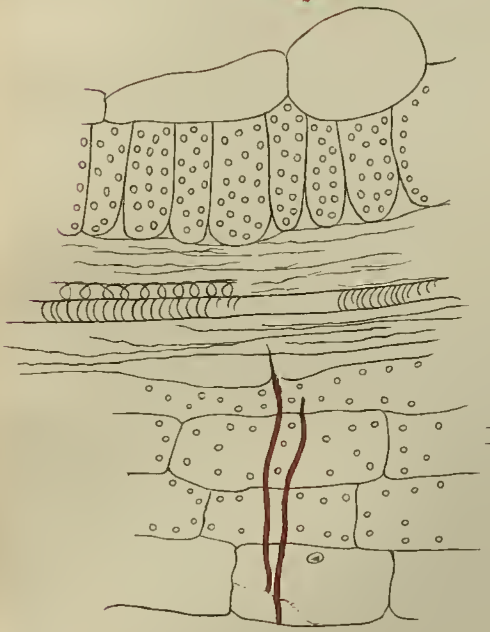

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18.

DALE — BACTERIAL DISEASE OF POTATO.

Huth, lith et imp.

A Contribution to the Life-history of Viola.

BY

MARY C. BLISS.

With Plates XVII–XIX.

INTRODUCTION AND METHODS.

THE present study is based upon an investigation of the chasmogamic flowers of *Viola odorata*, *Viola pedata*, *Viola fimbriatula*, *Viola cucullata*, and *Viola pubescens*. Of these *V. cucullata* and *V. pedata* were found to be the most favourable species for study. The ovules are larger than in the other species, and the structures of the embryo-sac are less difficult to determine. The cleistogamous flowers were studied only by way of comparison, and offered no interesting differences as regards the development of the embryo-sac up to the eight-nucleate stage. Beyond this stage, the development of the female gametophyte in these flowers was not observed. Material has been collected for a comparative study of the floral parts of the cleistogamic and chasmogamic flowers, and it is my purpose to determine, if possible, the causes and conditions which operate to produce the cleistogamic flowers or which tend to modify the structure of the chasmogamic flower. It is interesting to note that violet plants transplanted from the open to the greenhouse in early spring produced only a few chasmogamic flowers and many cleistogamous flowers, but often transitional forms were developed, cleistogamous flowers with rudimentary petals and with more than the usual two stamens. The details of these phenomena will be considered in a later paper.

The material for this present work was collected in Wellesley and Needham from March 10 to May 27, 1903, and in Philadelphia from March 25 to May 1, 1908. Long before the first signs of leaves appeared in most of the species, *V. odorata* was in bud. The buds of the other species appeared in the following order: *V. fimbriatula*, *V. cucullata*, *V. pedata*, and *V. pubescens*. Buds of various sizes were collected and classified under the following heads: smallest buds, colourless, embedded in the crown of the root-stock; buds green, medium; buds larger, but still wholly green; buds with corolla visible; buds just opening and buds half

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open. From this point, flowers from one to five days open and withered flowers were collected. The buds collected in Philadelphia were measured for greater accuracy.

In order to determine the exact age of a flower at a certain stage of structural development, the buds were tagged in the open field with a tiny square of white paper tied to the stem by a thread. For instance, fifty buds which were to open the next day were tagged with the number 1, and this number with date of tagging was recorded in a notebook. The next day some of the flowers were collected and fixed ; others from the same group were collected on the second and third days and so on. In this way about a thousand violets were tagged and collected. The following table shows stages of structural development and relative size of bud or age of open flower.

TABLE SHOWING RELATIVE SIZE OF BUD AND STAGE OF DEVELOPMENT.

Buds.	*Oogenesis.*
Buds smallest, colourless. *V. cucullata.*	Placenta differentiated. Ovular papillae not yet visible.
Buds medium, green. *V. pedata.*	Ovular papillae developed.
Buds small, green. *V. odorata.*	Ovule nearly anatropous; integuments visible, archesporial cell differentiated, tapetal cell cut off.
Buds large, corolla not visible. *V. cucullata.*	Second division of tapetal cell. Megaspore-mother-cell in prophase of division.
Buds large, corolla visible. *V. cucullata.*	Growth of functional megaspore.
Buds large, corolla not visible. *V. pedata.*	Two-celled embryo-sac.
Buds just opening. *V. cucullata.*	Four-celled embryo-sac.
Buds just opening. *V. fimbriatula.*	Eight-celled embryo-sac.
Buds just opening. *V. pubescens.*	Fusion of polar nuclei.
Flowers first day open. *V. cucullata.*	Presence of pollen-tubes. Stages in fertilization. Division of endosperm.

Two fixing fluids were used, Flemming's chrom-osmo-acetic acid and chrom-acetic-acid solutions. Both gave good results. The pistils were removed from the bud and the ovaries only fixed. The wall of the larger ovaries was cut away so that the fixing fluid might penetrate more quickly to the ovules. In some cases, the style was left in position in order that the path of the pollen-tube might be traced. Some of the buds collected were so small that it was impossible to dissect out the ovaries. These buds were fixed whole, and in section offer an interesting comparison of relative microsporangial and macrosporangial development. Two stains, Flemming's triple stain and Heidenhain's iron-alum haematoxylin, were used. Both gave good results.

ORIGIN OF OVULES.

The ovary of the Violaceae is unilocular with three parietal placentae. At a very early stage the placental line is recognized by a layer of closely set cells differing from the other cells in their denser and more deeply staining cytoplasm. Very soon the placenta becomes more conspicuous by the rapid growth of the cells directly back of the placental region, so that the line of placentation extends out into the cavity of the ovary. It is interesting to note that before the primordial cells of the ovule are visible, the anther lobes are well developed, the cells of the tapetal and the sporogenous tissue are clearly differentiated, and the spore-mother-cells are in the early prophase of the heterotypic division. The ovules arise as minute papillae on the placenta, and at first grow directly out into the cavity of the ovary. Soon, however, the ovule became anatropous.

DIFFERENTIATION OF ARCHESPORIUM AND TAPETUM.

The primary archesporial cell is hypodermal in origin, and is differentiated before the ovule has become anatropous. This cell differs from the cells of the surrounding tissue by its greater size, denser cytoplasm, and larger nucleus (Pl. XVII, Fig. 1). A transverse division soon cuts off a tapetal cell from the primary archesporial cell (Fig. 2). The tapetal cell may divide first by an anticlinal or by a periclinal wall (Figs. 2, 3). Further divisions of the tapetal cells are variable, but finally a cap of considerable depth is formed, and the definitive archesporial cell comes to occupy a central position in the nucellus (Fig. 4). In *Viola*, the nucellar cap persists up to the time of embryo formation, and at the time of fertilization the pollen-tube makes its way through this tissue in its passage from the micropyle to the egg.

The interesting phenomenon of twin nucelli was noted in several preparations. In one preparation observed, the embryo-sac was developed in each nucellus as far as the eight-nucleate stage, and the egg apparatus had been established. If in such cases fertilization should take place, we would have a case of polyembryony in *Viola*, or according to Ernst (4) a case of pseudo-polyembryony, brought about by the division of the nucellus at an early stage of development.

FORMATION OF THE AXIAL ROW.

During the formation of the tapetal tissue the definitive archesporial cell increases in size, its cytoplasm becomes scanty, and the nucleolus appears as a densely staining granular mass of chromatin (Fig. 3). At this time the cells of the nucellus surrounding the archesporial cell are actively dividing. In the nuclear plate stage of the first division, the macrospore-mother-cell has increased to several times its original size, and occupies a central position in the nucellus. Its cytoplasm is very scanty, except in the region of the spindle. In some cases the macrospore-mother-cell has elongated to twice its width at the metaphase of the first division, but often the cell is still spherical as in the early prophase of division (Fig. 4).

At the close of the first division a distinct cell-wall is laid down, giving rise to two cells of nearly equal size (Fig. 5). Each of these two cells divides, and the result is an axial row of four cells. The development of the axial row in *Viola* differs from that described for *Trillium* by Coulter and Chamberlain (2), for *Houstonia* by Lloyd (6), for *Larix* by Juel (5), and for the great majority of Angiosperms as described by other writers, in that the upper daughter-cell, the one nearest the micropyle, divides first.

The cells of the axial row in *Viola* are not always arranged in a vertical row as represented in Fig. 6, but the walls may be laid down obliquely (Fig. 8), resulting in a group of cells in which the arrangement of the macrospores more nearly approaches the tetrad arrangement characteristic of typical spore formation. In this respect, the arrangement of the cells in the axial row in *Viola* corresponds to the arrangement figured for *Delphinium* (Mottier, 7), and for *Fatsia japonica* (Ducamp, 3). In *Viola*, generally the lower megaspore, the one furthest removed from the micropyle, is the functional megaspore, and develops into the embryo-sac. This order of development, however, was not invariable. Cases were observed in which the third megaspore became the functional megaspore (Pl. XVIII, Fig. 10), and still others in which, although the second and third cells increased equally in size for some time, the second without doubt was to develop into the embryo-sac. That the other cells of the axial row are disentegrating at this time is evidenced by the densely staining irregular masses of cytoplasm (Fig. 11).

DEVELOPMENT OF THE FEMALE GAMETOPHYTE.

During the rapid growth of the functional megaspore, the dense cytoplasm of its early development becomes vacuolate. Preparatory to its germination the nucleus moves from a central position in the cell to the micropylar end. At this stage, the mother-cell of the embryo-sac extends nearly one-third the length of the nucellus. The changes subsequent to the formation of the axial row take place rapidly, the integuments very soon extend to the top of the nucellus, and in Fig. 12 are just beginning to curve, preparatory to enclosing it. In the two-celled embryo-sac shown in Fig. 12 A, the nucleus has divided and the two daughter nuclei have separated, one nucleus remaining at the micropylar end of the sac, while the other has passed to the extreme opposite pole. The two nuclei are connected by strands of cytoplasm, and there is a slight condensation of cytoplasm at both poles and at the periphery of the embryo-sac. In the central portion of the sac is an irregular vacuole, two-thirds as long as the entire embryo-sac.

The embryo-sac continues to lengthen and broaden, the nuclei at either pole divide simultaneously, and the four-nucleate stage is established. At this stage, the embryo-sac is twice as long as at the two-nucleate stage, but the comparative length and breadth are the same. The third division soon follows, and gives rise to the typical eight-nucleate embryo-sac of Angiosperms. Immediately after this division, the three cells which are to form the egg apparatus increase rapidly in size, surround themselves with a delicate cytoplasmic membrane, and begin to assume their characteristic shape. The egg extends from the apex of the sac about one-third its length. The protoplasm is condensed in the basal portion of the egg, and in it lies the nucleus, close to the basal line. Just above the dense cytoplasm is a large vacuole, which occupies fully two-thirds of the entire egg. The synergids are not quite as long as the egg-cell. They are pointed at the apex and broadly oval at the base. The upper portion of each is filled with dense cytoplasm, while the lower one-third of the cell is occupied by a large vacuole, characteristic of synergids in the majority of Angiosperms investigated. The nucleus generally lies in the middle of the cell, surrounded by dense cytoplasm, but quite close to the vacuole (Fig. 15).

While the egg apparatus is being formed in the micropylar end, various changes are taking place at the antipodal end of the sac. The antipodal polar may be distinguished from the other antipodal cells at an early stage by its greater size and larger nucleus. The usual number of antipodals in *Viola* is three. Their development and arrangement is varied. In some preparations the antipodals appear as definite cells with distinct limiting membranes, in others the antipodal nuclei lie naked in the general cytoplasm at the base of the sac. The antipodal cells are generally much smaller than those at the micropylar end, but their nuclei are large and, almost without exception, each contains a single nucleolus. These cells may be arranged in a row, side by side, or in a quadrant. As a rule the antipodals are not conspicuous, and in many cases when the embryo-sac is fully mature, the antipodals have already disappeared or are present merely as disintegrated masses at the basal end of the sac. In only one instance observed was there any suggestion of the haustorial development of the antipodals as described for the Rubiaceae (Lloyd, 6), *Aster* (Opperman, 8), and other genera (Fig. 17).

Soon after the egg apparatus has been established, the polar nuclei move towards each other. The antipodal polar moves more rapidly than the micropylar polar, consequently fusion is often completed just beneath the egg. At first nucleus only fuses with nucleus, and for some time the nucleoli remain distinct. Finally, however, the nucleoli fuse, and the result is a large endosperm nucleus twice the size of either polar nucleus. The network of this nucleus is extremely delicate, and only slight condensations of chromatin are visible at the periphery and in the meshes of the net. The endosperm nucleus is very easily recognized, when present, by the large size of its nucleolus. This nucleolus is always characterized by a conspicuous vacuole which very nearly fills it.

POLLINATION AND DEVELOPMENT OF THE POLLEN-TUBE.

Before the pollen-grain is shed the contents of the microspore divide into a larger and a smaller cell. The smaller or generative cell becomes lenticular and lies free in the cytoplasm of the tube cell (Fig. 18). Chamberlain (1) and Wylie (9) figure similar generative cells for *Salix* and *Elodea*. The pollen-grains lodge on the inner surface of the stigmatic cavity where germination takes place. Often the upper portion of the stylar canal is completely filled with these pollen-tubes, although I could trace comparatively few into the ovary. There is no evidence of branching in the pollen-tube as it passes down the stylar canal, and no penetration of the stylar tissue. The passage of the pollen-tube through the micropyle and between the tapetal cells was clearly observed many times.

The pollen-tubes stain densely at this period, compared with the egg, and in most cases their exact contents could not be determined. From careful observations, I am led to believe that the generative nucleus divides immediately after the entrance of the pollen-tube into the embryo-sac. In cases where the sperm nuclei could be distinguished lying in the densely staining mass emitted from the pollen-tube, they were small, very nearly equal in size, and spherical in outline, giving no suggestion of the spiral form described by so many authors. The nucleus proper stains lightly, but it contains a large, spherical, deeply staining nucleolus.

In *Viola*, the embryo-sac has reached its mature size previous to the entrance of the pollen-tube (Fig. 15). At this time it is broadest at its middle diameter, narrowing somewhat at the micropylar end, but tapering more conspicuously at the antipodal extremity ; the egg apparatus occupies nearly three-fourths the width and about one-third the length of the sac, and the endosperm nucleus has moved away from the egg, and occupies a central position in the embryo-sac. Following the entrance of the pollen-tube into the embryo-sac, one or both of the synergids disintegrate, the nucleus of the egg increases in size, and, in some instances, the growth of the antipodal cells is very conspicuous (Fig. 16). In such cases the size and shape of the antipodals recall the antipodal egg described by Chamberlain (12) and by Opperman (11) in *Aster*, but in no case was there any evidence of fertilization of these cells. Never more than two enlarged cells were found at the antipodal end of the embryo-sac, thus there was never found any structure suggesting an egg apparatus.

The sperm nuclei, small at first and spherical in outline, increase rapidly in size. One passes down and fuses with the endosperm nucleus. This fusion is clearly shown in Pl. XIX, Fig. 19, in which the endosperm nucleus is easily recognized by the characteristic vacuole of its nucleolus. The sperm nucleus is very large, nearly spherical in outline, and differs from the endosperm nucleus very conspicuously in appearance, in that masses of chromatin are held in the rather close network of the nucleus, and it contains a small densely staining nucleolus. The second sperm enters the egg at its apex. Fusion may take place in the central or in the basal portion of the egg. In both cases the two nuclei are surrounded by a mass of densely staining cytoplasm. Before fusion of the sperm and the egg nucleus is completed, and in some cases before the two nuclei are in contact, the so-called endosperm nucleus divides from one to several times (Figs. 21, 22). The young endosperm surrounds the egg and outlines a cavity beneath it which extends to the base of the embryo-sac.

This cavity persists in the seed of both the chasmogamic and the cleistogamic flowers when the embryo is fully formed and the seed is nearly mature. The formation of endosperm is very rapid at the last, and in the mature seed we find a solid mass of endosperm surrounding the tiny green embryo at the micropylar end of the seed.

The first division of the egg is transverse, dividing it into two cells nearly equal in size (Fig. 24). There is no suggestion of a suspensor. The oldest embryo figured (Fig. 27) shows a spherical mass of actively dividing cells at the apex of the embryo-sac; the endosperm is limited to a parietal layer lining the embryo-sac, and forming a sheath of cells which completely encloses the embryo, and separates it from the cavity beneath. In mature seeds of the chasmogamic flowers collected and studied in the spring of 1909, the embryo was an eighth of an inch in length, bright green in colour, and clearly differentiated into radicle, caulicle, and cotyledons.

SUMMARY.

The primary archesporial cell arises from a hypodermal cell of the nucellus.

A tapetal cell is cut off, which by vertical and transverse divisions forms a cap three or four cells in depth, directly beneath the micropyle.

The definitive archesporial cell divides into an axial row of four cells. The innermost of the four cells is generally the functional megaspore, but cases were observed where the second or third cell of the axial row was enlarging to form the embryo-sac.

The polar nuclei fuse in contact with the egg, generally before the entrance of the pollen-tube.

The synergids are pear-shaped with a large vacuole in the basal portion, and the nucleus lies just above the vacuole.

The cytoplasm of the egg is condensed at its base, and surrounds the large spherical nucleus.

Fusion of the second sperm with the endosperm nucleus was observed in *Viola cucullata.*

The endosperm nucleus divides several times before the sexual nuclei have fused, and in some cases before they are in contact.

The first division of the fertilized egg results in the formation of two cells nearly equal in size. There is no suggestion of a suspensor.

As the endosperm increases it forms a sheath around the embryo, and outlines a cavity beneath it which nearly equals in length that of the embryo-sac. This cavity persists until the seed is nearly mature.

In the mature seed the embryo is about an eighth of an inch long, and lies at the micropylar end of the seed surrounded by a solid mass of endosperm. It is bright green and is clearly differentiated into caulicle and cotyledons.

In conclusion, I wish to acknowledge to Professor Ferguson my sincere gratitude for her constant inspiration and invaluable aid throughout this work, and to thank Professor Macfarlane for the many courtesies extended to me during the weeks spent at the University of Pennsylvania.

WELLESLEY COLLEGE.

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EXPLANATION OF PLATES XVII–XIX.

Illustrating Miss Bliss's paper on the Life-history of *Viola*.

All figures were drawn with an Abbé camera lucida. The degree of magnification is indicated in the description of each figure. The portion of a figure nearest the micropylar end of the ovule is always towards the top of the plate. The abbreviations used in describing the figures are to be defined as follows: *ant.*, antipodals; *d.a.c.*, definite archesporial cell; *e.*, egg; *emb.s.*, embryo-sac; *emb.*, embryo; *end.*, endosperm; *g.n.*, generative nucleus; *i.i.*, inner integument; *m.m.c.*, megaspore-mother-cell; *meg.*, megaspore; *nc.*, nucellus; *n.*, nucleus; *nu.*, nucleolus; *o.i.*, outer integument; *p.t.*, pollen-tube; *p.g.*, pollen-grain; *p.meg.*, potential megaspore; *p.a.c.*, primary archesporial cell; *s.n.*, sperm nucleus; *s.nu.*, sperm nucleolus; *Syn.*, synergids; *t.n.*, tube nucleus; *tap.*, tapetum.

PLATE XVII.

Fig. 1. Section of upper portion of ovule, showing origin of archesporial cell. × 960. *V. odorata.*

Fig. 2. Section of upper portion of ovule, showing first division of primary archesporium to form tapetal cell. × 960. *V. odorata.*

Fig. 3. Section of nucellus, showing further divisions in tapetum and growth of definite archesporial cell. × 960. *V. cucullata.*

Fig. 4. Tip of nucellus, showing late telophase of first division. × 960. *V. pedata.*

Fig. 5. Second division of archesporial cell. × 960. *V. cucullata.*

Fig. 6. Axial row of four cells arranged in a vertical row. × 960. *V. cucullata.*

Figs. 7, 8. Axial row of four cells, showing oblique walls between the cells. × 960. *V. cucullata.*

Fig. 9. Tip of nucellus, showing axial row of four cells, upper three disintegrating, lower developing to form embryo-sac. × 960. *V. cucullata.*

PLATE XVIII.

Fig. 10. Axial row of four cells, third developing to form embryo-sac. × 960. *V. cucullata.*

Fig. 11. Axial row of four cells, second developing to form embryo-sac. × 960. *V. cucullata.*

Fig. 12. Section of ovule showing two-celled embryo-sac. × 960. *V. pedata.*

Fig. 12 *a*. Embryo-sac of Fig. 12. × 960.

Fig. 13. Four-celled embryo-sac. × 960. *V. cucullata.*

Fig. 14. Eight-celled embryo-sac. × 960. *V. fimbriatula.*

Fig. 15. Mature embryo-sac after fusion of polar nuclei, antipodals disintegrating. × 960. *V. pedata.*

Fig. 16. Unusual development of antipodals. × 960. *V. cucullata.*

Fig. 17. Antipodal portion of embryo-sac, showing slight haustorial development. × 960. *V. pedata.*

Fig. 18. Mature pollen-grain, showing tube-cell and fusiform generative cell. × 960. *V. cucullata.*

PLATE XIX.

Fig. 19. Fusion of sperm with endosperm nucleus. × 1,200. *V. cucullata.*

Fig. 20. Mature embryo-sac before fusion of sexual nuclei, showing telophase in the first division of the endosperm nucleus. × 960. *V. cucullata.*

Fig. 21. Embryo-sac, showing fertilization of the egg and four endosperm nuclei.

Fig. 21 *a*. Upper portion of Fig. 21. × 960. *V. cucullata.*

Fig. 22. Embryo-sac, sperm, and egg nuclei not yet in contact. Fourth division of endosperm completed. × 490. *V. cucullata.*

Fig. 23. First division of the egg. Late anaphase. × 1,200. *V. pubescens.*

Fig. 24. Two-celled embryo. × 960. *V. fimbriatula.*

Fig. 25. Three-celled embryo. × 960. *V. cucullata.*

Fig. 26. Four-celled embryo. × 960. *V. cucullata.*

Fig. 27. Multicellular embryo, globular in outline, showing sheath of endosperm. × 960. *V. pubescens.*

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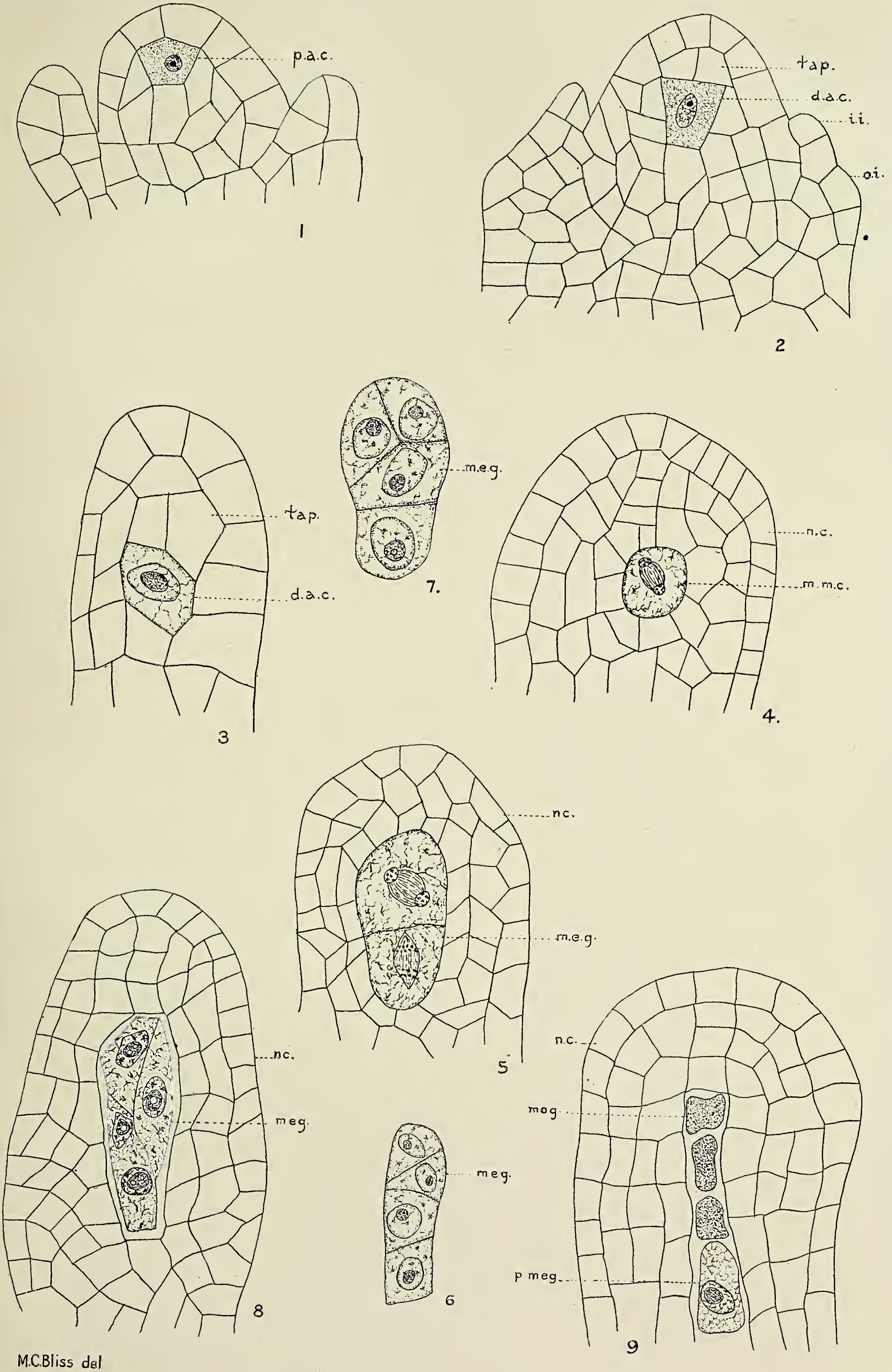

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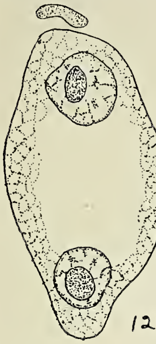
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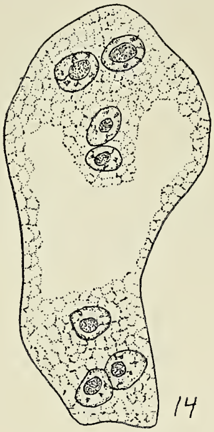
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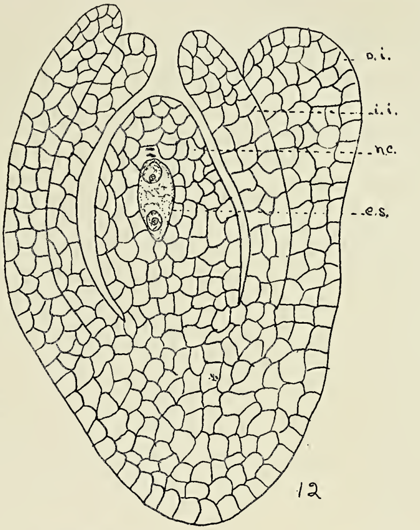

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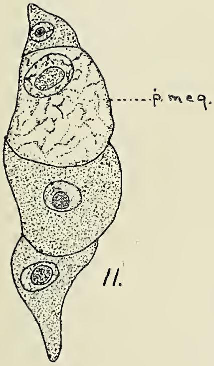

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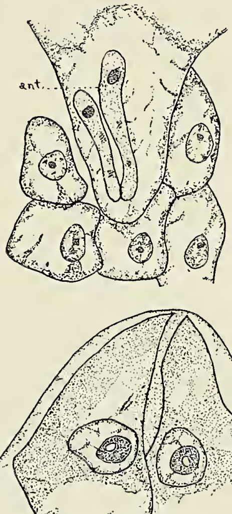

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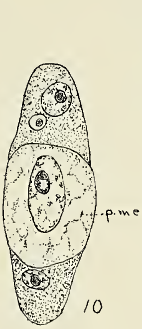

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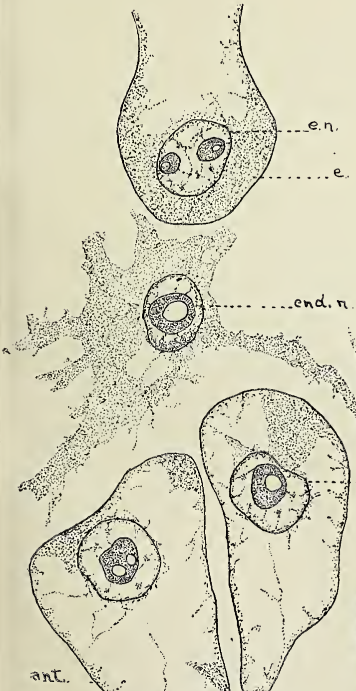

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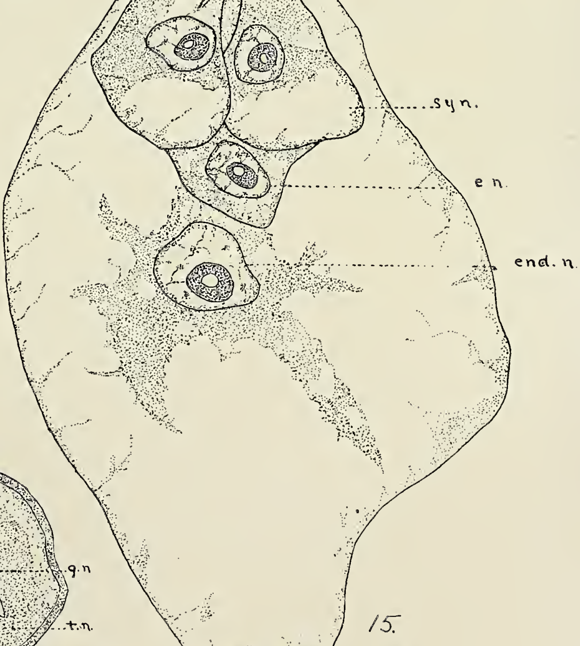

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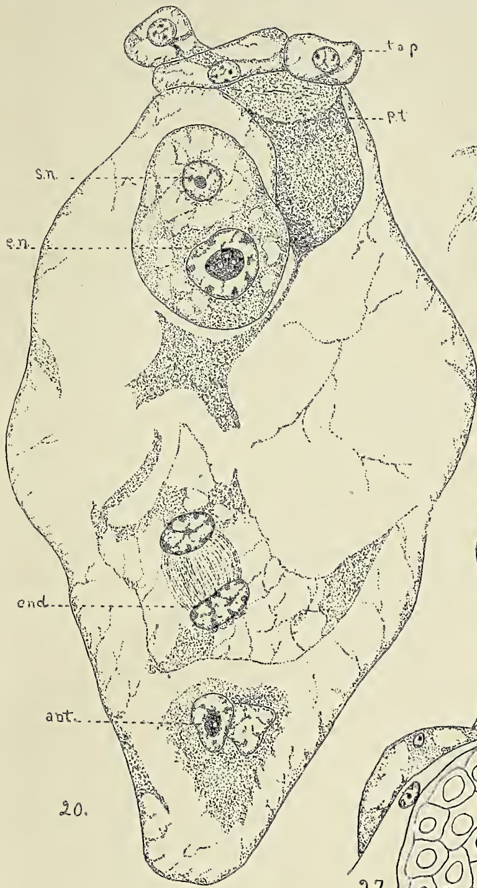

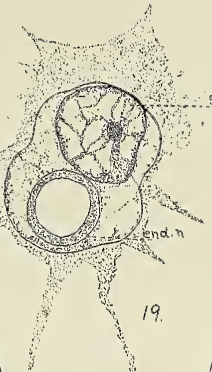

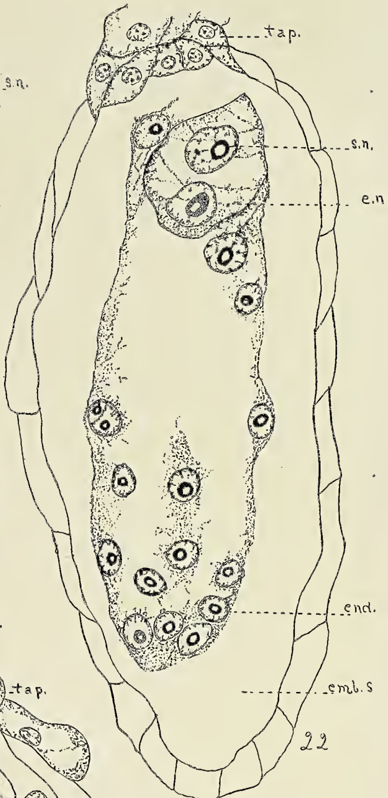

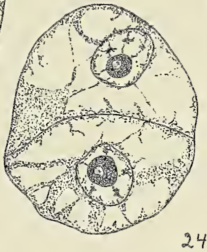

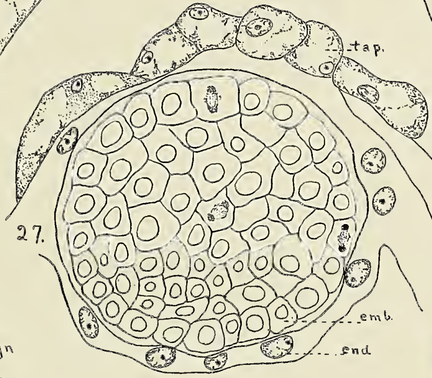

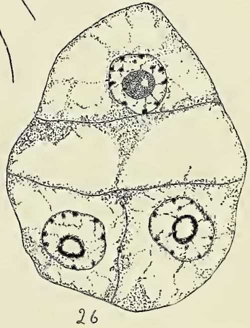

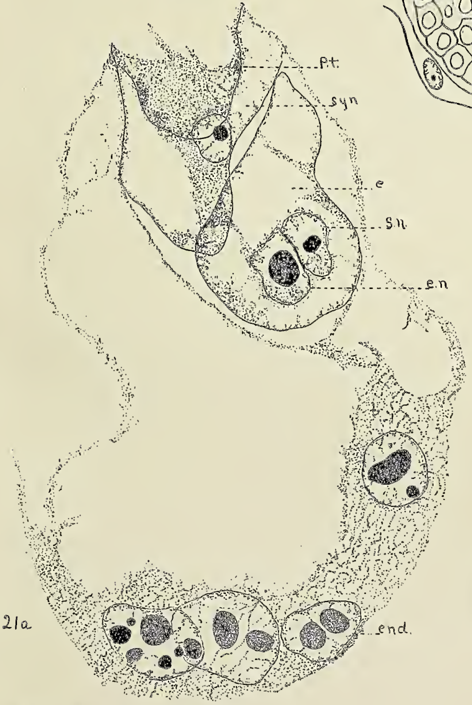

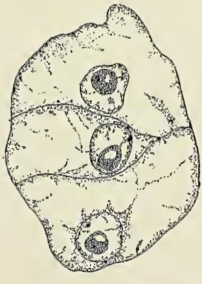

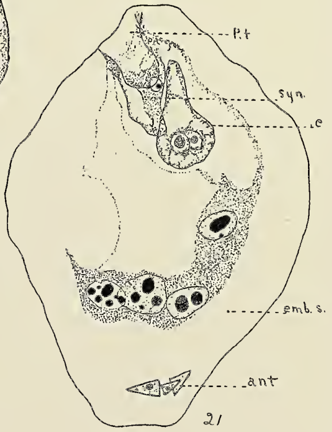

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Reduction and Reversion in the North American Salicales.[1]

BY

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Wilby Prize Student of Radcliffe College.

With Plates XX and XXI.

IN the accepted classification of the Angiosperms, as presented by Engler and Prantl in 'Die natürlichen Pflanzenfamilien', the Salicales are placed as the third alliance under the Archichlamydeae, the families below them being the Casuarinales and Piperales. A few families above them are the Juglandales and Fagales, with which the Salicales were formerly united to constitute the Amentiferae. The sequence of these orders is based on the development of the perianth and the character of the floral members. On this criterion the Salicales are certainly low in the series, having catkins of flowers which are naked in the axils of bracts. Even on floral structure there is one objection, however, to relegating them to this primitive group, viz. the nectaries of *Salix*. Insect pollination is generally conceded to be characteristic of the higher orders, yet the entomophilous *Salix* is placed below the anemophilous Juglandales and Fagales. Another objection to the existing classification is the fact that the Salicales are porogamous, while the Casuarinales below, the Juglandales and some of the Fagales above, are chalazogamous. Although there is some difference of opinion as to the relative primitiveness of porogamy and chalazogamy, it is a significant fact that chalazogamy is confined to lower families of the Archichlamydeae, while all the higher Dicotyledons and all the Monocotyledons are porogamous. Further, the step from the gymnospermous condition, where the pollen-grains fall on the ovule and burrow through the intervening cells to the archegonia, to chalazogamy is short, but from the gymnospermous condition to porogamy is obviously great.

Granting that insect pollination and porogamy are points against the primitiveness of the Salicales, geological evidence has been cited to prove their antiquity.[2] From the Potomac of Virginia, however, the lowest of the

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[1] Contributions from the Phanerogamic Laboratories of Harvard University, No. 44.

[2] Penhallow, D. P.: A Systematic Study of the Salicaceae. American Naturalist, vol. xxxix, No. 464, Aug. 1905, p. 525.

[Annals of Botany, Vol. XXVI. No. CI. January, 1912.]

Cretaceous, remains have been discovered not only of *Saliciphyllum* and *Populophyllum*, but also of at least twenty other Angiospermous genera.[1] Until more data are secured, then, it is impossible to prove anything regarding the phylogeny of the Angiosperms from palaeobotanical evidence.

In view of these conflicting standpoints, it seems desirable that something should be said regarding the anatomy of the Salicales. Before doing so it will be well to enumerate some of the principles upon which comparative anatomy rests at the present time. The fundamental one is Haeckel's law of recapitulation. According to this we should look for primitive conditions in the seedling and the first annual ring, as applied to plants. Investigation has shown the soundness of this principle and has added other primitive regions, viz. the tissue about outgoing traces in both root and stem, the reproductive axis, the leaf petiole, &c. Further, experimental work has shown that reversion to a primitive condition often takes place after injury. It is from a study of these regions then, that phylogenetic deductions must be drawn.

Turning now to the Salicales, Figs. 1, 2, and 3 (Pl. XX) represent the wood of a typical poplar, *Populus trichocarpa,* in transverse, radial, and tangential planes respectively. From these it is evident that the rays are one cell in width, or uniseriate, and the parenchyma only at the end of the annual ring, or ' terminal '. Another diagnostic feature of the wood of the Salicales is the end-wall of the vessel, which has one large pore constituting the so-called porous perforation, as opposed to the scalariform perforation characteristic of *Betula*, for example. The type of fibres is obviously not primitive, and the type of vessel-end-wall is likewise high.

Populus tremuloides has normally the same wood structure as that of *P. trichocarpa*; Figs. 4 and 5 show, however, transverse and tangential sections through the wood formed after injury. Here the rays are bi- and triseriate, and the parenchyma, instead of being terminal, is present also around the vessels.

Seedlings of *P. tremuloides* were examined and show that, while the rays are always uniseriate, the parenchyma around the vessels, as well as in the terminal position, is common. This is especially true of the transition region between stem and root—in the hypocotylary stem. Above and below, the vasicentric parenchyma tends to die out. In the stem it is always present to a greater or less extent in the first annual ring, while in the root it persists fairly abundantly throughout. Fig. 6 represents a section through the root at some distance from the hypocotylary region and shows most of the vessels with one or two parenchyma cells next their tangential walls. Fig. 7 represents at the same magnification vessels with parenchyma cells, and Fig. 8 represents at a higher magnification another vessel with one cell.

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[1] Fontaine : Potomac or Younger Mezozoic Flora. U.S. Geological Survey, vol. xv.

The last section includes the end-wall of the cell, and its simple pits prove conclusively that it is parenchymatous.

The other regions retentive of ancestral characteristics confirm the evidence supplied by the study of wounds. Fig. 9 represents a tangential view of the tissue subtending an outgoing leaf-trace; Fig. 10 an outgoing root-trace. Both show aggregations of bi- and triseriate rays.

Another species, *P. balsamifera*, may now be considered. The normal wood is similar to that of the two species already described, with uniseriate rays and terminal parenchyma. Figs. 11 and 12 are of wounded tissue and show the presence of biseriate rays. As in *P. tremuloides*, the wound also recalls the parenchyma around the vessels. In the former, however, wounds tend to bring back the parenchyma to a much greater extent than in the latter; as regards reversion in the rays, the condition is exactly opposite, i. e. a wound which in *P. tremuloides* would bring multiseriate rays only in the immediate vicinity of the injury, in *P. balsamifera* recalls the rays half-way around the stem. In the root of *P. balsamifera* the parenchyma is mostly terminal, with a slight tendency to the vasicentric condition; a very slight wound, however, suffices to bring it back in abundance around the vessels. Conditions similar to those of *P. tremuloides* were found in the first annual ring of the stem and under the leaf-trace.

P. nigra var. *italica* is another species with terminal parenchyma and uniseriate rays. Wounds in stool shoots bring back the parenchyma and rays in a striking way, an interesting feature being that the vasicentric parenchyma extends completely around the stem after a wound, while the multiseriate rays are confined to the immediate vicinity of the injury.

Among other poplars with terminal parenchyma and uniseriate rays are *P. grandidentata* and *P. laurifolia*. On investigation of the latter it was found that a very slight wound in the second annual ring is sufficient to recall the parenchyma to a moderate extent, while a severe wound further out brings back abundant vasicentric parenchyma and multiseriate rays.

P. deltoides presents a new condition. In this species the parenchyma is normally around the vessels, but the rays are uniseriate like those species already described. In the first two annual rings of a seedling the rays are practically all biseriate, dwindling thereafter to uniseriate, while vasicentric parenchyma in that region is extremely abundant. Slightly higher in the stem, where the leaf-traces go off, there is a comparatively large amount of parenchymatous tissue above and below each trace. A transverse section slightly above a trace shows a ray ten to twelve cells wide, which runs out for a short distance and then is dissected into multiseriate rays three to four cells wide. The other rays throughout the periphery of the stem are bi- and triseriate. Abundant vasicentric parenchyma is present in the first two annual rings, growing less so in the succeeding years of growth, but a transverse section in the plane of an outgoing trace shows that on each side of the trace the vessels are surrounded by a jacket of parenchyma. Higher up in the stem biseriate rays disappear entirely except in immediate relation to the leaf-trace, while vasicentric parenchyma is scantily present throughout.

Another poplar with the same type of normal wood is *P. rotundifolia.* Here the wound reactions are what might be expected—multiseriate rays and abundant vasicentric parenchyma, while a moderate amount of ray parenchyma subtends the trace.

One species remains to be mentioned, the cottonwood of California, *P. Fremontii.* The wood of this species, represented in Pl. XXI, Fig. 13, has normally biseriate rays, but the parenchyma is exclusively terminal.

To sum up, the normal wood of the majority of poplars has uniseriate rays and terminal parenchyma. Investigation of regions characterized by ancestral conditions shows that vasicentric parenchyma is retained by the root and seedling and recalled in post-traumatic tissue in the stem. Further, multiseriate rays are retained in the seedling, and in connexion with leaf- and root-traces, and are likewise recalled by wounding.

Conditions in *Salix* are to a large extent similar to those in *Populus.* Figs. 14, 15, and 16 show three planes of *Salix Nuttallii* with uniseriate rays and terminal parenchyma. Fig. 17 represents a radial view demonstrating the vertically elongated 'marginal' cells of the rays. These cells are a perfectly constant diagnostic feature, and serve to differentiate between *Salix* and *Populus.*

Seedlings of various species of *Salix* show more or less abundant vasicentric parenchyma near the pith, but no biseriate rays except in immediate relation to a leaf-trace. Roots show vasicentric parenchyma normally and an abundance of biseriate rays after wounding. Both leaf-trace, Fig. 18, and root-trace, Fig. 19, show abundant subtending multiseriate rays.

Wounds in the stem result in the appearance of parenchyma around the vessels and multiseriate rays. Fig. 20 shows the wood of *S. nigricans* var. *primulifolia* after wounding. Almost every vessel in the field has at least one parenchyma cell on its tangential wall and some have two. Fig. 21 shows a portion of the same under a higher degree of magnification, Fig. 22 under a still higher magnification. This species gives an especially good wound reaction, parenchyma about the vessels being formed to a less extent on the side of the stem opposite the wound.

Young twigs a year old show considerable parenchyma around the vessels opposite the protoxylem clusters. At the node a large medium trace goes off to the bud, and two lateral ones to the leaf. The bud-trace leaves an extensive gap, some distance above and below which are clusters of bi- and triseriate rays. The leaf-traces often have subtending them rays four or five cells wide, which, half-way through the annual ring, are dissected into biseriate rays by the intrusion of vessels.

Leaf petioles and the mid-rib of the leaf itself have such a small amount of vascular tissue that no rays are developed, but the vessels are surrounded with parenchyma.

Other species of *Salix* with terminal parenchyma and uniseriate rays are *S. Hookeriana*, *S. japonica*, and *S. daphnoides*.

There are a number of species with uniseriate rays and vasicentric parenchyma—*S. fluviatilis*, *S. sitchensis*, *S. purpurea*, *S. lutescens*, and *S. viminalis*. Fig. 23 represents a transverse section of *S. fluviatilis*, showing vasicentric parenchyma.

S. laevigata, Fig. 24, has biseriate rays and terminal parenchyma normally, while both *S. sessilifolia*, *S. lasiolepis*, and *S. taxifolia* have biseriate rays and vasicentric parenchyma.

To sum up the genus *Salix*—the wood of the majority of species has normally uniseriate rays and terminal parenchyma. Vasicentric parenchyma is recalled after injury and retained in the root, in the seedling, and usually in the first year's growth throughout; multiseriate rays are recalled after injury and retained in connexion with leaf- and root-traces.

Applying then the principles of comparative anatomy as enumerated at the beginning, it is evident that the Salicales were primitively characterized by multiseriate rays and vasicentric parenchyma. A few species retain these features, while others have been reduced and have lost them, except in certain regions especially retentive of ancestral characteristics. In considering the relative position of different species of *Populus* and *Salix*, Penhallow suggests that on the basis of its wide distribution *P. balsamifera* is the most primitive and represents an ancestral type from which many of the localized types have descended. In view, however, of the anatomical considerations, it is evident that such forms as *P. balsamifera*, *P. trichocarpa*, *P. tremuloides*, *Salix Hookeriana*, *S. japonica*, &c., appear to represent reduced types, while such forms as *Populus Fremontii* and *Salix sessilifolia* represent the primitive condition.

Professor Penhallow also regards *Populus* as more primitive than *Salix*, since (p. 808) 'the parenchyma in *Populus* is always terminal' and (p. 813) biseriate rayed forms are more common in *Salix* than in *Populus*. The first statement appears to be a mistake, *P. deltoides* and *P. rotundifolia* having vasicentric parenchyma, but if it were true it would only serve to reinforce the second in showing, not that *Populus* is more primitive, but that it is more reduced and therefore higher than *Salix*. On p. 820 he says that 'the general trend of evidence so far collected, geological, geographical, and anatomical, is all in one direction, and that is to show that the genus *Populus* is essentially the more primitive'. The geographical evidence consists in the fact that the willows are more widely distributed and less localized than the poplars—the latter being supposedly survivors of some ancestral type. This evidence, if unsupported by other more convincing testimony, is of little weight. The geological evidence consists in the fact that *Populus primaeva* is supposed to be older than any *Salix*, but the finding of both *Saliciphyllum* and *Populiphyllum* in the Potomac discredits any conclusions from fossil evidence. As for the anatomical evidence, there is only one species of *Populus* with biseriate rays, and there are only two with vasicentric parenchyma, as opposed to a dozen or more with terminal parenchyma and uniseriate rays. On the other hand, there are more species of *Salix* with vasicentric than with terminal parenchyma, and almost as many with biseriate as uniseriate rays. Accordingly, since both *Salix* and *Populus*, as shown by comparative anatomical evidence, are descended from forms with multiseriate rays and vasicentric parenchyma, it seems clear that *Populus* is more reduced than *Salix* and consequently higher.

The geographic distribution of the different species is of interest. The three North American species of *Salix* with vasicentric parenchyma and biseriate rays are indigenous west of the Mississippi, and the one species of *Populus* with biseriate rays is a native of California. On the other hand, the more reduced forms with uniseriate rays and terminal parenchyma grow in the eastern as well as the western part of the country. It is not uncommon to find that the western species of the United States are more primitive and less reduced than the eastern. For example, Mr. Bailey has recently investigated the western chestnut, *Castanopsis*, and finds here various structures, present in a greatly reduced form in the eastern *Castanea*.

Having discussed the anatomy of the Salicaceae, it is now desirable to discuss its bearing on their systematic position. Recent investigations in wood structure have shown that the most constant feature for diagnostic purposes is the distribution of the wood parenchyma, and the next best the width of the rays. The evolution of rays in the vascular plant series would be somewhat as follows: most Gymnosperms have uniseriate rays. In the epicotyledonary seedling stem of *Quercus* there are uniseriate rays near the pith which on passing out in certain segments become bi- and triseriate; then by approximation of the rays and elimination of vessels these segments become 'false' rays—the permanent condition in the adult Live Oak. Further on, by the elimination of the intervening tracheides the rays become solidly parenchymatous, as represented in the homogeneous masses of ray parenchyma in the mature wood of North American White and Black Oaks.[1] That rays of this type are made up of compounded multiseriate rays is proved by the wound reaction.[2] In genera slightly higher than *Quercus*, i. e. *Carya*, *Ostrya*, *Fagus*, &c., these rays, solid at the pith, break up as they pass out, until by the intrusion of fibres and vessels in both

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[1] Eames, A. J.: On the Origin of the Broad Ray in Oaks. The Botanical Gazette, xlix, pp. 161–7, No. 3.

[2] Bailey, I. W.: Reversionary Characters of Traumatic Oak Woods. The Botanical Gazette, l, pp. 374–80, No. 5.

horizontal and vertical planes they become the multiseriate rays characteristic of the mature wood of most Dicotyledons.[1] This process can be observed especially well in certain of the Ericaceae and Casuarinaceae, where the compound ray is present only near the leaf-trace. In fact, it has been shown that the leaf-trace has played the chief part in the formation of compound rays.[2] As these multiseriate rays go from the pith, they are apt to dwindle, as seen in the seedling of *Populus deltoides*, for example, until uniseriate rays are formed. This is the condition in the Salicaceae, *Aesculus*, and, as Mr. Bailey has ascertained, in *Castanea*, all of which have uniseriate rays normally in the adult, but multiseriate rays in the primitive regions.

Whether the direct ancestors of the Salicales ever attained compound rays like those of the oak, it is impossible to say. All that comparative anatomical evidence shows is that they are descended from ancestors which possess multiseriate rays, and adequate palaeobotanical evidence on their origin is lacking at present. It is evident, however, that the compounding process must have taken place in very remote geological time, since multiseriate rayed forms are common in the Upper Cretaceous of Japan.[3]

The development of parenchyma is somewhat as follows: In the Abietineae there is no parenchyma in *Pinus*; but in all the other members there is terminal parenchyma. In the Sequoineae and Cupressineae the parenchyma is diffuse, i. e. scattered through the year's growth. Since the Gymnosperms never developed vessels the parenchyma never got beyond the diffuse condition. It is likewise diffuse in the Gnetales and in the lower Angiosperms, as the Casuarinaceae, Betulaceae, Fagaceae, and Juglandaceae; while in the highest Angiosperms it is clustered around the vessels, as in the Oleaceae, Ulmaceae, Leguminosae, &c. Reduction then sets in and the Saliceae and Magnoliaceae have terminal parenchyma in the stem, but vasicentric in primitive regions. It is of interest to note that conditions in the Magnoliaceae are similar to those in the Salicaceae—the Asiatic genus, *Michelia*, has vasicentric parenchyma in the stem, and *Liriodendron tulipifera* has vasicentric parenchyma in traumatic tissue. Another interesting case of reversion is seen in *Osmanthus*, a member of the Oleaceae, where the parenchyma is exclusively terminal.

From this consideration of the evolutionary tendencies in the development of rays and parenchyma, it is evident that the Salicaceae, instead of coming low down among the Dicotyledons, as is assumed by systematic botanists, in reality represent, from the anatomical standpoint at least,

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[1] Thompson, W. P.: The Origin of Multiseriate Rays in Dicotyledons. Annals of Botany, vol. xxv, 1911, p. 1005.

[2] Bailey, I. W.: The Relation of the Leaf-trace to Compound Rays in the Lower Dicotyledons. Annals of Botany, vol. xxv, No. xcvii.

[3] Stopes and Fujii: Studies on the Structure and Affinities of Cretaceous Plants. Phil. Transactions of Royal Society of London, Series B, vol. cci.

a condition of reduction from a high type. The evidence for this view may be briefly stated as follows: The primitive distribution of wood parenchyma in the Salicaceae as shown by this investigation is obviously vasicentric or centred around the vessels, a condition uniformly characteristic of such high families as the Compositae, Oleaceae, &c. In ray structure, the Salicaceae are likewise clearly allied with the higher and not the lower Dicotyledons, since their uniseriate rays obviously represent a condition of reduction from the multiseriate type characteristic of the higher woody Angiosperms and not a primitively simple condition as suggested by the late Professor Penhallow.

CONCLUSIONS.

1. Most eastern representatives of the North American Salicales are characterized normally by terminal parenchyma and uniseriate rays.
2. In primitive regions vasicentric parenchyma and multiseriate rays are found.
3. Certain western North American representatives of the Salicales have normally vasicentric parenchyma and multiseriate rays.
4. The primitive condition for the wood of the Salicales is vasicentric parenchyma and multiseriate rays.
5. The low position assigned to the Salicales by systematists appears not to be justified.
6. Since their simple structure is due to reduction from a condition originally more complex, the Salicales have a high position in the dicotyledonous series.

In conclusion, I wish to express my thanks to Professor E. C. Jeffrey for his helpful advice and for aid in securing the photomicrographs accompanying this article; to Professor J. G. Jack of the Arnold Arboretum for seedling material he was kind enough to send me; and to Mr. I. W. Bailey for sections of various western species.

EXPLANATION OF PLATES XX AND XXI.

Illustrating Miss Holden's paper on Salicales.

PLATE XX.

Fig. 1. *Populus trichocarpa*: transverse section of normal wood, showing uniseriate rays and terminal parenchyma. × 300.

Fig. 2. Same: radial section, showing terminal parenchyma. × 300.

Fig. 3. Same: tangential section, showing uniseriate rays. × 300.

Fig. 4. *P. tremuloides*: transverse section of wounded wood, showing biseriate rays. × 300.

Fig. 5. Same: tangential section, showing biseriate rays. × 300.

Fig. 6. Same: transverse section of root, showing vasicentric parenchyma. × 400.

Fig. 7. Same. × 400.
Fig. 8. Same. × 700.
Fig. 9. Same : tangential section of stem, showing multiseriate rays under leaf-trace. × 300.
Fig. 10. Same : tangential section of root, showing multiseriate rays under root-trace. × 300.
Fig. 11. *P. balsamifera* : transverse section of traumatic wood, showing biseriate rays. × 300.
Fig. 12. Same : tangential section of traumatic wood, showing biseriate rays. × 300.

PLATE XXI.

Fig. 13. *P. Fremontii* : tangential section of normal wood, showing biseriate rays. × 300.

Fig. 14. *Salix Nuttallii* : transverse section of normal wood, showing uniseriate rays and terminal parenchyma. × 300.

Fig. 15. Same : tangential section of normal wood, showing uniseriate rays. × 300.

Fig. 16. Same : radial section. × 300.

Fig. 17. Same : radial section, showing marginal ray cells. × 300.

Fig. 18. *Salix* sp. : tangential section of stem, showing multiseriate rays under leaf-trace. × 300.

Fig. 19. Same : tangential section of root, showing multiseriate rays under root-trace. × 300.

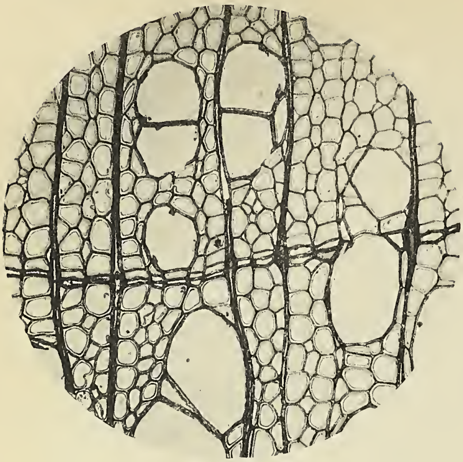
Fig. 20. *S. nigricans* var. *primulifolia* : transverse section of traumatic wood, showing vasicentric parenchyma. × 300.

Fig. 21. Same. × 400.

Fig. 22. Same. × 700.

Fig. 23. *S. fluviatilis* : transverse section of normal wood, showing vasicentric parenchyma. × 300.

Fig. 24. *S. laevigata* : tangential section of normal wood, showing biseriate rays. × 300.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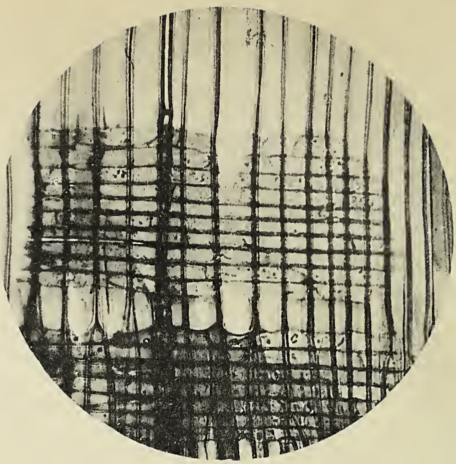
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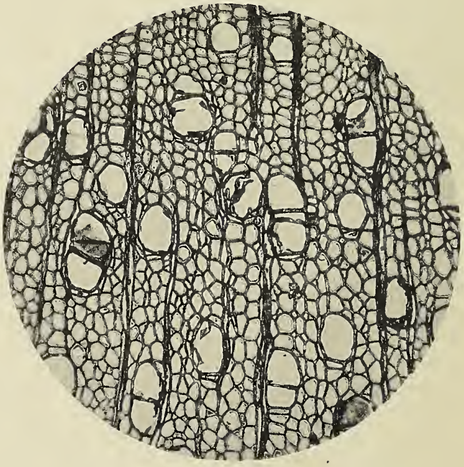
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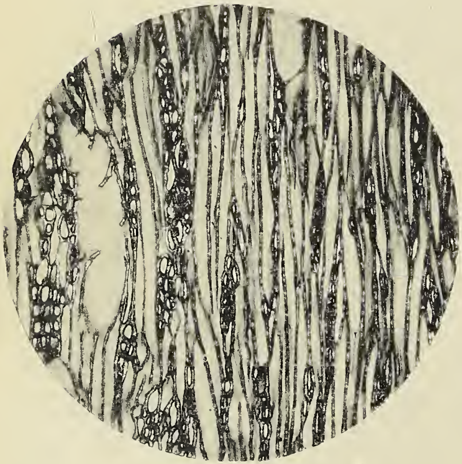
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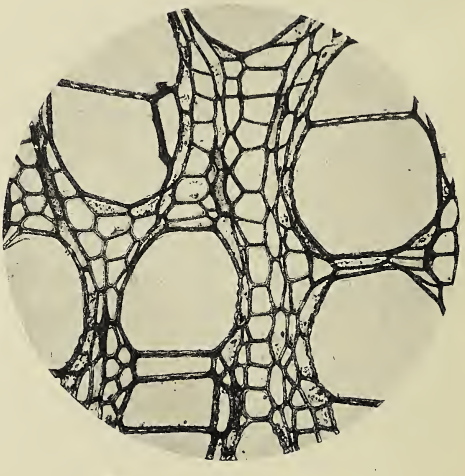
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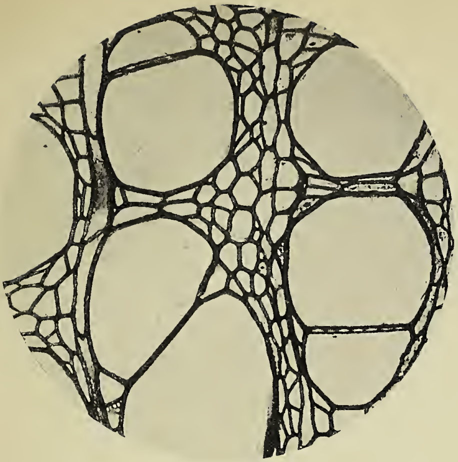

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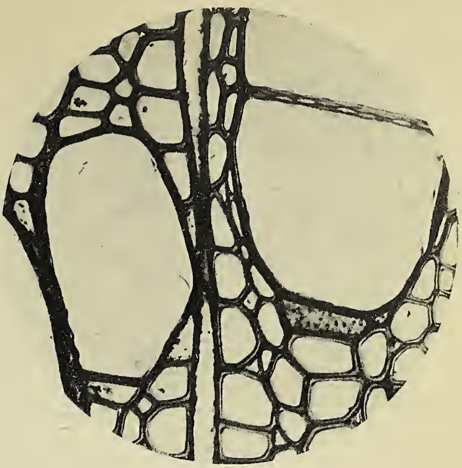

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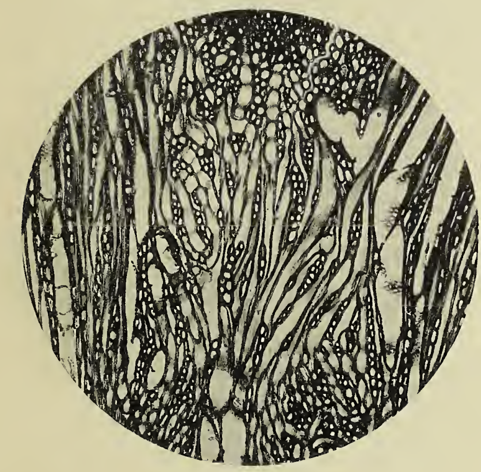

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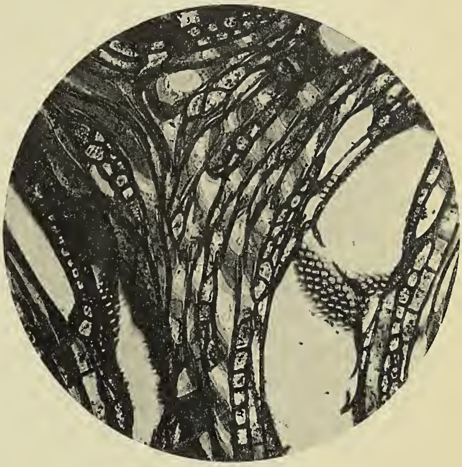

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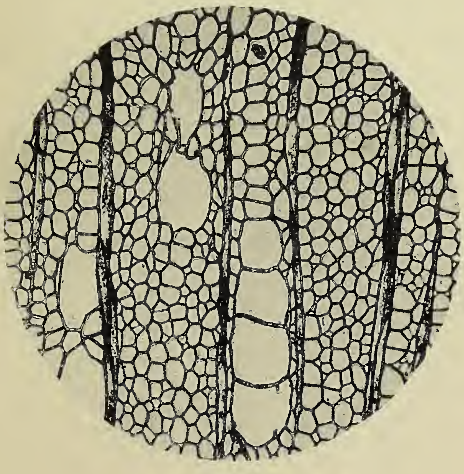

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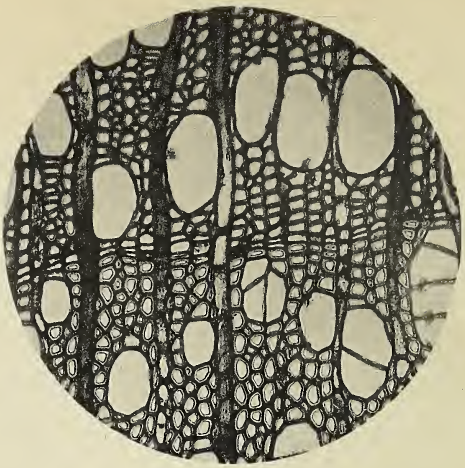

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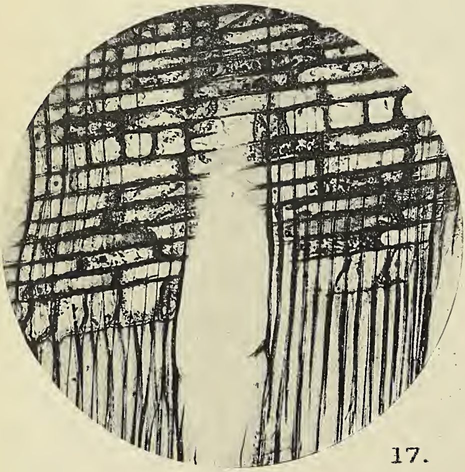

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HOLDEN—SALICALES.

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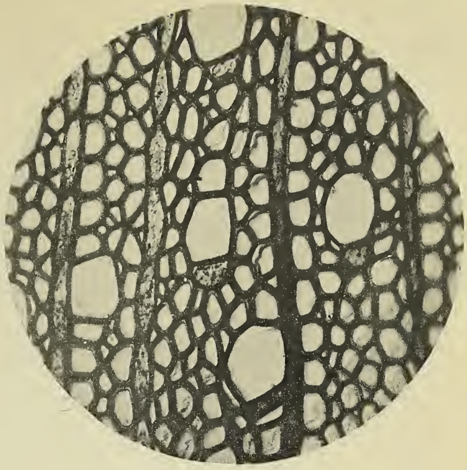

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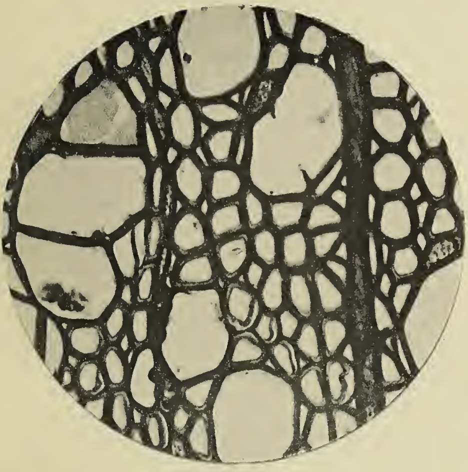

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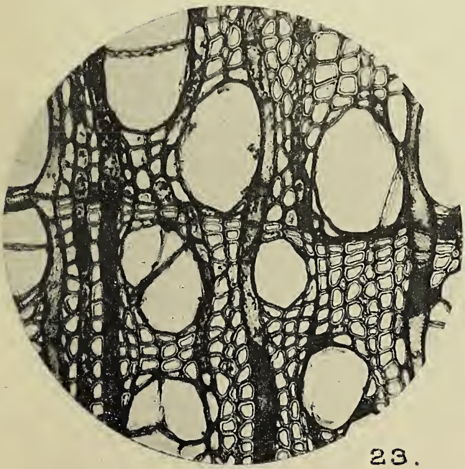

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On the Seedling Structure of certain Centrospermae.

BY

T. G. HILL,

Assistant Professor of Botany; University of London; University College,

AND

ETHEL DE FRAINE,

University of London; University College.

With eight Figures and seven Diagrams in the Text.

THE study of the seedlings of the Centrospermae[1] has resulted in no very striking results, the chief features of interest being connected more especially with the Natural Order Nyctaginaceae.

Unfortunately, we were unable to procure seeds of the Natural Orders Basellaceae, Batidaceae, and Cynocrambaceae, so that we have no information to offer regarding the structure of their seedlings. The same remarks also apply to certain genera of the other Natural Orders of the Cohort, but sufficient of the rest have been examined to give a general idea of the seedling anatomy of those orders centring around the Phytolaccaceae.

The characteristic mode of transition of the Centrospermae is Van Tieghem's type 3, and although this has been described in previous papers it appears desirable to draw attention to its features in one example, *Calandrinia Menziesii*, in order that much repetition, when dealing with the different plants, may be avoided.

PORTULACACEAE.

Calandrinia Menziesii, Torr. et Gray. The seedlings of this plant are very small, with fleshy cotyledons which show no internal differentiation into palisade tissue. In this species, and also in *C. amoena*, *C. speciosa*, *C. umbellata*, and *C. grandiflora*, a short cotyledonary tube is present. Each seed-leaf, which in transverse section is circular in outline, has in its upper part three bundles, one central and two lateral, of which the former is the largest. Tracing these strands downwards, the central one soon shows

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[1] T. G. Hill: On the Seedling Structure of certain Centrospermae. Brit. Assoc. Rep., Sect. K, York, 1906.

[Annals of Botany, Vol. XXVI. No. CI. January, 1912.]

signs of bifurcation; the phloem divides into two parts which gradually move away from one another so that the appearance of the strand, as viewed in transverse section, is like the letter V, the protoxylem being situated at the apex (Diagram 1, Fig. 2). This apparent movement is continued, hence the V becomes widely opened and the protoxylem comes into a central position and is bounded on each side by metaxylem and phloem; in other words, the bundle is bicollateral (Diagram 1, Fig. 3). Whilst these changes have been taking place, the lateral strands have gradually approached the central bundle, and finally they fuse with it. The condition now obtaining is seen in the third figure of Diagram 1, which arrangement is maintained for some distance downwards. The seed-leaves, which have no marked petiole, fuse laterally to form a short tube; at the cotyledonary node the cotyledon-traces are seen to have their protoxylems situated well towards the periphery, full exarchy being arrived at during the somewhat rapid passage towards the centre of the hypocotyl. In the axis a general centripetal displacement occurs and, concurrently, the opposing groups of phloem and metaxylem move towards each other and effect a junction (Diagram 1, Figs. 5 and 6); thus a diarch root-structure is organized. With regard to the fundamental transition phenomena, it will be observed that one bundle enters the axis from each cotyledon; each of these strands bifurcates and rotates so that

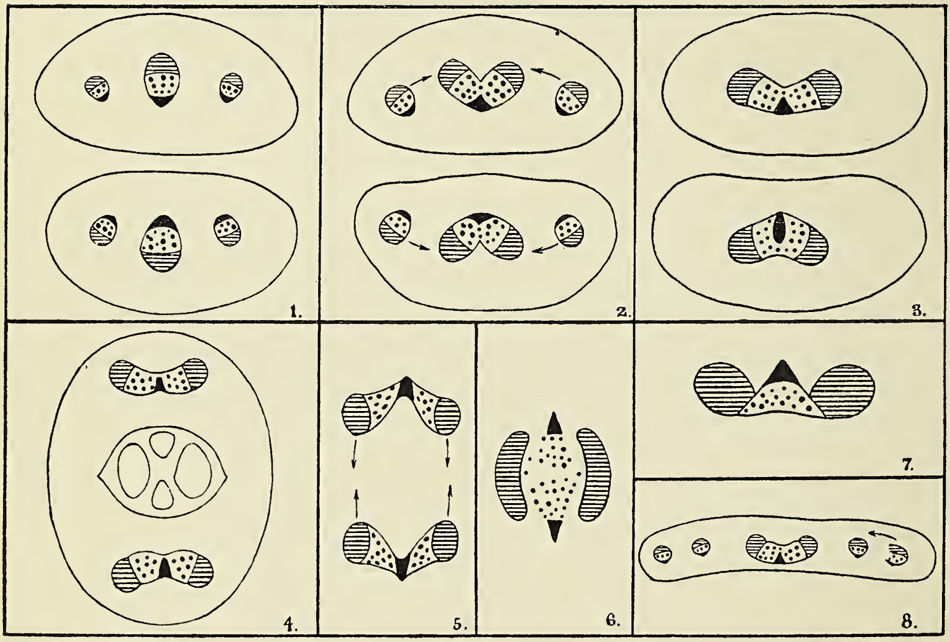

DIAGRAM 1. Figs. 1–7 *Calandrinia*, Fig. 8 *Portulaca*. In this and in the following diagrams the protoxylem is indicated by black areas, the metaxylem by round or elongated dots, and the phloem by shading.

the protoxylem is brought into the exarch position but still remains in the plane of the cotyledons; finally, the two groups of phloem- and of metaxylem-elements of one cotyledonary bundle fuse with the corresponding tissues of the other cotyledon, the resulting strands being situated in the inter-cotyledonary plane. A diarch root-structure is thus formed.

This is Van Tieghem's third type of transition, and it will be referred to in the following pages as type 3.

Calandrinia speciosa shows no important feature of difference from *C. Menziesii.*

Calandrinia amoena, Vis., in all essentials is identical with *C. Menziesii*; the cotyledons, however, are not so rounded and the bifurcation of their central bundles does not take place at so high a level.

Calandrinia grandiflora, Lindl., is chiefly remarkable from the fact that the rearrangements of the cotyledonary strands are more complete at a higher level than in any of the other species of the genus examined, the rotation of the bifurcated bundle being carried so far that the two groups of metaxylem elements are closed up, with the protoxylem in the exarch position, at a level just above the cotylar tube (Diagram 1, Fig. 7). This feature is also shared by *C. umbellata*, although not in so marked a degree.

Claytonia perfoliata, Don. The main features of dissimilarity between the transition phenomena in the very small and slender seedlings of this plant and those of species of *Calandrinia* are that the two lateral bundles of each seed-leaf of *Claytonia* early fuse with the larger central one; the main cotylar bundles are endarch and collateral through the whole length of the seed-leaf; and, finally, the vascular rearrangements which take place in a very short vertical distance leading to the diarch root-structure occur during the passage of the seed-leaf-traces from the cotyledons to the central region of the axis and in the upper part of the hypocotyl.

Portulaca oleracea, L. The seedling is very small and its cotyledons are much flatter than those of any of the preceding plants. The seed-leaves have several bundles in their blades, one large central one with two or three smaller strands on each side. The latter fuse on to the main central bundle, which shows bifurcation and rotation before the last of the laterals have effected a junction with it in the basal region of the blade (Diagram 1, Fig. 8). The transition is of type 3 and closely resembles that of *Calandrinia umbellata.*

CARYOPHYLLACEAE.

SILENOIDEAE.

Cucubalus baccifera, L. The transition resembles that of the foregoing plants in following type 3. Of the differences, relatively unimportant, which obtain between *Cucubalus* and *Calandrinia* for example, the following are the more striking: in the former plant, as in *Claytonia*, the lateral strands of each cotyledon fuse on to the larger central bundle in the upper region of the petiole before any vascular rearrangement is inaugurated in the median bundle; this strand, in transverse section, appears like a widely opened U with the xylem-elements, situated on the concave side, well dispersed owing to the development of much parenchyma, the presence of which makes it difficult to trace the movements of the protoxylem; finally, no cotyledonary tube is formed. As in the majority of the foregoing plants, the vascular rearrangements begin in the petioles of cotyledons, but in the basal rather than in the upper regions.

Lychnis Viscaria, L. The transition phenomena resemble those of *Cucubalus*; the central bundle of each cotyledon is, however, very small in *Lychnis* and the vascular rearrangements are more ill defined. A cotyledonary tube is formed.

Lychnis Githago. According to Gérard[1] the transition follows the same course as in *Silene inflata.*

Silene inflata follows type 3. From Gérard's account it appears that the transition phenomena commence in the upper regions of the petioles of the seed-leaves, but at a level just above the cotyledonary node the bifurcated bundles again close up. This is in accordance with our observations on *S. pendula.*

Silene pendula, L., shows no features of particular interest; the transition follows type 3, and the bifurcation of the main cotylar strand begins in the petioles of the seed-leaves before all the small laterals have fused with it. On tracing these half-bundles downwards, they are seen gradually to close up so as partly to sandwich the protoxylem-elements between them; thus at the cotyledonary node these bundles are closely apposed with the protoxylem towards the exarch position. There is a well-marked cotylar tube.

Silene Otites, Sm., closely resembles *S. pendula.*

Silene Schafta, Gmel ex Hohen., does not differ in any important feature from *S. pendula.* The bifurcation begins at a relatively high level in the cotyledons, and the lateral strands unite with the adjacent portions of the divided central bundles at about the same level.

Another example showed the unusual feature of a triarch root. The changes which led to this were as follows. One cotyledon was normal in having in the base of its blade one large central bundle and two small lateral ones; the other had two central ones and two laterals. The lateral

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[1] Gérard: Recherches sur le passage de la racine à la tige. Ann. Sci. Nat., Bot., sér. 6, t. xi, 1880.

strands fused on to the central ones, which in the petiole bifurcated and rotated in the usual way (Diagram 2, Figs. 1 and 2). In the case of the cotyledon *c.* 2 there are thus two pairs of phloem-masses and two groups of protoxylem-elements. Fusion takes place between the phloems which lie side by side, and the resulting strand remains in position, whilst the other two rotate inwards (Diagram 2, Fig. 3). The completion of these rearrangements in the axis results in a triarch root-structure (Diagram 2, Figs. 4 and 5).

Dianthus barbatus, L. The mode of transition is that of type 3. The lateral bundles in the base of the blade of the seed-leaves fuse on to the central bundles so that one strand only occurs in each cotyledon petiole. Although these bundles commence their division at the top of the cotyledonary tube, the major part of the transition takes place during the inward passage of these strands to the centre of the axis, and in the upper part of the hypocotyl.

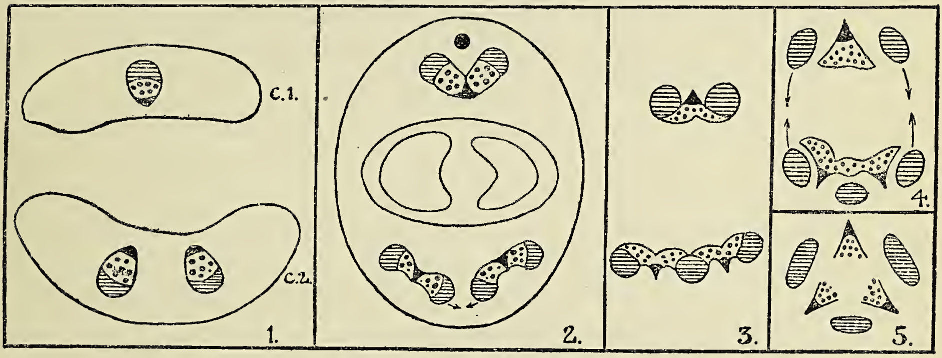

DIAGRAM 2. *Silene Schafta.*

Dianthus chinensis, L., differs from *D. barbatus* in two features: the main bundle of each seed-leaf commences its vascular rearrangements in the base of the blade before the lateral strands have joined on to it; also, the protoxylem of each seed-leaf-trace becomes completely exarch during its passage down the short but well-marked cotyledonary tube.

Dianthus arenarius, L., resembles *D. barbatus* very closely.

Gypsophila Saxifraga, L. The third type of transition is followed, and the vascular rearrangements begin in the base of the seed-leaves on a level with the top of the cotyledonary tube. The main changes take place in the upper part of the hypocotyl as in *D. barbatus.*

Saponaria cerastoides, Fisch. ex C. A. Mey. (Fig. 1, B), does not differ in any essential feature from *Gypsophila Saxifraga*, with the exception that a cotyledonary tube is not formed.

Saponaria Vaccaria, L. (Fig. 1, A), shows a few points of difference when compared with *S. cerastoides*. *S. Vaccaria* has much larger seed-leaves and a well-marked cotyledonary tube; also the petioles of the cotyledons each have three bundles, a main central one with a lateral on each side, which do not fuse until just above the cotyledonary node. Although the transition takes place as in *Gypsophila* and *Dianthus barbatus*, it is inaugurated at a lower level, namely at the base of the cotyledonary tube.

Saponaria vulgaris is practically identical with *S. Vaccaria* as regards the phenomena under discussion; the vascular changes, however, are initiated at a slightly higher level than in *S. Vaccaria*.

Fig. 1. A. *Saponaria Vaccaria*; B. *S. cerastoides*. Natural size.

Dangeard[1] has pointed out the similarity in the essentials of the seedling anatomy of *Saponaria*, *Atriplex*, *Chenopodium*, *Kochia*, *Salsola*, *Basella*, and *Rivinia*; and so far as our observations overlap we are in agreement with him.

Tunica prolifera, Scop., does not differ in any important feature from *Gypsophila Saxifraga*. One example recalled *Silene pendula* in the fact that the strand of one cotyledon bifurcated and then closed up at a slightly lower level.

The diarch root-structure is arrived at in a very short vertical distance.

ALSINOIDEAE.

Cerastium perfoliatum, L. The transition phenomena are practically identical with those of *Dianthus barbatus*; there is, however, in *C. perfoliatum* no cotyledonary tube, and the separation of the phloem-masses of the bifurcated seed-leaf-traces is more marked.

Stellaria graminea, L., also closely resembles *Dianthus barbatus*. Unlike *Cerastium perfoliatum*, *S. graminea* has a short cotyledonary tube.

Spergularia salina, J. et C. Presl.,[2] does not differ in any essential feature from *Dianthus barbatus*, the chief point of dissimilarity being in the fact that in *S. salina* the vascular rearrangements start at a higher level and the cotyledonary bundles enter the axis in a more advanced state of division and rotation.

Spergula arvensis, L., has a larger seedling than *Spergularia salina*, and its vascular rearrangements recall those of *Calandrinia Menziesii*; thus,

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[1] Dangeard: Recherches sur le mode d'union de la tige et de la racine chez les Dicotylédones. Le Botaniste, i, 1889.

[2] This may possibly be *S. rubra*, J. et C. Presl.

unlike *Spergularia salina*, the central bundle of each cotyledon bifurcates before the lateral strands have effected a junction with it, and this beginning of the transition phenomena takes place high up in the seed-leaf.

Polycarpon tetraphyllum, L., closely resembles *Spergula arvensis*. A cotyledonary tube is formed.

Corrigiola litoralis, L. The seed-leaves do not form a tube at their base. The resemblance to *Spergula arvensis* is very close.

AMARANTACEAE.

AMARANTOIDEAE.

Celosia cristata, L., *C. plumosa*, ?, and *C. spicata*, Spreng. The transition phenomena in these three seedlings are almost identical with those of *Calandrinia grandiflora*. The bifurcation of the central cotyledonary strand takes place at the top of the petiole or even in the base of the foliar part of the seed-leaf, and the so-formed 'double bundle', in its passage towards the cotyledonary node, is accompanied by two lateral strands which fuse on to the divided central bundle at a level a little above the insertion of the seed-leaves which do not form a tube.

One example of *C. spicata* had three cotyledons, of which one was markedly larger than the other two. The central bundle of this large seed-leaf was more massive than that of the others, and the corresponding pole of the root was similarly much stronger. The triarch root-structure was arrived at in exactly the same way as the diarch arrangement in normal dicotyledonous specimens.

Amaranthus caudatus, L. The vascular re-arrangements closely resemble those of *Celosia*. The central cotyledonary strands bifurcate in the blades of the seed-leaves and the protoxylems are fully exarch at the cotyledonary node. Also, as in *Celosia*, the lateral strands join on to the median bundle at a level just above the insertion of the seed-leaves. The only point of dissimilarity from *Celosia* lies in the fact that in *A. caudatus* the four groups of phloem-elements in the upper region of the hypocotyl derived from the two seed-leaf-bundles may postpone their union for some distance downwards; similar observations have been made in some Cactaceae.

Amaranthus abyssinicus, ?, *A. Dussii*, Spreng., *A. hypochondriacus*, L., *A. paniculatus*, L.—which has also been investigated by Gérard,[1] with whose observations we are in agreement—and *A. viridis* all closely resemble *Amaranthus caudatus*.

Amaranthus sylvestris, Desf., is remarkable chiefly from the fact that not only do the central seed-leaf-bundles bifurcate and rotate in the blades

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[1] loc. cit.

of their respective cotyledons, but they divide completely into two, leaving protoxylem elements isolated between them, a feature which is characteristic of *Phytolacca*, *Allionia*, *Mirabilis*, and other Nyctaginaceae; also it occurs in some species of *Lupinus* and *Dahlia*. A minor point to be remarked upon in *Amaranthus sylvestris* is that the union of the laterals with the central bundles of the cotyledons is effected at a much higher level than in *A. caudatus*, &c.

Pupalia purpurea, unlike any of the foregoing species of *Amaranthus*, does not show an early inauguration of the transition phenomena; in fact the bundle from each cotyledon enters the axis in an undivided endarch condition, all the changes leading to the formation of a diarch root-structure taking place rapidly in the upper part of the hypocotyl.

GOMPHRENOIDEAE.

Gomphrena haageana, Klotzsch, resembles *Amaranthus sylvestris* in the division of the central cotyledonary bundles and isolation of protoxylem elements in the blade of the cotyledon. A feature of difference is found in the fact that one of the laterals may delay fusion with the adjacent half of the central strand until the upper region of the hypocotyl is reached.

Gomphrena globosa, L., is, in essentials, identical with *G. haageana*; the isolation of the protoxylem, however, is not so well marked as in the latter plant. In both species a cotyledonary tube is formed, a point of difference from *Amaranthus* and *Celosia*.

CHENOPODIACEAE.

The anatomy of the seedlings of this Order has formed the subject of investigations by previous authors. Van Tieghem[1] has remarked upon the occurrence of diarchy in the primary roots of *Beta vulgaris*, *Spinachia oleracea*, and *Atriplex hortensis*.

Gérard[2] has shown that the transition of *Atriplex hastata* follows that of type 3, and that it is not by any means rapid.

The work of Dangeard[3] in this connexion has already been alluded to; it may, however, be further remarked that he considers that the venation of the cotyledons has an important bearing on the primary root-structure and concludes that the seed-leaves with pinnate venation have a diarch root, whilst those with a palmate venation have a tetrarch root-structure.

Fron[4] has made the, hitherto, most extensive study of the seedling anatomy of this Natural Order, and points out that the structure of *Atriplex hastata* is typical for the genus. He also draws attention to the similarity

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[1] Van Tieghem: Recherches sur la symétrie de structure des plantes vasculaires. Ann. Sci. Nat., Bot., sér. 5, t. xiii, 1870. [2] loc. cit. [3] loc. cit.

[4] Fron: Recherches anatomiques sur la racine et la tige des Chénopodiacées. Ann. Sci. Nat., Bot., sér. 8, t. ix, 1899.

between *Salsola Kali*, *S. Soda*, *S. vermiculata*, *Suaeda*, *Chenopodium*, *Blitum*, *Roubiera*, and *Beta*. He concludes that the transition in the Chenopodiaceae takes place at different levels of the hypocotyl: at the lower end in *Atriplex*, *Salsola*, and *Suaeda*; slightly below the level of the insertion of the cotyledons in *Beta* and *Spinachia*; whilst, finally, in *Blitum* and *Chenopodium* it takes place in an intermediate position.

CYCLOLOBEAE.

Beta brasiliensis, ?, *B. chilensis*, Hort., *B. hortensis*, Mill., and *B. vulgaris*, Moq. The details of the transition in these plants are like those of *Amaranthus caudatus*, the only differences being that in *Beta* a short cotyledonary tube is formed and there is no marked delay in the fusion of the phloem-masses in the hypocotyl.

Chenopodium ambrosoides, L., *C. Atriplicis*, L., *C. Bonus-Henricus*, L., *C. Botrys*, L., *C. calulatum*, ?, *C. capitatum*, Aschers, and *C. scoparium*, L., all resemble *Amaranthus* and *Beta*. A short cotyledonary tube occurs in *C. Bonus-Henricus*, *C. capitatum*, and *C. scoparium*. The bifurcation of the central cotyledonary strand takes place well within the blade of the seed-leaf, but in no case is the protoxylem entirely isolated as in *Amaranthus sylvestris*. The V-shaped bundle is accompanied downwards by two laterals, and their union occurs at different levels: in *C. ambrosoides* and *C. Botrys* this coalescence takes place at a level well above the cotyledonary node; in *C. Atriplicis*, *C. Bonus-Henricus*, *C. calulatum*, and *C. capitatum* the junction is at, or just above, the cotyledonary node; finally, *C. scoparium* is remarkable in the fact that the laterals make no junction with the central strand, but come to an end.

It may also be remarked that there is no particular delay in the fusion of the groups of phloem-elements in the axis, as obtains in *Amaranthus*.

Atriplex hastata, L., has its transition-characters in common with many species of *Amaranthus*, *Gomphrena*, &c. The main seed-leaf-bundle bifurcates at a relatively high level, and the separation of the two halves is so complete that the protoxylem appears in an isolated position at a level above the cotyledonary node. There is thus a close resemblance to *Amaranthus sylvestris*, the only difference, of degree rather than kind, in this respect being that in the last-named plant this isolation takes place at a much higher level. A minor feature of difference is that the main bundle may be accompanied in its passage down the petiole by two or three laterals on each side, not two as is the case in all the foregoing plants. These lateral strands join on to the adjacent halves of the divided central bundle at the cotyledonary node, and sometimes even in the top of the hypocotyl, a feature of resemblance to some of the foregoing plants, *Gomphrena haageana* for instance. Again, as in many species of *Amaranthus*, there is a marked delay in the union of the corresponding pairs of phloem-masses within the hypocotyl; indeed, considering the advanced state of preparation in the cotyledons for the vascular rearrangements, the assumption of a proper root-structure takes place at a surprisingly low level.

A well-marked cotyledonary tube is present.

Atriplex littoralis, L. With regard to the features under discussion this plant is practically identical with *A. hastata.*

Atriplex thamnoides, ?, which has a much smaller seedling, shows some differences. It has no cotyledonary tube, the lateral bundles fuse on to the central one in the base of the blade of the cotyledons, and there is no undue delay in the fusion of the phloem-masses within the axis.

FIG. 2. *Obione portulacoides.* Natural size.

Obione portulacoides, Moq. (Fig. 2). The seed-leaves are fleshy and the behaviour of their important vascular bundles, both in the cotyledon and in the axis, is precisely like those of *Atriplex hastata.* The laterals, however, vary pretty much; in the lower part of a seed-leaf there are, as is usual, two, one on each side of the divided central strand; of these some may die out, especially if very small, or may branch into two before coming to an end, or they may ultimately fuse with the central strand.

Spinachia oleracea, L., very closely resembles those species of *Beta* examined.

Kochia trichophylla, ?. The main features of the transition strongly recall those of *Calandrinia grandiflora.* Each seed-leaf, in its more basal region, has the usual bifurcated strand with two laterals on each side of it; of these, those situated immediately on either side of the central bundle fuse with it, whilst those placed on the outermost sides die out. As in some species of *Amaranthus*, *Atriplex*, and *Obione* there is a delay in the fusion of the phloem in the axis, but this is not so well marked in *Kochia* as in *Obione portulacoides.*

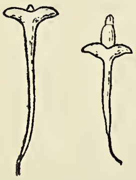

FIG. 3. *Salicornia europaea.* Two seedlings, one showing the epicotyl in a more advanced stage of development. Natural size.

Corispermum hyssopifolium, L., closely resembles *Kochia trichophylla.*

Salicornia europaea, L. The seedling of this plant (Fig. 3), and also of many other species of the genus, has two small fleshy cotyledons which fuse laterally towards the base to form a short cotyledonary tube or sheath. This tube becomes decurrent down the hypocotyl, forming a succulent 'cortex', a phenomenon exactly paralleled by the leaf-sheaths in the segments of the adult plant.

The distribution of the vascular bundles within the cotyledon is precisely similar to that in the leaves of the mature plant, and may be understood best by tracing the strands outwards from the cotyledonary node (Diagram 3, Fig. 6). A single trace (*c.* 1) leaves the node and branches into three portions; of these the median strand (*c.* 2) divides up and supplies the free leaf-tip, whilst the two lateral strands (*c.* 3 and *c.* 4) curve outwards and pass

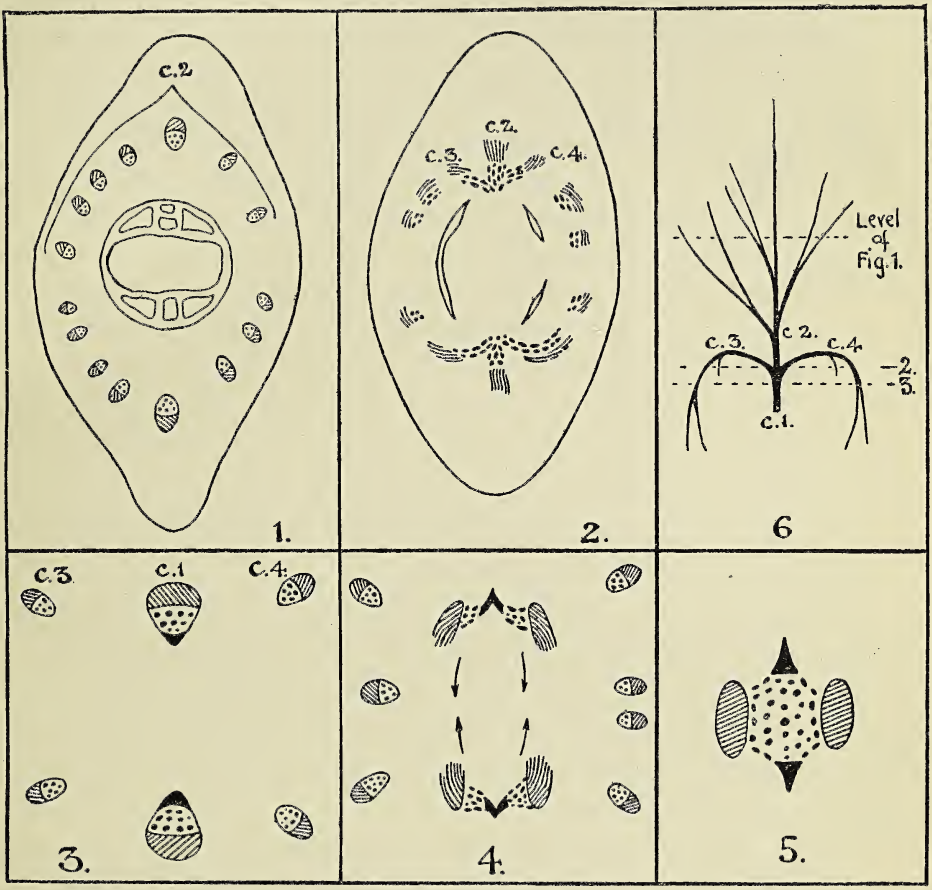

DIAGRAM 3. *Salicornia.* Fig. 6 illustrates the vascular skeleton of one side of the upper part of a seedling. The dotted horizontal lines indicate the level of the sections represented in Figs. 1, 2, and 3.

downwards, their branches supplying the cotyledonary sheath and ultimately ending blindly in the aqueous tissue.

Tracing the strands downwards, in order to follow the transition, a section through the united cotyledons (Diagram 3, Fig. 1) shows numerous bundles which are the branches of the central cotyledonary strands. Considering one side only, these bundles unite, as the sections are traced downwards, to form one strand (*c.* 2) which, at the node, is joined by the lateral trunks *c.* 3 and *c.* 4. Thus from each seed-leaf one endarch collateral bundle (*c*. 1) enters the hypocotyl; these strands soon bifurcate and a well-marked rotation rapidly follows, the adjacent phloems unite, and a diarch root is organized according to type 3.

The bundles seen in the outer regions are, of course, the branches of the lateral strands (*c*. 3 and *c*. 4 on the one side) already referred to.

The endodermis of the root is well marked, and immediately surrounding it is an aerating zone.

The primary condition of the root is maintained downwards only for a very short distance, anomalous secondary thickening beginning even in the roots of quite young seedlings.

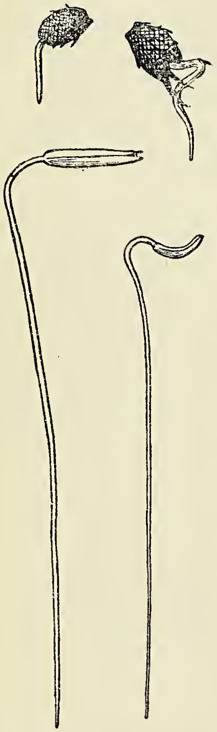

FIG. 4. *Salsola Kali.* In the two younger seedlings the cotyledons are still enclosed within the fruit. Natural size.

S. perennis, Mill., and *S. Smithiana*, Moss, are in all essential features similar to *S. europaea.*

SPIROLOBEAE.

Suaeda maritima, Dumort, is very like *Calandrinia Menziesii*, with the exception that the behaviour of the laterals is rather irregular; their number is variable and those adjacent to the central bundle may fuse with it, whilst others end blindly below. No cotyledonary tube is formed. As in *Kochia*, *Obione*, and others of the preceding plants, there is a delay in the fusion in the groups of cotyledonary phloem-elements in the axis.

Suaeda dendroides, Moq., has a much smaller seedling than the previous plant; the transition phenomena are, however, essentially similar. The only point of difference is that the lateral strands are disposed of at a higher level, and there is not much delay in the organization of a proper diarch root-structure.

Salsola Kali, L. Seedlings of this plant in different stages of development are shown in Fig. 4.

With regard to the transition phenomena, they are almost precisely similar to those of *Amaranthus caudatus*, the only difference being that in *Salsola* the protoxylems of the main seed-leaf-bundles are not so fully exarch at the cotyledonary node. With regard to other features, there is a well-marked cotyledonary tube in *Salsola*, and the palisade parenchyma, with its associated cells, of the upper part of the seed-leaves, which are centric in structure, is continued downwards in four columns to the cotyledonary node.

PHYTOLACCACEAE.

Phytolacca paraguayensis, ?, in most of its transition characters is very like *Amaranthus sylvestris*. There is the same division of the chief cotyledonary bundle and the isolation of the protoxylem-elements which takes place in the base of the blades of the seed-leaves. The only feature of difference is that in *P. paraguayensis* the union of the two laterals with the central seed-leaf-strand is effected at the extreme base of the petioles.

Phytolacca dioica, L., only differs from *P. paraguayensis* in the fact that the two halves of the main seed-leaf-bundle are more compact and their separation is less well marked.

Phytolacca acinosa, Roxb., which has a somewhat smaller seedling, does not show, so far as has been seen, the separation of the protoxylem of the cotyledons in the seed-leaves themselves; otherwise, its vascular rearrangements are like those of the two preceding species.

Phytolacca decandra. The transition phenomena of this plant, judging from Gérard's account,[1] do not appear to differ in any important feature from those of the foregoing species.

Rivina, which has been investigated by Dangeard,[2] also follows type 3, and so also does *Basella* (N.O. Basellaceae).

AIZOACEAE.

Mesembryanthemum. All the seedlings of this genus are of a fleshy habit, which is more marked in some than in others, and their cotyledons are more or less adnate. This union may be nothing more than the formation of a cotyledonary tube, as in *M. crystallinum*, L.; in other cases the adnation may be quite extensive, especially in the more fleshy forms, the seed-leaves being joined by their ventral surfaces for most of their length, as in *M. Bolusii*. Fig. 5 illustrates the two extreme forms of young seedlings; of the species examined, *M. linguaeforme*, *M. tigrinum*, Haw., and *M. truncatellum*, Haw., very closely resemble *M. Bolusii*, the chief differences being those of size; thus *M. tigrinum* is about half as large as *M. Bolusii*, and *M. truncatellum* about one-third or a quarter the size of this same plant, also it is more rounded. *M. crystallinum* resembles *M. tricolorum*, Haw., and *M. rhomboideum* is somewhat intermediate in appearance.

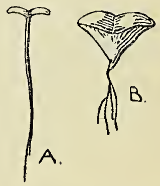

FIG. 5. A. *Mesembryanthemum crystallinum*; B. *M. Bolusii*. Natural size.

The transition phenomena are like those of many of the foregoing plants, *Calandrinia Menziesii* for instance. In *M. tricolorum*, *M. crystalli-*

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[1] loc. cit.

[2] loc. cit.

num, and usually in *M. linguaeforme* the protoxylems of the cotyledonary strands are exarch before the bundles enter the hypocotyl. On the other hand, in *M. filamentosa*, *M. rhomboideum*, *M. trigrinum*, and *M. truncatellum* the protoxylems referred to are not fully exarch, sometimes even practically endarch, although the bundles may be opened like a V. This feature, however, is variable; thus in one seedling, e. g. of *M. rhomboideum*, the central seed-leaf-bundle of one cotyledon may be well divided, at a level immediately above the cotyledonary node, with its protoxylem mesarch or partly exarch in position, whilst the other bundle may show no division at all and the protoxylem endarch in position.

Before passing on, attention may be drawn to a peculiarity in some species of this genus which is unique in the plants examined.

In *Mesembryanthemum Bolusii* and *M. linguaeforme*, the two species with the fleshiest and largest cotyledons, the main central bundles of the seed-leaves are, for some distance above the cotyledonary node, surrounded by an irregular endodermis, not necessarily consisting of a single layer of cells, but often of two or three. In the more upper regions, the endodermis may not entirely enclose the bundle, but at lower levels, especially in *M. linguaeforme*, it is complete and singularly convincing. In *M. filamentosa* and *M. rhomboideum* this endodermis is not so extensive, but it forms a more or less complete sheath around the main bundles of the seed-leaves at the cotyledonary node. In these cases an endodermis surrounds the vascular tissues of the axis before the root-structure is organized.

Tetragonia expansa, Murr. The transition phenomena do not differ in any essential feature from those of *Mesembryanthemum*. The main cotyledonary strands bifurcate in the base of the blades of the seed-leaves, or in the top of the petioles, and their protoxylems become exarch before the bundles pass into the axis. The fusion of the laterals with the central strands takes place at a low level, just above the top of the cotyledonary tube.

NYCTAGINACEAE.

Abronia. The seedlings of the species of this genus examined, *A. umbellata* and *A. villosa*, are of interest from the fact that the cotyledons are very unequal in size. Fig. 6 represents a number of seedlings of different ages of *A. villosa*; it will be observed that in the younger plants one of the cotyledons is extremely small, whilst in the older plants it approximates more closely to the larger seed-leaf. In the youngest seedling (A) the rudiment of one seed-leaf is inserted on the axis at about the same level as the well-developed cotyledon, but in older seedlings, owing to the more rapid growth of the large cotyledon, the small one appears to be inserted lower down the hypocotyl. Also it will be observed that the base of the hypocotyl, especially in the younger plants, is distinctly swollen and bears on one side a prominent peg or foot (*p*).

Charles and Francis Darwin[1] have drawn attention to these peculiarities in *A. umbellata* and *A. arenaria*, and have figured them in the former plant; they compare the swollen hypocotyl to a corm, and point out that the peg functions in rupturing the coat of the fruit.

Avebury[2] also has described the seedlings of *Abronia*, and remarks that in the seed one of the cotyledons is almost aborted and remains quite small for some time after germination. In *A. umbellata* it afterwards becomes the largest and grows like an ordinary leaf. He further remarks that the full-grown cotyledons of *A. arenaria* are very unequal in size.

Abronia umbellata, Lam. In the seedling about to be described there was one large and one small cotyledon. The large seed-leaf (Diagram 4, *c.* 1) has one large central bundle with many smaller ones on either side. Within the blade the central bundle divides into two and shows a certain amount of rotation, so that some, often only one or two, protoxylem elements become isolated between the two half-bundles which move apart (see Diagram 6, Figs. 1–4). This is comparable to what obtains in some of the preceding plants, *Amaranthus sylvestris* and species of *Gomphrena* and *Chenopodium* for instance. But in no case in these plants is the isolation so well marked as in *Abronia*, *Allionia*, and other Nyctaginaceae.

At the base of the blade the condition obtaining is shown in Diagram 4, Fig. 1; the laterals fuse on to the adjacent half-bundles, hence the petiole has two strands, the half-bundles referred to above, partly facing one another and with a small group of protoxylem elements between them (Diagram 4, Fig. 2). At a lower level the two large strands divide, each into two, and clearly are in the first stage of rotation (Diagram 4, Fig. 3). In this condition they enter the axis.

Fig. 6. *Abronia villosa.* Natural size.

Turning now to the small cotyledon (*c.* 2), which is not nearly so well

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[1] C. and F. Darwin: The Power of Movement in Plants. London, 1880.

[2] Lubbock: On Seedlings. London, 1892.

differentiated as the larger, the central strand behaves in exactly the same way as the corresponding bundle of *c.* 2 to begin with; that is to say, it divides into halves and isolates some protoxylem in the blade. The lateral bundles are not differentiated, consisting only of desmogen. Lower down (Diagram 4, Fig. 3), these desmogen strands fuse on to the adjacent halves of the central bundle, which do not divide as in the other cotyledon. Thus a transverse section through the top of the hypocotyl has the appearance indicated in Diagram 4, Fig. 4.

The bifurcated bundles derived from the large cotyledon undergo a vascular rearrangement, as a result of which each pair gives origin to a protoxylem pole situated in the intercotyledonary plane; then the two half-bundles derived from the small cotyledon fuse on to the adjacent bundles derived from the large seed-leaf, and thus a tetrarch root-structure is arrived at (Diagram 4, Figs. 5–8).

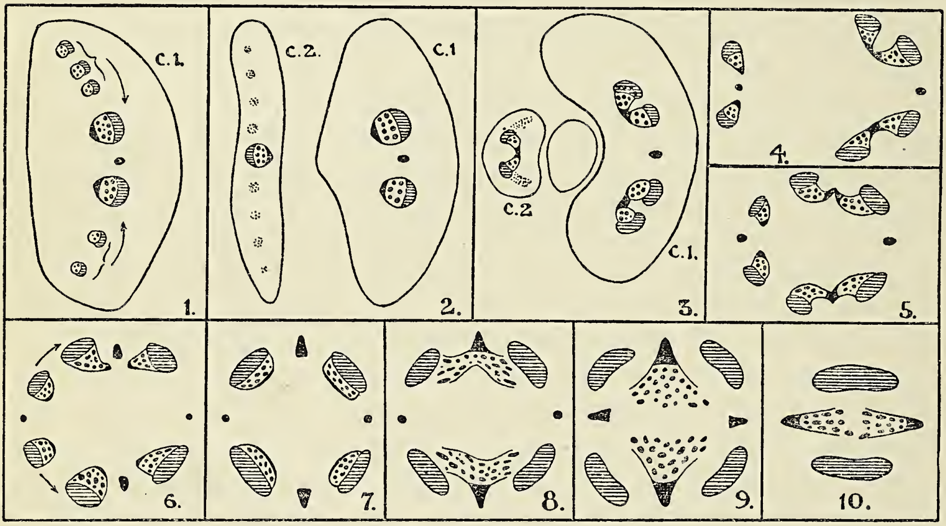

DIAGRAM 4. *Abronia umbellata.*

For a relatively long distance downwards the intercotyledonary xylem poles are much the stronger, but eventually a gradual addition of protoxylem elements is made to the poles in the plane of the seed-leaves, so that equality is ultimately arrived at (Diagram 4, Fig. 9). Reduction, however, may take place; in one example, below the level of the peg, the intercotyledonary protoxylem rays diminished in size and disappeared, the corresponding phloem strands united, and a well-marked diarch root-structure resulted (Diagram 4, Fig. 10).

Minor variations in these changes may occur; thus the division of the two half-bundles of the large cotyledon may take place in the upper part of the hypocotyl, and not in the petiole of the seed-leaf, and the union of the half-bundles derived from *c.* 2 may unite with the corresponding strands from the other cotyledon, immediately before, or simultaneously with, the bifurcation of the latter (Diagram 5, which compare with Figs. 4 and 5 of Diagram 4). Finally, the root does not always show a reduction from tetrarchy to diarchy.

Abronia villosa, Wats. The vascular rearrangements resemble those of *A. umbellata* very closely; in fact the only difference found was in one example of *A. villosa*, in which only the two innermost laterals fused on to the neighbouring halves of the divided central bundle of the large seed-leaf. The others, two on each side, united together to form two large strands, so that the result was exactly the same as in *A. umbellata* (Diagram 4, Fig. 3).

In all the examples of these two species examined, the bundles from the larger cotyledon gave origin to three poles of the tetrarch root, whilst the bundles from the smaller seed-leaf gave rise to the smaller pole; this irrespective of the age of the seedling. It may also be remarked that the continuity between the isolated protoxylem elements in the petioles of the seed-leaves and the corresponding poles of the root may be interrupted, so that they are almost if not quite impossible to trace. This is doubtless due to the growth in length either rupturing or pulling together the soft walls of the annular vessels.

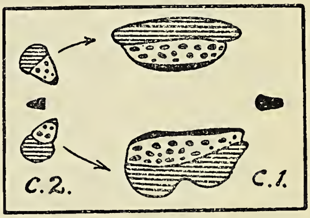

DIAGRAM 5. *Abronia umbellata.*

Allionia albida, Walt. The main features of the transition phenomena of this plant resemble those of *Abronia*, the chief differences which obtain being connected with the formation of those poles of the root situated in the intercotyledonary plane. The central bundle of each cotyledon gives rise, in the blade of the seed-leaf, to an isolated strand of protoxylem in the same way as in *Abronia* (Diagram 6, Figs. 1–4). In the upper regions of the petiole of each seed-leaf several strands occur (Diagram 6, Fig. 5): the two large bundles formed by the division of the main cotyledonary strand (*b.* 1 and *b.* 2); two or three laterals on the outer sides of each of these (*a*); a few small bundles situated between the first two (*e*), and the group of protoxylem elements (*c.* 1). On tracing these downwards their number becomes less; thus the small medianly placed bundles (*e*) unite with the adjacent large ones, the half-bundles of the main cotyledonary strand, and the lateral strands unite together until there are but one or two on either side. There is also in the centre a group of protoxylem-elements (*c.* 1 and *c.* 2) which are never numerous.

Either at, or immediately above, the cotyledonary node, or in the hypocotyl, the laterals *a* fuse on to the neighbouring half-bundles (Diagram 6,

Fig. 6), so that in the upper region of the hypocotyl (Diagram 6, Fig. 7) there occur two groups of protoxylem (*c.* 1 and *c.* 2), four large bundles derived from the cotyledons (*b.* 1 and *b.* 2, and *b.* 3 and *b.* 4), and six plumular strands which soon fuse to form two (*d.* 1 and *d.* 2, Diagram 6, Fig. 8). Tracing these bundles downwards, their behaviour is seen to be

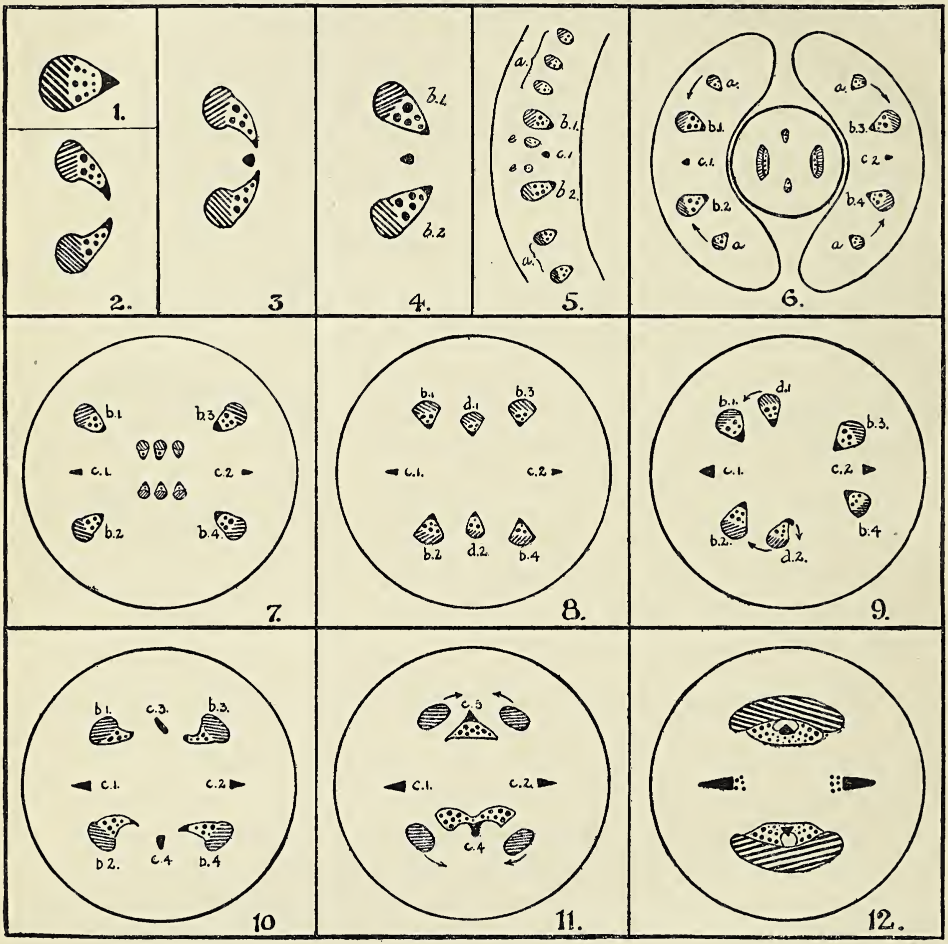

DIAGRAM 6. *Allionia albida.*

very complicated; the protoxylems *c.* 1 and *c.* 2 retain their position, but the bundles *b.* 1, *d.* 1, and *b.* 3 anastomose very freely, and so also do the corresponding strands on the other side. Concurrently the bundles *b.* 1 and *b.* 3, and *b.* 2 and *b.* 4, show certain rotatory movements which tend to bring their protoxylems into the intercotyledonary plane and into the exarch position, but for some space downwards these movements lead to nothing definite owing to the presence of the strands *d.* 1 and *d.* 2, which may give off branches which join on to the adjacent bundles. Eventually the plumular strands *d.* 1 and *d.* 2 join on to one of the bundles situated on their flanks (Diagram 6, Fig. 9), and a definite rotation of the protoxylems of the bundles *b* takes place (Diagram 6, Fig. 10); thus there is organized a tetrarch root-structure (Diagram 6, Fig. 11), which, however, in a very short vertical distance downwards gives place to a diarch arrangement. The phloem and metaxylem of *b.* 1 and *b.* 3, and *b.* 2 and *b.* 4, effect a junction and enclose the protoxylems which were derived from these same bundles (Diagram 6, Figs. 11 and 12). These protoxylems die out and thus there supervenes a diarch root-structure, the groups of tracheae *c.* 1 and *c.* 2, which have gradually been increasing in number, forming the permanent protoxylems.

Mirabilis longiflora, L. The seedlings of this plant (Fig. 7) are large and have prominent epigeal cotyledons, often unequal in size, with long petioles inserted on a rather massive hypocotyl, at the base of which there is a conspicuous peg (*p.*).

The essential features of the transition phenomena resemble those of *Allionia albida*; but the structure of the hypocotyl is exceedingly complicated and is illustrated in Diagram 7.

The vascular changes in the cotyledons are so like those of *Allionia* that no further description is necessary (see Diagram 6, Figs. 1–6). At the top of the hypocotyl, as in *Allionia*, there obtain two isolated groups of protoxylem-elements—four large vascular bundles (*c.* 1, *c.* 2, *c.* 3, and *c.* 4) derived from the cotyledons, and two masses of plumular vascular tissue (*p*). Tracing these bundles downwards, the plumular vascular tissue passes outwards between the strands *c.* 1 and *c.* 3, and *c.* 2 and *c.* 4, deploys on either side, and breaks up into isolated vascular strands (*p*), which sometimes consist only of phloem (Diagram 7, Figs. 2, 3, and 4). Concurrently the

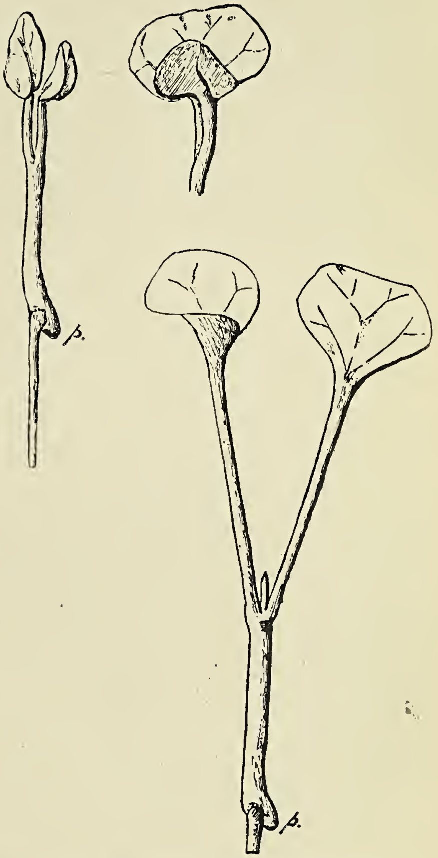

FIG. 7. *Mirabilis longiflora.* Natural size.

large bundles (*c.* 1–*c.* 4) give off branches of phloem and xylem which follow the path taken by the plumular tissue and reinforce it as it were. Hence, although these plumular bundles are marked *p* in the Diagram, in order to indicate their nature, it must be understood that at levels below the second figure of Diagram 7 they contain also vascular elements derived from the cotyledonary strands. During these changes the original bundles, *c.* 1, *c.* 2, *c.* 3, and *c.* 4, have each divided up into three; thus *c.* 1 gives origin to *c.* 5 and *c.* 6, *c.* 6 again dividing to form *c.* 15 and *c.* 16; on the other hand, *c.* 4 divides directly into three, namely *c.* 10, *c.* 11, and *c.* 12 (Diagram 7, Figs. 3 and 4). At this level (Diagram 7, Fig. 4) the plumular bundles are clearly defined, and form a zone surrounding the cotyledonary ring of bundles; that is to say, a similar anomaly of scattered bundles obtains in the hypocotyl as in the stem, although arrived at in a different way. Amongst the smaller cotyledonary bundles there is a general movement outwards so that they ultimately come into line with the plumular traces; *c.* 9, *c.* 11, and *c.* 15 are among the first to show this tendency, and in so doing fusion with certain plumular strands may take place. For instance, the two small bundles immediately above *c.* 15 (Diagram 7, Fig. 5) join together and then fuse on to *c.* 15; also *p.* 5 and *c.* 11 effect a junction. Meanwhile the centrally placed cotyledonary bundles show changes, the most interesting of which are the formation of bicollateral structures which result from a strand of xylem passing from the normal wood to a position on the outer side of the phloem. For example, in *c.* 7 such a strand passes between this bundle and *c.* 16 outwards, and rests on the outer side of the phloem (Diagram 7, Figs. 4, 5, 6, and 7). On the opposite side of the axis the corresponding xylem is derived from *c.* 13, and comes to rest on the adjacent bundle, *c.* 12. This appears to be the usual mode of origin of the abnormality, but exceptions occur; thus in another example of *M. longifolia* it was, on one side only of the axis, arrived at in a modified way. In Diagram 7, Figs. 12 and 13, there are represented three cotyledonary strands, *c.* 1, *c.* 2, and *c.* 3, and one plumular strand, *p.* This latter consists almost entirely of phloem, but xylem-elements pass outwards from *c.* 1 and *c.* 2 and divide into two parts, one of which accompanies and completes the bundle *p* (Diagram 7, Fig. 13), and the other for the moment is isolated, then it is joined by a strand of phloem from *c.* 2, so that there is formed, between *c.* 1 and *c.* 2, a collateral bundle showing reversed orientation, which quickly effects a junction with *c.* 1.

Directly after these bicollateral bundles have been organized there is a junction of three bundles to form one situated in the intercotyledonary plane: thus *c.* 16, *c.* 7, and *c.* 8 join to form *c.* 17, and *c.* 13, after its division into two, combines with *c.* 12 to form *c.* 18 (Diagram 7, Figs. 4, 5, and 6). Thus there are formed two large bicollateral bundles with xylem on both sides of the phloem, and in them the extension outwards of the normally

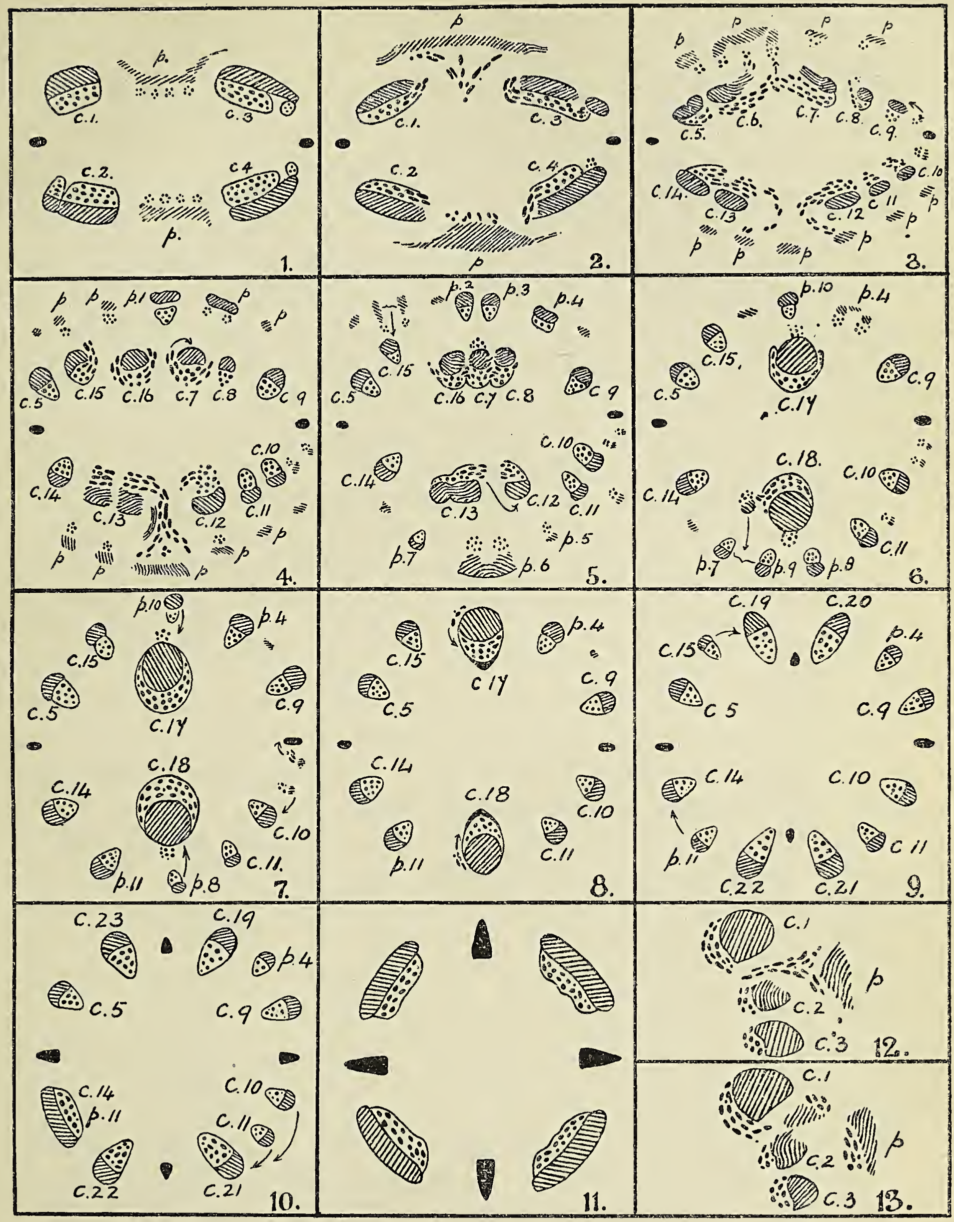

DIAGRAM 7. *Mirabilis longiflora.* Explanation:—*c.* indicates the bundles derived from the cotyledons; *p.* represents those of plumular origin. Whenever one important bundle fuses with another, or divides into two, the resulting strands are indicated, when necessary, by the next available higher figures. For instance, *c.* 1 (Fig. 1) divides into two; the next available numbers are *c.* 5 and *c.* 6, so these are used to represent the two branches (Fig. 3). Again, *c.* 17 (Fig. 8) divides into *c.* 19 and *c.* 20, then *c.* 15 and *c.* 19 join and so there is formed *c.* 23 (Fig. 10). Further than this the numbers are meaningless.

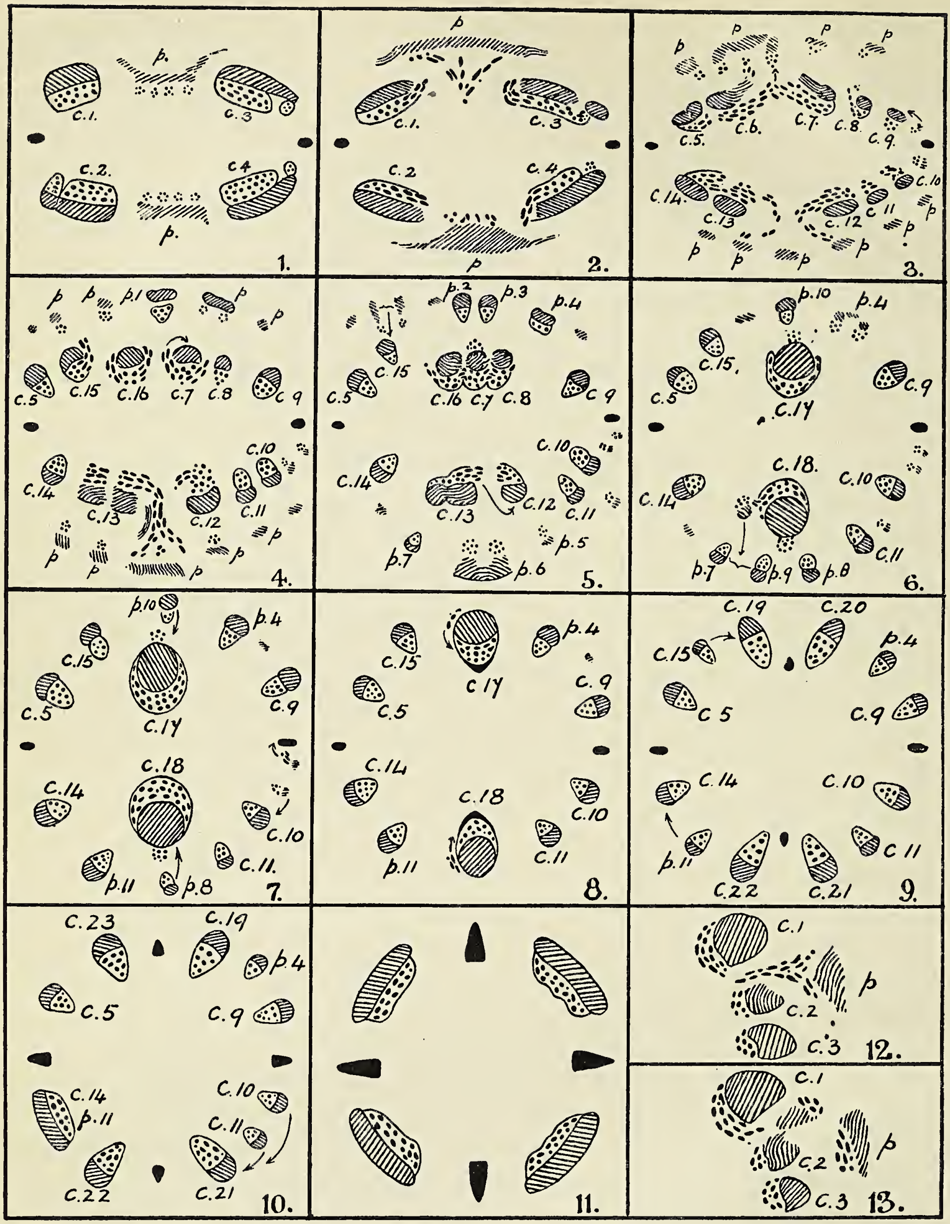

DIAGRAM 7. *Mirabilis longiflora*. Explanation:—*c*. indicates the bundles derived from the cotyledons; *p*. represents those of plumular origin. Whenever one important bundle fuses with another, or divides into two, the resulting strands are indicated, when necessary, by the next available higher figures. For instance, *c*. 1 (Fig. 1) divides into two; the next available numbers are *c*. 5 and *c*. 6, so these are used to represent the two branches (Fig. 3). Again, *c*. 17 (Fig. 8) divides into *c*. 19 and *c*. 20, then *c*. 15 and *c*. 19 join and so there is formed *c*. 23 (Fig. 10). Further than this the numbers are meaningless.

placed xylem may be so extensive as to make them almost concentric (Diagram 7, Figs. 6 and 7), a tendency shown also by the bundles of the stem.[1]

While these changes have been taking place the plumular strands have not been idle; *p.* 1 divides into two, *p.* 2 and *p.* 3, but only to rejoin (*p.* 10, Diagram 7, Fig. 6). Also, the strands *p.* 4 and *p.* 6 (Diagram 7, Fig. 5) branch into two; the two branches of the former recombine (Diagram 7, Figs. 6 and 7), but not so the latter (*p.* 8 and *p.* 9). The changes at lower levels are much less complex; the large strand *c.* 18 gives off a slender lateral branch (Diagram 7, Fig. 6) which fuses on to *p.* 7 and *p.* 9, these three forming the larger bundle, *p.* 11 (Diagram 7, Fig. 7). Further, there is a general clearing up of the smaller plumular traces; *p.* 10 joins on to *c.* 17, similarly *p.* 8 unites with *c.* 18, the small strand on the right side of *c.* 10 joins with this bundle, and the xylem of the similar strand just below the right-hand protoxylem joins on to the protoxylem, whilst its phloem comes to an end (Fig. 7). Finally, the abnormally placed xylem elements in *c.* 17 and *c.* 18 creep inwards and join on to the normal wood (Diagram 7, Fig. 8). Thus there is formed a ring of collateral bundles, some of which consist mostly of cotyledonary vascular tissue and two, *p.* 4 and *p.* 11, mostly of plumular vascular tissue, from which the root-structure is organized. Of the root-structure, two xylem poles, those in the plane of the cotyledons, are already in position; the other two are formed in this wise: the two large bundles, *c.* 17 and *c.* 18 (Diagram 7, Fig. 8), divide, each into two; the halves do not show any rotation comparable to what obtains in so many of the foregoing plants, but move apart, leaving the protoxylem isolated (Diagram 7, Fig. 9). There is then a general centripetal displacement, so that the bundles intervening between the four groups of protoxylem-elements fuse to form four large strands, and thus a tetrarch root-structure is organized (Diagram 7, Fig. 11).

Leaving out of consideration the extraordinary series of anastomoses, it will be seen that the type of transition is the same as in *Allionia.* In brief, two protoxylems are isolated in the seed-leaves themselves, the cotyledonary traces enter the axis and form four large bundles (*c.* 1–*c.* 4), the corresponding pairs of which give origin to the two protoxylem poles situated in the intercotyledonary plane.

The main differences between the two plants are these: whereas in *Allionia* the tetrarch arrangement gives place to a diarch root-structure, in *Mirabilis* the tetrarch structure, so far as our observations show, persists; but this is not constant for the genus, for in *M. divaricata* reduction to diarchy takes place, and the same also applies to *M. multiflora.* Further, in *Allionia* the anastomoses between the bundles in the hypocotyl, although

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[1] Solereder: Systematic Anatomy. Oxford, vol. ii, 1908, p. 647.

complex, are not nearly so complicated as in *Mirabilis*, nor do the bundles form two distinct zones.

M. divaricata, Lowe, in essential features is like *M. longiflora*, only two points of difference having been observed: in *M. divaricata* the bundles corresponding to *c.* 17 and *c.* 18 in Diagram 7 are never collateral, and the tetrarch root-structure becomes reduced to diarch.

M. multiflora, A. Gray. Out of a large sowing there was obtained but one seedling, which was of interest owing to the small size of one cotyledon (Fig. 8) and to the presence of a prominent peg. Unfortunately an accident happened to the series of microtomed sections, so that we have no observations to make regarding the structure of the upper part of the hypocotyl; sufficient preparations remained, however, to indicate pretty strongly that the fundamental features of the transition are essentially the same as in other species of *Mirabilis*. As has already been remarked, the tetrarch root-structure becomes reduced to diarch.

FIG. 8. *Mirabilis multiflora.* Natural size.

This also is true of *M. Jalapa*, for Van Tieghem[1] found that out of ten seedlings examined six had diarch roots and four tetrarch.

From Gérard's[2] account it is clear that the transition phenomena of *M. Jalapa* are of the same nature as those of the species described above, particularly *M. divaricata*, for *M. Jalapa* shows the same reduction of tetrarchy to diarchy.

With regard to the behaviour of the traces within the hypocotyl we are not in agreement. Gérard observed the double series of bundles (Diagram 7, Fig. 5), but he considered that those of the cotyledonary series, not the plumular, moved outwards to form the outer zone (see Diagram 7, Figs. 2 and 3). Apparently also Gérard did not make any observations on the behaviour of the bundles within the cotyledons, and therefore did not realize that the transition really begins—or ends, according to the direction the tracing is done—in the seed-leaves; consequently we do not agree that the protoxylems of the root play no part in the orientation of the bundles.

SUMMARY AND CONCLUSION.

1. The transition phenomena of all the seedlings examined of the Natural Orders Portulacaceae, Caryophyllaceae, Amarantaceae, Chenopodiaceae, Phytolaccaceae, and Aizoaceae follow Van Tieghem's type 3; whilst those of the Nyctaginaceae follow a course which at first sight appears distinctive, but is clearly a modification of this same type.

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[1] loc. cit.

[2] loc. cit.

2. In all the first-named orders the differences which obtain between the various species are chiefly connected with the levels at which the transition phenomena first appear and end, and the level at which the lateral strands of the seed-leaves effect a junction with the divided central cotyledonary bundle.

3. With regard to the first point, the plants may be divided roughly into three classes:

A. The bundle from each cotyledon enters the hypocotyl in an undivided state, with the protoxylem in the endarch position. Examples: *Claytonia perfoliata*, *Pupalia purpurea*, *Salicornia europaea*, *S. perennis*, and *S. Smithiana.*

B. The main seed-leaf-bundle bifurcates at a level just above the cotyledonary node. Examples: *Dianthus barbatus*, *D. arenarius*, *Lychnis Viscaria*, *Gypsophila Saxifraga*, *Saponaria cerastoides*, *S. Vaccaria*, *S. vulgaris*, *Tunica prolifera*, *Cerastium perfoliatum*, *Stellaria graminea*, *Mesembryanthemum* spp., *Cucubalus baccifera*, and *Spergularia salina.* Of these the first one is intermediate between Classes A and B, whilst the last two are intermediate between Classes B and C.

C. The main seed-leaf-bundle bifurcates at a much higher level, sometimes even in the base of the blade of the cotyledon.

All the other species examined, and these comprise the majority, fall into this class; of them, the following show the isolation of the protoxylem-elements either in the petioles, e.g. *Atriplex hastata*, or in the lower regions of the blade of the cotyledon, e.g. *Amaranthus sylvestris*, *Gomphrena Haageana*, *G. globosa*, *Phytolacca paraguayensis*, and *P. dioica.*

This feature is not constant in the genera; thus *A. sylvestris* was the only species of the genus *Amaranthus* which exhibited it; also it was not observed in *Phytolacca acinosa.*

Another character shown by some of the members of this class is the delay in the fusion of the phloem-masses of the otherwise normal diarch root-structure. Examples: All species of *Amaranthus* and *Beta*, *Atriplex hastata*, *A. thamnoides*, *Obione portulacoides*, *Kochia trichophylla*, and *Corispermum hyssopifolium.*

4. With regard to the second point, relating to the lateral bundles, it may be remarked that in very few cases are these strands corresponding in importance to the laterals, say, of *Dahlia Merckii*; also it is rather difficult to make a satisfactory classification, for much depends on whether the seed-leaves have well-marked petioles or not. Roughly, the plants examined fall into two categories:

A. Those in which the lateral bundles of the blade do not extend down to the cotyledonary node but fuse on to the main seed-leaf-strand at the top of the petiole or a relatively high level. This is constant in the Portulacaceae, and in the Caryophyllaceae with the exception of *Saponaria.*

S. cerastoides conforms to type, whilst *S. Vaccaria* and *S. vulgaris* fall into the next category. Of the Aizoaceae, *Mesembryanthemum*, the cotyledons of the species of which show no differentiated petioles, is to be included in the present class, whilst *Tetragonia* is to be placed in the next.

B. Those in which the lateral strands, generally one on either side of the main bundle, extend down as far, or nearly as far as the insertion of the seed-leaves, or in some cases even into the hypocotyl, before fusion with the central strand takes place. Examples: The Amarantaceae, with the exception of *Amaranthus sylvestris* and species of *Gomphrena*, which approach Class A; the Chenopodiaceae, with the exception of *Chenopodium ambrosoides*, *C. Botrys*, *Atriplex thamnoides*, *Kochia*, *Corispermum*, and *Suaeda dendroides*, which belong rather to the previous class; and, lastly, the Phytolaccaceae.

5. A comparative examination of these facts shows that differences sometimes occur in different examples of one species; the dissimilarities between some species of a genus may be greater than the differences between genera; further, *Pupalia*, *Claytonia*, and *Salicornia*, genera widely separated, show very close resemblances.

6. With regard to the Nyctaginaceae, the transition phenomena may be looked upon as a modification of type 3, for after each cotyledonary strand has bifurcated and isolated within the blade of the seed-leaf those protoxylems situated in the cotyledonary plane, corresponding bundles from opposite sides together form the two protoxylem poles situated in the intercotyledonary plane. To this, however, *Abronia* is an exception, three poles of the root-structure being formed from the bundles of the larger cotyledon, and the remaining pole from the vascular tissue derived from the smaller seed-leaf.

7. A connexion between the Nyctaginaceae and the other Natural Orders of the Cohort may be traced. For example, in *Amaranthus sylvestris*, *Gomphrena globosa*, *G. Haageana*, *Phytolacca paraguayensis*, and *P. dioica*, the protoxylem is isolated in the cotyledons; in *Amaranthus*, *Gomphrena*, and other plants there is, in the axis, a delay in the fusion of the four phloems; in other words, for a certain distance downwards the structure is incompletely tetrarch. In this connexion it will be remembered that it is not at all uncommon for the initial tetrarch structure in the Nyctaginaceae to be reduced to diarch.

8. Without entering into any theoretical considerations—they will be published later—it may be pointed out that the Nyctaginaceae have by far the largest seedlings as compared with the other Natural Orders of the Cohort; that the vascular bundles of the large cotyledon of *Abronia* form three of the four poles of the root-structure, the remaining one being from the traces of the small seed-leaf; and that in the much smaller seedlings of the orders other than the Nyctaginaceae a difference is often found in the

structure of two seedlings of a genus which are of different sizes. Thus the seedling of *Saponaria Vaccaria* is much larger than that of *S. cerastoides*; in the former plant the laterals fuse on to the central bundle of the seed-leaf at a much lower level than in the latter plant. *Spergularia salina* and *Spergula arvensis*, *Atriplex littoralis* and *A. thamnoides*, *Suaeda maritima* and *S. dendroides*, and *Phytolacca acinosa* and *P. dioica* are further examples. Also it may be mentioned that amongst the smallest seedlings examined were those placed in Class A (par. 3), and these exhibited the simplest structure. These facts, together with others of similar nature observed in other classes—for instance, the Gymnosperms—suggest that the transition phenomena may depend on the relative abundance and the distribution of the cotyledonary vascular supply, which is in turn influenced by various factors. This will receive more particular attention in our next contribution to the subject.

9. With regard to features of interest not directly connected with transition phenomena, the presence of an endodermis around the cotyledonary bundles of certain species of *Mesembryanthemum* may be referred to.

In conclusion we desire to express our thanks to Mr. Hales, the Curator of the Chelsea Physic Garden, for his never-failing kindness in rearing seedlings for our use.

Cordaites Felicis, sp. nov., a Cordaitean Leaf from the Lower Coal Measures of England.

BY

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With Plate XXII and one Figure in the Text.

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INTRODUCTION.

IT is not uncommon to meet with Cordaitean leaves in the petrified material from British Coal Measures. As a rule, however, the fragments are badly preserved, and beyond the fact that some of them bore a general resemblance to *Cordaites Wedekindi*, Felix,[1] little has been observed about them.

In two blocks recently presented to me by Mr. Sutcliffe from his mine at Shore, Littleborough, specimens have been met with which are better preserved, and were therefore photographed. A few of these photographs have been reproduced on Pl. XXII, and a camera drawing of a single bundle is reproduced as a text-figure.

Although these new specimens bear a somewhat close resemblance, as above said, to *C. Wedekindi*, other sections more closely resemble the other two forms described by Prof. Felix under the names of *C. loculosus* and *C. robustus*. For this reason, and because a closer comparison revealed constant minor differences from all three forms, it has seemed necessary to refer to the new specimens under a different specific name.

The name *Cordaites Felicis* has been selected because of the much closer affinity they show to Prof. Felix's forms than to any of the French forms.

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[1] Felix: Steinkohlen-Pflanzen. Preuss. Geol. Special-Karte (Abhandlungen), Bd. vii, 1885-7.

[Annals of Botany, Vol, XXVI. No. CI. January, 1912.]

All the micrographs on Pl. XXII (except Fig. 11) are taken from specimens in a single series from the same boulder. The expense of the work has been in part defrayed by a contribution from the Royal Society Government Grant Committee.

GENERAL ACCOUNT OF THE LEAF.

As may be seen by reference to the micrographs, the specimens exhibit a considerable range of structure, but there seems to be no ground for supposing that more than one species is represented. In the leaves of some recent Conifers, as was shown by Seward and Ford in their monograph on the Araucarieae,[1] the leaf bundles may be diploxylic near the apex, but show no centripetal xylem at the base. Maslen also points out[2] that the absence of centrifugal primary wood in the specimens described by Dr. M. C. Stopes[3] may be due to their having come from distal parts of the leaf, adding: 'Until sections of the lamina of *Mesoxylon Sutcliffii* have been identified, it will be impossible to determine to what extent the centrifugal portion of the bundles persists out into the leaves.'

In the specimens under consideration the thicker parts of the lamina contain bundles with a fair development of centrifugal wood and the bundles are frequently shown in the process of division (Figs. 5 and 6).

In thinner parts of the lamina (i. e. presumably more distal or more peripheral), the centrifugal elements become smaller, are relatively few in number, and in some cases are absent altogether.[4]

The distribution of the sclerenchyma is different in the thicker from that in the thinner parts of the lamina. In the former there is an almost, if not quite, continuous hypodermal layer beneath each surface. In the thinner parts of the leaf the hypodermal layer is interrupted, but both regions of the leaf show supporting plates vertical to the surfaces of the leaf. These plates form complete partitions running the length of the leaf between the bundles and alternating with them (Fig. 3).

They appear in transverse section like columns, and are the more interesting as they do not occur in any of the forms described by Grand'Eury or Renault. They are well represented, on the other hand, in the forms described by Felix, e. g. *C. Wedekindi*, *C. loculosus*, and *C. robustus*. As in most Cordaitean leaves so far described, the vascular bundle is surrounded by a sheath attached to both surfaces by thickening in

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[1] Seward and Ford: The Araucarieae, Recent and Extinct. Phil. Trans. Royal Soc., B., vol. cxcviii, 1906, p. 370.

[2] Maslen: The Structure of *Mesoxylon Sutcliffii* (Scott). Annals of Botany, vol. xxv, 1911, p. 406.

[3] Stopes: On the Leaf Structure of *Cordaites*. New Phytologist, vol. ii, 1903.

[4] It seems open to question whether this statement is strictly justifiable, as the inner sheath, which appears to represent the centrifugal xylem, is generally present even when the more central elements are undifferentiated (*vide* Figs. 8 and 10).

the hypoderm. In *C. Felicis* there are also thickened masses of hypoderm between the bundle strand and the partitions (Fig. 10). On the upper surface these masses are surrounded by but slightly differentiated palisade, while on the lower they are surrounded by the lacunar tissue connected with the stomatiferous epidermis.

The horizontal section shown in Fig. 3 is equidistant between the upper and lower surfaces of the leaf and thus escapes the hypodermal masses. The vascular bundles with their well-developed sheaths are approximately circular in section (Fig. 10), and relatively to the thickness of the leaf are larger than those of *C. Wedekindi*, Felix. In the parts of the leaf where division is most frequent, the bundle sheath is scarcely differentiated (Fig. 5). Correlated with the absence of the sheath the partition is more strongly constructed (Figs. 2 and 5), and the specimen approximates to the *C. robustus* type of Felix.

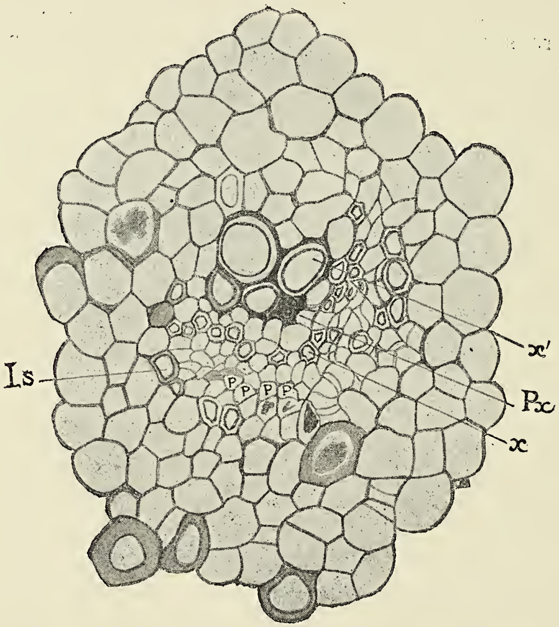
When present the sheath is composed of layers of longitudinally disposed fibres. In some cases pits are shown on their walls, but as a rule the later deposited thickening layers of the wall are dissolved before petrifaction, and the pits are then no longer distinguishable.

The innermost layer of the sheath is generally composed of cells of narrow calibre, and so the abaxial region of the bundle is partially composed of cells like those of the centrifugal primary xylem, which they slightly exceed in size. This sheath may be present even when no normal centrifugal elements may be differentiated (Fig. 8, *Is*).

To turn to the vascular elements proper, we note that the upper part of the xylem consists of a few large, porose, centripetal tracheides which increase in number as the leaf thickens. A considerable number may be present in the basal part of the leaf, especially when the bundle is dividing (Fig. 6). In the non-dividing bundle the centripetal wood radiates off from a small protoxylem group, which in turn is often separated from the centrifugal elements by a layer of parenchyma. In transverse sections the centrifugal elements, which are small and spiro-reticulate, lie in an irregular crescent which reaches to the periphery of the centripetal wood above and the small-celled inner sheath laterally. Thus, if we regard the tracheides of the inner sheath as primary transfusion tissue derived from xylem, there seems more reason to connect it with the centrifugal xylem than with the centripetal. It will be observed from the text-figure that the tracheides of the inner sheath (*Is*) are of slightly wider calibre than those intervening between them and the protoxylem (*Px*).

It is possibly the increasing development of this inner sheath in later forms that explains the more common absence of centrifugal wood in *C. principalis* as described by Dr. Marie C. Stopes. Below the centrifugal elements is a layer of xylem parenchyma which again is succeeded by the phloem (text-figure, *P*).

The specimens which are here regarded as representing the thicker parts of the leaf of *C. Felicis* correspond in structure much more closely to the forms called *C. robustus* and *C. loculosus* by Felix. That such forms represent the non-assimilating part of the leaf seems obvious from their structure, as there is no apparatus for gaseous exchange with the external atmosphere. Moreover, they occur with sections possessing a stomatiferous surface,[1] and all grades of intermediate structure occur. The treatment of such forms by Felix as different species has rendered it necessary to apply a new specific term to the new type, as it is impossible to identify it with any one by itself of the three forms described by Felix. Otherwise it might have been best to avoid introducing a new specific term on differences which do not amount to much more than that of size and robustness of development (*vide* next section).



TEXT-FIG. Transverse section of a single bundle surrounded by its sheath. × 225. The centripetal wood (x') is in marked contrast with the centrifugal (x). The protoxylem (Px) is separated by a single layer of parenchyma from the centrifugal wood. The elements of the centrifugal wood which contribute to the inner sheath (Is) are larger than the intervening elements. The inner sheath is limited to the abaxial part of the bundle, but the outer sheath is well developed all round the bundle. Some of the phloem elements are indicated by the letter P. Shore, C. N., 72. I.

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[1] Felix: loc. cit., p. 62. 'Other leaves of this species, i. e. *C. Wedekindi*, occur in the section from the Kgl. geol. Landesanstalt, No. 109.' It was in this section that the specimens called *C. loculosus* occurred (*vide* p. 71).

COMPARISON OF THE NEW SPECIMENS WITH C. WEDEKINDI, C. LOCULOSUS, AND C. ROBUSTUS, FELIX.

The dimensions of the various parts have been ascertained by my friend, Miss Lockhart, B.Sc., and comparison made with those cited by Felix for his forms. It is found that the leaf decreased in thickness from the parts where division of the bundles was taking place (Figs. 5 and 6) to the parts where division seems to have ceased (Fig. 10).

The thickness also decreased from the centre to the periphery. The margin of the leaf for about a width of 2 millimetres was not more than 19 μ in thickness. From the data available the whole width cannot have been less than 16 mm.—possibly more, but no complete transverse sections are available. The thickness ranges from 19 μ to 88 μ, but Felix reports for *C. Wedekindi* a range from 82 to 99 μ. The space intervening between the partitions ranges from 47 μ to 60 μ, whereas Felix reports a range from 55 to 66 μ. The partitions are seldom less than three fibres in width and often show five even in the upper parts of the leaf (Fig. 10, *Pa*).

In *C. Wedekindi*, on the other hand, even where division of the bundle is still taking place, the partitions are shown to be comparatively thin.[1]

The bundle sheath in the new species is also relatively better developed than in *C. Wedekindi*.

In *C. Felicis* the leaf parenchyma is built up of transversely running strands of cells which are slightly elongated in the direction at right angles to the bundles. These strands show very narrow crevices between them which are exaggerated by the shrinkage of the cells in Fig. 3. The lacunae do not show in transverse sections of the leaf, as the sections are thicker than the cells or the lacunae. They can be detected with difficulty in longitudinal sections taken vertically to the surface between the bundles (Fig. 4), but are always clearly seen in horizontal sections (Fig. 3).

No crystals have been observed, though the cells occasionally show blackened contents—a state of preservation which appears to correspond in the bundle sheath with an incomplete differentiation of the fibre.

Thus *C. Felicis* appears to be a leaf of relatively robuster build than *C. Wedekindi*. It has a much wider range of structure than was attributed to *C. Wedekindi* by Felix—the term being used to include forms which somewhat resemble *C. loculosus* and *C. robustus*, Felix. The latter, however, like the '*Wedekindi*' form, exceed the basal sections of *C. Felicis* in size, for the section of '*C. robustus*' described reaches a thickness of 115 μ as compared with the 75 μ reached by a section of *C. Felicis* in what I regard as a corresponding plane (Fig. 5).

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[1] Felix: loc. cit., Pl. III, Fig. 4 (the only published figure of this species).

COMPARISON OF C. FELICIS WITH RENAULT'S SPECIES, 'C. PRINCIPALIS', ETC.

Renault describes and figures six forms under the specific names *C. tenuistriatus*, *C. angulosostriatus*, *C. rhombinervis*, *C. crassus*, *C. lingulatus*, and *C. principalis*. The two last-named species have been further examined by Dr. M. C. Stopes.[1] These forms all differ from *C. Wedekindi*, Felix, and from the new species in having only incomplete partitions between the bundles. The horizontally extended strands of cells of the leaf parenchyma are thus continuous from one bundle to the next in the median horizontal plane of the leaf. Three of the species—*C. rhombinervis*, *C. lingulatus*, and *C. tenuistriatus*—show much better differentiated palisade than occurs in *C. Wedekindi* or *C. Felicis*.

The use of the specific term '*Felicis*' for the new species seems therefore doubly appropriate, as it is obviously much more nearly related to the species described by Prof. Felix than to any other described species, and possibly both may have to be placed in a new genus when attribution to their parent plants is possible.

That such an end may be achieved in the near future is rendered more probable by the announcement of a new genus of Cordaitales on stem characters by Messrs. Scott and Maslen in 1910.[2] It may also be worth while to put on record that at least ten specimens of the supposed Cordaitean seed *Mitrospermum compressum*, Will.,[3] occurred in close association with the specimens of the Cordaitean leaf described and figured in this paper.

DIAGNOSIS OF CORDAITES FELICIS, SP. NOV.

Leaf of the general Cordaitean type with numerous parallel collateral bundles. Each bundle is surrounded with a well-developed sheath except in the thicker, presumably basal, part of the leaf.

Alternating with the bundles are complete fibrous partitions also disposed longitudinally.

The centripetal elements of the mesarch xylem are better developed than the centrifugal, and consist of two or more tracheides with porose marking. The centrifugal xylem is more abundant in the thicker parts of the leaf and is shown best in the region, presumably basal, where the bundles are dividing. Elements similar to the centrifugal wood partially line the abaxial part of the bundle sheath, and it is suggested they may

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[1] Renault: Cours de Botanique fossile, pp. 90–3; with Pl. XII, Stopes: loc. cit.

[2] Dr. D. H. Scott and Maslen: Preliminary Note, On *Mesoxylon*, a new genus of Cordaitales. Annals of Botany, vol. xxiv, 1910, p. 236.

[3] Dr. Agnes Arber: On the structure of the Palaeozoic Seed *Mitrospermum compressum*, Will. Annals of Botany, vol. xxiv, 1910, p. 491.

represent an incipient phase in the formation of primary transfusion tissue. The palisade is but little differentiated, and the leaf parenchyma is but slightly lacunar even on the abaxial surface of the leaf, which is stomatiferous except at the base.

The whole leaf has a markedly xerophilous character, and shows in its upper part a considerable resemblance to the type named *C. Wedekindi* by Felix, while in the basal parts it more resembles the types called *C. loculosus* and *C. robustus* by the same author.

EXPLANATION OF PLATE XXII.

Illustrating Dr. Margaret Benson's paper on *Cordaites Felicis.*

The following abbreviations are used :—*H* = hypodermal strand of fibres between bundle and partition ; *Is* = inner and *Os* = outer sheath of bundle ; *L* = lacunae of leaf ; *P* = phloem ; *Pa* = partition ; *x* = centrifugal xylem. *x′* = centripetal xylem.

All the slides come from the R. H. C. Collection. The micrographs, with the exception of Fig. 11, were taken and the sections cut by C. H. Wells, the Botanical Laboratory attendant.

Fig. 1. Transverse section, probably in the upper part of the lamina, under a low magnification to show distribution of bundles and sclerenchyma. C. N., 365. 1. × 20.

Fig. 2. Transverse section, probably in the basal part of the lamina, under a low magnification to show distinctive character. C. N., 365. 2. × 20.

Fig. 3. Horizontal longitudinal section in the upper part of the lamina. The plane of section is about equidistant from each surface. The cells of the leaf parenchyma are collapsed and the lacunae between the strands are therefore exaggerated. C. N., 368. 2. × 20.

Fig. 4. Longitudinal section vertical to the surfaces in plane between the bundle and the partition. The minute size of the lacunae is shown well. The palisade is to the right of the figure. C. N., 365. 10. × 95.

Fig. 5. Transverse section in thicker part, to show weak development of bundle sheath and corresponding increase in strength of partition. This figure should be compared with the low-power photograph shown in Fig. 2, and with Felix's figure of *C. robustus*, to which reference is made in the text. C. N., 365. 8. × 95.

Fig. 6. A single bundle from a section in the basal part of the leaf. There is much centrifugal wood and division of the bundle is proceeding. C. N., 365. 19. × 95.

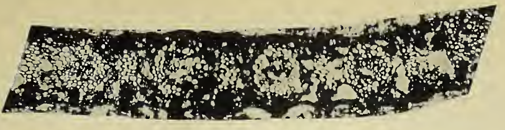
Fig. 7. Transverse section through a somewhat thicker part of a leaf, showing both centrifugal xylem and inner sheath. The compact nature of the leaf parenchyma is shown. C. N., 365. 12. × 95.

Fig. 8. Transverse section of a bundle in the upper part of the leaf, showing inner sheath but otherwise no centrifugal xylem. C. N., 365. 3. × 95.

Fig. 9. A transverse section showing incipient division of a bundle which sometimes occurs in the middle portion of the lamina. Divisions in this plane are carried out in bundles which have a normal sheath. C. N., 365. 4. × 95.

Fig. 10. Transverse section showing inner sheath and phloem. The hypodermal mass (*H*) intervening between the partitions (*Pa*) and the bundle strand is indicated; also the position of stomates is indicated on either side of the bundle strand. C. N., 365. 5. × 95.

Fig. 11. Photograph of transverse section from the same leaf as the text-figure is taken from. *P* = phloem, *x* = centrifugal xylem, and *Is* = inner sheath. C. N., 72. 1. × 225.



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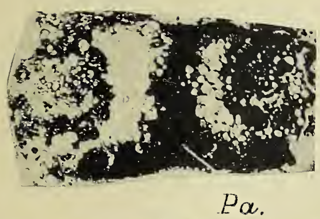

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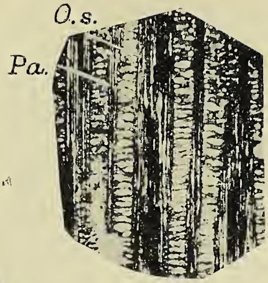

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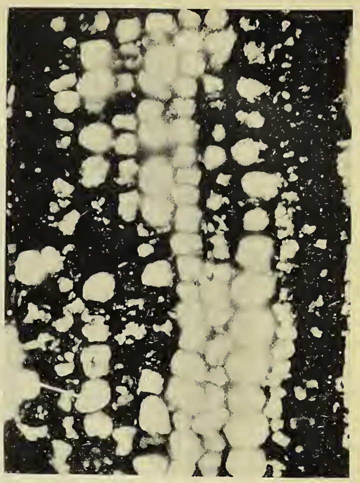

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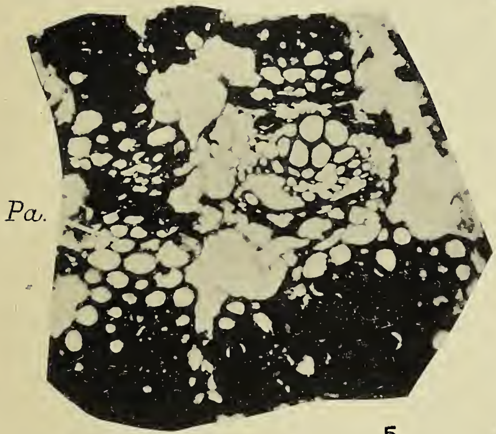

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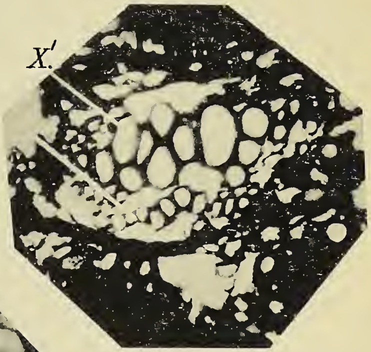

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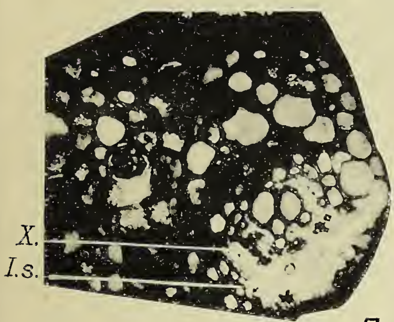

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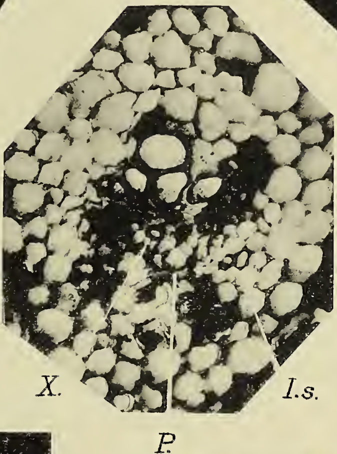

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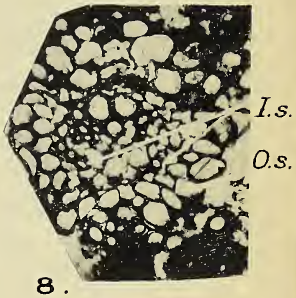

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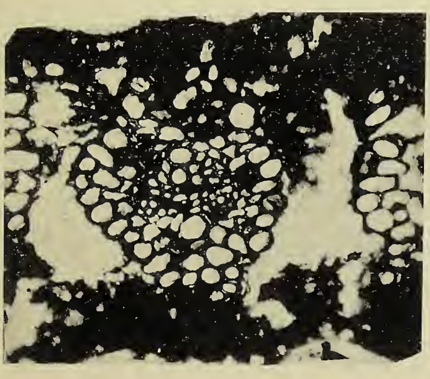

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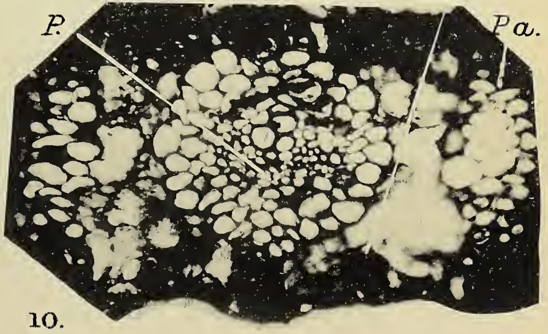

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BENSON—CORDAITES FELICIS.

Development and Sexuality of some Species of Olpidiopsis, (Cornu) Fischer.[1]

BY

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With Plates XXIII–XXVI.

1. INTRODUCTION.

TO students of the lower Fungi, there is an especial interest attached to the genus *Olpidiopsis*, because it is one of the few genera of the Chytridiales that possess a supposed sexual stage. There is also an interesting relation, historically, between the genus and other closely related forms, which emphasizes the danger of hasty conclusions made on imperfect and insufficient observations.

The genus *Olpidiopsis* was founded by Cornu (6), and although he did not diagnose the genus, it seems strongly probable that the main distinctive character was the presence in the life-history of its members of a resting spore to which was attached an empty smaller cell, called by him the 'cellule adjacente'. This term was, of course, indefinite, and although Cornu believed there existed a sexual relation between the two cells, he had no definite evidence.

Since the first recorded observation of a parasitic fungus on a member of the Saprolegniaceae by Nägeli (18), the same or closely related species have occasionally been described by various observers. In certain cases, the resting spores, which are known to be so necessary for the correct determination of the species, were not observed, hence we have no assurance what the forms were. However, the literature concerning these forms previous and subsequent to the founding of the genus *Olpidiopsis* is so related in one way or another, that it seems advisable to give a brief review of it here.

As stated above, the first reference to one of these organisms parasitic on the Saprolegniaceae was made by Nägeli in 1846. He observed in the swollen hyphae of what he called *Achlya prolifera*, now supposed to have been *Saprolegnia ferax*, elongated oval structures which eventually discharged their contents, as small motile bodies, to the exterior through

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[1] Contribution from Department of Botany, Cornell University, 142.

[Annals of Botany, Vol. XXVI. No. CI. January, 1912.]

a slender tube which perforated the wall of the enclosing hypha. These oval cell-like structures were considered by him as asexual propagative bodies of the *Achlya*. Cienkowski (5), who carefully illustrated and described the development of a similar organism in the swollen hyphae of *Achlya prolifera*, considered the oval bodies as a third form of sporangia of the host. He discovered and figured for the first time what he called 'Stachelkugeln', spiny spheres or resting spores. He did not observe their germination.

About the same time Alexander Braun (2) reported, after a long search, that he had succeeded in finding, in a few hyphae of a characteristic form of *Saprolegnia* on a sick but still living *Limneus minutus*, the oval bodies described by Nägeli. Some of these were empty, while others were still filled with dark granular protoplasm. He did not observe the discharge of the contents of any of these bodies, but from his own observations and from those previously made by Nägeli, he assumed that the structures concerned were not propagative organs, but parasites of the host. Braun placed the species in his new genus *Chytridium* with the name *C. Saprolegniae*, and considered the cell-like structures as zoosporangia and the motile bodies as zoospores.

Pringsheim (20), in his work on the sexual organs of the Saprolegniaceae, figures and describes the same or a similar organism. He, however, opposed the view of Braun in that he was inclined to interpret the oval or elliptical bodies found in the hyphae of *Saprolegnia ferax*, whose oogonia are unaccompanied by male organs, as antheridia, and the zoospores as motile spermatozoids. He also found accompanying the smooth sporangia the 'Stachelkugeln' of Cienkowski. Concerning these he says in part (p. 225): 'Diese Kugeln mit stacheliger Hülle treten entweder isoliert auf oder untermischt mit jenen anderen Körpern, welche eine glatte Hülle besitzen. Man könnte nun geneigt sein, diese Stachelkugeln als die zu den Körpern mit glatter Hülle gehörigen weiblichen Pflanzen zu betrachten, &c.' In his Fig. 15 *a*, Pl. XXIV, he illustrates a spiny resting spore with what appears to be an attached empty companion cell. He did not observe the germination of the resting spores.

Not until the appearance of Cornu's 'Monographie des Saprolégiées' was the question concerning the nature of these organisms again seriously considered. In the meantime it rested as left by Pringsheim for an interval of some twelve years. Cornu supported the view of A. Braun in considering the bodies in question as parasitic organisms. His careful observations did not include all stages of the life-history of the organisms, nor did he definitely determine experimentally their parasitic nature. He described five species parasitic on members of the Saprolegniaceae, three on species of *Achlya*, one on *Saprolegnia*, and one on *Aphanomyces*. These he included among the Chytridiaceae and in his newly established genus *Olpidiopsis*.

Cornu discovered in three of the species an apparently new and interesting type of resting spore, the large spiny cell being accompanied by a smaller smooth, or slightly echinulate, one which he designated as the 'cellule adjacente', as previously mentioned. In the diagnosis of *O. Saprolegniae*, (A. Br.) Cornu, he specifically states that the presence of an adjacent cell is a distinguishing character of that species. He comments upon the fact that, inasmuch as resting spores were not seen by A. Braun, it is impossible to determine the species described by him, but wishing to preserve Braun's specific name, Cornu used it for his species found on *Saprolegnia*.

Cornu attributed to this new type of resting spore a sexual character. He considered the large cell an oogonium, and the small one an antheridium.

Reinsch (21), some years later, observed a species of *Olpidiopsis* in the ends of swollen hyphae of a species of *Saprolegnia*. He described three types of cells occurring in the same filament. He observed the spiny resting spores with which were associated smaller spherical cells that eventually discharged their contents into the larger. Changes in the protoplasm of the cells were noted, and the actual transfer of the male protoplasm was observed. He states that Pringsheim's assumption as to the sexual nature of the spores is correct, but makes no reference to Cornu's work, which, apparently, he had not seen.

In 1883 Zopf (26) described an interesting species of *Olpidiopsis* parasitic in *Spirogyra*, which he named *O. Schenkiana*. This species is of particular interest in that it shares the peculiarity with one other species of the genus of possessing uniciliate zoospores.

It was observed that the sexual plant arose as a single mass of protoplasm which later put out an evagination to form the companion cell. At the time of fertilization no pore between the two was detected, and Zopf suggested that a diffusion of the male protoplasm through the wall itself took place. At any rate, the antheridium was completely emptied. Zopf had no doubt of the sexual nature of this spore.

The following year, Fisch (10) published an account of a new form, for which he erected the genus *Pleocystidium* and named it *P. parasiticum*. The species resembles very much *O. Schenkiana*, and was likewise found parasitic on *Spirogyra*. One important difference exists in the manner in which the sexual spores are produced. In *P. parasiticum* they arise, according to Fisch, from two or more closely associated masses of protoplasm representing separate individuals. One of these becomes what he terms the central cell, and the others the companion cells, or antheridia. Fisch was not certain whether the cellulose membrane eventually formed about the spheres was developed around each individual separately or around all as a whole. He was inclined to the latter view. The actual passage of the protoplasm from the antheridium into the oogonium was observed.

The investigations of A. Fischer (11) contain a very careful description of his studies on the development of what he supposed to be *Olpidiopsis Saprolegniae*, (A. Br.) Cornu. Unable, after much searching, to find an adjacent cell associated with the resting spores, he concluded that so far as *O. Saprolegniae* was concerned it was not present, and he strongly suspected that it was absent in Cornu's other species. Believing that Cornu was mistaken concerning this point and the diagnosis of his genus was incorrect, Fischer (12), in a later work, rejected the adjacent cell character and made the genus *Olpidiopsis* to include only those forms with resting spores which are not formed as the result of a sexual act. He accepts three of Cornu's species and considers the other two doubtful.

Schröter (22), in the 'Kryptogamenflora von Schlesien', accepted the change made by Fischer, and established the new genus *Diplophysa* for these parasites possessing an adjacent cell.

In 1892, in Rabenhorst's 'Kryptogamenflora', A. Fischer (13) gives an explanation of the cause of the confusion which his former work created. In the meantime he discovered a species on *Saprolegnia* which possessed a resting spore with an adjacent cell. This fact convinced him of the correctness of Cornu's observations, and he therefore restored the genus *Olpidiopsis* for the form with the adjacent cell.[1]

Inasmuch as the life-history of these organisms, especially the supposed sexual stage, is still imperfectly known, through the suggestion of Professor G. F. Atkinson, to whom I am very grateful for his continued advice and kindly criticisms, I am led to take up a study of a few of these forms.

2. STUDY OF LIVING MATERIAL.

A. **Methods and Material.**

The results of observations presented in this paper are based upon studies made on three species of the genus *Olpidiopsis*, Cornu (emend. A. Fischer), collected in the vicinity of Ithaca. Two are parasitic on *Saprolegnia*, the third on *Aphanomyces*. One of the former, found on *S. ferax*, agrees in every way with the species described and illustrated by A. Fischer in Rabenhorst's 'Kryptogamenflora' as *O. Saprolegniae*, Cornu, but which, for reasons to be stated later, I believe is not Cornu's species, but a new one for which I propose the name *O. vexans*. The other two species, one found on an undetermined *Saprolegnia*, and the other on *Aphanomyces laevis*, are strikingly similar in morphological characters, but because of their biological difference they are believed to

[1] l. c. p. 34. 1. *Pseudolpidium Saprolegniae* mihi. Synon.: *Olpidiopsis Saprolegniae*, A. Fischer, Schröter. *Olpidiopsis Saprolegniae*, Cornu pro parte. 2. *Olpidiopsis Saprolegniae*, (Cornu) mihi. Synon.: *Olpidiopsis Saprolegniae*, Cornu pro parte. *Diplophysa Saprolegniae*, Schröter. *Pleocystidium*, Fisch.

be distinct. The species found on *Aphanomyces* is considered new and is given the name *O. luxurians*, while the other occurring on *Saprolegnia* is believed to be Cornu's species *O. Saprolegniae.*

These organisms appeared in water cultures prepared for the purpose of obtaining terricolons and aquatic Phycomycetes. Water from streams and pools containing Algae, dead twigs, and débris of various kinds, was placed in small glass vessels in fresh water. When soils were used, about one half-inch was placed in the bottom of the vessel and covered with tap-water one-half to one inch above the soil. The vessels used were provided with glass covers, and measured four inches in diameter and two and a half inches in depth. As traps, various vegetable and animal tissues were tried, such as sections of boiled root of *Abutilon*, suggested by Butler (4), sterilized flies, millipedes, Dipterous larvae, the larvae and pupae of ants, aphids, boiled egg albumen, &c. Although all of these, with the exception of the *Abutilon* root and egg albumen, proved to be more or less serviceable, the sterilized flies, aphids, and ant pupae were more extensively used. Ant pupae and larvae served best when cytological studies were made.

Usually in cultures of this nature the most frequent forms secured are species of *Saprolegnia* and *Achlya.* In my cultures, however, *Aphanomyces laevis* proved to be more common than *Achlya* species, and was very commonly associated with some species of *Saprolegnia.* When any Chytridiaceous parasites were present, particularly on *Saprolegnia,* they could be seen with the naked eye within a few days after the culture had been started. Their presence is detected by the bright whitish specks seen among the radiating hyphae of the host (Pl. XXVI, Fig. 75). These specks are the swollen hyphae with the enclosed developing parasite.

When the parasite was once located by means of small scissors, the filaments containing it were cut off under a dissecting lens, washed in sterile water, and transferred to dishes containing pure cultures of the host. By this method reasonably pure cultures were maintained, and infection experiments with the host in various stages of development were carried on.

B. Zoospores.

The zoospores of the three species agree in all important features. They are oval to broadly elliptical, sometimes slightly reniform or anteriorly truncate. In motion they are usually slightly pointed at the anterior end, to one side of which are attached two cilia, rather close together and of approximately the same length. At the point of attachment of the cilia there is a prominent refractive spot frequently referred to as the hilum. With the aid of the high-power objectives, the protoplasm is seen to be slightly granular and not homogeneous as has been described for other species. This condition is more clearly brought out in zoospores killed with osmic acid.

There seems to be considerable variation among the species of this and other related genera in the position and number of the cilia on the zoospores. Cornu (6) observed in all species studied by him but one cilium. A. Fischer (12) found in the case of *Olpidiopsis* (*Pseudolpidium*), *Rozella*, and *Woronina* that the zoospores possessed two cilia, one placed in front and extending to the fore, the other attached laterally and extending to the rear. This same relation of the cilia was later (13) observed by him for the zoospores of his *O. Saprolegniae*, and was considered to be the normal condition for the genus.

Butler (4) records some interesting observations in reference to this point. He found the arrangement of the cilia as described by Fischer to exist in *Olpidiopsis minor*, A. Fischer; *Pseudolpidium* (? *Olpidiopsis*) *Saprolegniae*, (A. Br.) A. Fischer; *P. gracile*, Butler; and *Pleolpidium inflatum*, Butler. It is interesting to note that in the last-named species, he observed what appeared to be a variation in the position of the cilia. This he attributed to the varying position of the zoospore in swimming. In *Pseudolpidium Aphanomyces*, (Cornu) A. Fischer, and in *Pleolpidium Pythii*, Butler, the cilia are attached laterally and at the same point.

It is difficult, in many cases, to observe the attachment of the cilia on the living zoospore. However, this can be done without difficulty with the proper apparatus. Zoospores were allowed to escape from the sporangium into a small hanging drop of water in a Van Tieghem cell, and there observed with a 2 mm. Zeiss apo. obj. by the aid of strong artificial light. As the zoospores come to rest the attachment of the cilia was clearly seen to be at the same point. As has been frequently observed in other species, one cilium of the zoospore in motion trails behind. My observations revealed that this rear cilium crosses at an angle the upper end of the zoospore, and leaving it usually at the side gives it the appearance of being attached at that point. It was not until a number of observations were made that this peculiar condition was detected. When once seen it was easily verified for all the species of *Olpidiopsis* studied.

The same condition was found on careful examination in stained preparations of zoospores. In cases where the stain was not too deep, the rear cilium could be followed crossing the upper end of the zoospore to its point of attachment near that of the other. Occasionally both cilia extend out free from the body of the zoospore and leave no doubt whatever as to their relative position. In cases which present the appearance of a lateral attachment for one of the cilia, this cilium appears to be shorter than the other, but is actually about the same length (see Pl. XXIII, Figs. 1, 2, and 15). A more careful examination of the zoospores of other species of these closely related genera may reveal that the above-described relation of the cilia is common, if not the general rule.

As has been described for other species (Butler, 4) there occurs in the life of the zoospores two distinct motile periods. After their escape from the sporangium they swim about actively for a few minutes, two to five, in the vicinity, and then gradually come to rest upon the slide or cover-glass, as the case may be. There is now seen a waving rhythmic movement of the cilia, accompanied by a gentle rocking of the zoospore. These movements become slower and slower, the cilia gradually shorten, and the body of the spore assumes a spherical form. Finally, at the end of seven to ten minutes the zoospore has become quiet, and the cilia, when visible at all, appear as short thickened knobs or projections about the length of the radius of the spore.

The period of rest may last only a few or as many as fifteen or twenty minutes, during which time one, sometimes two, vacuoles are seen to appear and pulsate regularly—in one observed case, at intervals of six to eight seconds—until the second period of motion begins. The reverse condition now takes place. The short cilia begin to move and lengthen, the body of the zoospore resumes the rocking motion, and in a few minutes darts away. There is no change in the position of the cilia, and the fact that, in many cases at least, they do not lose their identity during the resting period makes such an assumption seem improbable. Butler (4) failed to observe cilia on zoospores of *Pseudolpidium Aphanomyces* during the first period of motion when this was followed by a second. The fact that such were seen attached laterally on zoospores killed in the sporangium at the time of discharge led him to state (p. 133): 'From this it would appear probable that there is no alteration in the position of the cilia during the two periods of motion, but it is possible that cases occur in which the first period is suppressed, and the sporangium figured may have been such a case.' He further states: 'As in *P. Saprolegniae*, the occurrence of two periods of motion is not, I think, a constant phenomenon.'

Butler suggests that such a condition may be compared to diplanetism in the Saprolegniaceae and *Pythium*. Inasmuch as diplanetism seems to prepare the zoospore for a more or less continued period of activity, and that in the cases just described the second period may persist for some time, it seems that, so far as it concerns the Saprolegniaceae, such a comparison is, at least, worthy of comparison.

C. Infection of the Host.

Inasmuch as the observations of the writer on the penetration of the host by the zoospores of the parasites differ immaterially from similar studies made by previous investigators, it seems unnecessary to give more than the most important facts as they apply to the species under consideration. No complete observations relative to this point have been recorded, so far as I am aware, for any *Olpidiopsis* species parasitic on the Saprolegniaceae.

Cornu (6) briefly describes this phenomenon for certain species studied by him, while A. Fischer (12), for *Olpidiopsis* (*Pseudolpidium*), *Woronina*, and *Rozella*, records his very careful observations as to both penetration and subsequent development of the parasite.

The zoospores, after a swimming period, the length of which seems to depend considerably on the age and condition of the culture in which it occurs, finally settle down on some favourable part of the host and either produce infection or die. There is at first a slight amoeboid movement, followed by a rounding up of the zoospore body and the formation of a wall (Fig. 3). After resting in this condition for fifteen minutes to one hour, the encysted spore produces from the side in contact with the host a short cylindrical tube which penetrates the wall of the hypha. During this process the zoospores become pushed slightly away from the filament. The penetration tube varies somewhat in length in the different species, being shortest in *O. luxurians*, and longest in *O. vexans* (Figs. 4, 8, and 9). After the formation of the tube, the appearance of a small vacuole in the spore body indicates that the passage of its contents into the host has begun. As observed by A. Fischer (12) and Butler (4) this tube is so small that it is impossible to observe the passage of the protoplasm through it. Occasionally it could be seen extending into the cell cavity beyond the limits of the wall (Fig. 9).

By far the majority of zoospores in all three species fail to infect the host. A greater percentage succeed in *O. luxurians* than in the species on *Saprolegnia*, due, no doubt, to the fact that infection in this species is not so nearly confined to the young hyphae. Those zoospores which fail to enter the host die sooner or later, either by a direct disintegration of the protoplasm, or after germination. In the presence of the host, and usually near or in contact with the hyphae, zoospores frequently put out long slender germ tubes. These have been observed in large numbers on old empty sporangia of *Aphanomyces* and on the old threads of *Saprolegnia*. The germ tubes sometimes entirely surround the hyphae; in other cases they may extend some distance parallel to them (Figs. 5, 6). As has been observed by A. Fischer, the life of the zoospore is much longer in the presence of the host than in pure water.

The germinated zoospores of *Aphanomyces* are frequently infected by *O. luxurians* (Fig. 10). As many as four developing sporangia of the parasite have been found in a very short germ tube. In no case was an infection of gemmae or reproductive organs of *Saprolegnia* observed.

Just how far, if at all, chemotropic stimuli set up by the host influence the behaviour of the parasite zoospores is impossible to state. The writer has carried on no controllable experiments in reference to this point, but is convinced from careful observations that there is some varying attractive force exerted by the host hyphae on the zoospores of the parasite.

De Bary was inclined to the view that by the secretion of some chemical substance the oogonia of *Saprolegnia* may be capable of inducing the formation of antheridial branches. This assumption is strengthened by the work of Kauffman (16), in which he showed how the development of sexual organs of *Saprolegnia*, especially antheridia, could, to a certain extent, be controlled by the use of chemicals.

A. Fischer (12) theorizes that different organs of *Saprolegnia*, such as young hyphae, gemmae, and oogonia, perhaps give off different metabolic substances, and further that such substances vary in different genera. These diffuse into the surrounding water and within a certain radius exert an attractive or repellent force on the zoospores of the parasite.

D. Development of the Sporangia.

Growth period. After the penetration of the zoospore into the host, it can frequently be observed for a brief time as a small, more or less refringent mass of the protoplasm (Figs. 4, 10). It exhibits slight changes in shape, and in the course of an hour or so is entirely lost to view. The young parasite is now more or less surrounded by the host protoplasm, at the expense of which it begins to grow rapidly, and at the same time it exerts upon the host a stimulus which causes, frequently in a few hours, an abnormal development of the infected hypha (Figs. 7, 11, 76). Usually the parasite undergoes its development at or near the point of entrance. Frequent exceptions to this condition have been observed, however, and are also noted by Fischer for *Olpidiopsis* (*Pseudolpidium*).

The change of position is very probably passive and due to currents of host protoplasm. In cases where a number of sporangia occur in the same intercalary swelling there seems to be no other explanation than that some of the young parasites migrated to that point. Growth of the infected hyphae beyond the position of the parasite usually ceases soon after its infection, especially when such takes place near the end.

In the early stages of development the parasite contains few granules scattered through the homogeneous ground substance, most of them lying about the periphery (Fig. 12). There soon appear a number of larger granules which grow rapidly and present the appearance of being of a fatty or oily nature (Figs. 12, 13, 17). They frequently exhibit a vacuolate character, and show marked changes in form (Figs. 13, 23). Similar granules have been observed by Zopf in *O. Schenkiana* and by Fischer for both species of *Olpidiopsis* (*Pseudolpidium*) studied by him.

The parasite now becomes surrounded by a hyaline wall. The protoplasm has become more dense and contains many small granules along with the increased number of oil bodies (Figs. 17, 78). At this time there is a marked radiating condition of the host protoplasm which decreases rapidly in extent as the sporangial development proceeds (Figs. 17, 18, 79, 80). Vacuoles of rather indefinite outline now appear in the parasite (Figs. 18, 81), which soon round up into one or several nearly perfect spheres. The protoplasm is now dense and granular. The large oil or fat-like bodies have disappeared. The sporangium is now said to be ripe and may enter directly into the maturation stages or undergo a limited period of rest before zoospore formation takes place (Fig. 82). I have observed sporangia remain in this condition for several weeks without losing their power of zoospore formation (Fig. 19). The only necessary condition, according to my observations, to start up the internal changes which bring about the discharge of zoospores is that fresh water and oxygen be supplied.

Maturation. Concerning the maturation stages of *Pseudolpidium Aphanomyces*, Butler (4) has given a detailed account. The changes which take place in the ripe sporangium of the *Olpidiopsis* species concerned, just previous to zoospore production, agree in the main with those described for the above species. I find, however, that the minute details in the behaviour of the protoplasm vary somewhat with the different sporangia of the same species.

Soon after the proper conditions are supplied to the ripe resting sporangium, one observes a change in the contour of the prominent spherical vacuoles. Usually the exit tubes begin to develop at this time and the protoplasm has the appearance of already being segmented (Figs. 14, 82). No heapings of the protoplasm such as described by Butler for *P. Aphanomyces* were seen to take place at this time. In a short time the vacuoles suddenly disappear, when the protoplasm presents a rather fine granular, homogeneous, and lighter appearance. It is at this stage, according to Butler, that the first shaping of the spore origins in the sporangium of *P. Aphanomyces* occurs. After a few minutes there may or may not appear a number of smaller spherical vacuoles surrounded by the now denser protoplasm.

After fifteen to thirty minutes the vacuoles disappear and the protoplasm becomes more coarsely granular. The zoospores take on a more definite outline and their movement increases. Suddenly the exit tube opens and their escape takes place.

The number and length of the exit tubes vary with the different species. In *O. luxurians* they usually number one or two, and extend some distance beyond the host wall (Fig. 16). In *O. Saprolegniae* the number of exit tubes is small, one to three, and they are comparatively short. In *O. vexans* they are usually more numerous, often numbering as many as six. Fig. 20 shows a sporangium of *O. vexans* which rested after a number of exit tubes had been produced. Several of the tubes show a peculiar coiled condition of the end, while one is branched. This latter condition, which seems rare, was also observed once by Fischer in another species. Other sporangia in a similar stage, when given the proper conditions, failed to discharge zoospores.

The exit tube secretes an enzyme which enables it to penetrate the wall of the host. In many cases, at least, mechanical pressure seems to play an important part. I have several times observed that the host wall was stretched out over the end of the protruding tube even when apparently some little distance through it. Very commonly, as has been previously observed, there is an enlargement of the tube just inside the hyphal wall. The escape of the zoospores takes place by the gelatinization and dissolution of the end of the exit tube.

E. Development of Sexual Spores.

The frequency of occurrence of the sexual spores of the organisms under consideration varies with the species and the condition of the cultures under which it develops. It has been assumed, in the case of resting spores belonging to species of a closely related genus, that they were formed only after a somewhat prolonged production of the zoosporangia, and then for the purpose of tiding the parasite over unfavourable conditions such as winter. They have been rarely found in the species of *Olpidiopsis* infecting members of the Saprolegniaceae, which fact, no doubt, partially accounts for our incomplete knowledge concerning their development.

The first of these so-called resting spores found by the writer appeared in a culture of *O. vexans* on *Saprolegnia ferax* growing on ant larvae. Several cultures had previously been made on sterile flies with the result that only smooth sporangia developed, and it was, therefore, impossible to classify the organism. Three days after starting the culture above mentioned, sexual spores were numerous in all stages of development. New cultures were immediately started with a view to securing material for cytological studies. After making several series of cultures, I was somewhat surprised that the desired spores failed to appear. Almost a month of culture experiments finally revealed the requisite condition. Instead of starting new cultures in separate dishes, as I had previously been doing, I kept the old ones, to which at certain periods I added a new supply of ant pupae. These were disturbed as little as possible, and the water remained unchanged. There were always a sufficient number of zoospores of both host and parasite present to start a ready infection of the new substrata.

By this method the otherwise rapid and vigorous growth of the *Saprolegnia* was somewhat retarded, and a large number of sexual spores of the parasite developed. It was detected that some contamination by Bacteria, protozoa, &c., seemed to be an advantage, but when carried too far would inevitably prevent the desired results. Not all cultures with this species gave results, and the successful ones needed to be carefully watched in order that their productiveness be maintained. After the sexual spores had attained the desired stage of development most of the larvae were transferred to other vessels in order that new ones could be added. It was necessary, at times, to deviate in one way or another from the regular method described, in order to overcome one or more difficulties.

The sexual spores of *O. Saprolegniae* were as difficult to obtain in quantity as those of *O. vexans*. Those of *O. luxurians* were secured in by far the greatest numbers and with the least difficulty. The host, *Aphanomyces laevis*, was grown on green aphids, a supply of which was kept sterile in test-tubes. The value of the aphids as culture media was not discovered until most of the cultures of the other species were brought to a close, but the few trials made showed that the aphids were equally serviceable for them. They furnish a very limited amount of nourishment, in that one can find them of all sizes, which fact seems to bear a direct and favourable influence on sexual spore production of the parasite. Correlated with this factor of a small amount of nourishment is the limited amount of water, which should remain unchanged. One disadvantage in the use of small bits of substrata is the reduced size of the sexual spores of the parasite that results. Another advantage obtains in the use of this method. Having the zoospores of both host and parasite in the culture provides for the immediate infection of the host hyphae as they emerge from the substrata. This enables the parasite to get a good start before the hyphae reach any length, and prevents the formation of a tangled mass of hyphae, so objectionable in the preparation of the material for cytological study. The result is a dense halo of short swollen filaments containing the parasites in all stages of development. In the case of the two species on *Saprolegnia* it is really necessary that infection take place when the hyphae are young.

So far as my observations with the three species of *Olpidiopsis* go, there seems to be no foundation for a view that a production of sexual spores need be preceded by a somewhat continuous production of sporangia, or that the two need alternate with each other. On the other hand, if the proper external conditions are provided, such spores are more or less easily obtained.

It should be stated here that the above-described method also provided good material of the sporangia in the resting stage.

The sexual spores develop in the same position as the sporangia, with which they may or may not be associated. Usually, however, one finds one or more sporangia in the same hyphal swelling containing sexual spores. The greater the degree of infection of the host, the greater the variation in the size of both kinds of reproductive bodies. In general, the manner of development of the sexual spores of the three species agrees. There are, however, some slight differences, especially between *O. vexans* and the other two whose development resembles each other more closely. For the reason stated the former will be described separately.

The developing sexual cells of *O. vexans* are first detected in the young stage by the darker and more dense appearing condition of the protoplasm of, especially, the larger, or female, cell. It does not require very high magnification to see that closely associated with the female plant are one or more smaller light-coloured protoplasmic bodies. These develop into the antheridia, while the larger become the oogonia. Such an early condition is shown in Pl. XXIV, Fig. 26. No walls have formed about the cells at this stage, and the very close association of the individuals might suggest a division of a single individual rather than the partial fusion of two or more. At this stage, were it not for the presence of the female cell, one could not distinguish the male elements from the young sporangia of the same age.

As growth proceeds, the two cells become more and more distinct and soon surround themselves by cellulose walls, which become fused at the point of contact. Usually one can clearly distinguish what appear to be brace-walls joining the two cells, as seen in Fig. 27. The wall of the oogonium shows early the formation of roughenings on the outer surface, which eventually take on a warty appearance, and resemble an encrustation of some kind. The male cell, or antheridium, remains smooth.

Noticeable changes also take place in the protoplasmic contents of both cells. In the young stages of growth these resemble those that take place in the young sporangia. There is at first in the oogonial cell a very much vacuolated, finely granular protoplasm in which a few larger irregular granules are distributed. Most of the protoplasm lies in a thin layer about the periphery, from which irregular strands extend into the interior. At this time the male cell shows a somewhat similar vacuolate condition, but contains more granular protoplasm.

Following this stage, which, owing to rapid growth at this period, lasts but a short time, is a stage in which the number of large granules have greatly increased in both cells, to such an extent as to make difficult the observation of protoplasmic changes within, especially in the oogonium. Soon the contents of the female cell become more or less clumped, while that of the male shows a fine granular condition with frequently one or more vacuoles. The cells are now ready for fertilization.

This takes place by the passage of all the antheridial contents into the oogonium. The beginning of this phenomenon is indicated by the appearance of a large central vacuole or, as is more usual, a shrinkage of the protoplasm from the wall of the antheridium opposite the oogonium. The whole act may require several hours or may be completed in less than one hour. I have frequently observed long strands of protoplasm still clinging to the opposite wall of the antheridium after most of the contents had passed over. Very rarely does any protoplasm remain in the male cell after fertilization.

Before the movement of the protoplasm of the antheridium into the oogonium begins, one only rarely detects a communicating pore. In fact, in many cases, it cannot be detected even while the passage of the protoplasm is going on. The pore in the fused wall is small and soon closes by growth of the surrounding wall, apparently after the act of fertilization is completed. Owing to the thick encrustation on the oogonial wall, together with the coarsely granular protoplasm, I have been unable to see the movement of the male protoplasm after it has passed through the communicating pore.

As a result of fertilization the oospore forms about itself a wall in close contact with that of the oogonium. This wall rapidly thickens and serves to protect its contents against unfavourable external conditions (Fig. 21). The now empty antheridial wall may persist for some time in its normal shape, but eventually becomes more or less collapsed.

When more than one antheridial cell is attached to an oogonium there is no apparent difference in their behaviour other than that their contents may or may not simultaneously pass into the female cell. Although one usually finds that the movement has at least started in all male cells before any one is entirely emptied, exceptions to this have been observed.

In the case of the sexual spores of the other two species, *O. Saprolegniae* and *O. luxurians*, what is said of one will apply equally well to the other. Those individuals which are to give rise to sexual cells can be detected early in their course of development by the difference in the appearance of their protoplasm from that of the young sporangial cells, and by the close association of the two or more individuals. This early condition is not so easily detected, however, as in the case of *O. vexans*.

The protoplasm of both the male and female individuals is much vacuolated and somewhat coarsely granular. The limits of the cells are less clearly defined than those of *O. vexans* because of the closer resemblance of the protoplasm to that of the host (Figs. 24, 83). Later their individuality and form is much more marked, as their protoplasm becomes more differentiated than that of the host (Figs. 25, 84, 85). The oogonium contains large vacuoles surrounded by more or less granular protoplasm, while that of the antheridium is finely granular and homogeneous. At this stage walls have formed, and there is observed a pronounced clear zone of protoplasm about the oogonium, which exhibits a strong contrast to the dense granular layer of the host on which it abuts. Dangeard (7) calls attention to this zone in the resting spores of *Olpidiopsis* (*Pseudolpidium*) *Saprolegniae*, Cornu, as interfering with his observations of the nuclei.

The host protoplasm beyond this layer forms a complicated system of radially arranged strands which communicate with a dense peripheral layer, and along which one can frequently observe a movement of granules towards the parasite. This gradually disappears as growth of the sexual elements proceeds, until at maturity these cells lie in an empty swelling.

The vacuoles of the oogonium now become less apparent and the protoplasm is more or less filled with large and small granules. A similar condition prevails also in the antheridium (Pl. XXIV, Fig. 28). Their development is rapid, as is the disappearance of the host protoplasm which surrounds them.

After fertilization the oospore develops a thick endospore. Fig. 30 shows a mature oospore of *O. luxurians* bearing two empty antheridial cells. The content is differentiated into an outer zone, more or less finely granular, and a central mass of large, more or less regular granules in which is usually one or more large oil globules. The oil globules are not represented in this figure. Occasionally in these species one finds the peculiar condition of one antheridial cell attached to two oogonia, one of which is usually provided with a separate male cell (Pl. XXVI, Fig. 88). Fig. 85 shows a young oogonium with three antheridial cells attached.

The fertilization pore in the sexual spores of these species is, so far as can be determined in the living material, not unlike that described for *O. vexans*. It has frequently been observed, and in some few cases the stream of male protoplasm could be seen passing through it into the female cell. In one interesting case, while observing this act, the weight of the cover-glass, because of evaporation of water under it, suddenly came upon the oogonium as the last of the antheridial contents was passing into it. Immediately a stream of protoplasm was forced through the pore and the male cell was soon refilled.

As yet, no case of germination of the oospores has been observed for any of the species studied. A. Fischer (11) witnessed the germination of the resting spores of *Olpidiopsis* (*Pseudolpidium*) and Zopf the same phenomenon for *O. Schenkiana*. In both cases germ tubes were formed through which zoospores escaped.

Reinsch (21) observed, in a species of *Olpidiopsis* (according to A. Fischer *O. minor*), that at the point of fusion of the two sexual cells a slit formed in the wall which permitted the passage of the male protoplasm into the oogonium. This opening, after fertilization, was closed by growth.

In *O. Schenkiana* Zopf (26) saw no communicating pore or opening between the two sexual cells. To him the contents of the antheridium seemed to diffuse through the wall into the oogonium. I have not had the opportunity to observe the condition in that species, but judging from the ease with which such a pore might be overlooked in the species studied, it seems probable that it occurs in *O. Schenkiana*.

3. STUDY OF SECTIONS.

A. Methods.

The material used for sectioning was secured as described under the heading 'Methods and Material'. The substratum bearing the organisms was washed in several changes of water in order to free it as much as possible from all contamination, then dropped into small phials of killing solution. The latter consisted of weak and strong Flemming's solution, medium and strong chrom-acetic acid, and Gilson's fluid. After killing and fixing, the material was washed either in slow running water or by making frequent changes by means of a pipette. Dehydration was secured both by carrying the specimens through the grades of alcohol, and by evaporating down in 10 per cent. glycerine. After clearing in cedar oil, the material was embedded in paraffin. Sections were cut 2–5 μ thick and stained on the slide.

The stains used were Flemming's triple stain, with the orange G. dissolved in the clove oil, Heidenhain's iron-alum haematoxylin, and Gram's stain followed by eosin in clove oil. For most purposes material fixed in medium chrom-acetic acid and followed by the triple stain gave the best results. The same stain applied to material fixed with Flemming's solutions served best for the maturation stages of the sporangium, and for staining the nuclei of the host. Iron-alum haematoxylin was of little service, while Gram's stain was excellent for bringing out the nuclei during fertilization stages.

Very little histologically and cytologically has been done on the members of the family Olpidiaceae, and nothing, so far as I have been able to learn, on the genus *Olpidiopsis* in the sense used in this paper.

Dangeard (7) studied the histology of two species of the genus *Pseudolpidium*, then called by him *Olpidiopsis Saprolegniae*, Cornu, and *O. Aphanomycis*, Cornu. Inasmuch as the two genera are very closely related, it seems proper to give briefly the results of Dangeard's observations on the above species. The material studied by him was stained *in toto*, which prevented the clearest definition of the nuclei, especially in the resting spores.

According to Dangeard, the sporangia of *O. Saprolegnia*, Cornu, present two principal phases in their development. In the young condition the protoplasm is very much vacuolated, and is composed superficially of broad meshes at whose nodes are located the nuclei. The nuclei are very small and appear as simple masses of chromatin. From a uninucleate to a multinucleate condition the change is rapid, there being present about twenty at the time the sporangium reaches its final size. Later, the nuclei which have increased in number space themselves, and show dense, rather large masses of chromatin, which are surrounded by nuclear hyaloplasm.

Successful examination of the histology of the resting spores was impossible. Dangeard observed that the dark zone of colourless protoplasm about the developing spore hindered observation. He states that they are multinucleate.

Concerning the study of the second species, *O. Aphanomycis*, Cornu, nothing of importance was added. Dangeard observed a spiny resting spore without a companion cell, which would place this species in the genus *Pseudolpidium* as *P. Aphanomycis*.

B. **Sporangia.**

Fig. 31, Pl. XXIV, represents a young parasite soon after its entrance into the host. It is uninucleate, much vacuolated, and not unlike Fig. 35, which is a zoospore from a discharging sporangium. The protoplasm, which is finely granular, exists in the form of a parietal layer. Growth of the individual has not proceeded far until the single nucleus divides.

Fig. 32 shows the binucleate stage. The nuclei are prominent and contain large deeply staining bodies which are presumably nucleoli. As is seen in Fig. 33, the nuclei divide mitotically. Fig. 34 is a section through a slightly older individual, showing three of its four nuclei and the very much vacuolated and granular nature of the protoplasm. It is assumed, from the condition of the protoplasm, that it is about this time in the life of the individual that it becomes lost to view in the host.

Nuclear divisions proceed rapidly, and we soon find the condition shown in Fig. 36. It will be observed that the protoplasm lies principally in the peripheral layer, in which are embedded the many still prominent nuclei. There is also observed a tendency for small granules to collect about the outside of the parasite, indicating, perhaps, the beginning of the formation of a wall, as a very little later one finds that a thin wall has developed. The large irregularly shaped fat bodies observed in the living specimens are in most cases not visible in stained sections.

Following the condition shown in Fig. 36, there is a gradual inward growth of the protoplasm, accompanied by an increase in size of the parasite and a continued rapid nuclear division. A little later the sporangium is almost filled with fine granular protoplasm in which are distributed a few large and numerous small irregularly shaped vacuoles. Fig. 37 represents a section of a small sporangium at that period of development with its nuclei undergoing their last division. The sporangial wall is very distinct and the sporangium has probably reached its maximum size. The large vacuoles have become regular in outline and at the same time the smaller ones show less distinctly.

Fig. 38 illustrates that condition. It shows the appearance of the nuclei after the last division, and around which the individual zoospores are to form. Chromatin masses are arranged about the nuclear membrane in more or less irregular areas. It is in this stage that the sporangium may undergo a period of rest.

I have observed no indication of an amoeboid or otherwise irregular stage of the parasite. A. Fischer (11) describes such a condition as common for *O.* (*Pseudolpidium*) *Saprolegniae* even after the parasite had reached some size (see Pl. X, Fig. 5, *a–d*). In the young living specimens it is sometimes very difficult to determine the limits of the host and parasite protoplasms, but in stained sectioned material such a difficulty disappears, for the reason that the protoplasm of the parasite has a different affinity for stain than that of the host. There is no indication whatever of a fusion of two or more parasites to form one, and it seems very evident that each zoospore after entering the host maintains its individuality and gives rise to a single sporangium. In other words, no plasmodium is formed. In abundantly infected material one frequently finds in section a number of very small individuals, sometimes still uninucleate, accompanying the larger ones in swollen hyphae whose protoplasm has been exhausted. In some cases these small parasites have taken on a mature state and may give rise to a small number of zoospores.

Fig. 39 represents a portion of a section of a sporangium in the vacuolate resting condition, and of the same age as that from which Fig. 38 was drawn. The former was killed in weak Flemming's solution, the latter in medium chrom-acetic acid. This section shows very clearly that the uninucleate spore centres are not only definitely formed at this time, but that fragmentation has, at least partially, taken place. The nuclei take a deeper stain than those shown in Fig. 38, in which lines of segmentation of the protoplasm are not visible. There is no apparent indication of vacuoles in the young spores, which indicates, it seems, that during the resting period of the sporangium the water of imbibition of the spore centres is at a low ebb.

From this and many other similar sections I think it is evident that the spore centres are formed before the resting period of the sporangium ensues. The changes which take place subsequently to the resting period, and which have been supposed to give rise to the fashioning of the spore centres, are merely the phenomena accompanying the final stages in the development of the zoospores.

So far as could be determined, fragmentation of the protoplasm is simultaneous throughout the sporangium much as described by Dangeard (7) for the primitive cell of *Synchytrium Taraxaci*. When exit tubes are present at this stage their protoplasm likewise shows segmentation into spore centres.

At the time of discharge of the sporangium the vacuoles disappear, while they are very evident in the zoospores themselves. The zoospores reach the exterior then, not, as Harper (15) described for *Synchytrium decipiens*, in the form of concentrated bits of protoplasm, but as definitely vacuolated structures (Fig. 35). Butler's assumption that an increase in the imbibition water is necessary to the final fashioning of the zoospores seems to be substantiated by my sections.

In sections stained with Gram's stain there are seen in the vacuoles of the sporangia spherical or oval faintly staining bodies the nature of which was not determined (Pl. XXIII, Fig. 22). They were not observed after the use of any other stain. They need further study.

C. **Sexual Organs.**

Very early in the development of the individual parasite, which is to become the oogonial cell, there is a noticeable difference in its staining reaction. While the protoplasm of all the parasites destined to give rise to sporangia and antheridia has a strong affinity for gentian violet, that of the oogonial cells shows very early a tendency to give up that stain and hold to the orange G. This fact enables one to recognize the young oogonial cell very early.

Most of the material studied in section was that of *O. vexans* because of the larger sexual individuals and their better response to stains. In the youngest oogonial cell of which I could be sure, the nuclei already numbered twenty-five to thirty. Some were distributed more or less irregularly throughout the contents, while the rest were arranged about the periphery embedded in a thin layer of finely granular protoplasm.

Fig. 40, Pl. XXV, represents the earliest stage seen in section in which both male and female cells showed. Enclosing walls have not yet formed and the two gametes are in close contact with each other. The nuclei of both individuals are undergoing mitotic division, and both show a marked vacuolate condition. The male cell possesses, as is the usual condition, proportionately more granular protoplasm. Most of the protoplasm of the oogonium is arranged about the periphery and contains many of the nuclei.

Following this condition there is a rapid increase in the size of the gametes and in the number of nuclei. By the time the cells reach their mature size they are enclosed in cellulose walls. The wall of the antheridium remains smooth, while that of the oogonium soon begins to take on a roughened character.

Fig. 41 shows portions of the two individuals with their walls fused and brace-walls formed. Already gelatinization of the common wall is taking place to form a fertilization pore. This can be detected by the deep stain of the wall at that point. The protoplasm is now more coarsely granular and much vacuolated, especially in the female cell.

Fig. 42 shows an advanced stage of gelatinization of the wall and its diffusion into the contents of the two cells. The nuclei of the oogonium are in the resting condition, while those of the antheridium are undergoing division. Fat globules are scattered throughout the female protoplasm, which is still sparse and exhibits a rather poor affinity for stain in contrast to that of the antheridium.

The next condition that one observes is the beginning of the passage of the protoplasm of the antheridium into the oogonium. Fig. 43 represents a section of the sexual cells of *O. luxurians* stained with Gram's stain, which shows this stage. It is interesting to note that the female nuclei located at the nodes of the protoplasmic meshes are very faint. This is due to the fact that, in order to bring out the male nuclei embedded in the dense, deeply staining protoplasm and gelatinized wall substance, it was necessary to decolorize to such an extent that the female nuclei lost their colour. The nuclei in different stages of division have different affinities for stains. In fact, many sections of antheridia fail to show them, while the nuclei of the accompanying oogonia are perfectly clear and distinct.

There seems to be no relation between the beginning of the passage of the protoplasm and the condition of the nuclei. They have been observed in various stages of division in both cells at the time the transfer of the protoplasm was taking place.

After the completion of the act of fertilization some change takes place in the fused protoplasm which alters its susceptibility to stain. The contents have a much greater affinity for safranin than previously and take up very little of the gentian violet. The nuclei now become distributed irregularly throughout the oospore in variously sized groups (Fig. 44), and are much smaller. The number of oil globules increases rapidly, the oogonial wall begins to thicken, and the communicating pore becomes closed. The nuclei appear under the lower powers as mere rings, but when examined with very high powers can be seen to possess peripherally arranged, deeply staining masses of chromatin (Fig. 46). They are much smaller than formerly.

There is no apparent difference in the male and female nuclei, and this fact, coupled with their extremely small size, makes it impossible to definitely follow their subsequent relation to each other. However, there are indications which strongly suggest a fusion of nuclei. Whether this fusion is always in pairs I am at present unable to say, but in the light of our present knowledge concerning compound fertilization this, it seems, would be assumed. Fig. 47 represents a portion of a section of an oospore of further development. The nuclei, although faint, are much larger than in previous stages and fewer in number. They are more regularly distributed throughout the interior, with a tendency to collect about the periphery. The endospore is now well developed.

In Fig. 48 are shown some nuclei from oospores, highly magnified;

d–g, as they appear soon after the two protoplasms have become fused; *b*, *c*, and *h*, in the fusion stage; *a*, large nuclei from the section represented in Fig. 47.

Unfortunately, no later stages of the oospores of this species were observed in section. In the other species, however, an interesting arrangement of the contents of the mature oospores takes place. Fig. 46 shows a portion of a section of an almost mature oospore of *O. luxurians*. The content is more or less differentiated into zones which show different staining reactions, especially with the triple stain. The outer zone is made up of fine granular protoplasm in which are embedded a large number of faintly staining nuclei. Inside of this is a zone of less definite outline composed of larger granules which stain rather deeply with gentian violet. The outer zone retains more of the safranin. In or near the centre is a large oil globule which stains with orange G.

Fig. 45 shows a section of a mature oospore. The same zonate character of the contents is evident. Fig. 90 is a photomicrograph of the same section. When iron-alum haematoxylin is used on such material the zonate character is less distinct and the nuclei can rarely be seen at all (Fig. 89).

The endospore becomes very much thickened in the mature oospore, while the hyaline spine-bearing exospore remains unchanged. The former stains slightly with orange G and eosin, the latter usually remains hyaline. In sections of *O. vexans* oospores there is only an occasional indication of a roughened or warty condition of the exospore, which would point to the assumption that such markings were due to some encrustation which dissolved in the preparation of the material.

There is no contraction of the oogonial protoplasm to form an oosphere and no apparent changes comparable to those taking place in oogenesis in the higher Oomycetes. After fertilization the oogonium becomes the oospore directly.

D. **Nuclear Division.**

The nuclei of both sporangia and gametes are small, measuring in the resting stage 4–5 μ in diameter. In the early stages of development of the sporangia, and up to the time of fertilization in the sexual spores, they have a rather strong affinity for stains, especially in division stages. The nucleoli and chromatin masses take on a brilliant red colour with safranin, while the spindle fibres stain very faintly with gentian violet. All material fixed in Flemming's solution proved much less satisfactory than that fixed with medium chrom-acetic acid for nuclear divisions.

The resting nucleus (Figs. 49 and 50) possesses a deeply staining, rather prominent nucleolus and a slightly granular nuclear plasm. A rather indistinct network with frequent deeply stained thickenings, which I take to be the chromatin material, is present. In cases (Fig. 51) where the nucleolus is absent, the chromatin masses are more prominent. They apparently become elongated and not infrequently assume the condition seen in Fig. 54. The conditions illustrated in Figs. 52 and 53 are frequent. They resemble somewhat the synaptic knot of higher plants, but I do not believe that to be their nature.

Following the condition seen in Fig. 54, the chromosomes become massed together into a large, deeply staining body from which the tips of the spindle appear to emerge. The poles finally extend to the nuclear membrane, which usually persists to the late metaphase or early anaphase.

Figs. 55 and 56 show spindles in the metaphase with large undifferentiated chromatin masses. Figs. 57–62 show indications of individual chromosomes of a rounded or globular form, which, as nearly as I could determine, number six (Fig. 66). In Figs. 57 and 59–61 the chromosomes have evidently divided, while in Figs. 63 and 65 they are passing in mass to their respective poles of the spindle. The nuclear membrane has practically disappeared.

Figs. 67–73 represent succeeding conditions in the anaphase and telophase stages. The conditions represented in Figs. 69–73 are not uncommon, especially in the sexual elements. Similar stages in dividing nuclei have been illustrated for the rusts as well as for a few other Fungi. Fig. 73 represents the still united daughter nuclei with their respective nuclear membranes partially formed. No indication of direct nuclear division was seen.

Very commonly, if not in the majority of cases, the nuclear spindle was more or less bent (Figs. 55–7, 60, 61, and 65). In every case of this kind the poles of the spindle were in contact with the ends of the oval or elliptically shaped nuclear membrane. There was no indication of centrosomes or any structure which would suggest nuclear polarity.

4. DESCRIPTION OF SPECIES.

In 1872 Cornu (5) described a species of *Olpidiopsis* to which he applied the name *O. Saprolegniae.* He was unable to determine whether or not it was the same form observed by A. Braun, but as he wished to preserve Braun's specific name he used it for his species.

In the description of this species Cornu specifically states that the resting sporangia are provided with a large number of very small spines, and are accompanied by an adjacent cell with a smooth wall. He further states: 'It is solely upon that species that the name *O. Saprolegniae* is to be applied. The form of the sporangia, however variable in the species of that group, is not necessarily spherical. The character of the species is not drawn from that, but from the presence of a smooth adjacent cell, coupled with an oospore provided with a large number of spines, isolated and not formed by ridges.'

Cornu's illustration (Pl. III, Fig. 10) of the oospore of this species certainly clearly shows definite spines on the mature oospore rather than warts or tubercles.

A. Fischer (13), in 1892, described what he considered as *O. Saprolegniae* as possessing resting spores with the outer wall thickly covered with hemispherical or blunt colourless warts up to 3 μ high. I am unable to reconcile this with Cornu's description. I believe that Fischer was dealing with a distinct and new species for which, as previously stated, I propose the name *O. vexans*.

Olpidiopsis vexans, nov. sp.

Synon. *O. Saprolegniae*, A. Fischer, 1892. Rabenhorst's 'Kryptogamenflora von Deutschland', I, lv, 38.

Sporangia, single or multiple, formed in terminal or intercalary swellings of the host hyphae, elliptical, oval, or spherical, very variable in size, up to 176 μ in diam., with smooth, colourless membrane, colourless contents, and one to several unbranched exit tubes; zoospores elongated, slightly inequilateral, with two cilia attached at or near the anterior end. Oospores dark grey, with one, sometimes two, antheridial cells; oospores surrounded by a thick endospore and a thin warty exospore, spherical to oval, sometimes elliptical, up to 116 x 84 μ, average 50 μ in diam.; antheridia spherical, less commonly oval, average diam. 20 μ, with a smooth, colourless wall. Germination of oospores not observed.

Zoosporangiis solitariis v. numerosis, in cellula matricis terminalibus v. intercalaribus viventibus, ellipsoideis, ovalibus, v. sphaericis, magnitudine variis, 50–176 μ diam., hyalinis, membrana tenui, levi; zoosporis numerosis, elongatis; ciliis duobus, apici v. proximis apici insertis; oosporis, griseis, sphaericis ad ovalia, interdum ellipsoideis, circ. 116 x 84 μ (saepissime 50 μ diam.), endosporio crasso, levi, episporio tenui, verrucoso; cellulis antheridiis hyalinis, sphaericis ad ovalia, membrana tenui, levi cinctis, 20 μ diam.

Hab. in *Saprolegnia ferax*, Ithaca, N.Y.

Olpidiopsis luxurians, nov. sp.

Sporangia single or multiple, formed in terminal or intercalary swellings of the host hyphae, oval to spherical, very variable in size, with one to three exit tubes extending considerably beyond the wall of the host; zoospores rather small, elongated, with two cilia attached at or near the anterior end. Oospores spherical, with one or two, rarely three, antheridia, with a thick endospore and a thin exospore provided with numerous conical spines up to 2·5 μ in length; oospores 25–50 μ in diam., contain one or more large oil globules; antheridia spherical, frequently oval to elliptical, 10–25 μ in diam., with a smooth colourless wall. Germination of oospores not observed.

Parasitic in *Aphanomyces laevis*, de Bary, Ithaca.

Zoosporangiis solitariis v. numerosis, in cellula matricis plerumque intercalaribus viventibus, ovalibus ad sphaerica, magnitudine variis, collo 1–3 extra cellulam matricis valde prominente ornatis; zoosporis minutioribus, elongatis; ciliis duobus, apici v. proximis apici insertis; oosporis sphaericis, 25–50 μ diam., endosporio crasso, episporio tenui, spinis numerosis, conicis, basi dilatatis, apice acutissimis; antheridiis sphaericis, saepe ovalibus ad elliptica, 10–25 μ diam., membrana levi hyalina inclusis.

Hab. in *Aphanomyces laevis*, de Bary, Ithaca, N.Y.

This species is made not unmindful of the fact that the form resembles rather closely in some respects *O. minor*, A. Fischer, especially as described and illustrated by Petersen (19, p. 405). The fact that the spines are much more slender than those illustrated by Cornu (6, Pl. IV, Figs. 3 and 4) and that this form could not be transferred to the hosts on which *O. minor*, Fischer, was found, seems to the writer to warrant the establishment of a new species.

Olpidiopsis Saprolegniae, Cornu.

Sporangia single or multiple, formed in terminal or intercalary swellings of the host hyphae, elliptical, oval to spherical, very variable in size, up to 150 μ in diam., with one, less commonly two, exit tubes; zoospores oval to elongated, with two cilia attached at or near the anterior end. Oospores spherical, with one or two, less commonly three, antheridia, with a thick endospore and a thin exospore bearing numerous slender spines up to 10 μ long in large specimens; oospores very variable in size, up to 107 μ in diam., provided with oil globules; antheridia spherical to oval, with a smooth colourless wall. Germination of the oospores not observed.

Parasitic in *Saprolegnia* species. Ithaca.

This species is in some respects similar morphologically to *O. luxurians*. Cross-inoculation experiments with the two species and their hosts revealed that neither could be transferred to the host of the other. This would indicate that they were at least biological, if not morphological, species. The spines of the latter are slightly more slender than those of the former.

The description as given above for *O. Saprolegniae* differs in some minor points from that prepared by Cornu, but on the whole seems too close to it to warrant the formation of a new species.

5. GENERAL CONSIDERATIONS.

The presence of true sexuality in the Chytridiaceae is, indeed, rare. *Urophlyctis* and *Olpidiopsis* have been supposed to possess such a stage, but no cytological evidence has been brought forth. Fisch (10) described the copulation of small motile gametes in *Reessia*, while Sorokin (22), according to Butler (4), reports a similar phenomenon for *Tetrachytrium*. A. Fischer (13) accepts neither of these results, but in view of the fact that Griggs (14) has recently reported a similar condition as existing in his new genus *Monochytrium*, their correctness seems more probable. An interesting sexual relation is found in the new genus and species *Zygorhizidium Willei* described by Loewenthal (17).

The one species, aside from that of *Monochytrium*, that is well known from a cytological point of view, is *Polyphagus Euglenae*, Now. Nowakowski described the external phenomenon of the union of the two individuals, but the internal behaviour relating to fertilization has until recent years remained unknown. The cytology of the form was first worked out by Wager (25). According to him the unicellular elements are uninucleate. After the fusion of the two protoplasms there ensues a period of rest, and it is not until the time of germination of the zygote that the sex nuclei fuse.

Dangeard (9) took up the study of the same organism and published a long memoir on it. He confirmed in general the results of Wager. He believes that copulation can take place with the male element before it has attained its complete development. This would appear to be another proof of his general theory of sexuality. According to this theory, living forms primitively asexual have become sexual on account of their resistance to unfavourable conditions where they live. In particular, the exhaustion of nutritive material brought about a sort of starvation which induced the appearance of sexual attraction.

The similarity of the mode of development and nuclear behaviour of the sexual elements of *Olpidiopsis* species, together with the relation of internal conditions to the development of the gametes, point strongly to the belief that they are morphologically equivalent to the sporangia. Many of the sporangial characters are retained by the male cells or antheridia up to the time of fertilization, while more marked differential changes in the oogonial forming individuals take place much earlier.

There appears to be no definite or proper time for the transference of the male protoplasm into the oogonium, at least so far as the behaviour of the nuclei is concerned. In other words, fusion of the sex protoplasm may take place before the maturity of one or both cells.

These characters, it seems, point to a primitive condition of the sexual stage. This view would, according to some students, be strengthened by the coenogametic nature of the sex individuals and the multinucleate character of the resulting oospore. This condition, however, is not confined to the lower forms. A similar condition was described by Stevens (24) for *Cystopus Bliti* in a genus showing what appears to be a sequence of species ranging, so far as numbers of fusion nuclei present in the oospore are concerned, from *Cystopus Bliti* to *Cystopus candidus* where only a single nucleus is present. Such a sequence occurring among closely allied species seems to indicate the gradual change from a primitive form to the more usual condition when only one nucleus is present. But when one considers that the sex cells of *Polyphagus Euglenae* are uninucleate, and the sex cells and resulting oospores of *Olpidiopsis* and *Ancylistes Closterii*, Pfit. (Dangeard, 7), are multinucleate, the importance of such conditions seems lessened. These facts concerning the nuclei of these forms indicate a relationship between *Polyphagus* and *Ancylistes* as close as between *Polyphagus* and *Olpidiopsis*, both belonging to the Chytridiales. The former probably represents an early type of the Zygomycetes, while the latter represents a primitive Oomycete. Similar types are found among the Ancylistales, as *Ancylistes* for the Oomycetes and *Zygochytrium* for the Zygomycetes, although the cytology of the latter form is not known. *Lagenidium* also shows a relation to the Zygomycetes, but here again nothing is known about its cytology.

The entire absence of an antheridial tube and the passage of the entire contents of the antheridium into the oogonium suggest, according to Atkinson (1), 'a primitive and generalized condition of sexuality rather than a condition of degeneration from forms in which it is more highly specialized.'

It would be interesting to know the cytology of the closely related genus *Pseudolpidium*, in which resting spores very similar to those of *Olpidiopsis*, but without a companion or antheridial cell, are produced. In view of the conditions found by Fisch (10) in *Reessia* and Griggs (14) in *Monochytrium*, it seems not altogether impossible that in that genus there may be a similar fusion of elements that has been overlooked.

6. SUMMARY.

1. The zoospores of all three species of *Olpidiopsis* studied possess two cilia of equal length attached at or near the anterior end of the elongated body. They have two motile stages separated by a brief period of rest, which suggests a primitive type of diplanetism.

2. The individuality of the zoospore after entrance into the host is maintained throughout its development, there being no plasmodium formed. Aside from a slight amoeboid movement immediately after entrance, the parasite does not undergo any noticeable changes in form.

3. Segmentation of the sporangial contents has, at least partially, taken place before the entrance of the sporangium into a period of rest, when such occurs, and is apparently simultaneous throughout. The zoospores on escaping contain vacuoles.

4. True sexuality probably exists, and takes place by the fusion of two sexually differentiated individuals and the subsequent passage of the protoplasm of the smaller, male, into the larger, female, cell. This is followed by a supposed fusion of nuclei.

5. Both sexual and asexual reproductive bodies develop rather rapidly from a uninucleate to a multinucleate condition. The oospore is likewise multinucleate.

6. External conditions play a great part in the determination of sex in these organisms. This fact adds evidence to the doctrine that sex in many plants is determinable by external or nutritive conditions.

7. Nuclear division is mitotic with the spindle intranuclear. The number of chromosomes is approximately six. No centrosomes nor any other indication of nuclear polarity was observed.

8. These forms seem to be primitive sexual organisms of the Oomycete type. The influence of external conditions on the development of the sexual stage, the mode of fertilization, the unequal size of the two gametes, and the apparent morphological equivalence of these gametes with the sporangia, seem to the writer to point to that assumption.

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EXPLANATION OF PLATES XXIII–XXVI.

Illustrating Mr. Barrett's paper on *Olpidiopsis*.

All figures were drawn with the aid of a camera lucida. The following combinations of objectives and oculars were used: Zeiss. apo. obj. 1·5 mm. N.A. 1·30, compen. oc. 18, Figs. 31–5 and 48–73; same obj., compen. oc. 12, Figs. 23 and 37–9; same obj., compen. oc. 8, Figs. 12–14, 22, 41, and 47; Zeiss apo. obj. 2 mm. N.A. 1·40, compen. oc. 12, Figs. 1–4, 8–10, 15, and 40; Zeiss. apo. obj. 2 mm. N.A. 1·40, compen. oc. 8, Figs. 28–30, 36, and 42–6; Zeiss apo. obj. 3 mm. dry, compen. oc. 12, Figs. 11, 17, 18, 24, and 25; 2/3 in. obj. and 1/2 in. oc., Fig. 26; 1/8 in. obj. and 1 in. oc., Fig. 27.

PLATE XXIII.

Fig. 1. Zoospores of *Olpidiopsis luxurians*. *a*, Drawn from a living zoospore. All others from stained preparations.

Fig. 2. Zoospores of *Olpidiopsis Saprolegniae*. *a*, From a living zoospore. All others from a stained preparation.

Fig. 3. Zoospore resting on an *Aphanomyces* hypha preparatory to entrance.

Fig. 4. Contents of the zoospore in the hypha, the empty spore wall outside.

Fig. 5. Germinated zoospore of *O. luxurians* on an empty sporangium of *Aphanomyces laevis*.

Fig. 6. Germinated zoospore of *O. luxurians* on an old, but still living, hypha of *Aphanomyces laevis*.

Fig. 7. A young developing sporangium of *O. luxurians* five hours after infection.

Fig. 8. Zoospore of *O. Saprolegniae* beginning to discharge its contents into the hypha of *Saprolegnia ferax*.

Fig. 9. Same zoospore as in Fig. 8, process nearly completed.

Fig. 10. Germinated zoospore of *Aphanomyces laevis* infected by *O. luxurians*.

Fig. 11. Young developing sporangium of *O. luxurians*.

Fig. 12. A sporangium of *O. luxurians*, showing the homogeneous ground substance containing small granules and a few fat globules.

Fig. 13. Same sporangium as in Fig. 12, three hours later.

Fig. 14. Same as Fig. 12, twenty-four hours later. Zoospores formed and exit tube beginning to develop.

Fig. 15. Zoospores of *O. vexans* from a stained preparation.

Fig. 16. Intercalarily formed sporangium of *O. luxurians* showing two characteristic exit tubes.

Fig. 17. Sporangium of *O. luxurians*, showing the recently developed membrane, vacuolate nature of the protoplasm, and the radiate character of the host protoplasm.

Fig. 18. Stage of developing sporangium showing the organization of regular vacuoles with diminished condition of the host protoplasm.

Fig. 19. Swollen hypha of *Saprolegnia* containing discharged and undischarged sporangia of *O. vexans*. The latter are in the resting condition.

Fig. 20. A sporangium of *O. vexans* in an old culture, showing a peculiar coiling of the long exit tubes.

Fig. 21. A slightly swollen hypha of *Saprolegnia* containing three empty sporangia and two mature oospores of *O. vexans*.

Fig. 21 A. An oospore of *O. Saprolegniae* drawn to the same scale as Fig. 21.

Fig. 21 B. An oospore of *O. luxurians* drawn to the same scale as Figs. 21 and 21 A.

Fig. 22. Portion of a sporangium of *O. luxurians* stained with Gram's stain. Shows the presence of faintly stainable bodies in the vacuoles.

Fig. 23. Large fat granule from a young sporangium. Much vacuolated.

PLATE XXIV.

Fig. 24. Young oogonium and attached antheridium of *O. luxurians*.

Fig. 25. An older stage of the sexual organs of *O. luxurians*. Two young sporangia are present in the same hyphal swelling.

Fig. 26. Young developing oogonium and two antheridia of *O. vexans*.

Fig. 27. Nearly mature oospore of *O. vexans* showing large oil globules and slightly roughened wall.

Fig. 28. Young oogonium and antheridium of *O. luxurians* slightly older than Fig. 25, which shows the developing spines and the large number of oil globules, also the granular protoplasm.

Fig. 29. Same specimen as in Fig. 28, two hours later. Spines are mature and the antheridial contents is passing into the oogonium.

Fig. 30. Mature oospore of *O. luxurians* bearing two empty antheridial cells.

Fig. 31. Young uninucleated sporangium of *O. vexans* soon after the entrance of the zoospore into the host.

Fig. 32. Binucleated sporangium of *O. vexans*.

Fig. 33. Binucleated sporangium of *O. vexans*, showing the nuclei in the act of division.

Fig. 34. Slightly older sporangium of *O. vexans*, showing its vacuolate character and the nuclei in division.

Fig. 35. Mature zoospores at the time of discharge from the sporangium, showing well-marked vacuoles.

Fig. 36. Sporangium of *O. vexans*, showing the peripheral arrangement of the protoplasm.

Fig. 37. Portion of a developing sporangium of *O. vexans*. The large vacuoles are forming and the nuclei are undergoing the last division before zoospore formation.

Fig. 38. A portion of a sporangium of *O. vexans* in the vacuolate stage following the condition shown in Fig. 37. Spore centres formed but not sharply marked off. Chromatin of the nuclei collected at the periphery. Fixed in weak chrom-acetic acid.

Fig. 39. A portion of a sporangium of the same age as that shown in Fig. 38, fixed in weak Flemming's solution. Segmentation of the contents into zoospores clearly visible.

PLATE XXV.

Fig. 40. Young oogonium and antheridium of *O. vexans*, showing the nuclei in a state of division. Both contain large vacuoles. Nuclei more or less irregularly distributed. No membranes apparent.

Fig. 41. Shows the wall between the oogonium and antheridium gelatinizing to form a fertilization pore. *O. vexans*.

Fig. 42. A later condition of Fig. 41. Nuclei of antheridium in division, while those of the oogonium are in the resting condition. *O. vexans*.

Fig. 43. Sex organs of *O. luxurians* showing actual passage of male nuclei into the oogonium. Nuclei of the oogonium poorly stained. Gram stain.

Fig. 44. Fertilized oospore of *O. vexans*. The nuclei, which are very small, are collected more or less in groups. Large granules, probably fatty in nature, scattered throughout the thin protoplasm.

Fig. 45. A section of a mature oospore of *O. luxurians*, showing the thick endospore, peripheral layer of protoplasm containing the nuclei, the large central granules, and an oil globule. Nuclei very indistinct.

Fig. 46. An earlier stage of Fig. 45. Nuclei more clearly shown, and appear as mere rings in the protoplasm.

Fig. 47. A segment of a section of a more mature oospore of *O. vexans*. Nuclei more evenly distributed throughout the contents and much larger than in earlier stages of the oospore.

Fig. 48. Nuclei of the oospores of *O. vexans*. *a*, Nuclei from the section shown in Fig. 47; *b*, *c*, and *h*, apparently fusing nuclei; *d*, *e*, *f*, and *g*, nuclei from such a section as shown in Fig. 44. Fusion has not yet taken place.

Figs. 49 and 50. Resting nuclei from a young oogonium.

Fig. 51. Early prophase.

Figs. 52 and 53. Conditions resembling synapsis.

Fig. 54. Apparent segmentation into chromosomes.

Figs. 55–62, and 66. Nuclei in metaphase stages. In some cases definite chromosomes are evident.

Figs. 63–5. Spindles in the early anaphase.

Figs. 67–70, 72, and 73. Late anaphase.

Fig. 71. Telophase.

PLATE XXVI.

Fig. 74. An aphid bearing *Aphanomyces* infected with *O. luxurians*.

Fig. 75. An ant pupa on which is growing *Saprolegnia ferax* infected with *O. vexans*.

Fig. 76. A very young stage of a sporangium of *O. luxurians*.

Fig. 77. A young sporangium of *O. luxurians*, showing the large refractive fatty granules.

Figs. 78–81. Successive stages in the development of *O. luxurians* sporangia.

Fig. 82. Sporangia showing large vacuoles and developing exit tubes.

Fig. 83. Early sexual stage of *O. luxurians*.

Figs. 84 and 85. Later stages of sexual organs, showing large granules and vacuoles. Fig. 85 shows three antheridia attached to one oogonium.

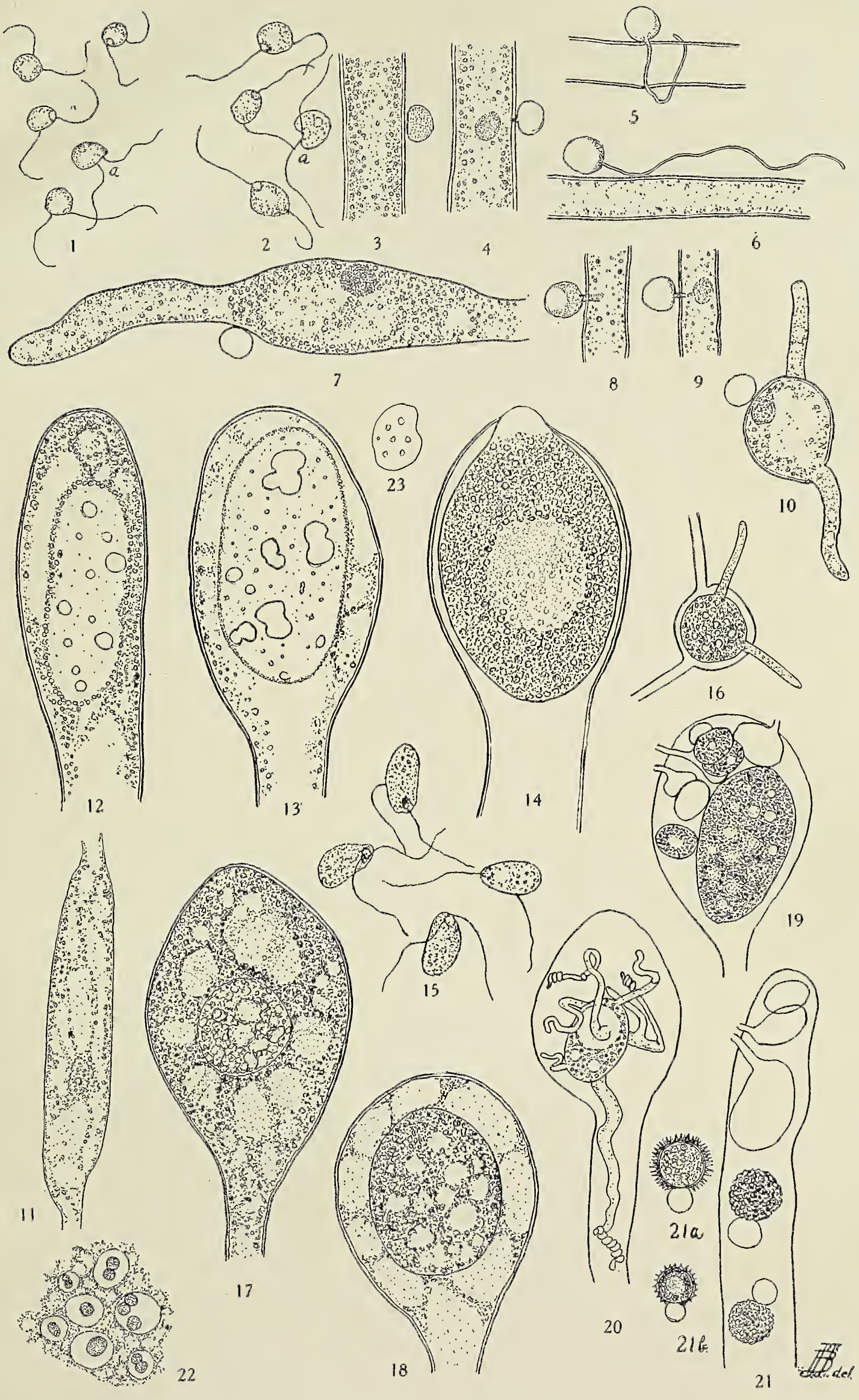
Fig. 86. Sexual cells of *O. luxurians* about ready for fertilization to take place.

Fig. 87. Contents of antheridium partially passed into the oogonium. *O. luxurians*.

Fig. 88. Two oospores connected by one empty antheridium.

Fig. 89. Section of a mature oospore of *O. luxurians* stained with iron-alum haematoxylin.

Fig. 90. Similar section stained with the triple stain.



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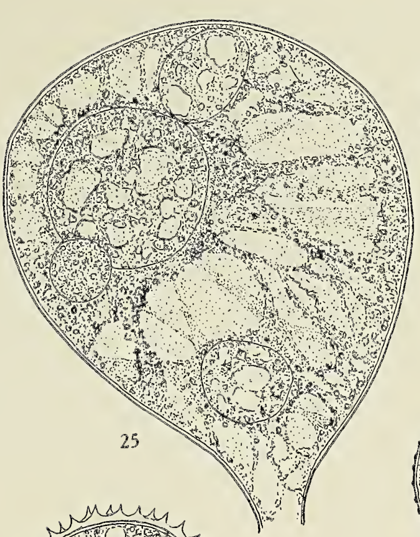

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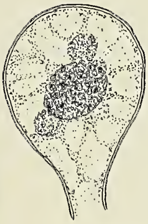

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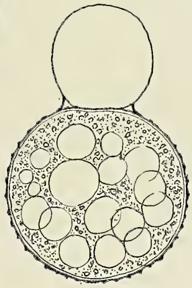

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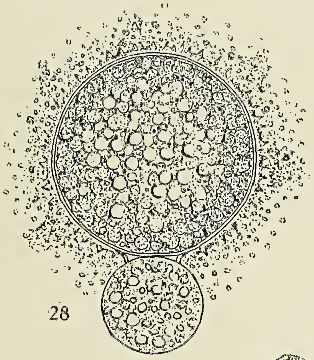

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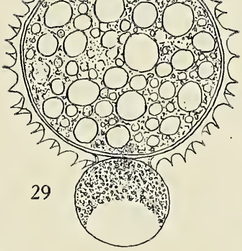

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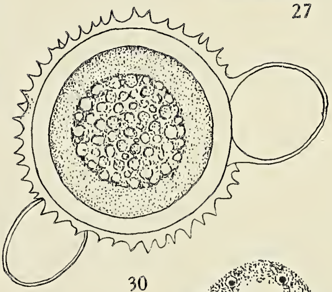

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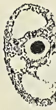

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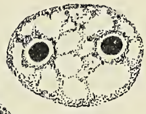

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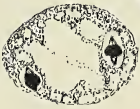

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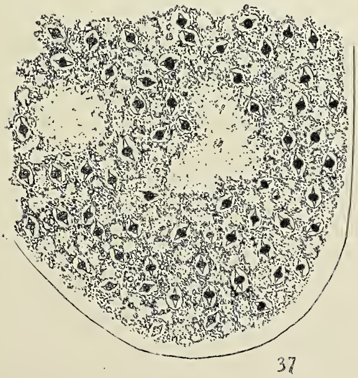

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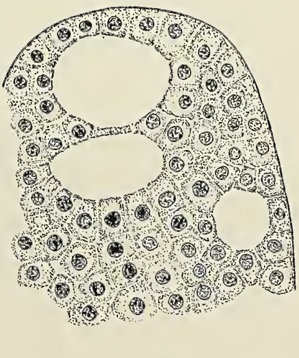

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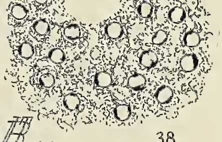

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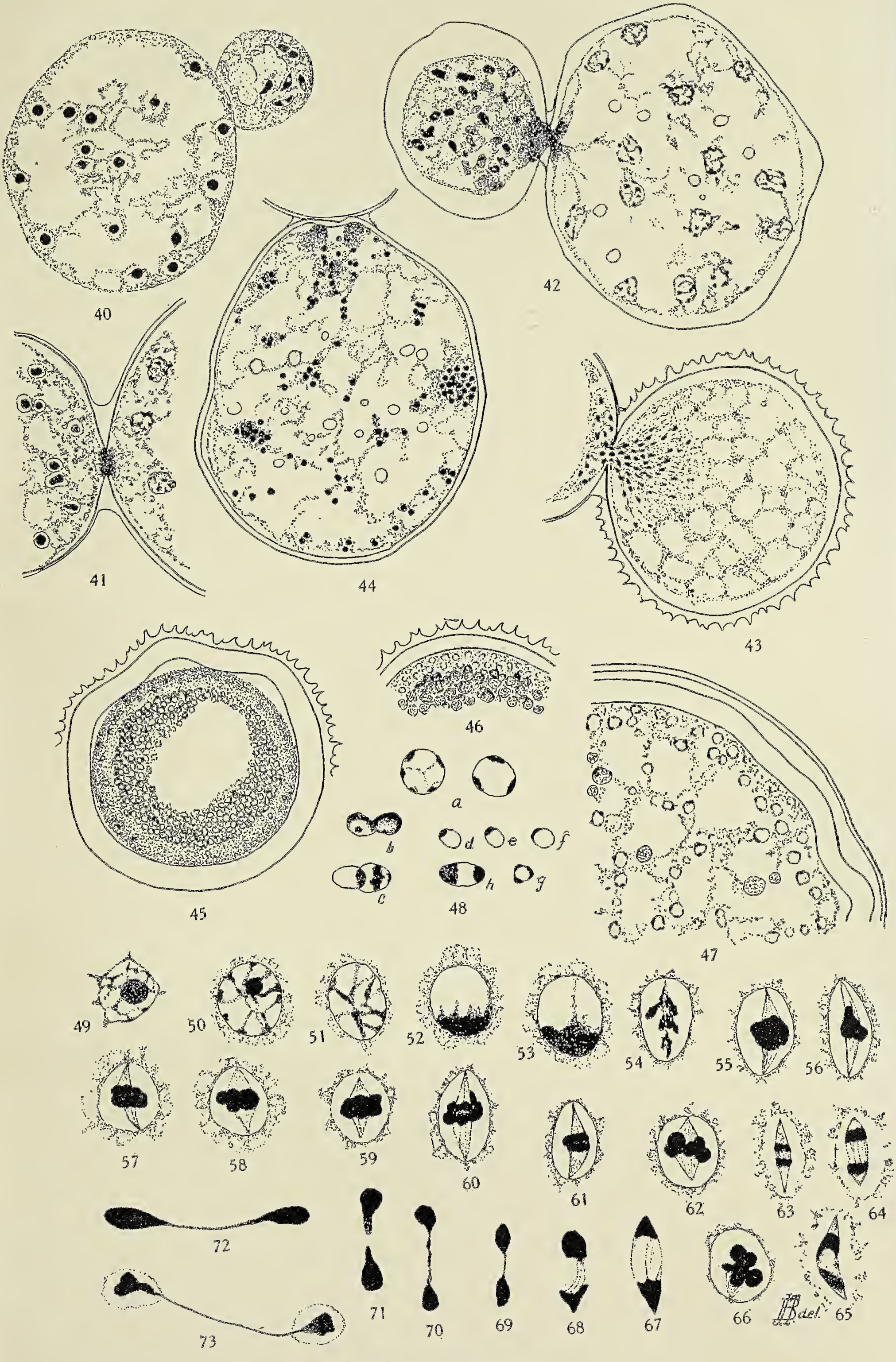

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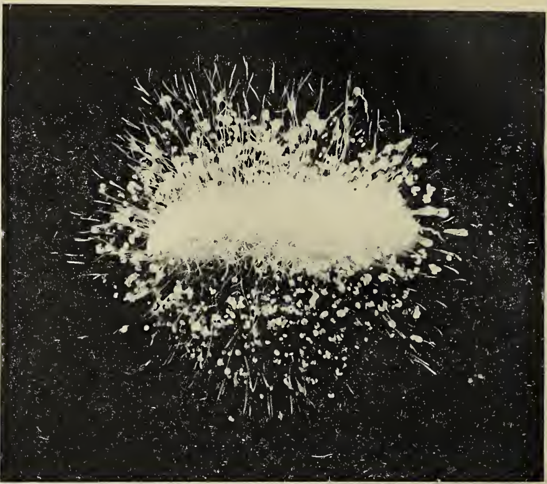
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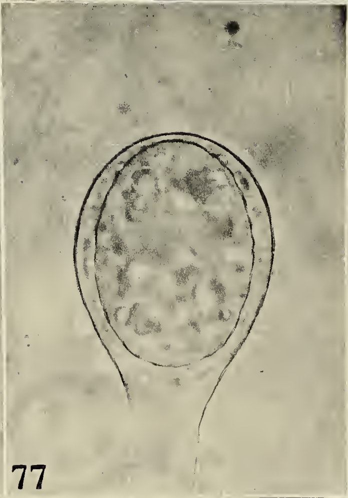
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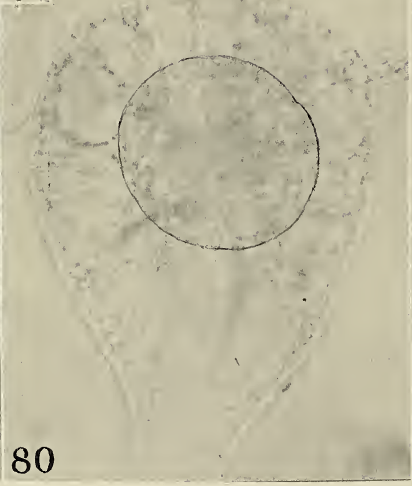
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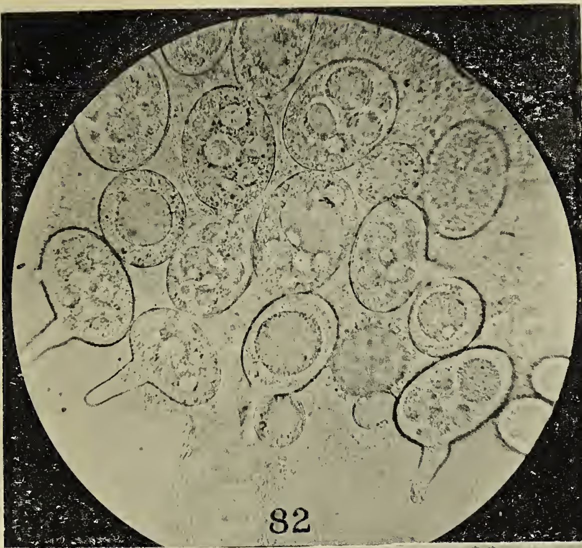
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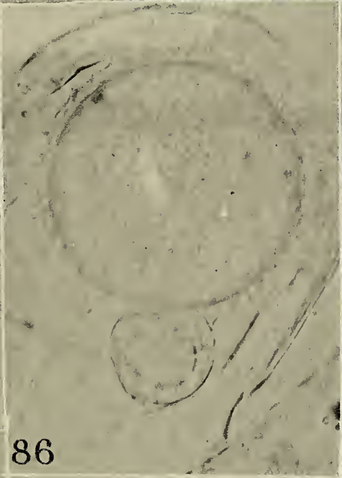
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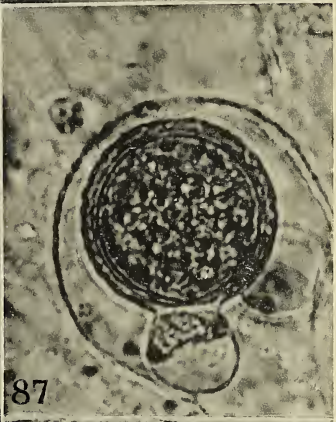
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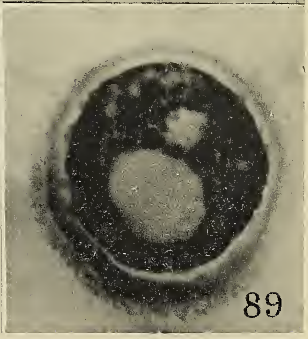
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Huth, coll

The Morphology of Trichodiscus elegans, Gen. et Sp. Nov.[1]

BY

E. J. WELSFORD, F.L.S.

Research Assistant, Department of Botany, University of Leeds.

OCCURRENCE. In March, 1910, whilst examining some *Azolla carolina* growing in a glass jar of rain-water in the greenhouse of the Royal Holloway College, I found a small green Alga which I was unable to identify. The plants were growing in small circular patches on the glass and also in a few cases on the *Azolla*, but in this situation they were exceedingly difficult to see.

The *Azolla* was bought in the previous November from Messrs. Ware and Sons, Limited, of Feltham, who had imported it from North Carolina, probably from Salem, and it is possible that the Alga was introduced from that district.

METHODS. Glass cover-slips were suspended in water against the side of a jar, and in a few weeks were covered with young Algae. Some of the slips were examined daily, and the development of the plant was studied in this way in the living condition. Material was also fixed in different stages of development in Flemming's weak solution and in 4 % formaldehyde. In the former case it was generally stained with haematoxylin or with brazilin, and mounted in Venice turpentine.

MORPHOLOGY. The Alga forms small circular green patches about 1 mm. in diameter, and is firmly fixed to the substratum. It consists of a flat disc bearing numerous erect branches and long septate hairs. The disc is one cell thick and is formed of branched creeping filaments which cohere to form a pseudo-parenchymatous thallus, the peripheral branches of which are more or less free, and sometimes curve upwards. All the cells of the disc, with the exception of the rapidly growing free apices of the filaments, are capable of giving rise to one or two short erect branches; these branches are more freely developed on old than on young plants. Hairs are not very numerous, and are usually found towards the periphery of the thallus. They are often 300 μ long, and

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[1] A short account of this work was read before Section K of the British Association, at the meeting held at Winnipeg in September, 1909, and was published in the report.

[Annals of Botany, Vol. XXVI. No. CI. January, 1912.]

consist of several cells; the basal ones contain chlorophyll, and the rest are colourless. The ordinary branches of the thallus arise as outgrowths immediately behind a septum. The hairs develop in a very similar way, but usually grow out from the middle of a lateral wall. The presence of protoplasm in every cell of the hair was clearly demonstrated in preparations stained with brazilin, and it seems probable that these hairs are really slightly modified branches.

The vegetative cells of the disc and branches contain a large parietal chloroplast with irregular lobes, a nucleus, and a pyrenoid.

REPRODUCTION. In young plants any cell is capable of producing zoospores, but as development proceeds a certain amount of differentiation takes place. The most common reproductive organs at this stage are large sporangia, which may be either terminal or intercalary, and which are always characterized by deep coloration. They generally occur in groups, and the various successive stages by which the contents of the sporangium divide into eight portions may often be found close together. The mature sporangium measures about $16 \times 12\,\mu$, and contains eight gametes which are set free through a pore or pores in the wall. When first liberated each gamete measures about $3\,\mu$ in length, but quickly becomes $6\,\mu$, and shows a distinct eye-spot and two equal cilia; the zoospores soon fuse in pairs and come to rest. The interval of time between fusion and germination was not determined, but some of the stages of germination were studied.

The zygote at an early stage appears to be firmly fixed to the substratum; a branch grows out from one end of it and is cut off by a cell-wall. As soon as this cell has developed into a short filament, another branch usually grows out from the opposite end of the zygote. Occasionally a zygote germinates at both ends simultaneously. The first-formed filaments grow rapidly, branch, and develop into the characteristic thallus. In many cases zygotes were found germinating so close to one another that their filaments combined together to form one plant or colony.

Sometimes the contents of the sporangium fail to form gametes, and while still within the cell-wall divide to form a filament of from 2 to 8 cells. The wall of the sporangium eventually ruptures, setting free the young plant. Occasionally cases were found in which the sporangium ruptured after the first division and liberated two non-motile daughter-cells which were capable of germination.

Besides these large sporangia, groups of smaller ones are found on poorly developed filaments. They measure about $6 \times 4\,\mu$, and contain from 8 to 16 motile cells which are liberated through an opening with a definite lid, which, however, is quickly lost. Owing to their small size and rapid movement their further development was not satisfactorily studied. Sometimes the contents of these small sporangia were liberated after one or two divisions as non-motile cells. Aplanospores are also formed by the rounding off of the contents of the cells of the disc and branches; when present they occur in large groups.

SYSTEMATIC POSITION. This plant does not appear to belong to any known genus of Chaetophoraceae, though it shows close affinity, in some respects, with *Chaetonema*, *Endoclonium*, and *Pseudochaete*. Like these it is apparently one of those many reduced epiphytic forms (Huber, 2) which are closely allied to *Stigeoclonium*, and of which they have sometimes been considered as species. As regards their epiphytic nature, a few plants only were found growing on *Azolla*; the others were on glass, a habitat on which epiphytes very easily develop (Cotton, 1). The relatively large disc and short upright branches are other epiphytic characteristics.

DIAGNOSIS. The name *Trichodiscus elegans* is proposed for the plant, and a diagnosis is appended.

Trichodiscus, gen. nov.

Thallus parvus, epiphyticus, matrici arcte adpressus, discum pseudoparenchymaticum e filamentis radiantibus ramosis inter sese coalitis, ad marginem autem liberis, constitutum efformans, ramis erectis brevibus numerosis et pilis longissimis septatis ornatus; cellulae uninucleatae chromatophoro singulo parietali lobato et pyrenoideo singulo praeditae. Reproductio per zoosporas, per isogametes biciliatas, per aplanosporas, et per cellularum palmelloidearum massulas.

T. elegans, sp. nov.

Characteres ut supra.

Hab. ad plantas aquaticas (*Azolla*) ad Salem in Carolina septentrionali.[1]

Trichodiscus, gen. nov.

Thallus small, epiphytic, closely adpressed to the substratum, and consisting of radiating branched filaments united to form a pseudo-parenchymatous disc with free filaments at the periphery. It bears numerous short erect branches and very long septate hairs. Cells with parietal lobed chloroplast, one nucleus, and one pyrenoid. Reproduction by zoospores, biciliate isogametes, aplanospores, and palmelloid groups.

T. elegans, sp. nov.

Characters of the genus.

Habitat. On *Azolla* collected at Salem, North Carolina.

DISCUSSION. Many Algae such as *Stigeoclonium* and *Ulothrix* have several methods of reproduction, and the occurrence of these has usually been correlated with changes in the environment. Klebs (3) described the variations which are produced by cultivating algae under different conditions. For instance, he records the fact that if plants of *Stigeoclonium* are grown on an artificial food solution the zoospores are not set free, but

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[1] My thanks are due to Mr. A. Gepp for help in drawing up the diagnosis.

germinate in the mother-cell. In *Trichodiscus elegans* the various phases of reproduction occur simultaneously, and sometimes very close together on the same plant. For instance, one of the figures represents a group of cells in which there is a mature sporangium containing gametes, and close beside it a younger sporangium, which after one division of its contents has ruptured and set free an asexual daughter-cell. Growing close to these is the group shown in another figure, in which there are three young sporangia before division, and one in which the contents, while still within the cell-wall, have developed into a thallus. In no case was it found possible to distinguish in any way between those cells which would eventually contain either gametes, asexual daughter-cells, or young plants ; the close agreement of their initial cells suggests the possibility that they are homologous structures.

The changes of environment which account for Klebs's results, and which Lang (4) suggests determines whether the sexual or asexual character of a cell is latent or patent, are in this case absent. Here we have both gametes and asexual cells arising under precisely similar conditions. In this case it appears, therefore, that we must look for some stimulus other than the ordinary environmental conditions suggested by Klebs. Such a stimulus is probably closely connected with the nutrition of individual cells, and may be due to some subtle osmotic change acting from within instead of from without, as Livingston (5) has suggested is the case in a species of *Stigeoclonium*.

SUMMARY.

1. The alga *Trichodiscus elegans*, a member of the Chaetophoraceae, is described.

2. In *Trichodiscus elegans* both sexual and asexual cells were found to be developed from similar initials under the same environmental conditions.

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1. COTTON, A. D.: On some Endophytic Algae. Journ. Linn. Soc., vol. xxxvii, 1906, p. 293.
2. HUBER, G.: Contributions à la connaissance des épiphytes et endophytes et leurs affinités. Ann. Sci. Nat., sér. 7, Bot., vol. xvi, p. 265.
3. KLEBS, G.: Die Bedingungen der Fortpflanzung bei einigen Algen und Pilzen. Jena, 1896.
4. LANG, W. H.: A Theory of Alternation of Generations in Archegoniate Plants based upon Ontogeny. New Phytologist, vol. viii, 1909, p. 5.
5. LIVINGSTON, B. E.: On the Nature of the Stimulus which causes Changes of Form in Polymorphic Green Algae. Bot. Gaz., vol. xxx, 1900, p. 289.

The Plate and description of Figures will follow in a subsequent number of THE ANNALS OF BOTANY. The delay is in no way due to the author.—EDITORIAL NOTE.

NOTE.

NOTE ON A CASE OF DOUBLING OF EMBRYO-SAC, POLLEN-TUBE, AND EMBRYO.—While examining material of a *Lychnis* hybrid with a view to discovering the processes which occur in the formation of gametophytes and embryo, a curious example of duplicity was observed in an ovule, and is perhaps worth recording. The plant examined was a member of the second generation from the cross *Lychnis alba*, Mill. × *Lychnis flos-cuculi*, Linn., and was a female with white flowers, showing a close resemblance to *L. alba*. Several flowers were pollinated by *L. flos-cuculi*, and an ovary was fixed forty-two hours later, one of whose ovules showed the structure to be described.

The ovule in question was about the normal size, and in the appearance of the nucellus, integuments, and sporophytic tissues generally showed no exceptional features when examined in a series of microtome sections. Within the nucellus, however, were present two embryo-sacs, roughly equal in size, lying in contact side by side in the usual position; each sac was of about the normal length, but of little more than half the normal diameter.

Into the apex of each embryo-sac had penetrated a pollen-tube whose nuclei had been expelled, and whose residual contents had degenerated in the usual fashion. An attempt was made to trace these pollen-tubes backwards, but this was found impossible, owing to crushing, except in the short gap between the micropyle and the apex of the nucellus; here there appeared to be two distinct tubes, but this was very difficult to determine and cannot be considered proven. The probability is strongly that two separate tubes entered the ovule, and it seems very unlikely that two functional apices should have been produced by the branching of a single tube. Moreover, in another ovule (with a single embryo-sac) two pollen-tubes were clearly present, though only one was functional, the other stopping short half-way through the peculiar specialized channel which leads from the apex of the nucellus towards the embryo-sac. The occurrence of two pollen-tubes in the ovule with two embryo-sacs may therefore be considered as almost certain.

Within each embryo-sac was found a single synergid, closely pressed against the apex of the pollen-tube; the second synergid had degenerated, as is usual. The antipodal cells had also disappeared.

Each embryo-sac contained a two-celled embryo of the type observed in other ovules of the same age. The cell towards the micropyle was already being differentiated as the basal cell of the suspensor. The other cell was, as usual, slightly elongated, the nucleus surrounded by dense cytoplasm being at its distal end, a large vacuole at the proximal.

Inside one of the embryo-sacs were found four endosperm nuclei—about the usual number at this stage of embryogeny. The other embryo-sac, however, still contained the large definitive nucleus with its enormous nucleolus, no formation of endosperm having taken place.

It is possible that if this ovule had been allowed to develop it would have produced two seedlings on germination. It may be recalled that Gaertner[1] grew two exactly similar individuals from a single seed of *Dianthus barbatus* × *D. superbus*, another hybrid in a genus closely related to *Lychnis*.

No similar case to the present appears to be recorded in the literature. The nearest example is described by Schacht[2] in *Cheiranthus Cheiri*. There the formation of more than one embryo-sac seems to be a frequent occurrence; and in one case Schacht mentions the entrance of two pollen-tubes into a single ovule. He remarks, however, that it appears that never more than one egg-cell is fertilized.

The fact that two pollen-tubes should enter and fertilize an ovule which had developed two embryo-sacs can hardly be a mere coincidence; rather it would seem to indicate a quantitative relation between embryo-sac and pollen-tube in the matter of chemotaxis, two embryo-sacs excreting sufficient of the chemotropic substance to attract two pollen-tubes.

R. H. COMPTON.

CAMBRIDGE.
July, 1911.

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[1] Gaertner: Bastarderzeugung. Stuttgart, 1849, p. 53.

[2] Schacht: Über Pflanzen-Befruchtung. Jahrb. f. wiss. Bot., i, 1858, p. 202.

EXPLANATION OF FIGURES IN PLATE XXVII.

(*To follow page* 242.)

Illustrating Miss Welsford's paper on *Trichodiscus elegans*.

Fig. 1. Mature plant of *Trichodiscus elegans*, showing the groups of short erect branches in the centre of the disc and the hairs towards the periphery. × 70.

Fig. 2. Part of periphery of disc, showing the free curving branches and the hairs. × 500.

Fig. 3. A group of short erect branches from the centre of the disc. × 1,000.

Fig. 4. The lower part of a hair, showing the two basal cells with chloroplasts; the remaining cells contain protoplasm, but no chloroplasts. × 1,000.

Fig. 5. Part of a young filament, showing branches arising close to the transverse walls. × 1,875.

Fig. 6. Group of large terminal sporangia from centre of disc. The following stages can be seen: one-celled sporangium, two-celled sporangium, eight-celled sporangium, a mature sporangium containing zoospores. × 1,000.

Fig. 7. A later stage of the same Two empty sporangia and a sporangium from which the zoospores are escaping are shown. × 600.

Fig. 8. A large intercalary sporangium, showing the pores through which four zoospores have already escaped. In the same group two-celled and four-celled sporangia are shown. One of the sporangia after dividing into two has set free one of its daughter cells. × 1,000.

Fig. 9. A zoospore. × 1,000.

Fig. 10. Two zoospores fusing (*from fresh material*). × 1,000.

Fig. 11. Resting stage of zygote (*from fresh material*). × 1,200.

Fig. 12. Three stages in the fusion of the gametes (*from material fixed in Flemming's fluid and stained with iron haematoxylin*). × 1,200.

Fig. 13. Stages of germination of the zygote. *a*, zygote germinating at one end; *b*, later stage of same; *c*, zygote germinating at both ends; *d*, later stage of same. × 1,200.

Fig. 14. Part of thallus, showing a young plant developed in a sporangium *in situ*; four young sporangia before division has taken place are also shown. × 750.

Fig. 15. A seven-celled filamentous plant lying within the wall of the sporangium, which has become separated from the plant. × 1,000.

Fig. 16. A five-celled filamentous plant which has begun to increase in size but is still surrounded by the sporangium wall, which has become gelatinous. × 1,000.

Fig. 17. A group of small sporangia, showing empty sporangia and also one which still contains four zoospores. × 1,000.

Fig. 18. A group of aplanospores. × 1,000.

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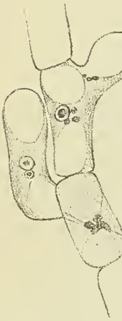

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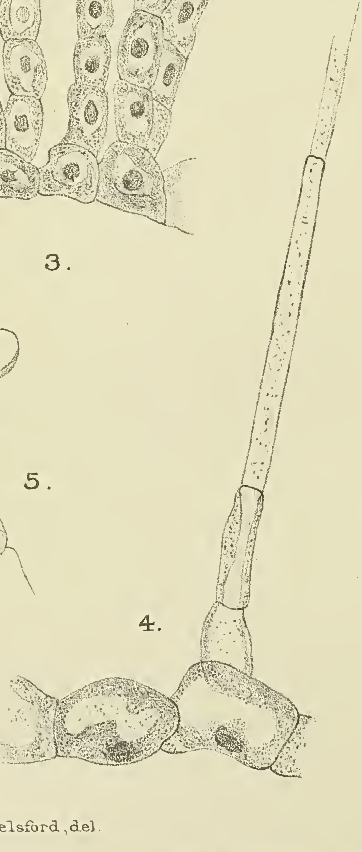

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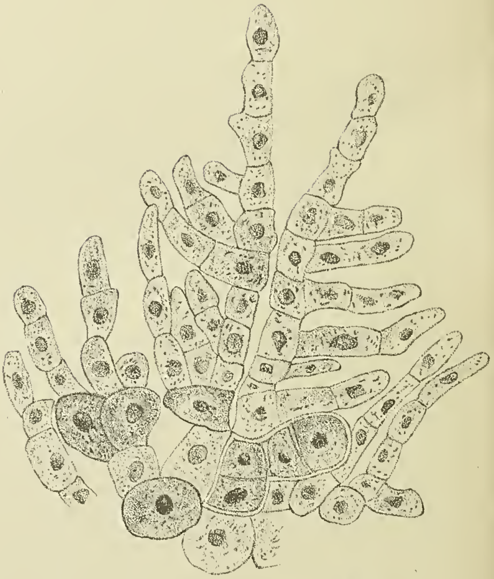

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WELSFORD — TRICHODISCUS ELEGANS.

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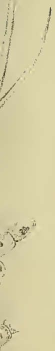

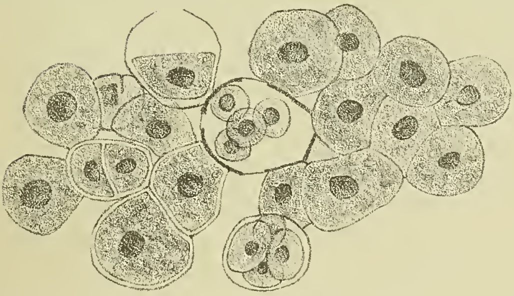

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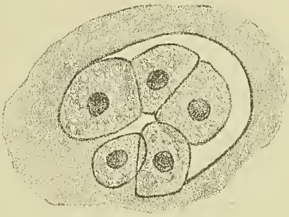

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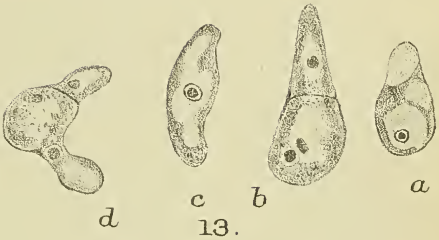

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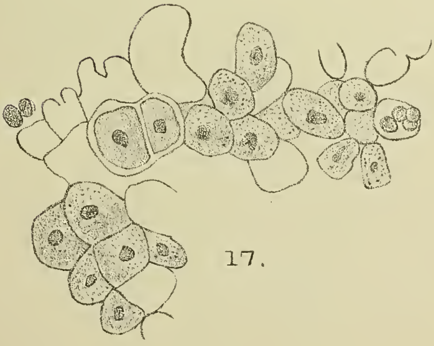

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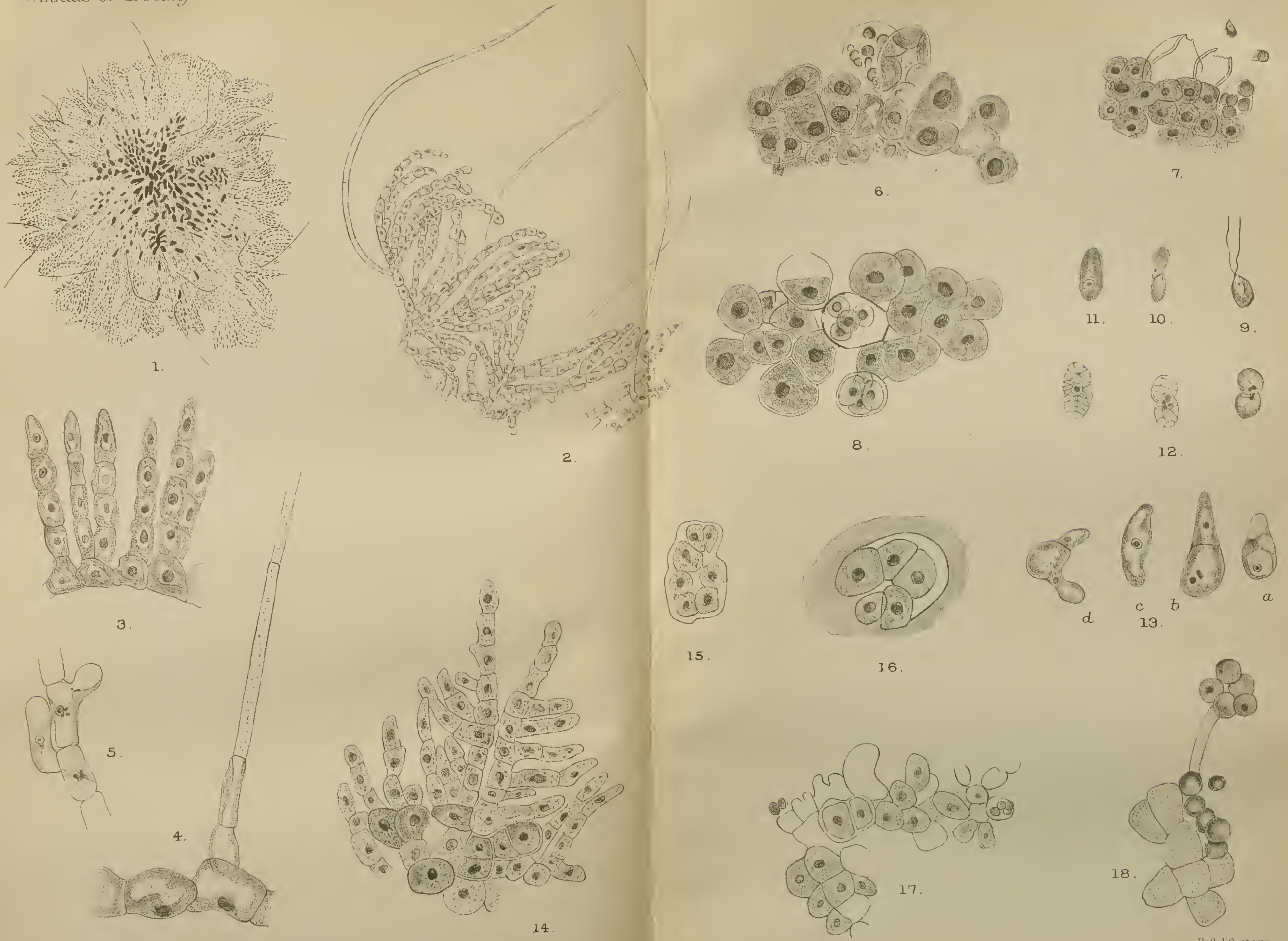

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WELSFORD — TRICHODISCUS ELEGANS.

FLORAL MECHANISM

By A. H. CHURCH, M.A., D.Sc.

LECTURER IN BOTANY IN THE UNIVERSITY OF OXFORD

The following statement has been drawn up by Professor Sydney H. Vines

THE object of this work is to provide the botanical student with a complete description of the development, morphology and mechanism of the principal types of flowers. Whilst giving the kind of information that is to be found in Payer's *Organogénie de la Fleur*, and in the late Professor Eichler's well-known *Blüthendiagramme*, it supplements this with an account of the ecology of the flower, including pollination and the formation of fruit and seed. Hence, when complete, it will be the most comprehensive treatise on the flower that has yet been published.

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Galanthus nivalis . .	Snowdrop.	*Narcissus Pseudo-Narcissus*	Daffodil.
Jasminum nudiflorum	White Jasmine.	*Erica carnea*	Heath.
Crocus vernus . . .	Blue Crocus.	*Ribes sanguineum*	Flowering Currant.
Richardia africana .	White Arum Lily.	*Cydonia japonica*	Scarlet Cydonia.
Daphne Mezereum .	Mezereon.	*Vinca major*	Greater Periwinkle.

In connexion with each type, two or three allied species are described for purposes of comparison.

The description of each type is illustrated by a full-page coloured plate, giving an accurate longitudinal section of the flower, and by a black-and-white plate giving the inflorescence, the floral diagram, and other structural details. As each subsidiary species has also a coloured plate allotted to it, the volume contains no less than forty coloured and fourteen uncoloured plates, in addition to a large number of figures, chiefly developmental, included in the text. It can be obtained at the original price of £1 1*s*. net by subscribers to Part II.

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The Structure and Affinities of Peranema and Diacalpe.

BY

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With Plates XXVIII and XXIX.

PERANEMA CYATHEOIDES, D. Don, was first collected in the mountains of Nepal, Sheopore, and Chandagiri by Wallich. It was included in his 'Plantae Asiaticae Rarae', and was sent in 1823 to the Honourable East India Company's Museum at the India House. Duplicates were most liberally distributed in May of the following year. One of these is in the Herbarium of the Royal Botanic Gardens at Kew. On this specimen, however, there is only a written label, bearing the name *Sphaeropteris barbata*, Wall. There is no description of the plant, not even a note of its marked characteristics. Wallich's description was not published until 1830, when his 'Plantae Asiaticae Rarae' was issued. Meanwhile, David Don, in 1825, had published the 'Prodromus Florae Nepalensis' and had described *Sphaeropteris*, though in no great detail, under the name of *Peranema cyathoides*. Thus on the basis of priority of publication, as the label on Wallich's specimen is not printed but written, the name of the Fern must be kept as *Peranema*. Hooker and Baker in the 'Synopsis Filicum' give the specific name as *Peranema cyatheoides*, D. Don. This is also done by Christ in 'Die Farnkräuter der Erde', and by Christensen in the 'Index Filicum'. It is, however, more accurate to retain this specific name than to revert to Don's own 'cyathoides'.

It is included by Presl in his 'Tentamen Pteridographiae' (1836), where it is placed with *Physematium*, Kaulf., *Thyrsopteris*, Kze., and *Cibotium*, Kaulf., in the Peranemeae, the first tribe of the Hymenophoreae, Cohort I of the sub-order Cathetogyratae, Bernhardi. This tribe immediately follows the Cyatheaceae and precedes the Aspidiaceae. In 1842 it was included in J. Smith's 'Genera of Ferns' in Hooker's 'Journal of Botany', vol. iv, p. 190. There it is placed in Section II of the Aspidieae,—the Orthophlebieae—a section characterized by their free venation, none of the

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venules anastomosing. It is separated from the Cyatheae (p. 181), 'because the pedicellate sori of *Sphaeropteris* cannot in my opinion be viewed as an elevated receptacle'. Smith adds, 'On viewing their[1] habit and characters in every other respect I do not hesitate in placing them in this tribe' (Aspidieae). In Hooker's 'Species Filicum' (1846) *Peranema* is again described under the name of *Sphaeropteris*, Wall., and is placed in the Woodsieae, the second sub-tribe of the Dicksonieae, Gaud. In 1856 it was described by Mettenius in his 'Filices Horti Botanici Lipsiensis' and by him put, with *Woodsia* and *Diacalpe*, in the Aspidiaceae, Group IV of the Polypodiaceae. R. H. Beddome in the 'Ferns of Southern India' (1863) places it in the Polypodiaceae, in the group Peranemeae. It there follows the Davallieae, with *Davallia* and *Microlepia,* and immediately precedes the Cyatheae, with *Cyathea* and *Alsophila.* Robert Brown in 1867 gave a full description of *Peranema,* placing it in his family Polypodiaceae. He remarks that it is clearly to be distinguished from several Cyatheae, but is closely related to *Woodsia mexicana*, R.Br. 'Hoc ultimo charactere et reticulatione involucri a Cyatheis nonnullis (involucro e medio venae orto) praesertim distinguenda; arctiori tamen affinitate nixa cum Woodsia, mediante specie mexicana (*W. mexicana*, Nob.).' Hooker and Baker in the 'Synopsis Filicum' (1874) place it in their Dicksonieae. H. Christ in 'Die Farnkräuter der Erde' (1897) places it in the Aspidiaceae. And Diels in Engler and Prantl's 'Natürliche Pflanzenfamilien' includes it in the Woodsieae-Woodsiinae group of Polypodiaceae—the group which immediately succeeds the group Cyatheaceae.

From the differing positions in which the Fern has been placed it is evident that its systematic position is one of difficulty and debate. The prevailing tendency has been to relate it to the *Woodsia* group, a group included in the Polypodiaceae by most systematists, in the Dicksonieae by Hooker and Baker. In Wallich's account of the Fern ('Plantae Asiaticae Rarae', p. 42) he says of his own specimen sent to the East India Company's Museum: 'In the Museum just mentioned the Fern was labelled "Cyathea ? barbata, Wall. vix non genus novum, Sphaeropteris, Nob. non Bernhardi".' This confusion of *Peranema* with *Cyathea* by the Museum authorities is also suggested in David Don's description in the 'Prodromus Florae Nepalensis', where the following concluding observation is made: 'Obs. Genus a Cyathea, cui affine, omnino distinctum: indusiis sphaericis indivisis pedicellatis.' Robert Brown's remark, already quoted, confirms this suggestion of an affinity with the Cyatheaceae. On the other hand, Mettenius and Christ place it in the Aspidiaceae, while C. B. Clarke in the 'Review of the Ferns of Northern India' ('Trans. Linn. Soc.', 2nd series, i, p. 435, 1880) describes *Peranema* as difficult to separate, when without fruit, from *Aspidium spectabile,* Wall. The intermediate position between

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[1] i.e. *Diacalpe, Sphaeropteris, Woodsia, Hypoderris.*

the Cyatheaceae and the Aspidiaceae, adopted for it by Presl, Robert Brown, and Diels, will be confirmed in this account of its anatomy.

The observations here to be detailed, and those later to be detailed for *Diacalpe aspidioides,* Bl., have been made partly on Herbarium specimens, but especially upon material preserved in spirit and upon living specimens, which were supplied from India, through the kindness of the Director of the Calcutta Garden. Strong plants of *Peranema* and *Diacalpe* from this source are under cultivation in the Glasgow Botanic Garden. The investigation was carried on partly in the Jodrell Laboratory, Royal Botanic Gardens, Kew. For facilities afforded there, I am much indebted to Lieut.-Col. Prain, F.R.S. To Mr. L. A. Boodle, F.L.S., and to Professor F. O. Bower, F.R.S., under whose direction the work has been carried on, I am indebted for much help and criticism.

EXTERNAL CHARACTERS.

The stock is distinctly upright, some four to six inches in length. The roots spring from every side of it and are branched and fibrous. The leaves are from one to three feet in length and of a bright green colour. Their stalks are long, sometimes a foot in length, convex at first and then furrowed on the adaxial side. Both stock and rachis are covered with paleae, which are lanceolate, with acuminate tips ; they have reticulate marking, almost entire or quite entire margins, and are of a light dusky red colour. These are persistent from the first on the young stalks. The leaves are tripinnate, broadly ovate, with acuminate apices. They have wide alternate pinnae, on the pinnules of which the leaf-segments are sessile. The leaf-segments are linear-oblong with rounded apices ; the lower ones are pinnatifid, the upper are broadly crenate. On the main rachis and on that of the branches are short, subulate, hyaline, incurved hairs, on which small glands are borne. Similar glands are also found on the rachis among the hairs, but arising directly from the epidermal cells of the rachis. The venation is open and simple ; no anastomosing veins are found in any part of the leaf (Pl. XXVIII, Fig. 1). On the under side the veins are provided with little stalked glands, the stalks of which are sometimes unicellular, sometimes multicellular. The sori are produced superficially on the lower side of the leaf. Each sorus is borne on a short stalk and arises upon the surface of a vein, but always some distance from the tip of the vein. The indusium is entire except for a small pit which is present on the under side and quite close to the junction of the stalk with the indusium. At maturity a vertical split occurs across the sorus, and the two sides of the indusium become reflexed and finally flattened out. The sporangia are very crowded on the elongated receptacle and are long-stalked. Each has a pale dusky red annulus. The spores are sub-globose and covered with warty markings.

INTERNAL STRUCTURE.

The stock is distinctly an upright one, widening gradually from the base upwards. It is covered by paleae of delicate texture and dark brown colour. Some of these are provided with numerous glands on the surface directed towards the stock. The whole stock is enveloped in a thick mass of sclerenchyma, several cell-layers in width. The ground parenchyma is compact, with few air spaces and those of small size, while the cells are filled with starchy and proteid contents. Throughout this parenchyma are scattered little masses of sclerenchyma, dispersed in irregular fashion, but of very firm texture. The leaves are inserted spirally on the stock, the divergence in the mature stock being one-seventh. A transverse section of the stock shows a series of meristeles, usually five or six in number, surrounded at their edges by a varying number of smaller strands (Fig. 2). One or two of the meristeles are elongated, and in transverse section show tracheides cut in an oblique or in a longitudinal direction. Other meristeles are rounded and have quite transversely-cut tracheides. If one of the elongated meristeles is followed up or down in the stock it is found to divide into two parts which assume a structure exactly like that of the remaining meristeles. Sometimes two elongated meristeles may be found opposite to each other. Generally, however, only one oval meristele appears in each section. This shows at once that the vascular system is dictyostelic. On dissection of the stock this dictyostele is found to consist of a series of strands united into a meshwork, in which the gaps have an oval form. It is indeed exactly like the advanced dictyostele generally exemplified in the vascular system of *Nephrodium filix-mas*, Rich. The gaps are found only opposite the entrance-points of leaf-traces into the dictyostele ; there are no perforations.

The leaf-trace is itself made up of three main strands and four or five subsidiary ones. Two of the main strands occupy the adaxial corners of the petiole, while the third occupies a median position on the abaxial curved side. Two smaller strands are found between each adaxial main strand and the median one, while often still smaller strands, of the nature of commissures, may be found between these subsidiary strands or between them and the main strands (Fig. 3). Sometimes a commissural strand may be nipped off from one of the adaxial strands, pass up to the next subsidiary strand, fuse with it on one side, then pass off on the other side, to unite with the next subsidiary strand a little further up the petiole. This process is continued up the petiole, the commissural strand passing from one strand to the next until it arrives at the second adaxial strand of the petiole.

When the leaf-trace strands are followed from the petiole into the dictyostele they are found to insert themselves along the sides of the oval gap. The strands from any one leaf-trace are inserted on the lower half of the two limbs of vascular tissue enclosing the gap opposite the leaf, while the median main strand is inserted at the lower junction of these limbs (Fig. 4, I, II). All the bundles from any one leaf-trace are inserted on the lower half of any leaf-gap. Evidently the upper half receives half the bundles from one leaf-trace which enters from a higher leaf and half the bundles from another. These two leaves are inserted on the axis obliquely above that of which the bundles enter on the lower half of the leaf-gap (Fig. 4, III).

This arrangement corresponds in the main with what occurs in the insertion of the leaf-trace on the dictyostele in *Nephrodium filix-mas*. But in the last-named Fern the adaxial bundles of the leaf-trace enter at the level of the bases of the gaps corresponding to the two leaves obliquely next above that to which the bundles belong. The median main strand enters at the fork on the lower half of the gap, just as in the case of *Peranema cyatheoides* (de Bary, 'Comp. Anat.', Fig. 132, and description, p. 285).

Histologically the meristeles of the stock are uninteresting. The xylem is made up of regular tracheides, interspersed with few parenchymatous cells and with no definitely differentiated protoxylem.

This is a characteristic of the meristeles of the stock among the Cyatheaceae, and except in that respect calls for no comment. The first lignification of the undifferentiated parenchyma in the young stock is on the inner side, as is represented in Fig. 5, which is made from a transverse section just below the apex of the young shoot. The stele is thus technically endarch as regards sequence of development, though no specifically marked tracheides of protoxylem appear to be found. Each meristele is enclosed in an almost complete sheath of phloem, which extends round the xylem on the inner and outer sides of the bundle, but is interrupted at the corners, so that two lenticules of phloem are found, one on each side of the mass of tracheides. The phloem is in turn surrounded by a parenchymatous pericycle of several layers and an endodermis with thickened cell-walls, often enclosing brownish contents. At the corners of the bundle the xylem comes directly into contact with the pericycle.

In the petiole the strands differ in structure from those of the stock. The leaf-trace strands leave the meristeles of the stock in quite simple fashion. In the case of the median leaf-trace strand, which departs first from the dictyostele at the fork below the gap, the oval strand shows a little hump or swelling on its outer surface. This gradually increases in size and rounds itself off from the parent stele as a plate of xylem surrounded by a sheath of phloem. The adaxial leaf-trace bundles leave the corners of two of the meristeles. They are at first simple in structure with a xylem-plate surrounded by a phloem sheath. In no case does a strand of the leaf-trace leave any gap in the xylem of the meristele from which it springs. Soon after the adaxial strands have passed into the base of the petiole their inner edges become extended, so that ultimately each of these strands is curved in form, with a dilated abaxial portion and a delicate strip of xylem projecting sometimes almost parallel to the lower surface of this, and forming a hook, with its back towards the periphery of the petiole (Fig. 6, *a*). Two or three protoxylem groups are found in the positions *prx.*^{1}, *prx.*^{2}, &c. The whole of the hooked mass of xylem is surrounded by phloem, which, however, thins out or altogether disappears on the side of the mass facing the centre of the petiole. A several-layered pericycle and a single brown-coloured layer of endodermis enclose each strand, while often a mass of sclerenchyma, one or two layers deep, encloses the whole bundle.

The median main bundle has a narrow plate of xylem with its long axis parallel to the adaxial side of the petiole, and with one or two protoxylem masses on the side facing the centre of the petiole. Phloem is present generally only on the side facing the periphery of the petiole. Sometimes a very small amount of phloem is developed also on the side facing the centre of the petiole.

The smaller subsidiary strands have the same structure.

Small groups of sclerenchymatous cells are here and there seen in the tissue of the petiole, generally between the subsidiary strands, but often also between them and the main strands. Other groups of sclerenchyma are found scattered in the ground tissue of the petiole.

When the supply to the pinnae is about to be given off, one of the adaxial strands of the petiole extends towards the adaxial corner of the petiole, lengthening the narrow strip of xylem on its adaxial side and pushing a tongue of xylem towards the pinna (Fig. 6, *b*). Some distance from the back of the hook two swellings occur in the xylem, one in the narrow portion, the other in the wide portion. These soon form a bridge, cutting off an island of parenchyma, the ring of xylem surrounding which has the shape of a signet ring in section, until at last the strand of the pinna-trace goes off as a ring of xylem with a larger amount of tracheides on one side than on the other (Fig. 6, *c*). Then the wider side of the ring pushes up a tongue of xylem, a gap appears, filled with parenchyma, the two halves of the strand draw apart to right and left, leaving a channel of parenchyma between them (Fig. 7, III). These take on the shape of the adaxial strands of the petiole. From one of them is nipped off a median strand of the shape of the early pinna-trace. And from it and from the other prosrachial[1] strand come two subsidiary strands. The final

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[1] As the faces of the rachis of the pinna are not orientated with regard to the stock but with regard to the rachis of the frond, the terms 'adaxial' and 'abaxial' cannot be used in connexion with the pinna-trace strands. It is therefore necessary to use new terms whereby these strands may be distinguished from one another. 'Prosrachial' means directed towards the rachis; 'aporachial', directed away from the rachis.

condition of the pinna-trace is that of a miniature petiole-trace, except that only two, instead of four or more, subsidiary strands are present. From the prosrachial strands the pinnules are supplied, as were the pinnae from the adaxial strands of the petiole (Fig. 7, IX, X). Through the rachis of the frond from the departure of the first pair of pinnae to the departure of the second pair there run five vascular strands and a commissural strand. To the second pair of pinnae the supply is given off from the prosrachial bundles in the same fashion as to the first pair of pinnae. The same process is repeated for the third pair of pinnae. Between pair 3 and pair 4 the number of strands is reduced to three, with a commissural strand. And pair 4 (pinnae VII and VIII) are given off again in the normal manner. Beyond pair 4 the vascular strands are only three in number with occasional fusions of the median aporachial strand and one or other of the prosrachial strands. Towards the tip of the leaf the strands are reduced in number to two, the pinna-supply going off in the normal fashion. Ultimately only a single strand remains in the rachis. This strand has a narrow plate of xylem, with no incurved ends, and from its edges the simple strands to the ultimate segments are quite simply nipped off. Here the plate extends in the direction towards the pinna to be supplied, and the marginal group of tracheides surrounds itself with phloem, pericycle, and endodermis, while the parent strand re-forms its simple plate. As this type of pinna-supply differs from that given to the basal pinnae, it is proposed to term it the 'marginal' type, while that found in connexion with the lower pinnae and already described may be termed the 'extramarginal' type. To these two types of pinna-supply and their significance reference will be made later on in the paper.

The root is diarch, with a single plate of xylem, composed of a set of four or six tracheides and two protoxylem groups, one at either end. The protoxylem elements abut directly on the pericycle, which is of two layers of cells at these points. Between the secondary xylem and the phloem is a series of parenchymatous cells, one or two layers thick. These have prominent nuclei and dense protoplasmic contents. Phloem surrounds this zone on the outer side. The endodermis has brownish cells, while the cortex is made up of thickened cells with deep brown coloured walls. The greater part of the piliferous layer breaks away, leaving the cortex as the outermost layer of the root. The stele comes off obliquely from the back of one of the meristeles of the stock, the root passing out into the soil in a slightly decurrent direction.

On the petiole and rachis of the mature leaf are innumerable paleae and hairs. The paleae are very similar to those on the stock, but their edges are quite entire. Each runs out into an acuminate apex (Fig. 8). Along the margins of a palea a few unicellular glands are inserted (cf. the paleae of *Blechnum occidentale*, Gardiner and Ito, '87), and these project directly out from the edges of the palea. Occasionally a gland may be found on the adaxial face of the palea, usually about its centre. Curious curved hairs are liberally distributed upon the surface of the petiole and rachis among the paleae. These hairs are inserted on distended boss-like cells of the epidermis. Each hair is made up of eight or ten cells of rectangular form. Two of these rise directly up from the boss-like cell, while the rest form a tapering series which bends over at its tip almost to touch the epidermis. At the top of the curved part two or three glands are inserted. These are of the unicellular type found on the paleae. Sometimes a gland may be inserted near the tip of the hair, but more usually a group is found on the curved portion of the hair (Fig. 9).

Here and there on rachis, petiole, and on the back of the veinlets of the pinnae and pinnules unicellular glands are also found simply inserted on the epidermal cells. In the young parts of the plant and on the petiole these glands have a hyaline margin, which appears to be filled with a clear fluid secreted by the gland (Fig. 10). Right over the top of this secretion passes a limiting membrane, which does not, however, appear to be cutinized, though it is continuous with the outer layer of the stalk of the gland. In the older parts of the plant and on the lower portion of the petiole the glands have no hyaline covering, but are limited externally by a thin cell-wall, and contain a distinct nucleus and dense cytoplasm (Fig. 11). Apparently the secreted fluid disappears from the glands as they grow older. The glands closely resemble those found in the tissue of the petiole and stock of *Nephrodium filix-mas* (de Bary, pp. 89 and 220, and Schacht, '63). They give to the plant a very distinct odour, somewhat recalling that of *Heracleum Sphondylium*, and also bearing some resemblance to that of *Lastrea Oreopteris*. In the case of *Peranema*, however, the glands do not show the golden appearance of those in *L. Oreopteris*, but rather a pearly white appearance by reflected light, while they are quite translucent by transmitted light.

On the lower surface of the veinlets in the pinnules are found short recurved hairs bearing no glands. They project from the epidermis and give the suggestion of little bent pegs hanging down from the pinnules. They are multicellular, but their cells are in a single series or row, and are some six or seven in number.

The segmentation of stem and leaf goes on in the usual fashion for Leptosporangiate Ferns. The wings of the leaf show the customary marginal series of cells (Fig. 12).

SORUS AND SPORANGIA.

The mature sorus and sporangium have been described by Mettenius ('Filices Horti Bot. Lips.', 1856, No. 40), and by Wallich ('Plantae Asiat. Rar.', p. 42). Drawings of sorus and sporangium after F. Bauer appear in

Engler and Prantl's 'Natürliche Pflanzenfamilien', where habit drawings of the sorus by Diels are also produced. In 'Phil. Trans.', vol. cxcii, 1899, p 57, Professor Bower has given a description of the sporangium from Herbarium material. F. Bauer's drawings in the 'Genera Filicum' and Professor Bower's drawing (loc. cit., Fig. 92) give representations of the sporangia. The sorus has never been adequately figured. Bauer's figures give no real idea of the nature of the receptacle, and, as Professor Bower remarks, 'suggest a regularity of orientation, which is not, however, to be recognized as constant in Herbarium specimens.'

The sorus is superficial in insertion and stalked. The stalk springs from one of the lateral veinlets on the under side of the pinna, and generally grows out for one millimetre or longer, vertically from the leaf. The sorus is spherical and, as Wallich remarks, 'about the size of a Coriander seed'. The youngest stages have, unfortunately, not been available. The material sent from Calcutta had upon it only mature or almost mature sori. And the living plant which accompanied this material, and which is now growing in the Botanic Gardens in Glasgow, has, during the two years it has been there, produced no sori. From the youngest sori available for examination it appears as though the indusium completely encloses the sorus. But at one point, close to the junction of the stalk with the indusium, a minute pore can be seen on the outside of the sorus. This forms a very narrow slit leading into the interior. Except at this pore, the indusium is quite entire, giving the outward impression of an unbroken covering for the sorus. It is essentially a cup, developed unequally on its two sides and contracted at its rim, which is turned inwards (cf. Fig. 15). When the sorus is mature a series of cells across the top breaks down, and an irregular crack is formed from one side of the sorus to the other. The two flaps of indusium thus formed bend back, exposing the sporangia within to the outer air. In this opened condition the resemblance to the sorus of *Cyathea* is remarkably close and convincing. It is this opened sorus which has been drawn by Bauer.

The indusium is made up of a single layer of cells, except just at the point of insertion on the receptacle, where it may be two layers in thickness. The cells are triangular or quadrangular in outline with triangular thickenings at their corners, giving the whole indusium a lace-like appearance. The receptacle is distinctly elongated, and occupies about one-half of the cavity covered in by the indusium. A series of tracheides runs into the receptacle from the stalk, and they then spread out in fan fashion just above the point of junction of receptacle and indusium (Fig. 13). This vascular supply passes right through the stalk of the sorus in a median direction, and merges into the vascular supply of the vein of the pinna.

On this elongated receptacle, which is distinctly of the Gradate type and strongly recalls the receptacle of the Cyatheaceae, are inserted some four hundred sporangia. Young and old sporangia are inserted promiscuously round the receptacle, a predominance of younger ones occurring towards the lower edges (Figs. 14 and 15). Young ones also occur at the apex of the receptacle among the stalks of quite mature sporangia (Fig. 14). The sorus is thus one distinctly of the mixed type, upon a receptacle characteristic of the Gradatae. The presence of younger sporangia towards the lower edges of the receptacle confirms the suggestion of the derivation of this sorus from one of a Gradate type.

There is no regularity of orientation among the sporangia in the sorus. Their annuli are arranged pointing in all directions when looked upon from above.

The sporangium itself is long-stalked, the stalk often taking a slightly curved form, the convex side of the curve being on the same side as the annulus (Fig. 16). Two or even three series of elongated cells are found contributing to the length of this stalk. In section the stalk consists of three cells symmetrically arranged about the centre, one rather larger than the other two (Fig. 17) as in *Nephrodium*. The capsule is rather elongated, much like the capsule of a typical Polypodiaceous Fern. The annulus consists of twelve to fourteen cells, indurated on the four sides, though, as usual, least strongly on the outer walls (Fig. 16). It passes right over the top of the capsule, and is continued always right back to the stalk as an unbroken and symmetrical series of typically indurated cells (Fig. 16). Frequently indurated walls are found in the cells past the stalk, though more commonly the induration stops in the one just at its centre. The series of cells of the annulus is, however, always continued past the stalk. As this stalk consists of three cells, they are arranged in a group of two on one side of the capsule, with the single one on the other side. The annulus is continued to one side of the single one, passing right across the tops of the cells in the other two (Fig. 18). It is thus slightly oblique in its insertion (Fig. 19).

The stomium consists of a series of wide cells, often seven in number, which continue and complete the hoop of the annulus round the sporangial head. Two of these cells are always strongly indurated, and are wider than the rest (Fig. 20). It is between these that the first break occurs, which allows the mechanism of the annulus to work and to secure the dispersal of the spores. The dehiscence of the sporangium takes place by a series of irregular cracks stretching across the sides of the capsule from the stomium.

The spores within each sporangium appear to number sixty-four. This number is characteristic rather of the Gradatae than of the Mixtae, though it is a feature to be anticipated in view of the elongated receptacle and the slight obliquity of the annulus. These three features of sorus and sporangium must be weighed against the long stalk of the sporangium and the mixed condition of the sorus in estimating the systematic position of *Peranema.* For the present it may be noticed that they all indicate that *Peranema* occupies an interesting position intermediate between Gradatae and Mixtae, and combines in its mechanism for spore production and dispersal the main characteristics of both groups.

The spores are very characteristic. They are of somewhat large size, and have on their surfaces very peculiar markings (Fig. 21). Each spore is surrounded by a membrane which encloses it rather loosely. At certain points, perhaps four or six or more, on its surface, the membrane is attached to the spore itself. These points take the form of little circular discs, each with a minute papilla in its centre, a portion of the membrane detached from the spore wall and filled with air. As a result of the presence of these circular pits and their included papillae, the surface of the membrane appears to be crossed by a series of folds of irregular shape. These are of course the portions of the membrane surrounding the circular pits, portions which are quite free from the spore. Such markings are found with some slight variation among many genera of Polypodiaceae, particularly in the genus *Aspidium.* They have lately been described by Hannig ('11). He finds that in the Cyatheaceae (loc. cit., p. 342), and in the genus *Polypodium* (p. 344), there is no loose membrane ('perispore') around the spores. From the characteristic appearance of the perispore in the genus *Aspidium* he has been led to distinguish spores surrounded by a similar perispore as 'aspidioid'. He remarks (p. 340) that the genera *Peranema*, *Diacalpe*, and *Woodsia* possess aspidioid spores.

Unfortunately the first set of germinated spores in the Glasgow Botanic Gardens damped off before the antheridia had developed upon the prothalli, and the second set have not yet produced antheridia. The first stages of development are quite normal. The later stages have not been available, and no conclusions can therefore be made from the characters of the gametophyte generation.

DIACALPE ASPIDIOIDES, Bl., was first described by C. L. Blume in his 'Enumeratio Plantarum Javae et Insularum adjacentium', published in 1828. From the genus *Woodsia*, R. Br., to which he says it bears close affinity, Blume separates it because the indusium is not 'dish-shaped and ciliated'. He places it in the third section of Kaulfuss's family Polypodiaceae, a section characterized by the sporangia being borne on receptacles, and by the indusium springing from below the sorus or being altogether absent. In the same section are included also *Cyathea*, *Alsophila*, and *Hemitelia*. *Diacalpe* grows, he remarks, 'in sylvis Javae altissimis'.

It is included in the Appendix to Presl's 'Tentamen Pteridographiae' (1836), with the query: 'An Cyatheacea, an Cathetogyrata stirps?'

J. Smith, in Hooker's 'Journal of Botany', iv, 1842, p. 191, assigns it, with *Peranema* and *Woodsia*, to the Aspidieae.

By Sir W. J. Hooker ('Genera Filicum', t. 99, and 'Species Filicum'), it is placed beside *Peranema* in the Dicksonieae, sub-tribe Woodsieae.

In the 'Synopsis Filicum' (Hooker and Baker, '74), it appears next to *Alsophila* and between it and *Matonia*, accompanied by the comments (p. 45): 'This genus seems more appropriately placed with the Cyatheae than with the Dicksonieae. The fronds much resemble those of *Davallia nodosa*' (which is the original *Acrophorus* of Presl). The close resemblance between *Diacalpe aspidioides* and *Davallia nodosa* is mentioned also by C. B. Clarke in the 'Review of the Ferns of Northern India' ('Trans. Linn. Soc.', 2nd ser., vol. i, pp. 434 and 445).

Captain R. H. Beddome in 'The Ferns of Southern India' (1863) separates *Peranema* and *Diacalpe* as the group Peranemeae of the Polypodiaceae. This group comes between the Davallieae (including *Microlepia* and *Davallia*) and the Cyatheae (with *Cyathea* and *Alsophila*).

Raciborski ('98) in the 'Pteridophyten der Flora von Buitenzorg' places *Diacalpe* between *Asplenium* and *Dicksonia* in the B. II group of Polypodiaceae.

Diels (Engler and Prantl's 'Natürl. Pflanzenfam.') associates *Diacalpe* with *Peranema*, and these two with *Woodsia*, *Hypoderris*, and *Acrophorus* in the Woodsieae group of Polypodiaceae.

By Wallich, *Diacalpe aspidioides* was named *Aspidium foliolosum* (Cat. no. 359) and *Sphaeropteris Hookeriana* (Cat. no. 775). Following on this Presl, though he puts '*Diacalpe aspidioides*, Bl.', among the insufficiently described genera, calls *Aspidium foliolosum* by the name *Cystopteris gigantea*, and places *Cystopteris* next to *Acrophorus*.

By Kunze ('Anal. Pterid.', p. 43) *Diacalpe* was named *Physematium aspidioides*. Another species of *Physematium* (*P. molle*, Kaulf.) is called *Woodsia mexicana* by Robert Brown ('Miscellaneous Works', ii, p. 547), who remarks that it is closely related to *Peranema cyatheoides*. These confusions of nomenclature all converge on a grouping together of *Peranema*, *Diacalpe*, *Woodsia*, and *Acrophorus*.

The hint of a relationship between *Diacalpe* and the Aspidieae given directly by J. Smith, and by Wallich in the name *Aspidium foliolosum*, is confirmed by Christ in a review of Christensen's 'Index Filicum' (Hedwigia, 1908, xlvii, p. 145): '*Peranema*, *Diacalpe*, *Acrophorus* einerseits, *Woodsia* und *Cystopteris* andererseits, werden wohl schliesslich auch zu den Aspidieae (bei *Lastrea*) gezogen werden müssen.'

EXTERNAL CHARACTERS.

The stock is upright, or at first creeping and becoming upright before more than twelve leaves have been produced. It is narrower than that of *Peranema*, and is not covered by a dense felt of paleae. The leaf-insertion depends on the size of the stock; in one case the divergence has been found to be a fifth, in other cases it is irregular.

The leaves are large, from two to four feet long, with a long petiole, and are decompound and herbaceous. The lower part of the petiole is covered with broad, brown membranaceous scales. Similar but smaller scales appear together with hairs as little tufts at the axils of the primary pinnae on the younger branches. The frond is tripinnate, the primary pinnae often being nearly opposite. The rachis of the frond and that of the pinnae bear short scales and hairs of a single long series of cells. Long hair-like scales also appear on the upper surface of the leaf above the veins. Unicellular glands are found on the petiole and rachis among the scales and hairs, but no glands are present elsewhere on the leaves. On older leaves the scales and hairs disappear from the raches, which are then quite smooth.

The primary pinnae are lanceolate, the secondary pinnae being obtuse and oblong-cuneate; the lower pinnules are deeply pinnatifid and somewhat decurrent, so that the rachis of the ultimate pinna is generally winged. The venation is open; the veins are simply pinnate, and the veinlets are undivided, not reaching quite to the margins of the leaflets.

The sori are produced superficially on the under surface of the leaf, usually on the lower anterior veinlet of each segment, at a point below its termination. Usually there is only one sorus on each veinlet. The sorus is apparently sessile, but it is not strictly so, being seated on a slightly elevated point, from which the entire sorus can be freely removed without rupturing the base of the indusium or receptacle. The indusium is at first quite entire, completely enclosing the sorus. Its texture is very coriaceous. It ultimately breaks irregularly at the apex, and opens by two or more valves. The receptacle is short and is covered by a large number of sporangia, irregularly arranged within the sorus.

The sporangium is long-stalked, with a somewhat bulky capsule, and an annulus vertical in insertion but slightly twisting to one side as it crosses the capsule. The spores have warty superficial markings.

INTERNAL STRUCTURE.

The transverse section of the stock very closely resembles that of the stock of *Peranema cyatheoides*. The meristeles are inserted in the same fashion, and the leaf-traces bear the same relation to the main vascular system as do those of *Peranema*. The vascular system is in *Diacalpe* also an advanced dictyostele, but all the gaps are here, too, leaf-trace gaps. Schlumberger (‘ Flora ’, cii, N. F. 2, 1911, p. 409) states that there are perforations in the dictyostele in *Diacalpe aspidioides* : ‘Ausser den Blattlücken tritt wie bei den Cyatheaceen eine ganze Reihe von “ Perforationen ” auf.’ In the stocks I have dissected I have been unable to discover the presence of any such perforations. In all respects the dictyostele of *Diacalpe* has been found to agree with those of *Peranema cyatheoides* and *Nephrodium filix-mas.* The leaf-trace is made up of three main bundles and a varying number of subsidiary bundles, with occasional commissural bundles uniting one main bundle with the subsidiary ones or with one of the other main bundles. The strands of the leaf-trace are inserted on the lower half of each gap opposite the entrance of the petiole to which the trace belongs, and the median main bundle of the trace enters the dictyostele at the lower fork of the gap. With regard to the histology of the supply of the leaf-trace from the dictyostele, the facts are the same for *Diacalpe aspidioides* as for *Peranema.* The adaxial main bundles of the leaf-trace have deeply incurved hooks on their adaxial faces. From the backs of these hooks the pinna-traces go off in the extramarginal fashion. The pinna-trace is at first a simple strand, but it soon breaks up, as in *Peranema*, into three strands. The pinnules are supplied from these on the extramarginal type. The ultimate pinna is supplied in marginal fashion from a dorsiventrally-constricted simple xylem-plate. The same process occurs in the supply of the ultimate pinnules from the pinna-traces.

Scales of three rows of parenchymatous cells, some six or eight in each row, occur on the leaves, usually above a vein or veinlet. Simple unicellular glands, but no multicellular hairs bearing glands, occur sporadically on rachis and leaves. Paleae of the type found in *Peranema*, but without glands on the edges, are present at the bases of the primary pinnae and on the raches and petiole in younger leaves ; few or none are found on fully-matured leaves.

The sorus is at first entirely covered by the indusium. There is in this indusium no such pit or pore at one side as is found in that of *Peranema.* Here the indusium forms an unbroken symmetrical covering for the sporangia, coriaceous in texture, with cells of polygonal outline possessing triangular thickenings at the corners. In section these cells are rectangular, often square, with thickened walls.

The receptacle is short but not flattened, and is supplied by a short fan of tracheides from the vascular bundle of the veinlet on which the sorus is seated. The sporangia are numerous. They are of all ages in the same sorus and are inserted on the receptacle in distinctly mixed fashion. There is no trace of a basipetal sequence of development. The full developmental stages have unfortunately been unavailable, but even in moderately young sori no traces of very young sporangia were found at the basal edges of the receptacle. The youngest sporangia, showing merely two or three segmentation-walls, were found at the apex of the receptacle close to fully developed sporangia. There are apparently 95 sporangia in each sorus. Three countings gave 93, 95, 97 for different sori.

The sporangium has a long stalk, scarcely so long as that of the sporangium of *Peranema*, with three rows of cells in the stalk. The annulus stops short of the stalk in its insertion (Fig. 22), but in passing across the head of the sporangium it twists slightly to one side and then back again into the vertical position before reaching the region of the stomium. This very slight obliquity of the annulus makes it possible to discriminate between the two sides of the sporangium, a fact referred to by Professor Bower (‘ Phil. Trans.’, vol. cxcii, p. 104) : ‘ In *Diacalpe* the two sides are so far dissimilar that it is possible still to distinguish the “ central ” from the “ peripheral ” face.’

Hairs are sometimes present on the stalks of the sporangia in *Diacalpe* (Fig. 23) as they are in *Nephrodium filix-mas*.

The spores in a single sporangium number about forty-eight. Each has an exospore and a perispore, attached at certain ring-shaped areas to the exospore and forming irregular folds and papillae on the surface of the spore. Here the circular pits with papillae in their centres are few in number ; the main portion of the perispore is thrown into irregular folds, causing its projection into a series of warty protuberances (Fig. 22). The spores of *Diacalpe* are characteristic ‘ aspidioid ’ spores (Hannig, ’11) and quite closely resemble those of *Nephrodium filix-mas*, where there are only warty protuberances on the surface of the spore. In *Peranema* the flanges of perispore are more prominent and numerous than the papillae ; in *Diacalpe* these flanges are almost entirely absent.

The prothallus has been described by Schlumberger (‘ Flora’, cii, N. F. 2, pp. 384 sqq.). On the under surface of the developed prothallus multicellular stalked glandular hairs are present. The stalk is composed of two or three cells, the middle one often showing a longitudinal division. The presence of multicellular hairs is a characteristic of the prothalli of the Cyatheaceae (Heim, ‘ Flora ’, lxxxii, 1896, pp. 360 sqq.). Schlumberger remarks for *Diacalpe* (p. 385) that ‘ in many cases, where the terminal cells are not developed as glands, these structures cannot be distinguished from the multicellular hairs of the Cyatheaceae ’.

The antheridium of *Diacalpe* has a segmented lid-cell, another characteristic feature of the Cyatheaceae (Bauke, ‘ Jahrb. für wiss. Bot.’, x, p. 72). It is therefore unmistakable that the closest relationship exists between the prothallus of *Diacalpe* and that characteristic of the Cyatheaceae.

An attempt may now be made to compare the features of *Peranema* and *Diacalpe* with those of other Ferns, with a view to locating these genera in a systematic position among the Filicales. They have so many characters in common that it will be quite possible to consider their position first together, and then to see how far the characters in which they differ may indicate special affinities for each of them with other Ferns which they resemble.

The ascending or upright stock which they show is shared by representatives of various groups of Ferns and cannot in itself be held as a strong diagnostic feature. It may none the less be noted that in *Athyrium filix-foemina* and in *Nephrodium filix-mas* the stocks are upright and of a similar type to that described for *Peranema* and *Diacalpe.* But in the latter pair there is a great development of sclerenchyma, which recalls the condition in the Cyatheaceae rather than that usual among the Polypodiaceae. In *Athyrium* and *Nephrodium filix-mas* definite sclerenchymatous sheaths are absent from the stocks.

The presence of both hairs and scales removes the two from such forms as those included in the (1) *Eudryopteris* section of *Dryopteris* (Christensen, '11) and relates them to (4) *Lastrea.*

In both cases the form of the leaf strongly suggests affinity with the Aspidieae. The tripinnate form is characteristic of the leaf in *Nephrodium* (*Lastrea*). The open, simply forked venation is a primitive feature, but it is shared by the members of the *Eudryopteris* section of *Nephrodium* and by numerous other members of the Polypodiaceae.

The hairiness of the leaves suggests a relatively primitive state, while the glands in the case of *Peranema* show resemblances both to those in *Eudryopteris* and in *Lastrea.*

When we look at the anatomical features we find that the general plan of the vascular system, with its advanced dictyostele, which shows no perforations but only leaf-gaps, and its subdivided leaf-trace, at once suggests affinity with *Nephrodium filix-mas.* The latter Fern appears, however, in the method of insertion of the leaf-trace strands on the gaps of the dictyostele to show an advance on the condition found in *Peranema* and *Diacalpe.* In these Ferns the leaf-trace bundles are inserted altogether on the lower half of the leaf-gap; in *Nephrodium filix-mas* the bundles are more spread over the sides of the gap. As in both cases the median main bundle of the leaf-trace is inserted at the lower fork of the limbs surrounding the gap, it would seem that this condition has been derived from that seen in solenostelic and in the simpler dictyostelic types by a breaking-up of the continuous horseshoe-shaped leaf-trace into a series of separate strands. The histology of the bundles would seem to confirm this. For the adaxial face of the leaf-trace in various Ferns, as illustrated in such types as *Loxsoma* (Gwynne-Vaughan, '01, Figs. 5, 6, 7) and *Davallia Speluncae* (Gwynne-Vaughan, '03, Fig. 26), shows incurved edges. And the adaxial leaf-trace bundles in *Peranema*, *Diacalpe*, and *Nephrodium filix-mas* also show such incurved edges. Protoxylem groups appear in these curved edges of the leaf-trace in *Loxsoma* and *Davallia*. The adaxial strands of the leaf-trace in *Peranema* and *Diacalpe* each possess two protoxylem groups in the hooked portion of the strand (Fig. 6). The facts in *Onoclea* have an interesting bearing on this point. Thus in *Onoclea orientalis* the leaf-trace bundles at their insertion on the leaf-gap are two separate strands with hooks at each end. They are inserted just at the base of the gap and symmetrically on its limbs (Fig. 4, V). At a point higher up in the petiole, however, and before the departure of the pinna-traces, this pair of bundles becomes joined together and forms a single curved trace with hooked ends, from which the supply to the pinnae is given off in extramarginal fashion. The same condition is found in *Onoclea sensibilis*.[1]

I find in my specimens of *Lomaria Spicant* (Fig. 4, IV) that three bundles enter the dictyostele at the base of the leaf-gap, one in a median position, the other two in adaxial positions.[2] The two adaxial bundles have hooked ends. The median one has a slightly curved plate of xylem, rather constricted in the centre and suggesting that it has been derived from the back of a triangular bundle of the type of *Gleichenia dicarpa* (Bower, 'Land Flora', p. 563, Fig. 314) through a suppression on either side of the central part of the limbs of the horseshoe.

Lastrea Oreopteris (Duval-Jouve, Pl. I, Fig. 2), another dictyostelic type, has a couple of strap-shaped leaf-trace bundles, which are inserted on either side of the leaf-gap, almost midway up the gap. In this case also the two widen and ultimately fuse to form a single curved strand in the petiole below the first pair of pinnae.

In *Athyrium filix-foemina* (Fig. 4, VI), on the other hand, the leaf-trace supply goes off from the base of the gap as a single triangular strand which almost at once breaks up into two strap-shaped bundles, and these below the first pair of pinnae fuse again into a single curved strand (Duval-Jouve, Pl. I, Fig. 1), a case recalling that of *Plagiogyria semicordata* (Bower, '10, p. 431), in which a single curved strand breaks up into three parts, to reunite below the first pair of pinnae into a single strand.

These facts would seem to indicate that the broken leaf-trace has originated from a single horseshoe-shaped or curved strand by a suppres-

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[1] In Annals of Botany, 1911, p. 177, Sinnott considers the Onoclean double leaf-trace as an advance on the 'ancestral triangular mesarch bundle'—e. g. of *Gleichenia Speluncae*—and the approximation of the two bundles as a movement towards the ancestral type. But the two bundles do come together in the petiole before the pinnae go off, and so make his 'ancestral character'. Still (p. 167) the base of the leaf-trace is 'the seat of ancestral characters'; 'here we should expect to find indications of what was the primitive foliar bundle in the ferns'; 'in this portion the effect of changing external conditions . . . must be felt last and least.' And yet the base of the leaf-trace in *Onoclea sensibilis* really shows an advanced condition (according to Sinnott's own hypothesis) on what appears higher up!

[2] This corresponds in substance, though not in exact detail, with the statement of Luerssen in Rab., Krypt.-Flora, vol. iii, p. 114.

sion of part of its xylem, or by its breaking up into a series of isolated strands, just as the leaf-trace of *Dicksonia Barometz* breaks up into a series of strands arranged in horseshoe fashion in the petiole (Gwynne-Vaughan, '03, Fig. 17). In some cases (e. g. *Onoclea* and *Lastrea*) the abaxial face has been suppressed, but later on resumes its position. In other cases short portions of the sides have been dropped out, causing a trace of several strands to result. These would seem at first, as in the type of *Lomaria Spicant*, to have been inserted right at the base of the gap. Later they became extended up its sides but kept to its lower half, as in the *Peranema* and *Diacalpe* type; while ultimately they became distributed well over the sides of the gap, extending into the upper half as well as occupying the lower, as in *Nephrodium filix-mas*. It is curious that no case has been found in which the several leaf-trace bundles, such as occur in *Nephrodium filix-mas*, fuse together in the petiole to form the single strand, while in cases with the Onoclean leaf-trace the single strand is regularly found in the petiole below the first pair of pinnae. But it would seem probable, at least, that development has gone along a line of breaking-up of the single horseshoe-shaped strand into several parts (Tansley, '08, p. 120), of the retention of these at first towards the base of the leaf-gap, and later of their extension up the arms of the gap. It would appear on this view that *Peranema* and *Diacalpe* come structurally very close to *Nephrodium filix-mas*, but that they show scarcely so advanced a condition of the arrangement of the leaf-trace bundles on the dictyostele as does that Fern.

The discussion of the relationship of the pinna-traces to one another in different groups of Ferns must be left for a future paper.[1] It will suffice to say at present that, while *Peranema*, *Diacalpe*, and *Nephrodium filix-mas* show exactly the same type of pinna-supply, the criterion of marginal or extramarginal departure of the supply cannot be held as valuable among the different groups. That there is some constancy in the type of pinna-supply in Ferns of the same group has been shown by P. Bertrand ('09) and Gordon ('11) for *Clepsydropsis*, *Diplolabis Römeri*, and *Metaclepsydropsis duplex*. The vascular details in the leaves in *Peranema*, *Diacalpe*, and *Nephrodium filix-mas* are, however, identical. Such a fact may have some weight in confirming an affinity of *Peranema* and *Diacalpe* with *Nephrodium*.

The presence of glands and of stalked hairs bearing glands would seem to indicate a relationship for *Peranema* with *Lastrea*. Glands are present internally in the rhizome and at the base of the petiole in *Nephrodium filix-mas* (Sachs, p. 439; de Bary, pp. 89 and 220), internally at the base of the petiole in *Aspidium spinulosum* (de Bary, p. 220), externally on petiole

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[1] The question has already been discussed for the Osmundaceae by Sinnott ('10) and Gwynne-Vaughan ('11).

and rachis in *Dryopteris pulvinulifera*, (Bedd.) O. Ktze.,[1] on the leaf of *N. molle* (de Bary, p. 89), and in *N. Sieboldii* (Goebeler, '86).

The fertile leaf and sorus in both *Peranema* and *Diacalpe* at once suggest relationships with Cyatheaceae and Polypodiaceae. In both the insertion of the sorus is superficial. The sorus is restricted to the veinlet and is inserted at a point short of its termination. This also holds for *Cystopteris*, for the Aspidieae, and for *Woodsia obtusa*.

The indusium is characteristically basal in the Cyatheaceae and in the Woodsiinae (Diels) group of Polypodiaceae. The indusium of *Diacalpe* corresponds in insertion exactly with that of *Woodsia*. In the case of *Peranema* the indusium appears to be unequally developed on the two sides, giving a suggestion of a Cyatheaceous sorus twisted to one side and with its rim tucked in beside the point of insertion (cf. *Allantodia*, which is to *Asplenium* as *Peranema* is to *Cyathea*).

But in the Cyatheaceae the sorus is distinctly of the Gradate type (Bower, 'Studies', IV, p. 54). In *Peranema* and *Diacalpe* the sorus is clearly a mixed one, though suggestions of a basipetal tendency in development have been found in *Peranema*, a fact confirmed by the presence of a distinctly elongated receptacle and a spore-output per sporangium of sixty-four. In *Diacalpe*, where the relationship on the ground of insertion of the indusium appears clearly to be with the Cyatheaceae, the sorus is a characteristically mixed one, with no signs of any basipetal tendency. It must be regretted, however, that developmental stages have been unavailable. In the absence of these, final conclusions cannot be drawn with regard to the sorus of these two Ferns.

The texture of the indusium and its insertion suggests unmistakable affinities with *Woodsia* and *Hypoderris*, in which (e. g. *Woodsia obtusa*) the sorus at maturity bears an exceedingly close resemblance to that of *Diacalpe*.

The stalked sorus of *Peranema* is almost unique, though it does find a parallel in the stalked sorus of *Marattia Kaulfussii*. But in the latter the details are quite distinct, while in its stalk there is no vascular bundle developed. A mixed sorus is found throughout the genus *Nephrodium*. Sections through the sorus of *N. molle* and *N. Sieboldii* show very close resemblances to the sorus of *Diacalpe*. The receptacle in *Dryopteris pulvinulifera*, which in habit closely resembles *Peranema*, is a little larger than that of *Diacalpe*. It is shortly stalked and is supplied by tracheides from the vascular bundle of the vein on which it is inserted. The sporangium is long-stalked, with three series of cells in the stalk. The annulus stops short of the insertion of the stalk. It generally passes vertically across the capsule, but occasionally it may take a slightly oblique course, twisting

[1] For the identification of this Fern (sent from India through the kindness of the Director of the Calcutta Botanic Garden) I am indebted to Dr. Carl Christensen.

a little to one side at its point of maximum curvature and then twisting back into the vertical position. This recalls the condition of *Diacalpe*, in which, though the annulus stops short of the stalk, it is so far twisted in its course that the two faces of the sporangium can be readily distinguished.

Peranema has an oblique annulus, which always passes the insertion of the stalk, though it is not always indurated throughout its whole length. The presence of an oblique annulus in a Fern with a mixed sorus is only paralleled by the cases of *Dipteris conjugata* (Miss Armour, '07) and *Plagiogyria* (Bower, '10). In *Peranema*, however, the obliquity is very slight and is accompanied by unmistakable vestiges of a Gradate condition. In the absence of developmental evidence it would seem that *Peranema* occupies sorally an intermediate position between the Gradatae and the Mixtae, combining the receptacle, the annulus, and the spore-output of the one with the sporangial succession of the other. *Diacalpe* is more distinctly related to the Mixtae, in receptacle, sporangial succession, and spore-output. The annulus, while vertical in insertion, still possesses a tendency to obliquity, which is seen, less highly developed, in the annulus of *Dryopteris pulvinulifera*. This affinity with *Nephrodium* is strengthened by the presence of hairs on the sporangial stalks in *Diacalpe* and in *Nephrodium filix-mas*.

The spores in *Peranema* and *Diacalpe* are distinctly 'aspidioid'. But those of *Diacalpe* are more closely related to those of *Nephrodium filix-mas* than are those of *Peranema*. In *Diacalpe* warty protuberances are much more numerous than perispore flanges and papillate pitted areas. The spores of *Dryopteris pulvinulifera* occupy an intermediate position between those of *Diacalpe* and those of *Peranema*. They possess both warty protuberances and perispore flanges, but the warts are rather more numerous than the flanges.

The characters of the gametophyte generation are only known for *Diacalpe*, but they make its affinity with the Cyatheaceae quite undoubted.

On the whole, then, *Peranema* and *Diacalpe* occupy an intermediate position between the Cyatheaceae and certain phyla of Polypodiaceous character. With the former they are joined on the characters of the prothallus of *Diacalpe* and of the receptacle and annulus of *Peranema*; with the latter they show affinity in the vascular anatomy, the sporangial succession in the sorus, the length of the sporangial stalks, and the characters of the spores.

The affinity with the Aspidieae, and more particularly with the genus *Nephrodium*, which is suggested by their habit, has been confirmed in their dictyostelic vascular system, in the scales on petioles and raches, in the glands on leaves and leaf-stalks, in the mixed sequence of sporangia, in the hairs on the sporangial stalks in *Diacalpe*, and in the spore-markings in both Ferns.

The natural position for *Peranema* and *Diacalpe* seems to be with

Woodsia and *Hypoderris*. The texture and insertion of the indusium are similar in all the four genera, while they all occupy an undoubtedly intermediate position between the Cyatheaceae and certain phyla of the Polypodiaceae.

It would seem that the very different habit of *Woodsia* on the one hand, and of *Peranema* and *Diacalpe* on the other, can be readily accounted for on the ground of environment, while the same remark applies to *Hypoderris*. *Hypoderris* has probably developed its hardly divided leaf in relation to conditions of shade. *Peranema* and *Diacalpe*, both inhabitants of mountainous tropical forest, have grown luxuriantly, while *Woodsia*, an essentially Alpine Fern, has reduced its vegetative system and elaborated the protective coverings of its indusium in a series of highly efficient scales.

Peranema and *Diacalpe* are undoubtedly very closely related to each other. It is hardly possible to say which is the more primitive, which the more advanced. The receptacle, sporangial succession, and annulus of *Peranema* are more nearly related to those of the Cyatheaceae than are those of *Diacalpe*. On the other hand, *Diacalpe* shows an undoubtedly Cyatheaceous prothallus. The spore-markings in *Diacalpe* most closely resemble those of *Nephrodium filix-mas*; those of *Peranema* are most nearly related to those of *Dryopteris pulvinulifera*. The presence of hairs on the stalks of the sporangia is common to both *Diacalpe* and *Nephrodium filix-mas*.

It would therefore seem probable that *Peranema* and *Diacalpe* fall naturally into the Woodsieae-Woodsiinae group of Polypodiaceae, and that this group occupies an intermediate position between the Cyatheaceae and certain other families of Polypodiaceae. There is very little doubt that both genera are closely related to the genus *Nephrodium*. It seems probable, then, that the Aspidieae sprang from a Gradate drift, of which the Cyatheaceae are near living representatives, and that *Peranema* and *Diacalpe* are related to that drift as relatively early examples. These indications make it seem probable that the mixed condition of the sorus of *Nephrodium* has arisen from a Gradate condition. This position traces one of the polyphyletic lines of the Polypodiaceae to the Cyatheaceae, and establishes a probably parallel series to that shown from *Dennstaedtia* to *Davallia* and its derivatives.

SUMMARY.

1. *Peranema cyatheoides*, D. Don., has an advanced dictyostele without any perforations; its leaf-trace is inserted on the lower half of the leaf-gap; the pinna-supply is 'extramarginal' to all but the ultimate pinnae, to which it is 'marginal'; the sorus, which is borne on a short stalk, is a mixed one; it has a Gradate receptacle, with traces remaining of a basipetal sequence of sporangia; the annulus is slightly oblique; the spores are 'aspidioid'.

2. *Diacalpe aspidioides*, Bl., coincides with *Peranema* in the details

of its vascular anatomy; the sorus is a mixed one, with no traces of an earlier basipetal sequence; the annulus is vertical in insertion, but slightly twisted in its course across the sporangial head; the spores are markedly 'aspidioid'; the prothallus is Cyatheaceous.

3. Both Ferns show relationships with the members of the genus *Nephrodium*, particularly with *Nephrodium filix-mas*, Rich., in vascular anatomy, the character of glands on the leaves, the sporangial stalks, and the spore-markings.

4. *Peranema* and *Diacalpe* are most nearly related to *Woodsia* and *Hypoderris*, and fall naturally into the Woodsieae-Woodsiinae group of Polypodiaceae, which is intermediate between the Cyatheaceae and the Aspidieae.

5. It seems probable that the Aspidieae sprang from a Gradate ancestry, and that *Peranema* and *Diacalpe* are relatively early members of a phyletic drift to the Polypodiaceae, of which the Cyatheaceae are near living representatives.

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DESCRIPTION OF PLATES XXVIII AND XXIX.

Illustrating Mr. Davie's paper on *Peranema* and *Diacalpe*.

Figs. 1–3, 5–21, are of *Peranema cyatheoides*.

Figs. 22 and 23 are of *Diacalpe aspidioides*.

Figs. 1, 2, 3, and 7 are freehand drawings. Fig. 4 is a diagrammatic representation of leaf-gaps and the subtending leaf-traces in various dictyosteles. Figs. 5, 6, 8–23 have been drawn with the aid of a camera lucida.

Fig. 1. Pinna of *Peranema*, showing open, simply forked venation. The veinlets terminate at points short of the margin. × 3.

Fig. 2. Transverse section of stock, showing the dictyostelic arrangement of the meristeles (*st.*), the leaf-trace bundles (*lt.*), and the very numerous sclerotic patches (*sp.*). × 4.

Fig. 3. Successive sections through the petiole, showing the departure of the vascular supply to the basal pinnae. *ad.*, adaxial strands; *m.*, median main strand; *ss.*, subsidiary strands; *cc.*, commissural strands; *pp.*, pinna-trace strands. × 8.

Fig. 4. Series of diagrams illustrating the insertion of the leaf-trace bundles on the dictyostele in various Ferns. I. *Peranema cyatheoides.* Portion of dictyostele seen from within, showing leaf-gap and the bundles from the leaf subtending this gap. II. The same leaf-gap viewed from without. *m.*, median main leaf-trace strand; *ad.*, adaxial main strands. III. Another leaf-gap with the bundles from the leaf opposite to it (*ll.*), and with parts of the traces from the two leaves obliquely next above (*aa*) and (*bb*). IV. *Lomaria Spicant.* Dictyostele at leaf-gap, with the three leaf-trace bundles. V. *Onoclea orientalis.* Dictyostele at leaf-gap, with the two leaf-trace bundles. VI. *Athyrium filix-foemina.* Dictyostele at leaf-gap, showing the insertion of the leaf-trace. It leaves the dictyostele as an undivided trace and in the base of the petiole becomes divided into two strap-shaped strands. All considerably magnified.

Fig. 5. Transverse section through the young shoot of *Peranema*, just below the apex, showing the first lignification in the stele. × 450.

Fig. 6. Transverse sections through the adaxial main strands of the leaf-trace, showing the 'extramarginal' type of supply to the basal pinna. All × 76. (*a*) Strand below the level of the pinna, showing the hooked end, from which the supply goes off to the pinna. (*b*) Pinna-supply in process of being given off. (*c*) Reconstituted adaxial strand (*ad*) with pinna-trace (*p*) passing out to the pinna.

Fig. 7. Successive transverse sections through the rachis of a basal pinna to show the development of the pinna-trace, from the point of departure from the petiolar adaxial strand (I) up to the fully constituted condition (XI). All about × 8.

Fig. 8. Palea from young leaf-stalk. × 3.

Fig. 9. Hair from the rachis of the frond, showing glands on the curved portion. × 76.

Fig. 10. Gland from the palea of a young frond. × 450.

Fig. 11. Glands from the surface of the mature petiole. × 450.

Fig. 12. Transverse section through a young pinna, showing marginal segmentation of the usual Leptosporangiate type. × 450.

Fig. 13. Longitudinal section through the stalked sorus, showing the elongated receptacle with its vascular supply and the mixed arrangement of the sporangia. × 76.

Fig. 14. Longitudinal section through the sorus, showing the elongated receptacle and the mixed succession of sporangia. The indusium is two-layered at its base. × 76.

Fig. 15. Longitudinal section through the sorus, showing the way in which the rim of the indusium is tucked in on the lower side, close to the point of insertion of the stalk. × 76.

Fig. 16. Section through a mature sporangium, showing the slight obliquity of the annulus. × 150.

Fig. 17. Section through the stalk of a mature sporangium. × 150.

Fig. 18. Almost mature sporangium, showing the continuation of the cells of the annulus past the stalk. × 225.

Fig. 19. Mature sporangium, showing the slightly oblique insertion of the annulus. × 150.

Fig. 20. Sporangium, showing the indurated cells of the stomium. × 150.

Fig. 21. Spores of *Peranema*, showing the papillate pits formed by the perispore. × 450.

Fig. 22. Section through a mature sporangium of *Diacalpe*, showing the insertion of the annulus and the markings on the spores. × 150.

Fig. 23. Mature sporangium of *Diacalpe aspidioides*, showing a hair on the stalk. × 150.

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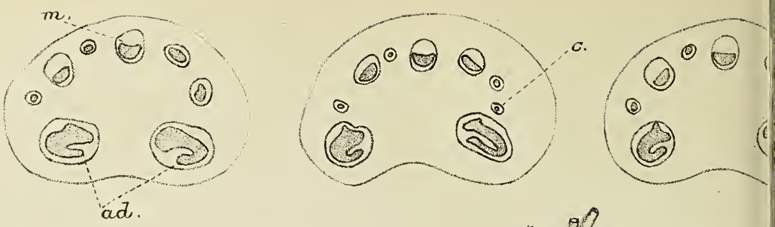

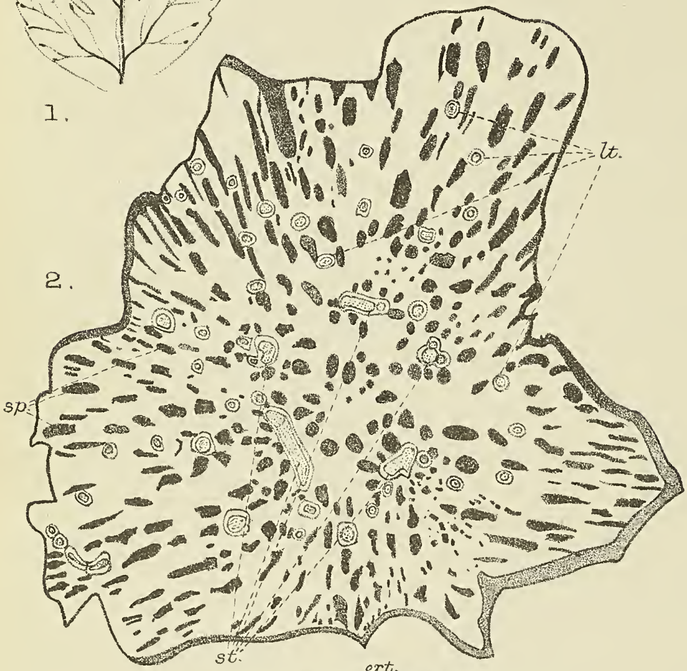

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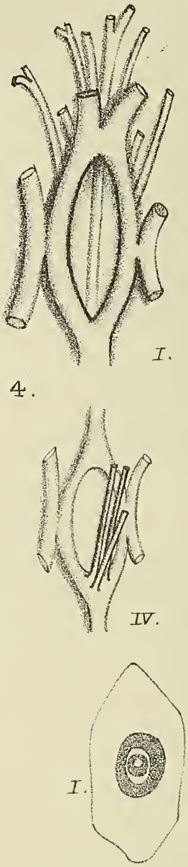

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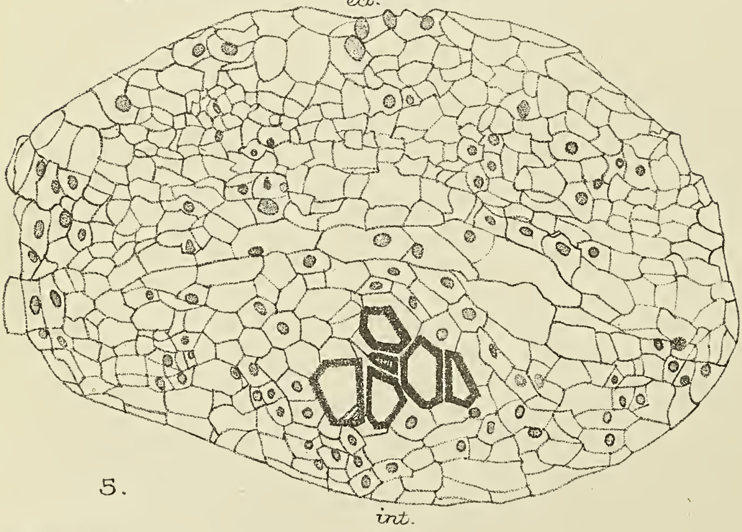

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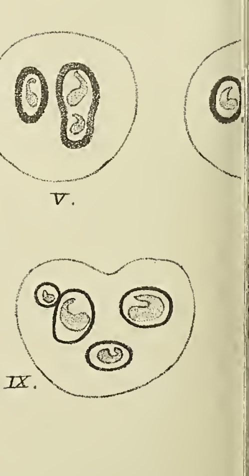

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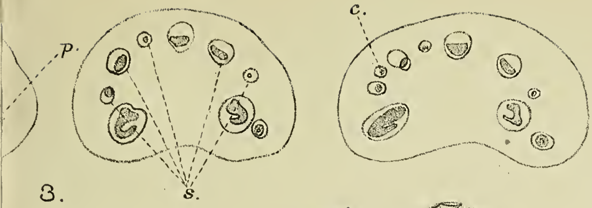
DAVIE—PERANEMA AND DIACALPE.



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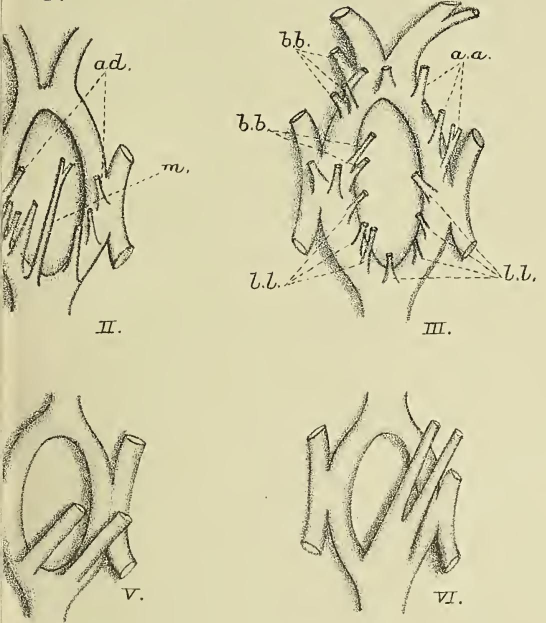

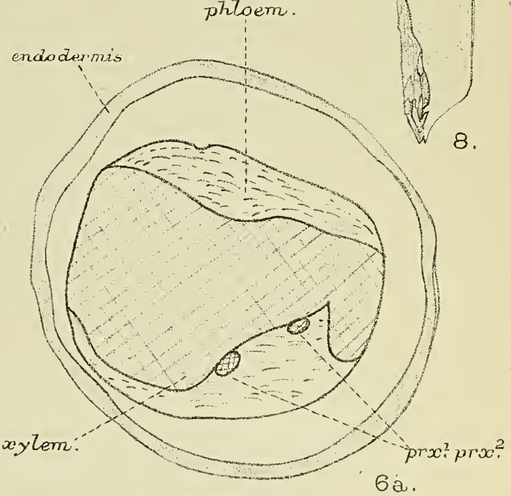

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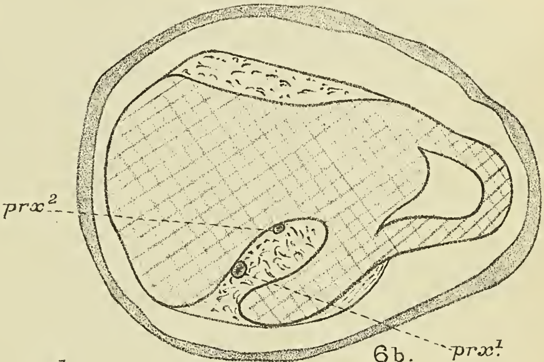

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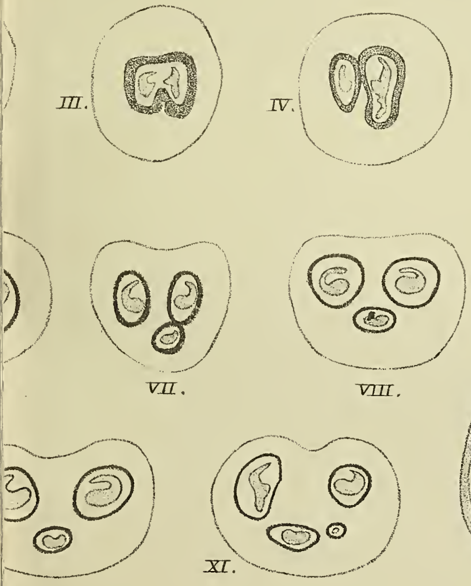

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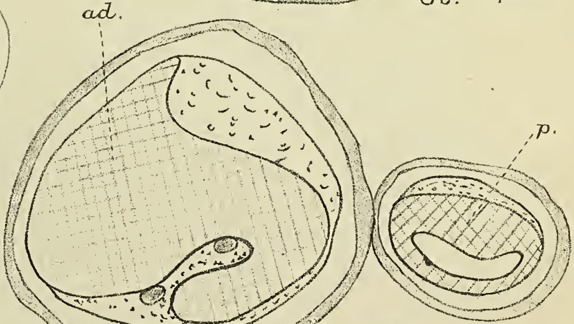

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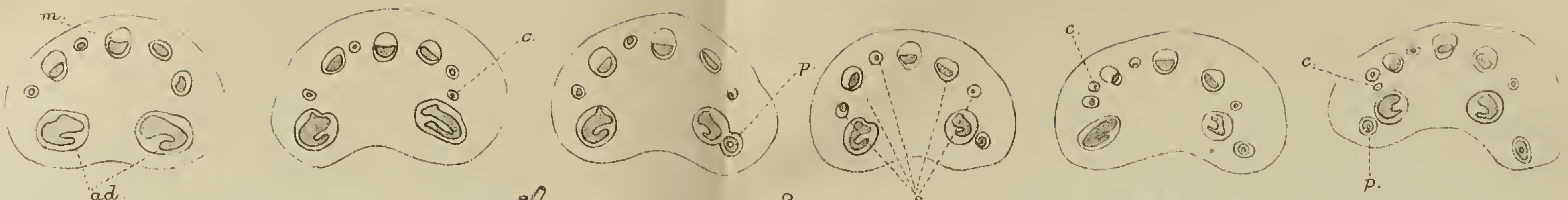

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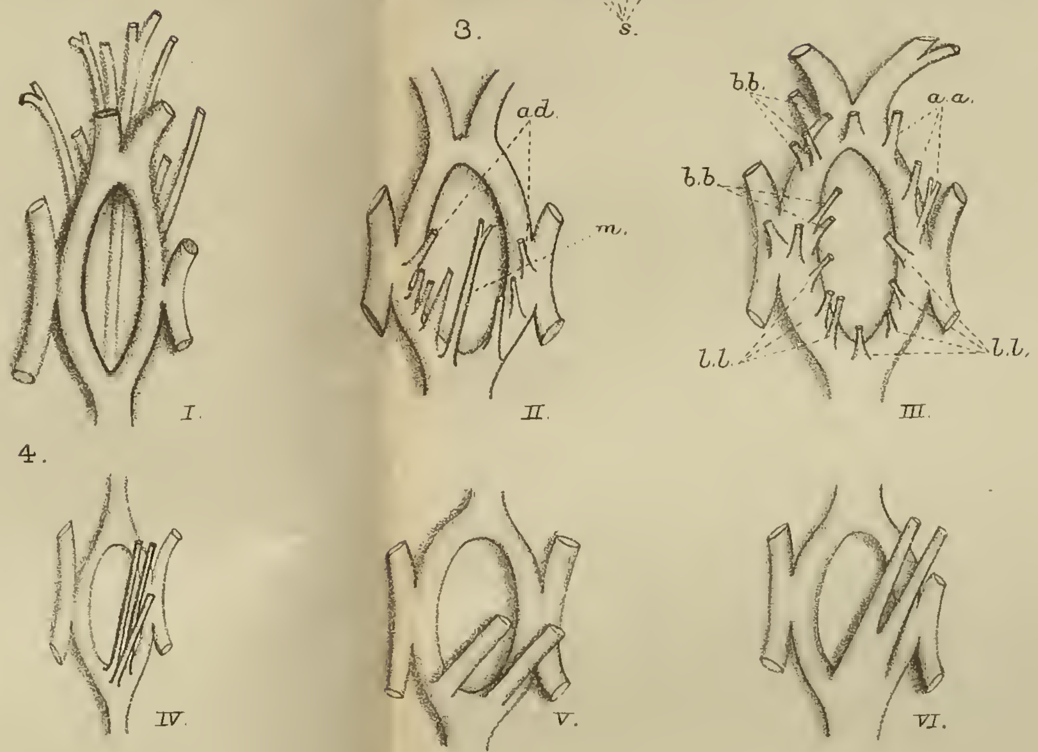

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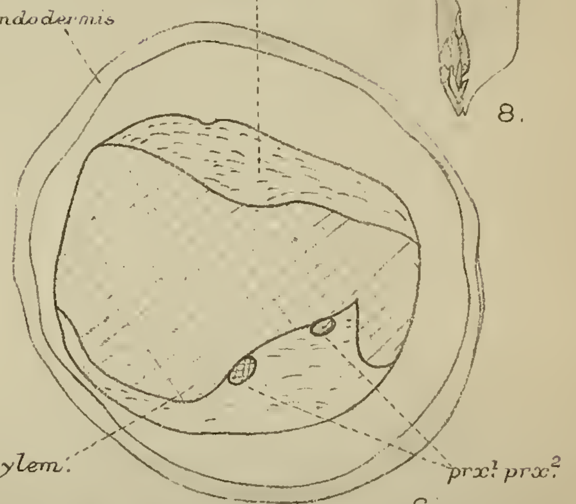

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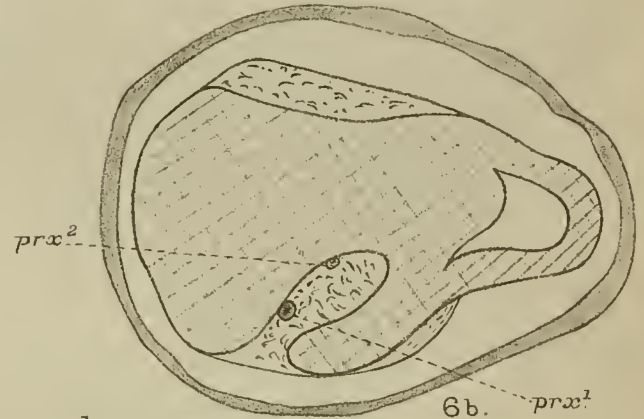

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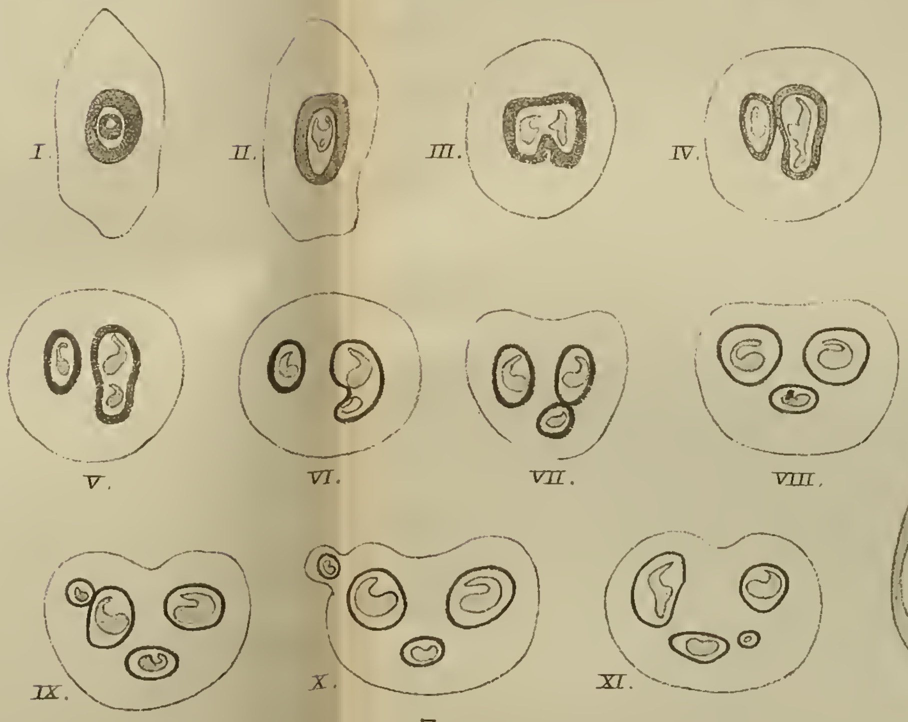

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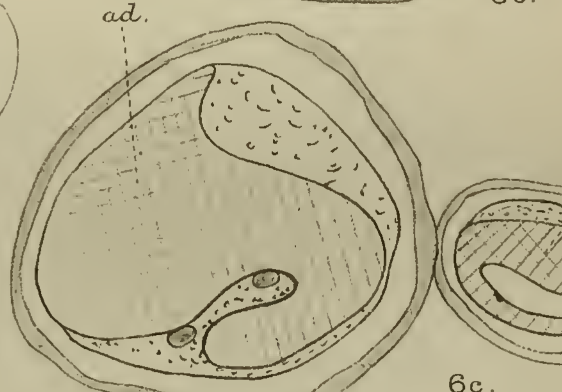

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DAVIE—PERANEMA AND DIACALPE.

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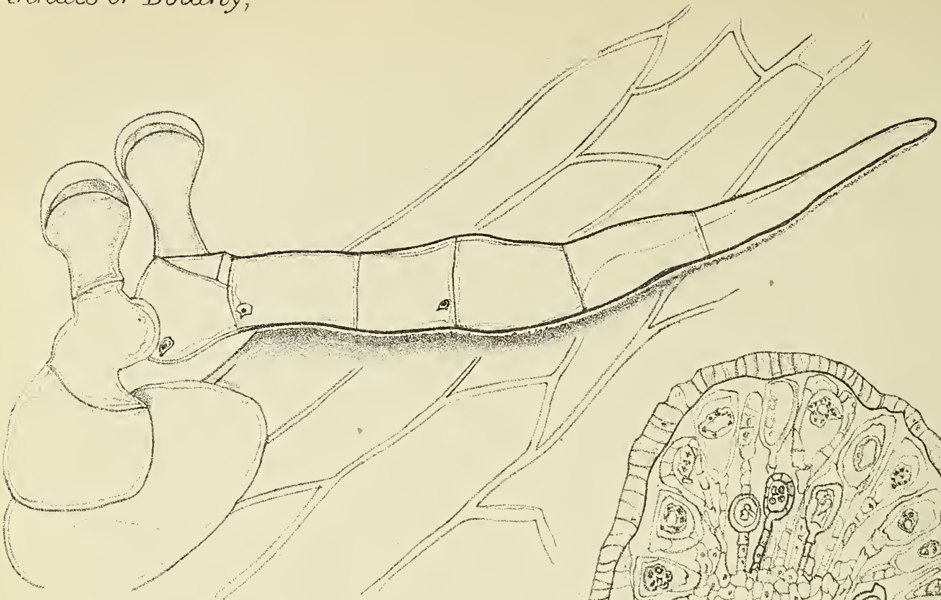

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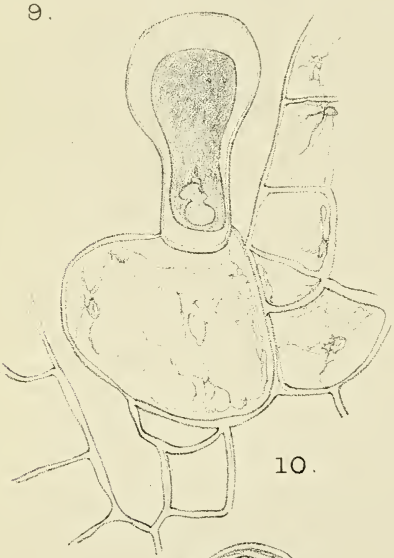

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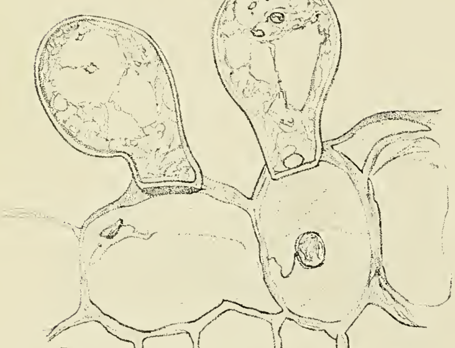

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DAVIE — PERANEMA AND DIACALPE.

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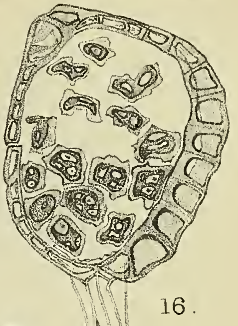

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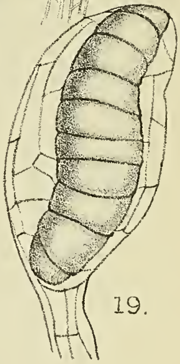

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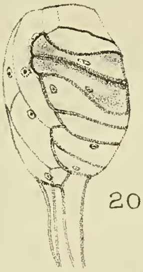

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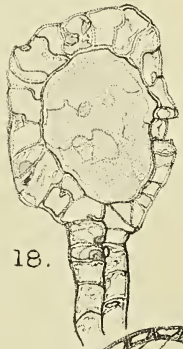

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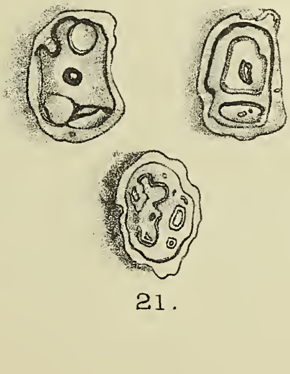

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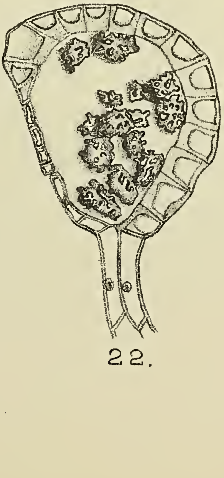

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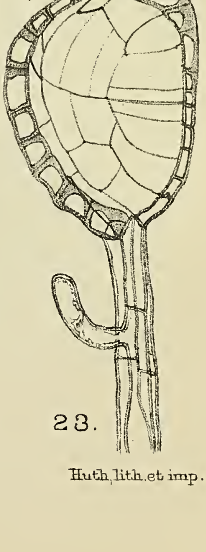

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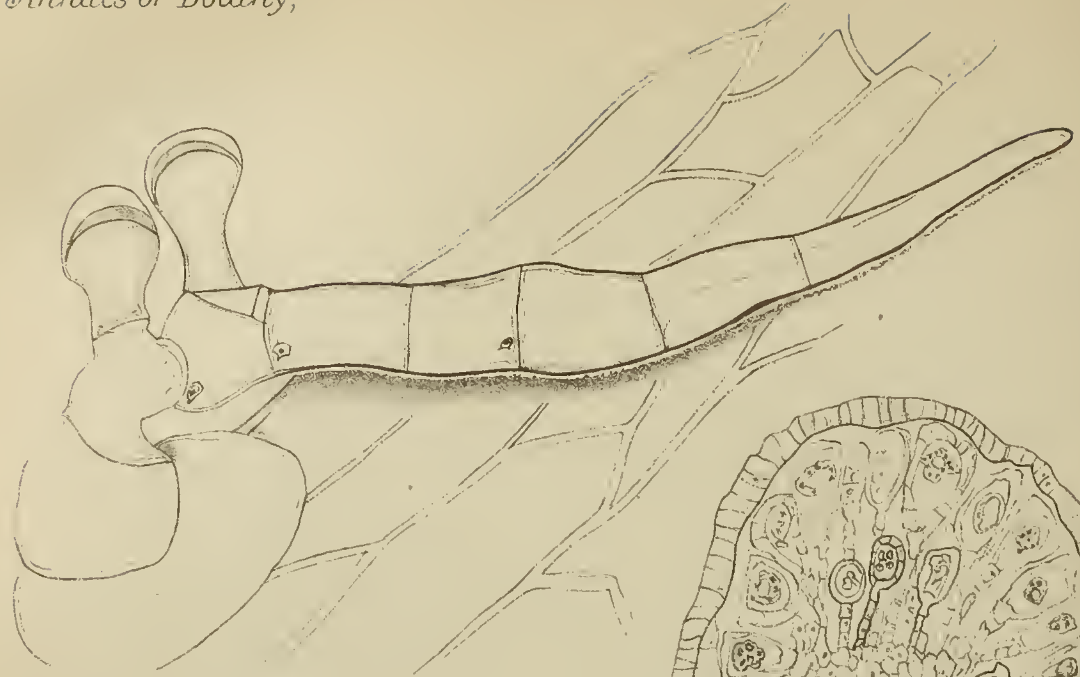
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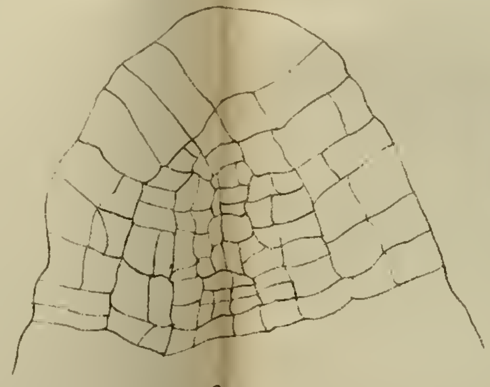
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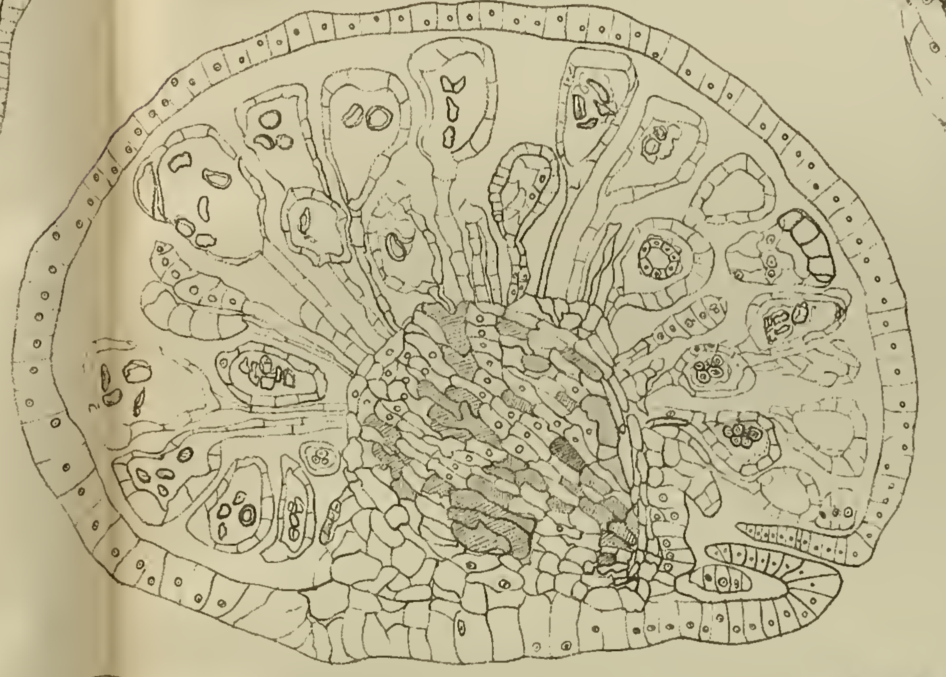
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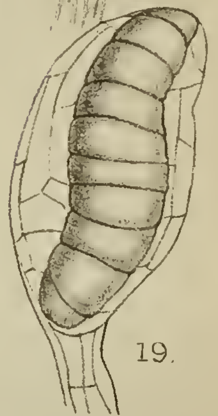
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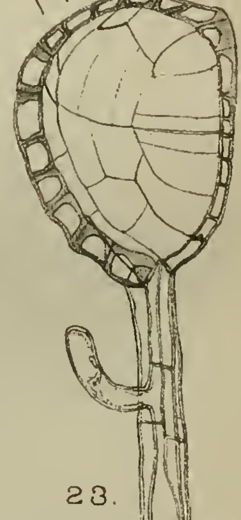
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R. C. Davie del.

DAVIE — PERANEMA AND DIACALPE.

Huth, lith. et imp.

Studies in the Phylogeny of the Filicales.

II. Lophosoria, and its Relation to the Cyatheoideae and other Ferns.

BY

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With Plates XXX–XXXVI.

IN my 'Studies in the Morphology of Spore-producing Members, No. IV. Leptosporangiate Ferns' ('Phil. Trans.', 1899, vol. cxcii, p. 122), the distinction was drawn according to the mode of development of their sori between the Ferns designated as the Simplices, the Gradatae, and the Mixtae. These were held to illustrate three steps in the evolution of the sorus. It was specifically stated that there was no intention to assert that the living Ferns of any one of these categories were the actual ancestors of those of any other. Nor was it suggested that the progression was by any single line of descent. Parallel development along a multiplicity of lines was distinctly contemplated, involving in any one of them progressions from the Simple to the Gradate, or from the Gradate to the Mixed state. It was even held as possible that a progression might have been effected directly from the Simple to the Mixed state (l. c., p. 124), a transition which has since been demonstrated to have occurred within the genus *Dipteris* (Miss Armour, 'New Phytologist,' 1907, p. 238), and it is highly probable that it has occurred also in the descent of *Plagiogyria* ('Annals of Botany', vol. xxiv, p. 438).

Perhaps the most obvious suggestion of transition from the Simple to the Gradate sorus that could be made is that from some Gleicheniaceous type to the Cyatheaceae. Already in 1899 it was pointed out (l. c., p. 123) that '*Gleichenia dichotoma* has a naked sorus' differing 'little in general construction from that of *Alsophila atrovirens*'. This case was not quoted then as indicating a true line of descent, but as an illustration how near one type may be to another in point of arrangement within the sorus. The widening of the basis of comparison, especially in respect of the vascular anatomy, led in 1908 to a more explicit statement ('Land Flora', p. 610), that 'there seems good reason to see in the Cyatheaceae a series having

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probable genetic relations with the Gleicheniaceae, but advanced on the one hand to the basipetal succession of the sori, and on the other to a high complexity of the vascular system'.

The degree of closeness of those 'genetic relations' was left undefined, though certain species were indicated as outliers on either side of the gap separating the two families. On the one hand are *Gleichenia linearis*, Clarke (= *dichotoma*, Hk.), and *pectinata*, Pr., distinguished as relatively advanced species, both by their soral and their anatomical characters; on the other hand, *Alsophila quadripinnata*, C. Chr. (= *Lophosoria pruinata*, Pr.), and *A.* (*Metaxya*) *blechnoides*, Hk., were indicated as lying anatomically at the base of the Cyatheaceous series. It seems desirable, as material is at hand, and as some further advance has already been made in the anatomy of the more complex Gleichenias, to examine more closely the probable relations of the two families, and to widen as far as possible the basis of the comparisons. For it is only in this way that a dependable conclusion can be arrived at. In discussing such genetic relations in Ferns, soral characters are not sufficient, nor are anatomical data sufficient. Nor should we be satisfied even with a combination of them. To obtain a fully trustworthy result the characters of external form, of the superficial appendages, and of the gametophyte and sexual organs should be also used, together with the details of the ontogeny, while some attempt at seriation of the species within the genera will also assist in giving point to the comparisons. Many phyletic conclusions which are generally accepted fall short of this comprehensive foundation, and it will not be possible in the present case to carry out the programme fully. But it is well at the outset to visualize what is to be aimed at, even though a complete demonstration may not be actually attained at the moment. As the comparisons to be drawn will centralize themselves round the species above named, though with general references from time to time to these and to other genera as a whole, it will be well at the outset to become acquainted with the history, systematic position, and characteristics of them. Often it happens that plants which have a peculiar interest from a comparative point of view have in the past been given diverse systematic places by various authors. This will be seen to have been the case with one at least of the Ferns in question. Such a history at once suggests that the systematist has encountered a synthetic type. In fact the puzzles of the earlier systematists will often provide the opportunities of the phyletic morphologist.

GLEICHENIA.

The two species *Gleichenia linearis*, Clarke (= *Gleichenia* (*Mertensia*) *dichotoma*, Hk.), and *Gleichenia* (*Mertensia*) *pectinata*, Pr., have long been regarded as outstanding in the genus to which they belong, and recent anatomical observations have accentuated their aloofness from the rest. But they share the general characteristics of *Gleichenia* so fully that there is no question of their real affinity with it. Both have the creeping rhizome, bearing upon it at intervals leaves which are endowed with the continued apical growth so characteristic of the genus. As in other species, the circinate apex of the leaf rests from active growth at intervals, appearing as a bud dormant between the last developed pinnules, and then resuming again its activity (Goebel, 'Organography,' ii, p. 318). In the disposition and development of the ultimate branchings of the leaf, these species have been recognized by systematists as forming each a separate section of the genus. Their segregation has also been based upon the fact that in the number of sporangia in the sorus they exceed the rest. Of *G. pectinata* Sir Wm. Hooker wrote that 'it is the only species of this section (§ 3), and not to be confounded with any other'; while he further remarked of his § 4, that 'as *G. pectinata* is a solitary species in its section, so is *G. dichotoma* of the present one' ('Syn. Fil.', p. 15).

Branching of the axis may be in *G. pectinata* either dichotomous in a horizontal plane, or monopodial in a vertical plane. Of the former branching Boodle and Hiley remark that 'it appears not to be known to occur in the rhizome of any other species of the genus' ('Ann. of Bot.', 1909, p. 426). It is true that collectors and systematic botanists have commonly neglected to provide evidence of, or to describe such features. But I have specimens of very perfect dichotomy of the axis in *Dicranopteris fulva*, Desv. Underw. (= *Gleichenia pubescens*, H. Bk.), and the same branching exists also in *G. flabellata*. Probably dichotomy of the axis is frequent within the genus. In the leaf, on the other hand, it is stated by Goebel ('Organography', ii, p. 319, footnote) that 'no species in *Gleichenia* has a dichotomous leaf', and these species appear to be no exceptions. Moreover, as Campbell has observed ('Ann. Jard. Bot. Buit.', 2e série, vol. viii, p. 98), 'the cotyledon in *G. pectinata*, *G. dichotoma*, and *G. laevigata* shows a prolonged apical growth like that found in the adult leaves of the young sporophyte'. He notes (l. c., p. 94) that 'the cotyledon of *Gleichenia*, especially in the section *Mertensia*, differs from that of most Ferns. In the majority of Ferns the cotyledon is fan-shaped, the result of an early dichotomy. In *Gleichenia* the original apex seems to persist.' The complex leaf-forms of the genus are the result of repeated pinnation, modified, but not in any genetic sense altered, by unequal development of the parts, varied distribution of intercalary growth, and periodic arrest of apical activity. In *G. linearis* the unequal development of parts of the leaf makes itself apparent in the specialization of certain pinnules as a protection to the resting apex of the leaf. Goebel points out that these are neither 'adventitious structures' nor 'vestiges', but only pinnules which are developed for the protective function; and that they vary in different species according to the size of the resting apex, being absent where it is small, or where scales or hairs form an efficient covering. In *G. linearis* (=*G. dichotoma*) they are highly specialized for the protective function (Goebel, 'Organography,' ii, p. 319, Fig. 206). In general form the leaves of these two species are very widely expanded, and are among the largest of the *Mertensia* division of the genus. They are thus in strong antithesis to the leaves of the *Eu-Gleichenia* division, which have the leaf-area reduced, probably in relation to a xerophytic habit.

The surface appendages have been too much neglected as a basis for comparison in Ferns. In many systematic treatises they are ignored. Even in the excellent work on the Filicales by Diels (Engler u. Prantl, 'Nat. Pflanzenfam.,' i. 4) their occurrence and peculiarities are often left unnoticed in cases where, as in *Gleichenia* and *Alsophila*, they have a comparative value. Long ago, however, the value of surface appendages for phyletic comparison was noted by Prantl (' Schizaeaceen ', p. 37), who concluded that simple hairs were the primitive type, but that in many circles of affinity they developed in a flattened form. The ramentum or scale is thus derivative or secondary. This distinction of hair and scale was adopted by Max Kühn as the basis of a classification of the Polypodiaceae, which he divided into *Chaetopterides*, with hairs, and *Lepidopterides*, with flattened scales. Against the use of this as a leading criterion, which resulted in an unnatural juxtaposition of genera, Prantl protested (' System der Farne ', ' Arb. K. Bot. Gart. z. Breslau,' p. 13). But, nevertheless, he adopted the existence of the scale as an expression of a progressive step of organization. This is the way in which the hair-scale criterion should be used as an indication of a relatively primitive or of a relatively advanced condition, which may be brought with other progressive features into a general argument; but with the full knowledge that the advance from hair to scale has probably been made in various phyletic lines, and that it does not necessarily progress coincidently with other features of advance.

An examination of the dermal appendages of *Gleichenia* brings some interesting results. Of the section *Eu-Gleichenia* three species have been examined, viz. *G. polypodioides*, Sm., *G. dicarpa*, R. Br., and *G. circinnata*, Sw. In all of them broad scales were found protecting the dormant leaf-apices, as also on the leaf-bases and the rhizomes. But with them are associated hairs. In the first two species these were pale and soft, but in *G. circinnata* they were stiffer, and very beautiful transitions can be found between simple brown hairs and those which are branched and tufted, and finally to broadened scales, with stiff hairs projecting from their margins. It is as though the bases of the hairs had broadened to form the scales, and this is their probable origin. The hairs and scales fall off early from rhizome and leaf. It appears thus that both hairs and scales are present, probably with constancy, in *Eu-Gleichenia*, and this is biologically in accordance with their xerophytic habit.

In the *Mertensia* section observations were made on *G. Bancroftii*, Bl., *glauca*, Hk., *flabellata*, Br., *Cunninghamii*, Hew., *furcata*, L., *owhyhensis*, Hk., *flagellaris*, Spr., *vestita*, Bl., and *hirta*, Bl. In all of them both scales and hairs were present, the former especially upon the circinate leaf-apices. The scales vary, being large and broad in *G. Bancroftii*, but only narrow in *G. flagellaris* and *vestita*, while in the latter the hairs are soft and fluffy, of the type to be described in *G. linearis*. Thus, though *Mertensia* is, as a whole, of less xerophytic habit than *Eu-Gleichenia*, both hairs and scales are general, especially on the circinate leaf-apices.

But in § 3, *Gleichenia pectinata*, and § 4, *Gleichenia linearis*, no scales are found. They stand in this character apart apparently from the whole of the rest of the genus. Hairs are present in both, and they are of the tufted type, branching near to the base. In *G. linearis* these are of a softer texture, but in *G. pectinata* they are stiff and brown, with their peg-like branches often reflexed. These hairs, especially those near the insertion of the leaf on the axis, are long and curved, and coarse in texture. Each of them with its tuft of stiff branches is borne on the end of a rather massive emergence, which is a firm multicellular outgrowth. But the hair is readily broken away from its tip; the emergence then remains as shown in Pl. XXX, Fig. A, in the case of the two lower emergences. And as these are numerous and relatively large, they give to the rhizome and the base of the leaf that prickly character which is often seen about the base of the leaf in *Alsophila*, *Hemitelia*, and *Cyathea*, though here it is on a smaller scale. The prickles in these latter plants bear each a ramentum when young, and this may be seen with special clearness in the case of the large, more or less isolated scales borne on the young leaf of *Hemitelia horrida* (Pl. XXX, Fig. B). There can be little doubt that the structures in question in *Gleichenia pectinata* and in the Cyatheaceae are essentially comparable one with another; in that case, those of *Gleichenia* may be held as a prototype of the larger emergences of the Cyatheaceae. See Pl. XXXIII.

It appears from the above observations that the two species of *Gleichenia*, which are outstanding in other characters from the rest of the genus, are also exceptional in showing the more primitive type of dermal appendages only, viz. hairs and no scales. Their aloofness from the rest of the genus is further indicated by their anatomy. It will suffice here to quote briefly from the conclusions which have been arrived at on the basis of comparison of their vascular tissues.[1] Tansley sums up his discussion of the anatomy of *Gleichenia* thus (l. c., p. 46): 'We are therefore led to the conclusion that such a form as *G. flabellata*, on the whole, represents the most primitive type of the genus.' As regards *G. linearis* he remarks (p. 41) that it 'stands apart from the other Mertensias', being 'intermediate

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[1] Tansley: Lectures on the Evolution of the Filicinean Vascular System, iv, p. 40, &c. Boodle: Ann. of Bot., xv, p. 703, and xxiii, p. 419.

between the *Mertensia* and *Eu-Gleichenia* types'. The latter are generally accepted as being forms showing reduction from the typical *Mertensia* type, as represented by *G. flabellata*; they are in fact a side branch. Thus, as Boodle points out (l. c., p. 432), *Mertensia* includes not only the species of *Gleichenia* which is held to be the most primitive anatomically, viz. *G. flabellata*, but also the most advanced, viz. *G. pectinata*. The solenostelic structure which it shows is to be regarded as derived from a protostelic *Mertensia* type, like that of *G. flabellata*. And thus it appears that, putting *Eu-Gleichenia* aside as a line of xerophytic specialization, *Mertensia* illustrates a sequence in which *G. linearis* takes an intermediate place, leading from a protostelic to a fully solenostelic structure of the axial stele. It will be seen that this runs parallel with certain soral characters.

The structure of the sorus in certain species of *Gleichenia* has been described elsewhere (Studies, IV. Leptosporangiate Ferns; 'Land Flora', p. 555, &c.), and the conclusion is drawn that the sorus and sporangium of *G. flabellata* are probably the most primitive of the forms examined, a conclusion based partly upon the construction of the sorus, which is radiate and uniseriate with about 3–5 sporangia ('Land Flora', p. 554, Fig. 309), partly on the large output of spores from each sporangium. The species of the *Eu-Gleichenia* section show a diminution in number of the sporangia, and in their individual output of spores, as compared with *G. flabellata*, and they present no feature of special interest for our present comparison. But the two species which are outstanding from the rest of the genus in other respects, viz. *G. linearis* and *G. pectinata*, both show indications of advance beyond the condition normal for the *Mertensia* section. This is apparent in the larger number of sporangia in the sorus, in their arrangement, and in the diminished output of spores per sporangium. The facts have already been observed for *G. linearis*, where the number of sporangia in the sorus may be as large as ten, and the central area of the sorus, which is usually vacant in other species, is occupied by sporangia (Pl. XXX, Fig. C). Hooker ('Species Filicum', i, p. 13) mentions 'capsules 10–12'. The sorus being thus more crowded than in the usual *Mertensia* type, the question of dehiscence of the sporangia becomes a critical one. In *Gleichenia* the dehiscence is by a split in a median plane of the sporangium, and in order that the opening may be effective elbow-room is required right and left of each sporangium, and it is therefore only suitable for a laxly arranged sorus. The difficulty will be appreciated by examination of the sori in Fig. C, or better of the detailed drawing (Fig. D). From this it appears that even in a sorus with seven sporangia their close juxtaposition has led to an oblique opening of one of them. But the question is not here one of acute difficulty. As regards the spore-output the number of spores per sporangium counted in specific cases for *G. flabellata* were 794, 695, 838, 634, all these figures lying between the typical numbers 512–1024. For *G. linearis* the numbers were in two specific cases only 251 and 319. This smaller number is in accord with the smaller size of the pear-shaped sporangia of the latter species, but it is nearly matched by the output in species of *Eu-Gleichenia*, where the relatively large sporangia produce large spores, but their numbers are in near proximity to the typical number 256.

A more special interest, however, attaches to the case of *G. pectinata*, which is sorally, as it is also anatomically, the most advanced species. It has long been known that the sori of this species contain the largest number of sporangia in the genus. Hooker (' Syn. Fil. ', p. 15) mentions ' 8–10 capsules '. The drawing (Pl. XXX, Fig. E) shows that this is clearly under the mark, for the numbers vary from 10 to 15 in this specimen from Jamaica not specially selected. The sporangia, being long-stalked and all seated upon a central and slightly raised receptacle, are obviously crowded. They form two tiers, the upper tier fully occupying the central space, which in *Mertensia* is usually vacant. The enlarged drawing of a single sorus (Pl. XXXI, Fig. F) shows that the sporangia are even flattened against one another, a fact which is readily seen in their flattened sides when the sporangia lie separate and free. They are also far from uniform in size, and the orientation of the annulus is occasionally reversed. It is plain that shedding of the spores will here be a matter of difficulty if the median dehiscence is maintained. That the difficulty is a real one is shown by the fact that, though the sporangia arise and mature all simultaneously, many of them remain full long after other sporangia of the same sorus have been emptied. It appears then that in *G. pectinata* the Gleicheniaceous type of sorus has reached the point of ineffectiveness in its increase in number of the sporangia. There are four possible ways out of the difficulty, and they may be adopted singly or in combination: (1) by increasing the length of the sporangial stalk, (2) by adopting a lateral in place of a median dehiscence, (3) by extending the area of the sorus, and (4) by elongating the receptacle. But none of these has been adopted by the genus *Gleichenia* itself. Other Ferns have, however, adopted them, and have succeeded.

The development of the sorus of *G. pectinata* has been followed out for comparison with that of other species. The receptacle is present as a considerable upgrowth before the development of the sporangia begins (Pl. XXXIV, Figs. 1, 2). The sporangia themselves are almost simultaneous in their appearance, but not perfectly so. In Fig. 3 the middle sporangium is just dividing off its cap-cell, whereas those right and left have them complete. There is still more discrepancy in the state of development of the three sporangia in Fig. 4. But there does not appear to be any rule or method of succession. The sporangia themselves show a segmentation which, though variable in detail, conforms to that already described for other species of the genus.[1] In most cases it ranks most nearly with the type seen in the Schizaeaceae

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[1] Compare Studies. IV. Phil. Trans., vol. cxcii, pp. 32–8, and Plate II.

('Land Flora', Fig. 349, *d*), but some show relations also with the types *c* and *e* of Fig. 349. In any case the segmentation is more massive than that of the Polypodiaceae. The result is a relatively bulky sporangium, with a stalk of several rows of cells (Pl. XXXIV, Fig. 5). When mature it closely resembles that of *G. linearis* both in shape and in average size. But there appears to be a smaller number of spore-mother-cells in each sporangium than in this species, and considerably less than in *G. flabellata.* Fig. 6 shows the definitive sporogenous group as seen in a section transverse to the axis of the sporangium, and the number of spore-mother-cells is fifteen, arranged roughly in four plates. Fig. 7 shows a section of a similar sporangium in a median longitudinal section, and the number of the spore-mother-cells traversed is twenty. Fig. 8 shows the condition where the spore-mother-cells have separated and rounded off. These drawings provide an interesting comparison with my drawings for *G. flabellata* and for *G. linearis* (Figs. 24–26, Pl. II of my Studies, V, and Figs. 27–30, Pl. III). From a comparison of such sections as those shown in Figs. 6 and 7 the total number of spore-mother-cells in *G. pectinata* may be estimated at some figure probably in excess of 64, which if all were matured would lead to an estimated output of spores something in excess of 256 per sporangium. Actual countings for sporangia of *G. pectinata* gave the result of 422 and 429 for large sporangia, and 228 and 236 for small sporangia. The larger are thus seen to approximate to the typical number 512, though falling a good deal short of it; the smaller approximate to, but fall short of the typical number 256.

A comparison of these facts with those for *G. linearis* and *G. flabellata* indicates clearly a sequence of reduction from the large sporangia of *G. flabellata*, which are few in a sorus, each with an individually large spore-output approximating to the typical number 1,024, through *G. linearis*, with its smaller sporangia, more numerous in each sorus, and with spore-output approximating to or exceeding 256, to *G. pectinata* with sporangia variable in size, but still more numerous in the sorus, and with a variable spore-output, approximating in large sporangia to 512, but in smaller falling to below 256. The variability in size of the individual sporangia makes precise statement precarious. An important point is that the variability exists in the species which is in various respects the most advanced in the whole genus.

Turning to the gametophyte of *Gleichenia*,[1] it presents characters of importance as indicating affinity with relatively primitive types. The massive form of the prothallus with a marked midrib is characteristic, and suggests comparison with *Osmunda*. It bears in some species 'marginal

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[1] Rauwenhoff: La Génération sexuée des Gleicheniacées. Arch. Néerland., 1890. Campbell: Mosses and Ferns, 2nd Ed., p. 336; also The Prothallium of *Kaulfussia* and *Gleichenia*. Ann. Jard. Bot. Buit., 2^{e} sér., vol. ix, p. 80.

leaf-like lobes', seen especially in *G. laevigata*. These are compared by Campbell with those of *Fossombronia*, or *Dendroceros*. But the most important characters for comparison are provided by the sexual organs. Campbell has shown that the neck-canal-cell of the archegonium (except in *G. polypodioides*) commonly divides into two cells. This is regarded as a primitive character. As regards the antheridium, Rauwenhoff examined various species of *Eu-Gleichenia*, but of the *Mertensia* section only *G. flabellata*, and found in all that he examined a general correspondence of the antheridium with that of the Polypodiaceae. But other species belonging to the *Mertensia* section examined by Campbell are characterized by the much larger size of the antheridium, this being especially marked in *G. laevigata* and *G. pectinata*.

The number of spermatocytes traversed in a median longitudinal section gives a numerical basis for comparison. From Campbell's published drawings the numbers appear as follows: *G. polypodioides* (§ *Eu-Gleichenia*)—19. *G.* (*Mertensia*) *laevigata*—95, 35, 76. *G.* (*Mertensia*) *linearis*—30, 42. *G.* (*Mertensia*) *pectinata*—46, 23, 60, 62. From this it appears that there is considerable variability not only within the genus, but also in specimens of the same species. It is a criterion which must be used with caution. The chief point is that the numbers are high compared not only with those of other Ferns, but also with Pteridophyta at large. From published figures (mostly taken from Campbell's 'Mosses and Ferns') it appears that typical numbers of spermatocytes seen in section are for *Ophioglossum* 87, *Kaulfussia* 61, *Marattia* 31, *Osmunda* 32, *Onoclea* 21, *Nephrodium* (Kny) 16, *Woodsia* (Schlumberger) 17, 18, 16, 14, 14, 13. In other Pteridophytes the numbers are for *Equisetum* 56, and *Lycopodium* 46.

From a comparison of these figures it follows that the antheridia of *G. laevigata* are exceptional in showing in specific cases a larger number of spermatocytes in median section than any of the Pteridophytes quoted. On the other hand, the figures for the *Mertensia* section of *Gleichenia* as a whole are in excess of those for the Leptosporangiate Ferns. *Nephrodium* with about 16, and *Woodsia* with an average almost the same, may be taken as typical for the Leptosporangiate types. And so it is seen that the *Mertensia* section of *Gleichenia*, together with the two outstanding species *G. linearis* and *G. pectinata*, occupy a position altogether exceptional among Ferns, while the latter species are extremes even among Pteridophytes at large, in respect of the number of their spermatocytes. This, going along with the large output of spores in the *Mertensia* section, and other relatively primitive characters, indicates in an unmistakable way that it is among the most primitive types of the living Filicales.

But the characters upon which this general conclusion is based do not always run parallel. Thus *G. flabellata*, which in its anatomy, its sorus, and its spore-output appears as the most primitive species, has not the largest antheridia (cf. Rauwenhoff). Unfortunately, the extreme type in the latter respect (*G. laevigata*) has not been examined as regards its spore-output. *G. linearis,* which anatomically and sorally is more primitive than *G. pectinata*, is on the antheridial criterion more advanced. These discrepancies find some explanation in the irregularity of size of the individual antheridia of *G. pectinata*, which shows that this criterion must not be pressed too hard. It has long been a matter of note that the extent of projection and the bulk of sporangia and antheridia show some degree of parallelism, the more bulky and deeper sunk being held as the more primitive condition. It has also been pointed out at length elsewhere ('Land Flora', p. 641, &c.) that the numbers of spores produced in a sporangium give a rough numerical measure of the relative bulk of the sporangium for comparative purposes. It is also the fact that there is a parallel between these characters and the segmentation of the apices of axis, leaf, and root in the plants in question. To these criteria is now added that of the spermatocyte-production of the antheridium. But while we recognize these points, they must not be driven to the length of exact numerical comparison. It is becoming apparent that while such parallels are true in general, indicating conditions of relatively greater or less bulk of fundamental organization, still they are not invariably exact, and are open to exceptions.

From the details and comparisons advanced in the above pages the conclusion may be drawn with some degree of certainty that the Gleicheniaceae occupy a place among the most primitive of the Filicales; that *Mertensia* is more primitive than *Eu-Gleichenia*; and that in many characters, though not in all, *G. flabellata* is a central and an early type. Perhaps it may share this position with *G. laevigata*, which is as yet imperfectly investigated. From such forms as central, lines of specialization appear to have led, resulting on the one hand in *G. linearis*, which show various features of advance; on the other hand there is *G. pectinata,* which may be held as the most advanced species of the genus. This appears especially from its vascular anatomy, and from its sorus, the latter having arrived at the limit of development which can be practically effective in the Gleicheniaceous type of soral construction. This species shows, however, in its hairs (scales being absent), in the relatively large spore-output of its larger sporangia, and in the large number of spermatocytes of its largest antheridia, traits that are primitive. But the variability of size of both its sporangia and of its antheridia indicates the readiness with which the type might advance progressively to characters shared by most Leptosporangiate Ferns. The question of genetic relations between the Gleicheniaceae and the Cyatheaceae is thus centred definitely upon *Gleichenia pectinata* above all other species of the genus. The next step will be to consider the question from the other side, and to see whether any outstanding species of the

Cyatheaceae show primitive characters, such as compare in any degree with those of *G. pectinata.*

LOPHOSORIA PRUINATA, PRESL.
(= *Alsophila quadripinnata*, Gmel.).

The Fern so named has passed under various designations, and has commonly been regarded as an outstanding species. It was called *Polypodium griseum* by Schkuhr. Swartz named it *Polypodium pruinatum.* In 1848 Presl isolated it as the only species of a distinct genus, under the name of *Lophosoria pruinata*, but Sir William Hooker reduced it to *Alsophila*, and it appears in the 'Species Filicum' (vol. i, p. 47) as *A. pruinata*, Kaulf. It is, however, there remarked that 'in habit and appearance this is extremely distinct from any other *Alsophila*, and the receptacles are very slightly elevated, so that it must be considered but a doubtful species of the genus'. It bears the same name in the 'Synopsis Filicum', where as species 34, not only is it not accorded the dignity of a special section of the genus, but it is ranked indiscriminately among the ordinary species. Diels ('Nat. Pflanzenfam.', i. 4, p. 135) treats it in somewhat the same way, but remarks that it is a peculiar species. Christ ('Farnkräuter', p. 236) passes it under the name *Alsophila pruinata*, Kaulf., but places it in the section *Lophosorus*, Presl. Grisebach ('Flora of the British West Indies', p. 703) names it *Lophosoria pruinata*, Pr., and goes so far as to place it as a substantive genus, with *Cyathea* intervening between it and *Alsophila.* Finally, in Christensen's Index it appears as *Alsophila quadripinnata*, (Gmel.) C. Chr.

The best description hitherto given is that by G. S. Jenman, in his 'Synoptical List of Jamaican Ferns'. Ranking the plant under *Alsophila*, as species 6, *A. pruinata*, Kaulf., his description is as follows:—

> Stem short, rarely 3 ft. high, clothed densely with laniferous scales (*sic*); stipites 3–6 ft. long, or more, arching, faintly impressed, rather polished, naked, except at the base; fronds 4–6 ft. long, 3–5 ft. wide, subdeltoid, tripinnate, pale green above, frost-coloured beneath, coriaceous, costae and costulae pubescent above, the pinnulae lanate on the ribs beneath; pinnae large, apart, petioled, lowest pair deflexed and a little reduced, next above 1¼–2½ ft. long, 6–10 in. wide; pinnulae lax, petiolate, 3–6 in. long, 1–1½ in. wide, serrate-acuminate, the lowest segment situated on the superior side; tertiary segments ½–¾ in. long, 1½–3 in. wide, acute, deeply cut into deltoid lobes in which the veins are pinnate; sori at the base of the lobes, at the apex of the lowest venule on the upper side near the crenulated sinus; sporangia few, rather large, roundish, mixed with copious lanate scales (*sic*). *Polypodium*, Swartz, *Lophosoria*, Presl, Plum-Fil. t. 33.
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> Very plentiful in forest shade from 3,000 ft. alt. to the highest ridges. The caudex, which is 3–4 inches in diameter, buds and throws up from the base a number of minor stems about half the size of the primary one. The petioles are sometimes as much as 10 ft. long, giving the fronds an immense

spread. In shape of frond, character of vestiture, and scant number of capsules, it differs materially from all the other species.

There has never been any question of the identity and distinctness of of the plant, the uncertainty has been how it should be treated systematically; and this is an indication of its aloofness, which gives it an added interest in relation to the present inquiry.

As regards the details, beyond such facts as are used by descriptive botanists quoted above, little is known of this Fern, except what has been stated by H. Karsten ('Vegetationsorgane d. Palmen', p. 123). He described and figured transverse sections of the stem and leaf-bases, and of the adventitious buds. From his drawings and description it is clear that the axis is solenostelic, and that the leaf-trace is at its base a continuous horseshoe (compare 'Land Flora', p. 605, where Karsten's figures are reproduced). This relatively primitive anatomical structure is so clearly divergent from that usual in the Cyatheaceae that the facts fix the attention still more forcibly upon this outstanding type.

Lophosoria is a widely spread Fern of the Western Tropics. It is found on the West Indian Islands as well as on the mainland, where it extends southwards as far as Patagonia. Its general characters, its habit, and large dimensions will be sufficiently gathered from Jenman's description, above quoted. Associated with each leaf is usually a bud, which may be only initiated in the case of the upper leaves, or it may be developed in the case of the leaves at the base of the plant; the bud takes then the form of a runner, which after growing horizontally, for a distance usually short, then turns upwards. The leaves on those parts that are underground may be arrested, but those at the distal end of a runner develop in the full form typical for the plant. The position which the buds hold is median at the base of the leaf that bears them, and on its abaxial side. There is in this way a very profuse provision for the vegetative expansion of the individual (Pl. XXXI, Fig. G).

As implied by the name '*quadripinnata*' which has been given to the species by some authors, the leaf is highly divided, and of the Cyatheaceous type, with a clean rachis without either basal pinnae or thorny emergences; except that in large leaves some low emergences may appear at the extreme base (Fig. G). The rachis, together with the axis, is covered by a dense felt of hairs—not 'scales' as Jenman described them. They persist till maturity on both axis and the base of the leaf-stalk, as shaggy rufous masses. They are present also on the under side of the pinnae and pinnules, giving a rusty appearance to the veins, while the rest of the under surface is glaucous. The upper surface of the mature pinnule is free from hairs. Numerous hairs are also associated with the sori. There are no scales on any part of the plant, though such appendages are a marked feature of the Cyatheaceae. In this *Lophosoria* resembles *Gleichenia pectinata* and *linearis.*

The pinnules themselves are narrow, with crenate margin curved downwards, and their texture rather stiff. The venation is forked, without fusions (Fig. H), just as is the case in *Gleichenia.*[1] The sori are circular and compact, inserted with great regularity upon the lowest anadromic veinlet of the ultimate pinnule. It will be noticed that this corresponds to the position of the sorus in *Gleichenia linearis* (Pl. XXX, Fig. C), and also in *G. pectinata.* The number of sporangia in the sorus is small; they are seated upon a circular and slightly raised receptacle, and show a regularity of orientation of their sporangia, indicated by their oblique annulus, which resembles that in *Gleichenia* (Pl. XXXI, Fig. I). The actual numbers as shown in Fig. H range from 7 to 10, and their arrangement is more or less obviously in two tiers: a lower tier of about 6 or 7, which form a circular series, and are so orientated that the annulus is in a plane almost vertical to the surface of the leaf, or forming an angle with it by sloping outwards from the centre of the sorus. In fact, the orientation is as in *Gleichenia.* The upper tier of sporangia, consisting it may be of only one or of more sporangia, shows less regular orientation, much as is seen in *G. linearis* or *pectinata.* But in *Lophosoria* the crowded nature of the sorus presents no difficulty in dehiscence, for it will be seen later that the slit is not here in a median plane, but lateral.

It thus appears from the external characters that *Lophosoria* holds a peculiar position between the Gleicheniaceous and Cyatheaceous types. The internal structure and development will now be described, with a view to testing the validity of these indications.

Anatomy of Axis.

The axis and leaf-bases of *Lophosoria* are covered externally by a dense band of hard black sclerenchyma, without any of those lenticel-like perforations which are so marked a feature in the dendroid Cyatheaceae (Fig. G). There is also a sclerotic tissue within the solenostele, which in the young plant is a solid core as in *Gleichenia pectinata*, but in larger plants it expands into a hollow cylinder filled with softer pith, and lining the tubular stele internally. At the leaf-gaps it extends outwards in close relation to the leaf-trace, and a portion separates with it as a supply of mechanically effective tissue into the leaf-base. The gap in the sclerotic ring is then closed by a commissure, and the ring is thus completed again before the closing of the foliar gap. In fact, the sclerotic tissue accommodates itself to the conformation of the vascular system (Pl. XXXIV, Fig. 9).

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[1] The figure of *G. pectinata* (Pl. XXX, Fig. D) appears to suggest fusions of the veins; but an examination of the pinnule from which the drawing was made, after removal of the sporangia, showed that no fusions were present.

Karsten's drawings and description of the structure, so far as they go, accurately represent the facts (see 'Land Flora', p. 605, and Fig. 336, where reference is made to the original paper). *Lophosoria* shows in certain sections a perfectly solenostelic state, the vascular ring opening in the usual way to give off a broad segment of the ring as a foliar strand. This is shown in Pl. XXXIV, Fig. 10, in which it is seen that the separation of the strand is not effected synchronously on both sides. One margin separates as a rule before the other, and this is in fact usual in Ferns with a creeping axis. After both are clear, a wide gap is left in the solenostele, which ultimately closes again. Sometimes the leaf-gaps almost overlap. A case is shown in Fig. 9 in which in a very large axis a foliar gap is seen still open, while on the obliquely opposite side the solenostele is already thinning out in preparation for the formation of the next succeeding leaf-trace. A clear idea of the behaviour can be obtained by comparison of a succession of sections, such as those shown in Fig. 11, which are similarly orientated, and are ranged in succession from below upwards. Fig. 11, i, shows the solenostele open on the side opposite to a leaf-trace which has just come off; the internal sclerotic ring has already closed, but not the stele. It may be noted that the leaf-trace shows the unusual state of having joined its margins, so as to form a complete ring; it may be remarked, however, that the leaf was a stunted one, the axis being a pushing adventitious shoot. Fig. 11, ii, shows a point higher up, where the foliar gap is closed, but the stele is still thinner there than the average. On the side almost opposite, the stele is thinning out for the formation of the next leaf-trace, but it is still a complete solenostele. In the next section, Fig. 11, iii, the leaf-trace thus prepared for is just separating at one margin, but connected by the other. In Fig. 11, iv, that leaf-trace is quite separate, and it shows again the closing in on the adaxial side, so as to form a complete ring. In the last section of the series, Fig. 11, v, the solenostele has closed completely, though two thinner points are seen, the one marking where the ring has closed after the departure of the last leaf-trace, the other showing where the next will come off, and this is already indicated also by the slight outgrowth of all the tissues on the side obliquely opposite to the preceding leaf. It is thus evident that the leaf-gaps do not actually overlap, though they may follow in rapid succession, and the structure is accordingly solenostelic, but with a rather compact arrangement of the leaves, which is specially marked where the plant has settled down to its normal vertical growth, with a terminal tuft of leaves.

The validity of these results is shown by Pl. XXXII, Fig. K. This does not represent any idealized conception of the vascular system, but it is an accurate delineation of an actual dissection of a large axis, from which the cortex has been removed, and the vascular system laid bare. The outer surface represented in the drawing is the solenostele itself, and it is shown of the natural size. The leaves are at considerable distances apart, so that in transverse section the leaf-gaps do not overlap, and transverse sections of the parts lying between them would show the complete solenostele. It may be noted that in this case the adventitious buds are absent from the leaf-bases.

It has been pointed out that roots and adventitious buds are borne by the axis. The attachment of the roots is shown in Pl. XXXIV, Fig. 11. Their vascular supply comes away from the outer margin of the stele in the usual way. The adventitious buds are placed with regularity below the leaf-bases. Frequently there is one at the base of each, but their presence is not constant. In order to trace the vascular connexion of the bud and its related leaf, sections were cut transversely to the parent shoot, as in Fig. 12. Of these (i) is the lowest; it shows a foliar gap of a preceding leaf just closing, while almost opposite the thinning out and extension of the stele is beginning preparatory to the departure of the next trace. But already the related bud (*b*) is traversed, though the section was below the level of its vascular supply. In Fig. 12, ii, the bud is traversed in median section, and it is seen that not only does the vascular tissue extend directly out into it, but the sclerenchyma also; in fact, the vascular tissue of the bud arises as a diverticulum of that at the base of the related leaf. Closely above it, as shown in Fig. 12, iii, the leaf-trace itself comes off in the usual way. These results were checked by tangential sections, as shown in Fig. 13. Of the sections there shown (i) is a transverse section of the bud close to its base, showing its solenostelic structure, with numerous roots. A tangential section just below its insertion is seen in (ii), which shows the continuity of the sclerenchyma and pith of the main axis with that of the bud. It is also seen how the vascular supply of the bud arises from the leaf-trace strand itself. A section still lower is seen in (iii), where the ring has widened out, and merged into the curved strand of the trace.

It is thus seen that the adventitious buds may be solenostelic from the first. It is not improbable that small and weak runners may be protostelic at their base, but none have been observed. Unfortunately the youngest plants available from those collected in the open forest showed already the solenostelic structure; and as the cultures from spores did not come through to the formation of sporelings, the ontogenetic origin of the solenostelic structure in the plant must for the present remain in doubt.

Pl. XXXII, Fig. L, represents the vascular skeleton of a shoot of moderate size, showing the relation of the supply to the adventitious buds to that of the leaves. In this case most of the leaves bear an adventitious bud, but in some cases the bud was absent.

As regards the details of structure, the solenostele is of a very usual type. It is limited on either side by an endodermis with brown coloured walls, succeeded by a pericycle of two or three layers, and the broad band of phloem on either side of the xylem presents no characters for remark. The xylem varies in its thickness, thinning off especially where a leaf-trace is about to depart. It consists of tracheides mixed with parenchyma. The tracheides are of uniform character, and, as is often the case in the Cyatheaceae, there appears to be no protoxylem. Not only is this absence of protoxylem seen structurally, but it is also shown in sections of young stems by the fact that the tracheides mature almost simultaneously over the whole transverse area.

Though the axis is often very bulky, no medullary vascular system has been seen.

Anatomy of the Leaf.

The leaf, which is slightly dilated at the base, is continued upwards with an almost cylindrical stipe, marked on either side by pale lines similar to those in the Bracken, and often accompanied by dark streaks of sclerenchyma. These are interrupted, just as in the Bracken, at the first pinnae, which are inserted in large leaves at two or three feet from the base.

The leaf trace has been seen to come off as a simple meristele, which soon assumes a complicated horseshoe outline, with lateral involutions. In small leaves it may remain throughout as a single continuous strand; but in large leaves it commonly divides close to the leaf-base into three distinct portions, which in very large leaves may be widely separated (Pl. XXXIV, Fig. 9). In leaves of smaller size the margins of these several portions may remain in close relation, or the strands may even fuse with one another from time to time. But sooner or later they finally join again to form a single band. This was found to be completed in a specific case within nine inches of the base of the leaf (Fig. 13 *bis*, *a*, *b*).

Very soon after leaving the axis protoxylem-groups make their appearance in the vascular strand, which had previously been without them. They are characterized in the usual way, but are very numerous, forty-seven being counted in a specific case. Each protoxylem acts as the centre of a 'divergent', and lies apparently nearer the periphery of the section than the metaxylem; but in actual fact the protoxylem elements, accompanied by cavity parenchyma, are really on the adaxial face of the xylem of the meristele. Since this arrangement impresses itself on the whole contour of the strand, a characteristically corrugated outline results (Pl. XXXIV, Fig. 13 *bis*; Pl. XXXV, Fig. 14). This condition is continued with little change in form of the trace, or even in number of the protoxylems, up to the level of the first pinna.

Opposite the involutions of the horseshoe-shaped strand a break in the continuity of the band of sclerenchyma which bounds the section may be seen on either side. The epidermis is there thin, with numerous stomata, while a lacunar ventilating tissue lies below it. This structure constitutes those lateral lines already observed from without (Fig. 13 *bis*).

The origin of the pinna-trace from the rachis has been followed. For purposes of description reference must again be made to the strand of the rachis. It is in outline like the Greek omega (Fig. 13 *bis*, *b*), thus corresponding to the chain of separate strands so characteristic of the leaf-trace in the Cyatheaceae. There are two internal bays on each side: these may be designated according to their position as the adaxial and abaxial bays. It is in relation to these that the pinna-trace arises. An example is seen in Pl. XXXV, Fig. 14, i–vii (left-hand side). The first sign is a deepening of the abaxial bay on the side where the pinna arises (Fig. 14, i). The xylems then come together a little short of the extreme curve and fuse, forming a loop of xylem, with conjunctive parenchyma enclosed (Fig. 14, ii). A constriction then takes place, so as to nip off the xylem-ring with its attendant tissues, while the phloem and sheaths close in so as to abstrict off an island of vascular tissue from the angle of the bay. Meanwhile the sheaths at the angle of the adaxial bay begin to widen out towards the island, and, joining with its sheaths, receive it as it separates from the abaxial bay (Fig. 14, ii, iii). After the junction has thus been made with the adaxial bay the xylem-ring connects with its xylem, and then opens out; the conjunctive tissue and the sheaths curve into the cavity, and the island thus takes its place as part of the adaxial bay of the trace (Fig. 14, iv, v). But meanwhile a constriction, and finally an interruption, appears further along the bay, on the adaxial side (Fig. 14, v); enlargements then appear facing one another upon the two sides of the adaxial bay: these meet and fuse (Fig. 14, vi), but shortly the bridge of fusion divides down its middle; when that is completed the pinna-trace becomes isolated, while the trace of the rachis resumes its original form (Fig. 14, vii). These successive steps are illustrated on the left-hand side of the sections seen in Fig. 14. It will be seen that similar steps are initiated on the right-hand side also; these are for the next pinna, which is seated almost opposite to the first, but slightly higher.

A comparison of these steps in the formation of a pinna-trace may be made with the description and drawings given by Tansley for the secondary pinna of *Cyathea excelsa* ('The Filicinean Vascular System', p. 118 and Fig. 97). There are differences of detail, partly in relation to the interrupted nature of the vascular tracts in *Cyathea*. But the essentials are the same, and the chief point is that in either case the pinna-trace is connected with both bays of the folded trace of the rachis. The median part of the pinna-trace is derived from the abaxial bay, and the lateral parts of it from the adaxial bay.

On the other hand, a comparison should be drawn with what is seen in *Gleichenia*, and a series of sections has been made from the rachis of *G. linearis*, at the level of insertion of a pair of pinnae. At first sight there does not appear to be much similarity. But in any such comparison allowance has to be made for the habit. In *Gleichenia* there is a more restricted area of the transverse section, and consequently a greater compression of the vascular tract. This is evident in the absence of the lateral involution between the adaxial and abaxial bays, as well as in the stronger curving inwards of the hooks of the horseshoe, which actually meet and fuse (Fig. 15, i). Thus the changes which lead to the separation of the pinna-traces start from a simpler and more compact state than in *Lophosoria.*

The first changes are the separation of the hooks from one another, while the loops formed by them are completed (Fig. 15, ii). Each of the loops then becomes constricted in the middle, and a C-shaped vascular tract is separated off from its distal end (Fig. 15, iii–v). Meanwhile, two projections arise from the concave side of the main bundle, opposite to these C-shaped tracts (Fig. 15, iii–v), while the latter alter their form by producing processes opposite to these (Fig. 15, v). The pairs of processes then fuse, and subsequently each bridge of junction splits down its middle into equal halves (Fig. 15, vii). The result is the formation of three C-shaped tracts of vascular tissue lying in a row, which supply respectively the main rachis and the two pinnae. Putting aside the differences of detail, the points of similarity with *Lophosoria* are, (i) that the pinna-trace is intra-marginal in its origin, and (ii) that its separation is effected by the splitting down the middle of a bridge of junction between the internal faces of the curved trace. On the other hand, as points of difference may be noted, (i) the greater simplicity of the initial steps in *Gleichenia,* consequent on the absence of the lateral involution, (ii) the difference of behaviour of the margins of the trace, consequent on its constriction, and (iii) the equality of size of the traces of the rachis and the pinnae. Thus the comparison brings out fundamental similarities, while the differences which appear may be attributed to the peculiar habit of the Gleicheniaceous leaf, and the extreme contraction of its vascular supply.

It may not be out of place here to remark that notwithstanding the well-known contraction of the meristele at the base of the leaf in *Gleichenia,* which is correlated with its continued apical growth and straggling habit, the meristele may open out above into a widely curved horseshoe. Sections from the upper regions of the leaf of *G. flabellata,* and especially of *G. linearis,* show this clearly. In them the xylem widens out into a dilated curve of one to three layers in thickness, while the protoxylems, with their cavity parenchyma, lie on the adaxial face. The structure is in essentials like that of *Lophosoria*; the chief difference is in the much lower number and less prominent identity of the divergents.

Hairs.

The only dermal appendages found on the shoot of *Lophosoria* are simple hairs, composed of a single row of cells. They are thin at the base, becoming thicker upwards, and narrowing suddenly near to the apex. They do not bear any glands, and are not branched (Fig. 16). Such hairs are of a very primitive type, and the absence of any broadening into ramenta or scales is. in strong antithesis to what is seen in many related genera, and especially those of the Cyatheaceae. But the condition here seen compares in essentials with the condition of those species of *Gleichenia* (*G. linearis* and *pectinata*) with which comparisons have been drawn on other grounds.

The Sporangium.

It has been seen that the sorus of *Lophosoria* consists of about 7–10 sporangia, seated upon a slightly raised receptacle. Not only does observation of young stages show that the sporangia of a single sorus arise simultaneously, as in the relatively primitive Simplices, but there is an extraordinary simultaneity of development of the sporangia on the same pinnule, and even on the whole leaf. This condition, so different from what is usual in the more advanced Ferns, was entirely unexpected when the collection of material was made. The result was that only certain stages were at first secured, and those did not include the earliest. The difficulty as to material was, however, surmounted through the kindness of Mr. Harris, of the Hope Gardens, Jamaica, who made a second collection for me so as to secure the earliest stages of the sorus and sporangium. It is to be remembered that the condition thus shown by *Lophosoria* is characteristic rather of the lower than of the higher Filicales, and is conspicuously the case in the Gleicheniaceae.

The mature sporangium is of large size, and of almost spherical form. It is attached by a short stalk, which in transverse section shows six or more cells (Fig. 17). This corresponds to what is seen in *G. linearis* (Studies, IV, Fig. 9), and is distinctly more complex than is seen in *Alsophila excelsa* (l. c., Fig. 89). There are no internal cells such as are seen in the massive stalk of *Todea* (l. c., Figs. 48–50), or in *Lygodium* as clearly indicated by Binford's drawing (' Bot. Gaz.', vol. xliv, Fig. 18, p. 217), or in *Gleichenia circinnata* (l. c., Fig. 5), or in *Matonia* (l. c., Fig. 59), and occasionally seen in *Mohria* (Prantl, ' Schizaeaceae,' Fig. 143). In this detail, which is in itself an indication of the massive character of the sessile sporangium, *Lophosoria* takes a middle position between the larger sporangia of some Simplices and the smaller sporangia of the Gradatae.

The external form and structure of the sporangium is shown in Figs. 18–20, from three different points of view. The oblique annulus is well marked, and continuous past the stalk, thus marking off two areas of thin-walled cells, corresponding to those described as the ' peripheral ' and ' central ' faces in the case of *Gleichenia* (Studies, IV, p. 34). The peripheral face is shown in frontal view in Fig. 18, and is the face of the sporangium which is directed obliquely outwards from the centre of the sorus in the normal orientation; this orientation we have seen to be habitual in those of the lower tier in *Lophosoria.* The central is the opposite face, which is directed obliquely inwards, to the centre of the sorus, just as in *Gleichenia.* It is shown for *Lophosoria* in Fig. 19. In Fig. 20, where the sporangium is seen from the side, showing clearly its turgid form, the two opposite faces are indicated by words. The annulus consists as a rule of a single row of cells of regular sequence; but in the neighbourhood of the stomium irregularities appear. In Figs. 19, 20, certain cells are divided, while in close proximity there appears a break in the regularity of the sequence. It is as though the distal part of the annulus were more nearly vertical than the proximal part. Such facts indicate an instability of exact position of the annulus such as might be anticipated in a case where that structure is in course of change from an inherited to a novel position; in fact, swinging from an oblique towards a vertical position.

The stomium is lateral in position—a marked difference from the case of *Gleichenia*, where the dehiscence is median. It is not exactly differentiated, and appears to be variable in the number as well as in the exact position of its cells. It serves, however, as a structural definition of the lateral dehiscence, though not with the constancy of structure seen in more advanced Ferns. Such a condition is what might have been expected in a type in which a recent change of the point of dehiscence from a distal to a lateral position had occurred.

The number of tabular cells covering the faces of the sporangium is relatively large, and it is markedly so when a comparison is made with the sporangia of the Cyatheaceae. Thus, over fifty cells are shown on the peripheral face of Fig. 18 of *Lophosoria.* In Studies, IV, Fig. 83 of *Cyathea* there are only six, in Fig. 86 of *Hemitelia* there are eight. The correspondence is, however, a closer one with the large sporangia of *Gleichenia.* Having noted thus the large size of the sporangium, and its complexity of structure, it was a matter of some surprise to find that the output of spores is low. Several countings gave figures closely approximating to, but not exceeding sixty-four, which appears to be the typical number in each sporangium.

The development of the sorus and sporangium has been studied in sufficient detail for bringing out the points that are essential for comparison. As in *Gleichenia* the receptacle makes its appearance on the lower surface of the pinnule, at some distance from the margin, as a multicellular upgrowth of rounded form (Figs. 21–2). Certain of its superficial cells begin to grow out as hairs (Figs. 24–6), while others become soon recognizable as the parent cells of sporangia (marked × in Fig. 23). These undergo divisions by walls inclined to one another, leaving a pyramidal cell at the apex of the upgrowing sporangium. From this by a periclinal wall the cap-cell is cut off. The number and relations of the cells which thus go to form the stalk of the sporangium do not appear to be precisely the same in all cases. The stalk which results is of a massive type (Figs. 25, 26). The whole segmentation of the sporangia, as well as their position on the receptacle, and the structure of the latter are strongly reminiscent of what has been demonstrated for *Gleichenia linearis* (= *G. dichotoma*) some years ago (Studies, IV, Pl. II, Figs. 15–17). In both cases the relatively thick stalk is indicative of a primitive sporangial type. On the other hand, a comparison may be instituted with the young sporangia of the Cyatheaceae, as shown for *Cyathea* (l. c., Figs. 78–80), for *Alsophila* (l. c., Fig. 87), and for *Onoclea* (l. c., Fig. 90). It is, however, to be remarked that in these the sporangium is of a rather more attenuated form, and projects more in its early stages of segmentation, which is an indication of its more advanced state.

A later state of the sorus and sporangia is shown in Figs. 28, 29, the former being cut so as to follow the course of the vein over which it is seated, while in the latter the vein is cut transversely. From the convex surface of the receptacle arise numerous hairs, which show a basal intercalary growth. The sporangia are few, and are always of uniform age in the sorus. There is no evidence of any succession in their appearance. Thus *Lophosoria* would be properly ranked, as *Gleichenia* is, among the Simplices. It may be noted that the sporangia in *Lophosoria* are raised further from the surface of the leaf than in *Gleichenia* (compare Figs. 28, 29 with Figs. 22, 24 of Studies, IV). This is owing to the greater height of the receptacle, a fact which accords with its relation to the Cyatheaceae, where the receptacle is higher still.

As regards the sporangia, their position when young naturally corresponds with that which they hold when mature. In Fig. 28 two are traversed in positions corresponding to those of *Gleichenia linearis*, as seen in Fig. 15 of Studies, IV. In Fig. 29 three are seen corresponding in their disposal to those of *G. linearis* shown in Studies, IV, Fig. 16. The sporangia themselves have their short massive stalk segmented in a way that would lead to a structure as seen in Fig. 17. It corresponds to that of *G. linearis*, rather than to the more massive structure seen in *G. circinnata*. The sporangial head has as usual the single peripheral layer of cells that will form the sporangial wall, lined with the tapetal layer, which surrounds the single sporogenous cell. The structure and proportions thus seen for *Lophosoria* are of a type common for the more primitive Ferns where the sporangium is large, such as *Gleichenia linearis*, *Matonia*, or *Loxsoma*, but not of the very largest size. As it grows older the annulus begins to be differentiated by enlargement of certain cells of the wall (Figs. 30–2), the tapetal layer divides in the usual way into two, and the sporogenous cell divides into usually sixteen cells. These are as a rule arranged in two tiers of eight, so that in transverse sections that number is shown (Fig. 32). In one case, however, nine were found (Fig. 33). It would thus appear possible that a larger number than sixteen spore-mother-cells were present. But as this was an isolated example among many others, and as the countings of the spores showed consistently figures not exceeding sixty-four, stress should not be laid on this single divergent fact. Moreover, it is possible that a division may have been left out in the other tier of cells, so that the total of the spore-mother-cells would still be only sixteen. Then follows the tetrad division, and maturing of the spores in the usual way. The result is shown in a section from an almost mature sorus in Fig. 33. Here the position of the sorus relatively to the curved surface of the pinnule is seen, with the mass of tracheides which enters the receptacle. Of the four sporangia traversed, those right and left show the regularity of orientation characteristic of the Gleicheniaceous type. A comparison of these with the sporangia as seen from outside (Figs. 18–20) explains the relation of the large cells of the annulus to the body of the sporangium, and to its peripheral and central faces. But the two sporangia which occupy the centre of the sorus show irregular orientation, as would be anticipated from the drawing of the sorus as a whole (Pl. XXXI, Fig. I).

It has been seen that the stomium is lateral, and therefore the annulus on dehiscence behaves in the manner characteristic of the Gradatae, as seen in the Cyatheaceae. This makes no demand for 'elbow-room' as in the median dehiscence of the Gleicheniaceae. It is an arrangement which is practically effective in closely crowded sori, and it is evident from the relation of the sporangia, as seen in Fig. 33, that the mature sporangia of *Lophosoria* are in close contact.

It appears from the data thus given that the sorus of *Lophosoria* presents features of correspondence with, as well as of strong divergence from, that of the Gleicheniaceous type. The points of similarity are: (1) the superficial position, (2) the insertion on the anadromic branch of the forked veins, (3) the raised receptacle with vascular supply, (4) the simultaneous origin of the sporangia, (5) the disposition of the sporangia after the type of *G. linearis* and *pectinata*, (6) the segmentation of the sporangia, and (7) the oblique annulus. The points of difference are: (1) the lateral dehiscence, and (2) the smaller spore-output from the individual sporangium. As to the latter, the condition seen in *Lophosoria* is in some degree approached by the smaller sized and less prolific sporangia of *G. linearis* and *pectinata*, while in the last-named species a marked degree of variability of size of the individual sporangium is seen. There is, however, an obvious break in the series, leaving *Lophosoria* isolated. As to the dehiscence there is little evidence on either side of transition from the median to the lateral type. The want of strict definition of the stomium in *Lophosoria* may, however, be held as some indication that shifting of its position had occurred. It is, on the other hand, quite possible that the Cyatheaceous type of dehiscence may have existed *ab initio.* This, however, seems to me to be the less probable reading of the facts.

Taking all the points of correspondence and of difference into account, it appears probable that *Gleichenia* and *Lophosoria* are genera which are descendants of a common stock which had a superficial sorus, of the type of the Simplices ; that in certain of the forms a departure was made from the originally radiate uniseriate type by formation of sporangia in the centre of the sorus, as in *G. linearis* and *pectinata*, the latter of which reached the limit of crowding of the sorus which could secure effectiveness of dehiscence by the median rupture ; that this difficulty was avoided by another related stock, represented in its simplest form by *Lophosoria*, in which, either as an original character, or more probably as a result of later adaptation, the lateral dehiscence is now seen.

Spore and Prothallus.

The mature spore of *Lophosoria* is a very characteristic one. It is tetrahedral, and shaped like a kettle-drum. There are three flattened faces, separated by lines which converge to the central apex and marked by irregular spots. These represent the skin of the drum. The margin of the drum-head is expanded into a projecting circular band, while the rest of the rounded body of the spore is covered by a thick wall, marked by minute rounded bosses (Fig. 34).

On germination the rupture takes place along the three converging lines, and the three apical flaps of the outer wall come apart. The prothallus is at first filamentous, and in crowded cultures may continue so for some length. But sooner or later the apex widens out into a spathulate form, with its lobes ill-defined and unequal, as is usual in *Alsophila* (Fig. 35). My cultures have progressed to the stage of producing antheridia, which are of a usual type. They rupture by the extrusion of a single cell. Unfortunately, the first cultures at this stage damped off. But attempts are being made to raise fresh cultures from spores kindly supplied from Jamaica by Mr. Harris. A fuller statement on the gametophyte and observations on the young sporophyte will have to be deferred to a later communication.

Morphological Discussion.

A considerable number of characters have been mentioned in the above pages, according to which the Fern described as *Alsophila* (*Lophosoria*) *pruinata* occupies a peculiar position of aloofness; characters which were for the most part unknown to Sir William Hooker, or at least not regarded by him as important when he merged it as a species of *Alsophila*.

It will be shown that these characters indicate that *Lophosoria* is a more primitive type than the true species of *Alsophila*, and point with all the strength of parallel comparison to its position at the base of that series, where in point of fact it has been naturally placed by most systematists.

On the other hand, a somewhat similar aloofness has been indicated within their own genus for two species of *Gleichenia*, viz. *G. linearis* and *G. pectinata*. This has been commonly recognized by writers. It has been shown that their peculiarities mark them off as an advance upon the genus *Gleichenia* in general. By further comparisons between this advanced guard of the ancient genus *Gleichenia* and the *Lophosoria* type as the most primitive of the Cyatheoid Ferns, it appears that the two large groups approach one another. In fact, on the basis of the foregoing observations, together with other considerations now to be advanced, the conclusion may be drawn that the primitive and creeping Gleicheniaceae resembled the ancestry of the Cyatheoid series, and that of living types they most nearly represent the forerunners of that great body of dendroid Ferns.

The criteria which may be used for the purpose of this comparison are the following:—

(i) The position and branching of the axis.
(ii) The form and pinnation of the leaf.
(iii) The dermal appendages.
(iv) The characters of the vascular system.
(v) The sorus, its position and constitution.
(vi) The sporangia and spores.
(vii) The prothallus and sexual organs.

Each of these will now be examined with a view to an opinion on the phyletic relation of the Ferns named.

1. In chapter xvi of the 'Land Flora' I have dealt broadly with the question of symmetry in the sporophyte, and drawn the conclusion that 'the radial mode of construction was primitive for the sporophyte at large, and that where dorsiventrality occurs it is a secondary condition'. If this were accepted in its baldest form, one might proceed to classify all radial types as relatively primitive, and all dorsiventral types as derivative. But that could only be done by making the quite gratuitous assumption that changes of symmetry could only occur once in any phyletic line. For this there is no warrant. It would seem probable rather that according to circumstances the change from radial to dorsiventral, and also from dorsiventral back to the radial condition of the shoot, may have occurred repeatedly in the course of descent. This subject has been already broached by Prantl in relation to the Hymenophyllaceae and the Schizaeaceae, and similar suggestions have been advanced by Boodle as explaining the anatomy of certain Hymenophyllaceae ('Annals of Bot.', xiv, p. 482). He contem-

plates it as probable for them that though the original ancestry may have been radial, all the living members of the order may have been evolved from creeping forms; some species retaining that character, and others having changed to the upright habit, and back again. It is necessary to keep an open mind on such questions, and to let opinion follow fact and comparison.

Applying this point of view to the present case, though we may contemplate the ultimate origin of the Gleicheniaceæ from more primitive Ferns with radial upright axis, we see them to-day as characteristically a creeping family, with dorsiventral shoot. But if the facts indicate that any derivatives from them became in the further course of descent upright, and attained to a radial axis, there seems no reason to dissent from such a conclusion. On grounds especially of their sori reason has been found to contemplate the derivation of the Cyatheaceae from a source resembling the Gleicheniaceae. Our point now is, that if a careful examination of widespread character indicates that that was so, there is no inherent reason to doubt that the columnar upright axis, which is the most striking feature of the Cyatheoids, was itself a secondary character.[1]

The question will largely turn upon the existence of intermediate types. There are no relatively advanced Gleichenias which have adopted a definitely upright, self-supporting habit. The two most advanced species, viz. *G. linearis* and *G. pectinata*, are both creepers. But on the other side, though the more advanced genera *Hemitelia* and *Cyathea* show no creeping species, such are seen in the more primitive *Alsophila*, in the species *A.* (*Metaxya*) *blechnoides*, and in less pronounced degree in *A.* (*Lophosoria*) *pruinata*. Moreover, both of these proclaim themselves as primitive by other characters. A detailed account of *Metaxya* will be held over to a later memoir of this series. It may suffice here to say that with its creeping habit go a solenostelic structure of the axis, an undivided leaf-trace, and hairs only as dermal appendages; all of these being relatively primitive features as compared with other Cyatheaceae. The same may be said for *Lophosoria*, which, however, as regards the mature plant has an upright, though short axis. But the buds which originate at the bases of many of the leaves give rise to runners, which at first take a horizontal course, with their leaves coming off at intervals right and left. The creeping habit with isolated leaves is thus actually shown in the early condition of these shoots. Thus, as regards position of the axis, *Metaxya* pronouncedly, and *Lophosoria* in a less degree, bridge over the gap between

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[1] The question here considered, of the upright habit in the Cyatheoid Ferns being a secondary derivative from the creeping habit, has already been stated briefly (Ann. of Bot., xxv, pp. 567–8). The detailed evidence for the opinion is here stated, and it is essential that it should be, for the theory of medullation stated in the paper above quoted could not hold, if the vertical character were primitive. But the comparative evidence from external form, from dermal appendages, from anatomy, and from sorus and sporangium alike, as stated in the above and in the succeeding paragraphs, indicates that it was not.

the creeping habit of the Gleicheniaceae and the upright dendroid types of the Cyatheaceae.

In this connexion it may be noted that terminal branching occasionally is seen in the dendroid Cyatheaceae. An example of this is shown in Pl. XXXII, Fig. M, in a plant in the Glasgow Botanic Garden. Here there is an apparent dichotomy, the two resulting branches having continued their development equally. Anatomical examination would be necessary to prove that the branching was really dichotomous in this case, and naturally that cannot be undertaken. So it must suffice for the present to state that all the external indications are favourable to the branching having been a true dichotomy. If this were really the case, it would be a further point in common with the Gleicheniaceae, in which it has been shown that dichotomy is frequent.

2. As regards the form of the leaf the Gleicheniaceae and Cyatheaceae seem at first sight widely divergent, and *Lophosoria* does not appear to help any transition. But if a comparison be made between the ultimate pinnules of *Lophosoria* (Pl. XXXI, Fig. H) and those of some species of *Gleichenia* (Pl. XXX, Figs. C and E) a similarity of outline, of venation, and even of texture is apparent ; the soral similarity is, however, the most important feature of all. The divergence is then in the general habit of the leaf rather than in the details. But even in general habit there are features which link the two families together. The most marked characteristic of the leaf of *Gleichenia* is its apical growth, which may be continued indefinitely though interrupted at seasonal intervals. Looked at broadly, however, the leaf is constructed upon the ordinary pinnate type, and its peculiarities depend upon irregularities in time and extent of the intercalary growth of the rachis, and of the branches of higher order. For the most part the intercalary growth between the pairs of pinnae is strong, so that they are far apart, and usually uniformly removed from one another. In the Cyatheaceae it is less strong, and the pinnae are more closely aggregated. But as a rule the pinnae stop short at some distance from the base of the mature leaf, intercalary growth having been strongest below the lowest pinnae. A special interest, however, for comparative purposes lies in those cases where this rule is not maintained, with the result that basal pinnae are left behind at the base of the leaf, the intercalation of the petiole having taken place above them. These basal pinnae have long been known as the so-called 'aphlebiae', and the example commonly quoted is that of *Hemitelia capensis.* But as a matter of fact it is a feature which exists sporadically in all the three genera of the Cyatheaceae. Pl. XXXIII, Fig. N shows a very pronounced example of *Hemitelia setosa*, Mett., from the Edinburgh Botanic Garden. Fig. O shows the case of an *Alsophila* from Jamaica, growing in the Glasgow Botanic Garden ; and Christ has figured a very marked case of basal pinnae in a species of *Cyathea* ('Geographie der Farne', Fig. 24). It thus appears

that the existence of basal pinnae, left behind owing to the higher localization of intercalary growth having produced the petiole above them, is a feature in all the genera of the family of the Cyatheaceae. But in other Ferns it is virtually unknown. It seems to me that the existence of 'aphlebiae' thus exclusively in this family of upright Ferns is strongly reminiscent of an ancestry where the localization of intercalary growth between the pinnae and not exclusively below them was the rule. The unlimited growth and vigorous intercalation seen in the Gleicheniaceae would be impossible features in plants of dendroid habit. But, supposing these dendroid types to have sprung from an ancestry of Gleicheniacous habit, what more probable than that they should still retain some traces of that unusual distribution of intercalary growth? The absence of basal pinnae in other Ferns to which a near relation to the Gleicheniaceae is not ascribed gives strength to this interpretation of the 'aphlebiae' of the Cyatheaceae.

3. Comparisons based upon dermal appendages have mostly been neglected in the phyletic treatment of Ferns, or they have been put to too strenuous a use. The latter was the case when Kühn proposed to divide the Polypodiaceae into two groups on the basis of the simple hairs, as against flattened paleae ('Die Gruppe der Chaetopterideen unter den Polypodiaceen'). A middle course is, however, gradually coming into use, having been initiated by Prantl, who indicated very clearly in the Schizaeaceae how the hair is the more primitive, and the flattened scale, ramentum, or palea the derivative and later type. Other characters being in accord, the presence of hairs only may be held to indicate a relatively primitive condition; the existence of scales, with or without simple hairs as well, may be held to indicate a derivative or later condition.[1] There is reason to believe that a parallel transition from hair to scale was effected in several distinct phyletic lines.

Applying this criterion in the present case, the facts show that *G. linearis* and *G. pectinata* bear hairs only, whereas scales are widely present in other Gleichenias, and especially among the xerophytic types. Now *Lophosoria* and *Metaxya* also have hairs only, and no scales, whereas the genera *Alsophila*, *Hemitelia*, and *Cyathea* are characterized by scales, often of very large size. This superficial feature thus brings together in a very striking way those species upon which our comparison specially turns. But there is a further point to be noted in this connexion. The scales in many of the larger species of the Cyatheaceae are often borne each upon a massive conical emergence, which may persist after the scale itself dries and falls away, as one of those large spines which are specially developed about the bases of the leaves. A like condition has been found in *Gleichenia pectinata* (Pl. XXX, Fig. A), and the existence of such conical

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[1] This line of comparison, based on dermal appendages, is being carried further into detail by C. Christensen (On a Natural Classification of the Species of *Dryopteris*, Biologiske Arbejder, Nov. 1911).

emergences about the leaf-bases justifies the comparison of the branched hairs which they bear with the broad scales of the Cyatheaceae. Similar emergences also exist in *Lophosoria* (Pl. XXXI, Fig. G). The progression of characters from the Gleicheniaceae to the Cyatheaceae through *Lophosoria* is in this case based upon a minor detail; but the existence of such a detailed progression supports all the more strongly the comparisons based upon more important characters.

4. By a general consensus of opinion the non-medullated stele is recognized as the primitive stelar type. The characteristic elaboration of this in Leptosporangiate Ferns was connected with the formation of foliar pockets, which intruded above the insertion of each leaf. This led to complete solenostely, with leaf-gaps. When the latter overlap one another the complete dictyostele is the result. No better illustration of these successive steps is to be found than in the Ferns under consideration. The sequence of stages of the elaboration is as follows: In *Gleichenia* in general the stele of the rhizome has a solid xylem-core; it is a protostele, from which the leaf-traces come off in some cases without any disturbance of the underlying tissues. The types of the genus described fall into a rough series, as regards their vascular characters, extending from the simple forms of *Eu-Gleichenia*, through *G. linearis*, to the typical Mertensias, and finally to *G. pectinata* (Tansley, 'Lectures,' p. 44). There is, however, reason to think that the first named owe their simplicity of structure to reduction in connexion with their xerophilous habit, and that *G. flabellata* may be regarded as a central and primitive example. It has a solid protostele, which gives off to each leaf a C-shaped trace, with a pocket of internal phloem, endodermis, and sclerenchyma, which is continued into the stele, but does not intrude far. *G. pectinata* is, however, a solenostelic form, which shows certain complications noted by Boodle and Hiley ('Ann. of Bot.', xxiii, p. 419). From the known facts it seems still uncertain what the exact steps were which led to the solenostelic structure there presented. This, however, is not the present question. What interests us is that, however produced, the solenostely exists in *G. pectinata* with a centrally placed sclerenchymatous pith, surrounded by endodermis and internal phloem. The internodes are long, so that the solenostelic structure is only interrupted by leaf-gaps at long intervals. Passing to *Lophosoria* the solenostelic structure is essentially the same as in *G. pectinata*, but the whole axis is on a more bulky scale. Though in the young plant of *Lophosoria* the sclerenchyma within the solenostele is at first solid, as in *G. pectinata*, in the older plant a parenchymatous core replaces the central region of that sclerenchyma, the remainder of which appears as a dark shell lining the solenostele internally, as is usual in the Cyatheaceae. The latter shows the usual amphiphloic structure, but there is no marked protoxylem: in this it differs from *G. pectinata*, but it shows a condition

which is common in the Cyatheaceae. The internodes are longest in the creeping lateral buds of *Lophosoria*, while in the upright shoots the leaf-gaps sometimes overlap, giving an elementary state of dictyostely. This is exactly what would result if a shoot of the type of *G. pectinata* were to adopt an erect habit. The leaves themselves, being of necessity mechanically self-supporting, must have relatively broad bases of insertion. The axis would necessarily be shortened, and become more bulky to accommodate the more crowded and relatively broad bases of the leaves. That condition is already seen in *Lophosoria*, but the leaf-gaps do not usually overlap. But they do overlap in the typical species of *Alsophila*, which are thus dictyostelic. *Lophosoria* forms accordingly, in the vascular characters of its axis, an intermediate step between the two types, as it does also in habit, and in other features.

The leaf-trace of *Lophosoria* comes off as a single broad strap, and often passes undivided up the leaf. But where the leaf is large it commonly divides up into three parts, though this separate course does not continue far, the three joining again into a single strand below the lowest pinnae. A very similar condition is seen in *Plagiogyria semicordata* ('Ann. of Bot.', xxiv, p. 431), and in some other Ferns. Such cases have their interesting bearing upon general views of the leaf and the relative primitiveness of its parts, a subject which will be held over for the present. From the comparative point of view *Lophosoria* in this detail again takes a middle place between *Gleichenia*, in which no such partition of the trace has been recorded, and the Cyatheaceae, where the dissolution of the trace into separate strands is present in much higher degree, and extends much further along the leaf than in *Lophosoria*. Further, in the horseshoe form of the trace, with its deep lateral depressions, as well as in the origin of the pinna-trace, *Lophosoria* also takes an intermediate place, but with a decided leaning to the Cyatheaceae. This comes out particularly in the large number of the 'divergents', and in the corrugation of the trace in accordance with their position. Thus the general conclusion from an anatomical comparison is that while the affinities of *Lophosoria* are distinctly with the Cyatheaceae, it shows in a number of anatomical features, which may themselves be held as primitive, a condition intermediate between that family and the Gleicheniaceous type. And among the latter it approaches most nearly to *G. pectinata*.

5. But it is upon the characters of the sorus, its position and its constitution, that the most frequent comparisons have been made. In the Gleicheniaceae, as in the Cyatheaceae, the venation of the fertile pinnule is without any fusions, except occasionally in *Hemitelia*. The veins fork, but not very profusely, and the branches run out free to the leaf-margin. In fact, in both the venation is of the primitive, Pecopterid type. In *Lophosoria*, as also in *G. linearis* and *pectinata*, the sorus is constantly seated upon the anadromic veinlet of the ultimate pinnule. This uniformity in position, com-

bined with the similarity of circular form, the absence of any indusium, the approximate uniformity in number of the sporangia, the simultaneity of their origin, and the likeness of their relation to the slightly raised receptacle, are characters that at once establish a sound basis of comparison between the plants named. Moreover, the two species of *Gleichenia* stand out from their genus in the arrangement of their sporangia in the sorus. There is little doubt that the 'radiate uniseriate' type of sorus, as it is seen in *G. flabellata*, is a primitive one—comparing as it does with what is seen in the Marattiaceae on the one hand, and in *Matonia* on the other. Here the centre of the sorus is unoccupied by sporangia. But *G. linearis* has the centre of the sorus occupied by one or more sporangia, while in *G. pectinata* it is habitually crowded with them, so that they are flattened against one another. The constitution of the sorus of *Lophosoria* is very like these, while in point of time of origin of the sporangia those of each sorus are in both cases simultaneous. Both belong technically to the Simplices, while *Lophosoria* is the only case hitherto recorded of this from the Cyatheaceae, which are typically Gradatae.

6. Turning to the sporangia themselves, both the Gleicheniaceae and Cyatheaceae have an oblique annulus, but they differ in that the dehiscence in the former is in the median plane and distal, while in the latter it is lateral. It has been pointed out above (p. 275) that *G. pectinata* with its median dehiscence, which requires lateral space to be effective, has in its crowded sorus reached fully to the limit of possible efficiency. A modification of its type of sorus or of its sporangium is necessary if any larger number of sporangia is to be accommodated, and each is still capable of dehiscence. One modification would be the adoption of a lateral dehiscence. Another would be an elongation of the receptacle. The best result would be by a combination of both. In *Lophosoria* the lateral dehiscence is adopted, but not the elongation of the receptacle. In the rest of the Cyatheaceae both modifications are carried into effect, together with a basipetal sequence of sporangia, which the elongation of the receptacle makes possible. A circumstance which is an indirect support of the view that the sorus of *Lophosoria* is a modification of the Gleicheniaceous type is that, though all the sporangia are simultaneous, the lower sporangia retain the orientation seen in *Gleichenia.* This orientation is of no practical importance where the sporangia are all simultaneous in origin, and the dehiscence is lateral. It may be held as one of those useless survivals which are on that account all the more cogent as phyletic evidence.

From the above considerations it would appear probable that, in the production of *Lophosoria* from some Gleicheniaceous source, a phyletic shifting of the point of dehiscence of the sporangium from the distal to a lateral position has taken place. This suggestion is supported by the fact that the stomium of *Lophosoria* is not highly differentiated, while there is inconstancy in the numbers and relations of the cells which compose it.

In this respect it shows a marked difference from other Cyatheaceae, in which the stomium shows a high degree of differentiation. In point of size also, and in the number of tabular cells forming its lateral walls, the sporangium of *Lophosoria* corresponds rather to the Gleicheniaceae than to the Cyatheaceae. But in the spore-output it is otherwise. The number per sporangium in the Gleicheniaceae is large. In *Lophosoria* it is only 64, a number which tallies with that of *Alsophila* and *Cyathea medullaris*, though that in *C. dealbata* is much lower. Taking all these soral characters together it seems clear that *Lophosoria* holds a very interesting middle position between the Gleicheniaceae and Cyatheaceae, and the characters are such as accord with the view that its sorus and sporangium were originally of the Gleicheniaceous type; but that while the plant has retained the sorus of the Simplices, it has parted with the primitive mode of dehiscence, and the originally large spore output of the individual sporangium is here represented on a greatly reduced scale.

7. The details of the prothallus and sexual organs of *Lophosoria* being still deficient, comparison on these points must be deferred.

OTHER GENERA OF FERNS WITH SUPERFICIAL SORI AND BASAL INDUSIUM.

There are a number of genera of Ferns, containing each relatively few species, individually of moderate or small size, which share with the Cyatheaceae the possession of a superficial sorus with a basal indusium; some of these prove even to have also like them a basipetal sequence of origin of their sporangia upon the receptacle. They have been ranked by various systematists as related to the Cyatheaceae, and they are placed by Diels immediately after them (Engler u. Prantl, 'Nat. Pflanzenfam.,' i. 4, p. 159). They are as follows: *Struthiopteris* and *Onoclea*, *Peranema* and *Diacalpe*, *Woodsia* and *Hypoderris*, *Cystopteris* and *Acrophorus*. Their characters show that these genera are naturally grouped together in pairs, as above, each of these being more nearly related to one another than the pairs themselves are *inter se*. In order to arrive at some conclusion as to the phyletic relations of these pairs of genera to the Cyatheaceae, and to one another, comparisons must be instituted so far as possible on the basis of those criteria which have been employed above. None of these Ferns, however, are essentially primitive types. This is indicated by the fact that they all show a more or less advanced state of dictyostely in the stock, in some cases with the peculiar basket-like structure owing to deep involutions of the superficial tissues. Their leaf-trace is divided, but not usually in an advanced degree. The venation is in most cases open, but in *Onoclea* and *Hypoderris* it is reticulate. Broad scales are common, in place of or in addition to the more primitive hairs. Some of them have a mixed sorus, while the sporangia usually have the annulus interrupted at the insertion of the stalk. The sum of these characters indicates that we are dealing with Ferns of a middle position in the phyletic scale, a fact which makes the question of their suggested relation to the Cyatheaceous series all the more interesting. It will be convenient to take first the genera *Struthiopteris* and *Onoclea*, for they have for long been regarded as having some near relation to the Cyatheaceae.

Struthiopteris and Onoclea.

The genus *Struthiopteris* (= *Matteuccia*) includes two species, *S. germanica* and *S. orientalis*, which have obliquely ascending or upright stocks covered with the bases of the closely grouped leaves, and *Onoclea* with its single species, *O. sensibilis*, L., having a creeping rhizome bearing isolated leaves.[1] Of these three species *Struthiopteris orientalis* is the least familiar. It shares the leading characters of them all. It will be briefly described here, from observations made partly on plants obtained from India through the Calcutta garden, and my thanks are due to the Director for them; partly from plants living in the Glasgow Botanic Garden, originally obtained from Messrs. May of Edmonton. The points in which this Fern differs from the other two better known species will be noted as they arise.

S. orientalis is a rather coarse-growing Fern, which may attain about the same dimensions as *S. germanica.* It has an obliquely ascending, or upright stock, which is completely covered by the bases of the densely tufted leaves. There were no runners on any of the plants examined, such as are a conspicuous feature in *S. germanica.* The leaf-bases are enlarged as they are in the other species, and in *Plagiogyria*, while they bear rough brownish, rounded outgrowths, mostly along their margins, in appearance like the pneumatophores of that Fern. The leaf-bases and stalks are thickly covered by broad, brown, chaffy scales. The simply pinnate leaves are dimorphic, the broad pinnae of the sterile leaves being deeply pinnatifid, and showing the Pecopterid venation without any anastomoses (cf. Engler u. Prantl, i. 4, Fig. 90 B, of *S. germanica*, which it strongly resembles). But in *Onoclea* the venation is reticulate, a condition which is held to be one of phyletic advance. Frequent middle forms occur between the sterile and fertile leaves. The latter are also simply pinnate, but the segments are narrow, and their margins strongly turned downwards so as to protect the sori, just as in *Blechnum capense*, L., a plant which this species closely resembles.[2]

The massive stock is solid, and does not show that basket-like structure, due to deep pockets immediately above the insertion of the leaves, described by Gwynne-Vaughan for *S. germanica*, and in less degree for *Onoclea sensibilis* ('New Phyt.', vol. iv, p. 211). It is traversed by a dictyostele

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[1] Dichotomy of the axis may be observed in *O. sensibilis*.

[2] It may be remarked that several species now placed in the genus *Blechnum* have from time to time been ranked in the genus *Struthiopteris*, a clear evidence of the similarity which the genera bear to one another. The significance of this will appear in a subsequent paper of this series.

with proportionately large meshes, from the lower margins of which are given off two large strap-shaped strands to the subtending leaf. There is a central pith which is very voluminous in proportion to the other tissues, so that the ring of meristeles lies very near to the periphery. It is to be noted that sclerenchyma is absent from it, in marked contrast to what is seen in the Cyatheaceae. In fact, the structure of the stock is strikingly similar to that of those species of *Nephrodium* which have a binary leaf-trace, such as *N. Oreopteris.*

The general character of the fertile leaf of *S. orientalis* resembles that of *S. germanica* and *Onoclea sensibilis.* The venation of the fertile pinna is on the same plan as that of the sterile pinna, but the branches of each vein from the midrib are only 5–6 as against 10–12 in the sterile. Anastomoses are again entirely absent in *S. orientalis.* The lowest branch of each vein is katadromic (Pl. XXXVI, Fig. 36). Each branch may bear a single sorus, as in *S. germanica,* but whereas there the sori of the lower branches are slightly nearer to the midrib than those of the upper, in *S. orientalis* they are all at an equal distance, so as to constitute a regular intra-marginal row. This will be found to be matched by *Blechnum capense* (see Mettenius, 'Filices Horti Lipsiensis,' Pl. IV, Fig. 21). Each vein is continued a short distance beyond the insertion of the sorus, but stops short where the margin of the pinna curves over as a continuous protective flap, which in this species is firm, and coloured brown. In addition to this protection each sorus is, as in *S. germanica* and *Onoclea,* covered by a shell-shaped indusium, attached at the side next the midrib. It is structurally of the nature of a ramentum or scale. A section through a sorus from one of Messrs. May's plants shows how the leaf-margin overlaps the scale indusium, the protection of the sorus being a very efficient one (Fig. 37). But in the specimens from India the indusium does not appear to be present.

The receptacle in *S. orientalis*, as seen in specimens from the Glasgow Garden, is a rather flat one, being hardly raised beyond the general level of the leaf-surface (Fig. 37). A tract of tracheides rising from the vascular strand which underlies it brings supplies to the sporangia. These are relatively few in number, as seen in the Glasgow specimens ; but in the normally grown specimens from India the sori are larger, the receptacle more raised, and the sporangia more numerous. There is an evident basipetal succession of the sporangia, as has already been demonstrated for *Onoclea sensibilis* and *S. germanica* (Studies, IV, Pl. V, Fig. 90). This is apparent even from the drawings from garden plants, for the central sporangium at the apex of the convex receptacle is the most advanced, and the lateral sporangia younger.[1]

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[1] The absence of an indusium in the specimens from India, together with the much larger receptacle and more numerous sporangia, suggests further inquiry as to specific identity of the specimens. The Glasgow plants correspond most nearly to the specific descriptions for *Struthiopteris orientalis*.

The mature sporangium of *Struthiopteris orientalis* is long-stalked, and it is specially worthy of note that in the Indian specimens a glandular hair is, as a rule, attached to the stalk of each. This corresponds in character and position to the gland which accompanies the sporangium of *Nephrodium filix-mas.* These glands are absent in *S. germanica* and most of the other related Ferns. The annulus is nearly vertical, and it is interrupted at the stalk, being in this point similar to what has been shown for *S. germanica* (Studies, V, Fig. 91). It consists of about fifty cells, a larger number than is shown in *S. germanica.* It is to be noted that frequent cases occur where the series of cells of the ring is irregular, taking sometimes a zigzag or contorted course in place of the usual regular hoop-like series. Such irregularities may be expected in those forms where a transition between the oblique and the vertical annulus is in progress, according to our general hypothesis. It is in such Ferns that these irregularities have been observed.

The characters of *Struthiopteris orientalis* thus described correspond in essentials with those of *S. germanica* and *O. sensibilis.* The differences are in details, and there is no doubt of the naturalness of the group. But in *O. sensibilis* the venation is the most advanced, being reticulate, while in the rest it is an open, Pecopterid venation. The Onocleae appear to be nearly related on grounds of habit, of venation, of anatomy, and of sorus to the Cyatheae, and probably *Struthiopteris,* with its upright habit and open venation, is the nearest. These Ferns have, however, adopted the dimorphic frond, though the existence of middle forms in both genera shows that the distinction has not been definitely impressed. The fertile leaf has rolled up its margin, as in *Plagiogyria* and the Pterideae, for the better protection of the sori. These, however, are all distinct, and have the *Hemitelia*-like indusium present in addition. This, with the gradate, superficial character of the sorus, stamps the relationship with the Cyatheoid Ferns. They may be held to be a series which has carried the Cyatheoid type into boreal and alpine climates, where additional protection is required. Further, *Onoclea* shows in its reversion to the creeping habit, and all of them in their deciduous foliage, evidence that they are the outstanding colonists of the family which have become modified in order to meet the conditions of the habitats to which they have spread.

Cystopteris, Bernh.

This genus was founded by Bernhardi in 1806, and includes thirteen species. Presl ('Tentamen', p. 93) recognized its affinity with *Aspidium* and *Asplenium*, and placed it in close juxtaposition with *Acrophorus* in his Aspleniaceae. Hooker ('Syn. Filic.', p. 103) places it between *Davallia* and *Lindsaya*, but remarks that it is 'allied to *Woodsia* and *Microlepia*'. Diels (Engler u. Prantl, i. 4, p. 163) places it in his 'Woodsiinae', in close relation to *Acrophorus*. Christ ('Farnkräuter', p. 280) ranks it next to *Woodsia*. Influenced by the example of Sir W. Hooker, and after insufficient personal observations, I had in 1899 assented to the relation with *Davallia*, § *Leucostegia* (Studies, IV, p. 76, and 'Land Flora', p. 655). It will now be shown, on the basis of wider comparisons, that the relation is rather with *Struthiopteris* and other Ferns which have a superficial sorus, basal indusium, and a basipetal succession of sporangia.

The anatomy of the stock of *Cystopteris fragilis* has been examined by Gwynne-Vaughan, who found it to have, like *Struthiopteris*, a dictyostelic structure. The leaf-trace also consists of two vascular strands which arise separately from the dictyostele ('New Phytologist', vol. iv, p. 215). He remarks, with regard to the peculiar involutions above the insertion of the leaves, that its stem furnishes almost as good an example of the development of epidermal pockets as *Struthiopteris* (*Onoclea*) *germanica* itself, although the whole structure is on a smaller scale. Thus there is a substantial anatomical similarity with the stock of *Struthiopteris*. If the pockets be left out of account the structure corresponds also with that of *Woodsia*; though in *Cystopteris* the meristeles are smaller and further apart, the disposition of them is essentially the same.[1]

Sections of the young sorus of *Cystopteris* show conditions resembling those of *Struthiopteris*, but on a reduced scale, and modified in relation to the flattening of the receptacle under an indusium which lies parallel to the leaf-surface. The formation of the sorus begins slightly back from the apex of the leaf-lobe, as an upgrowth subtended below by the scale-like indusium. The first sporangium, which Schlumberger (l. c., p. 408) describes as being prior to the indusium, appears with regularity on the middle of the convexity (Fig. 38, *a*), several large cells intervening between it and the insertion of the indusium. Transverse sections show that the first sporangium occupies also a median position in relation to the indusium (Fig. 38, *b*); in fact, it occupies the apex of the small flattened receptacle. A comparison may be made with what has been seen in *Struthiopteris* (Fig. 37). Later other sporangia appear right and left (Fig. 39), while the cells which intervene between them and the base of the indusium give rise also to a regular gradate succession in basipetal sequence (Fig. 40). A comparison of the latter drawing with Fig. 90 of my Studies, IV, shows how similar this condition of the sorus is to that of *Onoclea sensibilis*. A comparison with *S. orientalis* also shows correspondence, due allowance being made for the relatively small number of sporangia shown in the garden-grown specimens on which the observations were made. But whereas in these Ferns the

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[1] This account does not accord with that of O. Schlumberger (Flora, 1911, p. 410), who states that only a single strand enters each leaf. His observations seem to have been made on a young plant ('Keimpflanze'), and it may be true for the first leaves; but the leaves of mature plants are supplied by two distinct strands, as in *Struthiopteris* and in *Woodsia*. This is already recorded by Luerssen (Rab. Krypt.-Fl., iii, p. 447.)

receptacle rises symmetrically from the leaf-surface, here it is compressed below the indusium, and develops its basipetal sequence as a rule only on the side away from the leaf-surface. None the less is it a gradate sorus, and, contrary to my statement of 1899 (l. c., p. 76) (which was made from old sori), irregularly intermixed stages of sporangia have not been observed in the young state. The segmentation of the sporangia is also a matter for note. It will be seen that the stalk is a relatively massive one with irregularly inclined walls (Fig. 38, *b*). This corresponds closely with what is seen in *Onoclea sensibilis*, and with the Cyatheoid series, and is in sharp contrast to the simple transverse segmentation of the stalk seen in *Davallia* (Studies, IV, Figs. 134, 135). In form the mature sporangia of *Cystopteris* correspond to those of *Struthiopteris* in having their annulus continued to the stalk, but there interrupted; while the stalk itself is relatively massive, and consists of three rows of cells. But the number of cells of the annulus is smaller than in *Struthiopteris*, being only about thirty. The conclusion is, then, that sorally *Cystopteris* is in near relation to *Onoclea* and *Struthiopteris*, and is essentially of the Gradate type; while the characters of anatomy and of habit support the alliance with the Cyatheoids, and especially with certain of their derivatives. Schlumberger, however, considers that the facts of development of the sorus indicate a relationship of *Cystopteris* to the Davallieae (' Flora ', 1911, p. 408), rather than with the Cyatheoid series. This opinion I do not share, preferring to regard the peculiarities of the sorus as a consequence of a flattening of the receptacle between the indusium and the leaf-surface, together with an absence of the sporangia on the side of it next to the leaf-surface.

The characters of the prothallus of *Cystopteris*, as described by Schlumberger (l. c., p. 386), are of some importance in considering the relation of the genus to the other Ferns mentioned above. The hairs which it bears are only unicellular glandular hairs, as in the ' Polypodiaceae ', not multicellular as in the Cyatheaceae. Further, the lid-cell of the antheridium is undivided, which is again a Polypodiaceous as against a Cyatheoid feature. These characters, together with the flattened type of the sorus above alluded to, accentuate the aloofness of the genus. But its gradate sorus and its anatomy and habit all indicate a relationship to the Ferns with a basal indusium. It may be held to occupy a middle position linking the Woodsieae, especially *Struthiopteris*, with the Aspidieae.

Acrophorus, Presl.

This genus was founded by Presl in 1836, to receive the single species now designated *A. stipellatus*, (Wall.) Moore. It was placed by him in close relation with *Cystopteris*: but the species had previously been styled *Aspidium nodosum* by Blume. Sir Wm. Hooker merged it in *Davallia*, but evidently with some doubt, as it stands in the ' Species Filicum ', vol. i, p. 157, under the name *D.* (?) *nodosa*, Hook. In the ‘Synopsis Filicum’, however, the query is dropped, and it appears (p. 92) as *Davallia* (*Leucostegia*) *nodosa*, Hk. Diels (Engler u. Prantl, i. 4, p. 164) upholds its generic distinctness, and names it *Acrophorus nodosus*, (Bl.) Presl, giving it a place in close relation to *Cystopteris*. Christ (Farnkräuter, p. 285) also upholds the genus, but places it in near relation to *Struthiopteris*.

It is clear from these diverse opinions that this isolated species shows characters apparently in common with two widely different affinities, both characterized by superficial sori, protected by an indusial structure attached on the side of it nearest the midrib. The *Davallia*-type is, however, a derivative of the Dicksonioid stock, with marginal sori, while the affinities of *Cystopteris* and *Struthiopteris*, as long recognized and as specially indicated on the widened basis of comparison in the above pages, are with the Cyatheoid stock with superficial sori. The former approaches the latter in appearance by a gradual phyletic shifting of the sorus from the margin to the surface of the leaf, a process clearly indicated by the comparative study of *Davallia*. Consequently the position of the sorus, supported merely by the comparison of habit, will not serve to determine the true affinity in any critical case. A careful examination of the anatomy, the superficial appendages, and especially of the structure and development of the sorus and sporangia will be required for a decision in any given example.

This seems specially necessary in the case of *Acrophorus*, and in the absence of material for detailed study I am not prepared to express an opinion whether its relation is truly with *Cystopteris* or with *Davallia*. There is, however, one feature which seems to make the former affinity doubtful. In all the Gleicheniaceae and Cyatheaceae, as well as in the other allied genera with basal indusium, the sorus is seated on a vascular strand, which is continued beyond its insertion. In *Acrophorus*, as its very name implies, the vascular strand stops at the sorus, which is described as being on its tip. This is the usual, though not invariable character of Ferns with marginal sori (Hymenophyllaceae, Dicksonieae, &c.), and is mostly retained, at least in cases with relatively simple venation, even where the sorus has become superficial. This fact appears to strengthen the Davallioid alliance of *Acrophorus*, but no definite expression of opinion on the point is here given.

This is an example of many like questions which must necessarily arise in the phyletic treatment of the more advanced Ferns; for they, perhaps as frequently as any well-represented group of plants, show converging lines of specialization. So that the most advanced representatives of stocks quite distinct in origin assimilate closely to one another, and can only be disentangled by minute comparative study, bringing all possible criteria which may serve to guide a final decision.

Peranema and *Diacalpe.*

I have left the detailed examination of these genera to Mr. Davie, whose paper on these Ferns precedes this (p. 245 above). I would merely remark here that the position which has been assigned to them at the base of the Aspidieae appears to be justified on the ground of their dictyostelic structure, divided leaf-trace, open venation, abundant chaffy scales, mixed sorus, with indusium of the nature of a highly modified scale covering the vascular receptacle. Lastly, the sporangia have an annulus, at least partially interrupted at the insertion of the stalk, but showing some indications of obliquity. These features, combined with the general habit of each, so like that of certain types of *Nephrodium*, indicate a relationship which has been already recognized on less extended grounds of observation by various writers, and specially by Christ (Farnkräuter, p. 286, and preface, p. vii).

On the other hand, the character of their sorus has from their first description been held to relate the Ferns to the Cyatheaceae, and this view is stereotyped in the name *Peranema cyatheoides*, D. Don. Though the detail of the correspondence now proves not to be so close as it was earlier thought to be, still there is sufficient indication that it is in the neighbourhood of the Cyatheaceae that their natural place is to be found. This follows from the position and general character of the sorus, and especially of the indusium; from the vascular receptacle, and from the indications of a basipetal sequence in *Peranema* in the appearance of the sporangia, as described by Mr. Davie. But perhaps the most convincing evidence of a Cyatheoid affinity lies in those characters of the gametophyte which have recently been described for *Diacalpe* by Schlumberger ('Flora', 1911, p. 385). In the first place, hairs of the Cyatheoid type are present upon its prothallus, while further, the lid-cell of the antheridium is divided as it is in the Cyatheaceae, but not in the 'Polypodiaceae'. These indications, in characters which are apparently isolated and not of any great biological importance, may on that account be held to be all the more important as evidence of the Cyatheoid affinity.

Woodsia, R. Br.

This genus was founded by Robert Brown in 1815, and now includes twenty-five species, which mostly inhabit cold or temperate zones of the Northern Hemisphere, and are of small size. The axis is short and ascending, the leaves show an open Pecopterid venation, and the shoot bears superficial scales, which are a specially prominent feature in *W. polystichoides*, var. *Veitchii*. Supplies of this Fern were given from the Edinburgh Botanic Garden. The superficial sori have a basal indusium, which is often reduced to mere hairs united at their base to form a Cyatheoid cup. The details of the indusium are, however, variable in different species.

The anatomy of the stock is on the same plan as in the preceding genera. It is traversed by a dictyostele. In *W. polystichoides* the foliar gaps are relatively narrow, and from the lower region of each two leaf-trace strands arise.[1] A distinct involution of the outer surface is found just above the insertion of each leaf, similar to those in *Struthiopteris*, *Onoclea*, and *Cystopteris*.

The leaves are densely covered with broad chaffy scales; these almost hide the sori, which form regular series near the margin. Vertical sections through them show that, as in other Ferns of this relationship, the vein does not terminate in the receptacle, but may pursue a further course towards the leaf-margin. This may be quite clearly seen in sections of *Woodsia obtusa*. It is a point of difference from *Acrophorus*. The receptacle itself is only slightly raised above the leaf-surface, and from its base springs the indusium; this consists of usually four overlapping scales, which may be separate down to their very base. They are similar in structure and origin to the protective scales of the leaf-surface. Upon the receptacle the sporangia are seated. They are not numerous. Such evidence as is available goes to show that the sorus is essentially a gradate one. This conclusion coincides with the observations of Schlumberger ('Flora', 1911, p. 407, Fig. 13) on *W. ilvensis* and *W. obtusa*; for though he makes no mention in his text of the order of succession of the sporangia, his drawings show the centrally lying sporangia as the earliest and the most advanced, while the peripheral are the lower and smaller. This is also indicated by Fig. 42, which represents a drawing of the sorus of *Woodsia ilvensis* in surface view, kindly sent me by Prof. Goebel.

In my Studies, IV, p. 59, the statements as to the condition of the sorus in the genus were rather indefinite. It was stated that 'some species (*W. mollis*, J. Sm.) suggest that the central sporangia mature first, and the peripheral ones later, but this is not borne out by *W. obtusa*, Torrey, from the Edinburgh Garden'. And in a later passage the sorus of the genus was held to be a 'mixed' one. Examination of a large number of sections from *Woodsia obtusa*, of which additional material was obtained from the Edinburgh Botanic Garden, has convinced me that the sorus of that species is a gradate one. No interpolation of sporangia has been seen. But the receptacle is not raised far beyond the general leaf-surface, and the basipetal sequence of sporangia is very short (Fig. 41). It thus appears that my earlier statements with regard to this genus were incorrect, being based on insufficient observation. The sorus is probably a gradate one throughout the genus. The sporangia themselves show no special point of interest, and have the usual structure of stalk, with three rows of cells.

Observations have lately been made by Schlumberger on the prothalli

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[1] See Schlumberger, Flora, 1911, p. 409, and Fig. 14, where the vascular skeletons of *W. ilvensis* and *obtusa* are described. Compare also Luerssen, Rab. Krypt.-Fl., vol. iii, p. 498.

of *Woodsia*, which bring forward some welcome characters for comparison from the gametophyte generation. In particular he draws attention (l. c., p. 385) to the characteristic multicellular hairs on the under side of the cushion in the Cyatheaceae, and shows that similar hairs are present in *Diacalpe*, and also in *Woodsia*. A still more interesting point is that the antheridia both of *Woodsia* and of *Diacalpe* have a divided lid-cell, which is the condition shown by the Cyatheaceae, while the 'Polypodiaceae' have the lid-cell undivided. When these separate and apparently immaterial features of correspondence are put in relation to the characters of the sporophyte, which also indicate a Cyatheoid affinity for these Ferns, the evidence of such a relation appears to be very strong indeed. *Woodsia* may in fact be held to be a boreal and alpine type, derived from, but closely connected with, the Cyatheoid stock. In relation to its less favourable habitat we may note the small size of the plants themselves, and the small number of sporangia in the sorus. The latter fact carries with it the flattened receptacle. But still the basipetal sequence characteristic of the Cyatheaceae is retained.

Hypoderris, R. Br.

Hypoderris is a genus founded by Robert Brown in 1830. Mettenius included it in the genus *Woodsia* ('Fil. Hort. Lips.', 1856, p. 98), from which it is, however, properly separated by its habit, and by the copious anastomosis of the veins. It is represented by three species, of which the best known is *H. Brownii*, J. Sm., a Fern which grows upon damp and shaded rocks in the Antilles. The rhizome is creeping, and bears solitary leaves right and left, while leaves and axis are invested by soft brown scales. The leaf is broadly lanceolate, with two lateral lobes at its base, which are variable in their proportion to the central lobe. The venation is reticulate, and the numerous sori, with basal indusium, are 'inserted in lines or series parallel with the primary veins upon the confluent angles of the reticulate veinlets' (Hooker, 'Sp. Fil.,' vol. i, p. 57). The general habit and appearance resembles that of *Aspidium trifoliatum*, while a comparison may also be drawn as regards habit with another shade-loving Fern, viz. *Kaulfussia aesculifolia*. As in the latter Fern, the sori of *Hypoderris* show frequent and various examples of fission (compare 'Land Flora', Fig. 281).

The vascular system of the rhizome of *Hypoderris* has been examined by Schlumberger ('Flora', 1911, p. 409, and Fig. 14, 5). He found that it is traversed by a dictyostele, with large leaf-gaps and additional 'perforations'. Four vascular strands pass from each leaf-base to the subtending foliar gap, while the upper and under strands between the laterally placed leaf-gaps show wide-meshed perforations. But Schlumberger's account leaves open the relation between the four-stranded leaf-trace of *Hypoderris* and the two-stranded trace of related Ferns. I have examined the vascular system of *Hypoderris* in material kindly supplied to me by Professor Goebel. Tangential sections of the leaf-base make it clear that the two larger strands, which are inserted higher up upon the foliar gap, represent the margins of the trace as seen in *Woodsia* or *Struthiopteris*, for they show the hooked xylems characteristic of that position. The strands inserted lower down upon the foliar gap represent the more median portions of the trace. In fact, each pair of strands right and left is representative of one of the two strands of *Woodsia*, which has become divided, and its two parts have in *Hypoderris* widely diverged. This condition, which is an advanced one, harmonizes on the one hand with the stele of the axis, with its numerous 'perforations'; and on the other it occurs together with the profuse reticulation of the veining of the lamina. In all these respects *Hypoderris* shows evidence of phyletic advance on the related genera, an advance which is also matched by the condition of its sori.

An analysis of the sorus of *Hypoderris* was published by Sir W. Hooker in his 'Genera Filicum' in 1842, Tab. I. The drawings show its characters, with the cup-like basal indusium surrounding a dense mass of sporangia. Individually the sporangia are of small size, and long-stalked. A feature which is clearly brought out by the drawings 3 and 4 of his Plate is that the sporangia of different sizes are intermixed without order, a fact which has since been frequently ignored.

The sorus of *Hypoderris* is seated with a flat receptacle upon a vein of the reticulum which is so marked a feature of the leaf in this genus. The position of the sorus relative to the vein-endings is variable. Commonly it is seated upon an arch of the reticulum. At other times it may be seated upon a twig of the reticulum, which is not terminated at the receptacle. Occasionally, however, the vein appears to end below the receptacle. But the common rule appears to be that the vein does not terminate in the receptacle of the sorus. At first the receptacle is almost even with the general surface of the leaf, and it is never much raised above it. It is shielded by the cup-like indusium before the sporangia appear. These arise sporadically, and without any obvious order, upon the receptacular surface. But as fresh sporangia continue to arise, the sorus becomes crowded. The heads of those sporangia which mature first break away from the stalk just below the capsule, leaving the lower part of their stalks as a protection to the sporangia which follow. A section through an old sorus shows accordingly a crowded condition quite similar to that of certain Mixtae of quite different affinity from *Hypoderris*.

The sporangia themselves are relatively small and long-stalked. Vertical sections through the sorus show that the stalks are not of uniform structure, and when the sorus is cut transversely the sections of the stalks may show three cells, or only two, or one (Fig. 43). A comparison of young developmental stages of sporangia shows that this is connected with the initial segmentation. Three different types of segmentation are illustrated in Fig. 44, *a*, *b*, *c*. In *c* there is a relatively massive stalk, such as might be found in any of the related genera above described. But in *b* the whole sporangium is more attenuated, while at the very base of the stalk it consists of a single cell-row with transverse segmentation. In *a* this condition is still more pronounced, and doubtless the three sporangia of Fig. 44 would correspond roughly in stalk-structure to the three sections shown in Fig. 43. But the condition does not appear to be uniform throughout the length of the stalk of any given sporangium; thus in Fig. 44, *b*, the stalk would consist at its base of a single cell-row, and in its upper parts of two or even three rows. Examination of sporangia dissected out separately from a sorus show that this is actually the case, one or two of the cell-rows stopping short before the base of the stalk is reached. In fact, the condition is closely similar to that shown by Müller's drawings of *Asplenium trichomanes*, quoted by Engler u. Prantl, i, 4, p. 81, Fig. 57.

The sporangium itself is of relatively small size. The annulus is indurated on one side of the sporangium right down to the insertion of the stalk, but there interrupted. On the other side is the stomium, composed of the usual group of four cells. Attached to the stalk of most sporangia is a hair, which often shows a glandular terminal head, similar to what is seen in *Nephrodium*. This fact acquires greater point when it is remembered how like *Hypoderris* is in habit to *Aspidium trifoliatum*.

The interest of the facts thus related for *Hypoderris* lies in the comparison of like conditions in other Ferns. No one has doubted that the natural place of *Hypoderris* is with *Woodsia*, and in less near relation with other Ferns with a basal indusium. But it stands apart from them in its relatively disintegrated vascular system of stock and petiole; its broad leaves with reticulate venation; in its mixed sorus, and reduced type of sporangium. The progression has been parallel along these distinct lines of character, and the changes from type thus shown here in an isolated genus of a well-defined affinity are matched very closely by similar progressions in other types of Ferns believed to be phyletically distinct. The more frequently such parallel progressions are traced the greater is the confidence in believing them to be indications of true phyletic advance. But the question why they should run so strictly parallel remains awaiting its full physiological explanation. As to *Hypoderris* itself, it may be held to be a member of the *Woodsia* affinity, modified as a tropical shade-form. The broad reticulate leaf follows on this habit; but not so clearly the disintegration of the stele, or the mixed character of the sorus. The reduction seen in the sporangium is, however, to be related with the continued production of sporangia in the mixed sorus.

Discussion of the Phyletic Relations of the Ferns described above.

Comparison has now been made between *Lophosoria* and those Ferns with which its relationship has habitually been recognized by earlier writers in respect of various leading characters, external and internal. The result has been in the first place to confirm on every count the recognition of its place at the base of the Cyatheaceous series, while the newly acquired facts have materially strengthened the relation of these Ferns with the Gleicheniaceae. It is not necessarily to be concluded that any present Cyatheaceae were directly derived from any Gleicheniaceous ancestors such as we now see them. What is concluded is that a stock of Ferns with the creeping habit, vascular structure, and soral characters essentially like those of the living Gleicheniaceae gave rise on the one hand to the Gleicheniaceae themselves, and on the other to the Cyatheaceae.

Among the living species of *Gleichenia*, we have concluded that *G. flabellata* represents a central type, with its protostelic creeping axis, its deeply pinnate *Mertensia*-type of leaf, and its superficial, uniseriate sorus, of few, laxly-grouped sporangia, each with a large spore-output. A line of xerophytic specialization resulted in the *Eu-Gleichenia* section of the genus, still with protostelic axis, and with sorus of the same type, but with the ultimate pinnules much smaller, like shells concave downwards, protecting the sori. There is, moreover, a profuse protection of the young stems and leaves by scales, shared in less degree by *G. flabellata*; this is probably a further sign of xerophytic adaptation.

But another line of specialization was without these xerophytic signs, while it retains the more extended pinnule, and shows only hairs and no scales as dermal coverings. It is represented by *G. linearis* and *G. pectinata.* The former is anatomically and sorally the nearer to the central *Mertensia*-type. But *G. pectinata* shows complete solenostely, while it has been pointed out that its sorus had reached the practicable limit of elaboration, so long as the receptacle remains short, and the sporangium retains the distal dehiscence in a median plane. This circumstance probably blocked further progress of an otherwise successful line ; in fact, *G. pectinata* may be held to represent the ultimate realization of its own type. On this view, then, the Gleicheniaceae in their modern developments represent a blind evolutionary branch.

But either an extension of the receptacle or a lateral dehiscence of the sporangium, or better, a combination of both these factors, would make further advance possible.[1] The latter only is realized in *Lophosoria,* but both are combined in the typical Cyatheaceae. All these Ferns have, however, the same position and general type of sorus as the Gleicheniaceae,

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[1] A third possible factor would be an enlargement of area of the receptacle. This was probably realized in *Metaxya,* a Fern to be specially investigated later, together with other phyletic consequences believed to have eventuated along this line of advance.

as well as many other characters of structure and conformation above noted, which clearly point to a relationship. The nature of this relationship has probably been that they sprang from a common stock. But while *Gleichenia* was conservative in its sorus, it specialized in its foliar development, so as to rival even *Lygodium*. On the other hand, the Cyatheaceae adopted an extension of the receptacle, and lateral dehiscence of the sporangium, combining these advantages with reduced spore-output per sporangium. In point of habit they became upright, and many of them definitely dendroid.

It is here that the phyletic interest of *Lophosoria* comes in. Its features mark it out as a synthetic type, both anatomically and sorally. Its solenostelic structure and creeping adventitious rhizomes link on with the similar structure seen in the most advanced Gleichenias. Its undivided leaf-trace comes off like theirs from the stele. But its internodes are short in the upright axis, though the leaf-gaps rarely if ever overlap, so that there is not actual dictyostely. Further, the meristele of its larger leaves may show a simple but temporary division into three parts, which anticipates the greater subdivision of the leaf-trace in *Alsophila* and *Cyathea*. The dermal appendages of *Lophosoria* are hairs only, like the most advanced Gleichenias. Scales are associated with the hairs in the definitely dendroid Cyatheaceae. There is thus a parallel progression in this apparently trivial character also. But it is most clearly in the sorus that *Lophosoria* shows its synthetic character. For while retaining the simple sorus of *Gleichenia* without extension of the receptacle in height or in area, it shows the lateral dehiscence of the sporangia. Thus anatomically and sorally it occupies an intermediate position.

It may accordingly be held that *Lophosoria* is a synthetic type, and represents a phyletically early condition of Cyatheoid development. In relation to it the three leading genera are readily seriated phyletically, *Alsophila* being the nearest to *Lophosoria*, *Hemitelia* taking a middle position, and *Cyathea* representing the most advanced type. This conclusion may be based partly on the sorus, partly on the anatomy, and the following brief statement will show that there is a parallelism in the progression in both of these respects.

The description given by Gwynne-Vaughan of the anatomy of the young plant of *Alsophila excelsa* shows that certain stages of structure believed to indicate a natural phyletic progression are passed through with varying rapidity by the young plant of that species ('Annals of Botany', xvii, pp. 709–11). It is at first protostelic; it then becomes solenostelic; this again merges into the dictyostelic structure by overlapping of the leaf-gaps; and finally the first indication of the internal vascular strands makes its appearance 'at about the tenth leaf'. Comparing this ontogeny with the vascular structure in the series we are contemplating, the first stage finds its correlative in *G. flabellata*; the second in *G. pectinata,* and also in

Lophosoria, with a nearer approach in the latter to dictyostely; the third stage, that of dictyostely, is not reached in this plant, though it is rapidly arrived at by *Alsophila* and both of the other genera of Cyatheaceae. These also show the fourth stage of complexity, viz. the addition of internal vascular strands, which are absent in the solenostelic *Lophosoria*. Gwynne-Vaughan remarks (l. c., p. 712) that 'it should be noted that in this plant (i. e. *A. excelsa*) the internal strands do not appear at all until the ordinary stelar cylinder has become more or less dictyostelic'. The fact that they are absent in *Lophosoria*, but present in the mature axes of *Alsophila*,[1] of *Hemitelia*,[2] and in *Cyathea*, appears to indicate that where the stem is massive and dictyostelic there accessory strands are liable to be present; where the leaf-gaps do not overlap, accessory strands are absent, even though the axis may be of large bulk as it is in *Lophosoria*. It thus appears that the phyletic progression we are contemplating is in direct accord with the progress in complexity of the vascular structure of the plants involved.

A similar phyletic progression, which shows parallel steps to those of anatomy, is to be traced in the sori, which are throughout superficial in position. And this will be specially evident in the details of their protection. In the protostelic *G. flabellata* the sorus is radiate-uniseriate, with 3–5 sporangia, and a spore-output of typically 512–1,024. In *G. linearis*, which is still protostelic, the sporangia are more numerous (10–12) and smaller, the centre of the sorus being usually occupied by one or more; the spore-output is 236 or more. In *G. pectinata*, which is solenostelic, the sorus is crowded with 10–15 sporangia, which are variable in size, and the output of the larger approximates to, but does not reach 512, while that of the smaller approximates to 256. In none of these are hairs a feature of the sorus. The reduction of the spore-output runs roughly, though not with numerical exactness, parallel to the increasing number of the sporangia in the sorus, and to the anatomical advance.

There is a marked break in passing to *Lophosoria*. Its axis is solenostelic as before, but much more massive, while after a short horizontal course the buds become upright and subdendroid. The sorus is constructed on the same plan as that of the more advanced Gleichenias, and all the sporangia are simultaneous in their appearance. But the sporangia have lateral dehiscence, and their spore-output is only 64, while numerous simple hairs are associated with the sporangia. *Alsophila* is the Cyatheaceous genus which is most nearly allied to *Lophosoria*. Its sorus has no protection, excepting numerous hairs which resemble those of *Lophosoria*. It has, however, a more or less pronounced basipetal succession of its more numerous sporangia, which are individually smaller, have lateral dehiscence, and

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[1] This has been observed in *Alsophila crinita* in addition to cases already recorded.

[2] This has been observed in a large specimen of *H.* (*Amphicosmia*) *Walkerae* from Ceylon.

a spore-output of approximately 64. *Alsophila* has a definitely dendroid habit. The protostelic and solenostelic stages are quickly passed over in the young axis, and a dictyostelic structure is adopted, with internal accessory bundles. Superficially it bears broad scales as well as hairs, and the former at the leaf-bases are, as in *G. pectinata*, borne on peg-like emergences. These characters collectively indicate for *Alsophila* a state of advance upon what is seen in *Lophosoria*.

Hemitelia shares in the main the anatomical and soral characters of *Alsophila*, but its distinguishing feature is the 'indusium'. Christ ('Farnkräuter', p. 329) describes the genus appositely as a small one, 'which as regards the indusium is midway between *Cyathea* and *Alsophila*, since the sorus is in fact naked above, but is provided at the base with a scale-like rudimentary indusium'. This indusium is described as variable in size by various writers. In form and structure it is very similar to the scales found on the surface of stem and leaf, and the probable view which follows from this fact is that it simply represents one of these scales turned to a special protective use.

In *Cyathea* the most specialized type of the series is seen, with lofty dendroid habit, complex structure of the axis, profuse covering of the surface of axis and leaf with chaffy scales. The sori are gradate, and are protected and sometimes completely covered in by the basal, cup-like indusium. This is clearly comparable with that of *Hemitelia*, but it has lost its scale-like character, and is usually developed equally all round the sorus. The sporangia are of the same type as in the other genera, but in *C. dealbata* they are of specially small size, and show a spore-output reduced to the unusually low figures of 16, or even 8. These characters collectively indicate that *Cyathea* is the most specialized genus of the whole series.

When the parallelism of the external, the anatomical, and the soral facts for the sequence of Ferns thus laid out is fully taken into account, it seems impossible to doubt that in essentials it illustrates a true phyletic progression which has taken place. It is not asserted that any modern *Gleichenia* was the progenitor of *Lophosoria* or of any other Cyatheoid Fern. Probably the Gleichenias as we know them are a blind branch. What is concluded is that from an essentially Gleichenioid type long ago the Cyatheoid type of Ferns originated, and an essential link in the progression is supplied by *Lophosoria*. The progression involves a gradual change of habit from a creeping type with isolated leaves borne by an elongated rhizome, to an erect type which includes the largest of the Tree-Ferns. It necessarily follows that here at least the upright axis is a secondary result of evolution. A similar comparison applied among the members of the quite distinct sequence of the Dicksonieae leads to a like result; this will be taken up in detail on a subsequent occasion. From both sources the result is arrived at that the dendroid character is secondary for the Gradate Ferns.[1]

It also appears from the sequence in question that a natural progression is illustrated from the protostelic to the solenostelic, and finally to the dictyostelic type of vascular arrangement. Lastly, there is the further step to a dictyostelic state with accessory strands in pith and cortex. There is probably no series of nearly related Ferns which shows such progressions as these so clearly.

It is also indicated that while the hair is present in the lower members of the sequence, broad scales become a later feature, and that though these at their first appearance in *Alsophila* do not form an 'indusium', in the more advanced types they become specialized for that duty.

Finally, the sequence illustrates the progression from the simple to the gradate sorus, and from sporangia with large spore-output and median dehiscence to those with greatly restricted output and lateral dehiscence. It is the consistent parallelism of all these progressions, in characters physiologically quite distinct from one another, which gives them their special cogency as evidence, and forbids any of the progressions being read in converse.

Unfortunately the evidence from the fossils, which would be so valuable an adjunct to comparison, and especially in deciding whether or not the sequences contemplated were really progressive, is not of a distinctive character. As to the Cyatheaceae, Seward states ('Fossil Plants', vol. ii, p. 366) that 'it is not until we reach the Jurassic Period that trustworthy data are obtained'. The evidence as to the existence of the Gleicheniaceae, such as we at present know them, earlier than the Jurassic Period, where they are certainly represented, is not convincing. It turns upon the interpretation of specimens of *Oligocarpia*, which bear sori with a small number of sporangia, and have been referred by some authors to the Gleicheniaceae. But opinion as to their Gleicheniaceous character is divided. Zeiller and Scott (see Seward, l. c., p. 352) uphold the presence of an annulus such as would be indicative of a Gleicheniaceous affinity. But this is not admitted by Solms-Laubach, Stur, or Schenk. It cannot therefore be used as direct evidence here. Nevertheless the comparative considerations which have been advanced would make the occurrence of ancestral representatives of the family earlier than the Jurassic Period seem probable. And the character of the sorus of *Oligocarpia* suggests at least some such ancestral form, whether or not the sporangia have the exact type of annulus now recognized as characteristic of the Gleicheniaceae. There is no doubt that they had the median dehiscence; this is clear from specimens shown me by Dr. Kidston. There is, however, reason to think that the annulus in these early types may not have consisted of a single row of

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[1] Compare footnote on p. 293, above; also Ann. of Bot., xxv, pp. 567–8.

cells, as is the case in the modern Gleicheniaceae, but of several rows. This has already been suggested by Dr. Kidston (‘Les Végétaux houillers’, Bruxelles, 1909, p. 35).

As regards the remaining genera with superficial sori and basal indusium, the details described above have only served to strengthen their relation to the Cyatheaceae, a relationship which has never been in doubt except in the case of *Acrophorus* and *Cystopteris*. The position of the former must remain uncertain until it has been properly re-examined. But of the Cyatheoid affinity of *Cystopteris* there can now be little doubt. Its sorus is superficial in origin, as it is in all of these Ferns. The demonstration of its basipetal sequence of sporangia upon the receptacle, which has itself become lop-sided in accordance with the lateral attachment of the indusium, combined with the superficial position, is distinctive; while the anatomy of the stock and petiole, so like that of *Struthiopteris*, gives independent and cogent support to the relationship suggested.

The importance of this conclusion lies, however, in the consequences which follow from it. The comparison of *Cystopteris* with the Aspidieae dates back to the time of Swartz and of Presl. The similarity of habit is unmistakable; in point of vascular structure *Struthiopteris* and *Cystopteris* compare very closely with such a type as *Nephrodium Oreopteris*. The sori of these Ferns are alike in position, and in their indusium, to those of the Aspidieae. Both have also a vascular receptacle. The similarity of the young sorus of *Nephrodium Filix-mas* when cut in median section to that of *Cystopteris* appears clearly from a comparison of Figs. 45, 46 of the former Fern with Fig. 38, *b*, of the latter. But while we thus compare its sorus with that of *Nephrodium*, it is to be borne in mind that the one is gradate in the sequence of its sporangia, the other mixed. The conclusion which naturally follows on such considerations is that there has been a further phyletic progression—the recognition of which is facilitated by the living genera *Struthiopteris* and *Cystopteris*—from Cyatheoid derivatives to certain Aspidieae, by the assumption of a mixed character of the sorus. Such a progression has already been recognized within this very sequence, in *Hypoderris*, while *Peranema* and *Diacalpe*, which are shown in Mr. Davie's paper (p. 245) to have mixed sori and a vascular system closely resembling *Nephrodium*, have in the form of the sorus and in the basal indusium clear Cyatheoid characters. As Mr. Davie justly points out, between them *Peranema* and *Diacalpe* seem to unite all the intermediate features between the Cyatheaceae and the Aspidieae. A similar transition from gradate to a mixed type of sorus is believed to have occurred in several other evolutionary lines. This change appears in the present case to have gone along with a flattening of the receptacle between the indusium and the leaf-surface. A further step was the lateral extension of the sporangium-bearing surface right and left, in the direction of the midrib of the pinnule, so that it took a horseshoe form, the indusium following suit. And so the characteristic Nephrodioid sorus may be held to represent a flattened modification of the Cyatheoid type. A further step is seen in *Polystichum*, by the junction of the two lateral arms of the receptacle on the side next the midrib of the pinnule to form a complete ring. The indusium again following suit, it now appears in the well-known shuttlecock form. The last step is in the abortion of the indusium in certain derivative forms, giving again a 'Polypodioid' sorus of simple circular outline, almost like that of *Alsophila*. It would be out of place here to attempt to trace the various evolutionary lines within the Aspidieae.[1] It must suffice to have recognized the probability that they constitute a great group of Filicales, phyletically related with the Cyatheaceae, and ultimately with the Gleicheniaceae.

The biological advantages of the Nephrodioid type of sorus over the Cyatheoid are obvious. First there is the general advantage which a mixed sorus has over a gradate one, that while it spreads the physiological drain over a long period, it makes the best possible use of a limited length of receptacle. But a second advantage not hitherto recognized is the greater convenience in packing during the circinate condition of the young leaf. In the gradate sorus pressure of the next outer coil of the spiral on the apex of the sorus would tend to crush the first formed sporangia, and evidence of this is sometimes to be seen. This is avoided by the flattened form assumed by the sorus, as seen in *Cystopteris* or *Nephrodium*. But a sacrifice of sporangial space is made in *Cystopteris*. This is amply made up for by the ring-like extension of the receptacle in *Nephrodium* and *Polystichum*, as well as by the continued production of sporangia in their mixed sorus. Thus it appears that the modifications, which are believed to have been phyletic advances, show that physiological advantage is gained by them. This increases the probability that they are rightly so recognized.

The relation of *Hypoderris* to *Woodsia* has always been admitted, and the facts here given tend to strengthen the affinity. But *Woodsia* has a gradate sorus, though the sequence is not long continued, while *Hypoderris*, which is more advanced in vascular structure, in venation, and in foliar character, has a mixed sorus. There can be little doubt that a like soral progression from the gradate to the mixed state has occurred here to that in *Peranema* and *Diacalpe*, or in *Nephrodium*. There is, however, no reason to think that any of these genera had any place in the descent of *Hypoderris*. The conclusion must then be that *Hypoderris* illustrates another and a distinct progression from the gradate to the mixed sorus. It is, in fact, becoming plain that this transition has happened not once only, or twice, but by many parallel progressions.

There does not appear to be any sufficient reason for holding that any

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[1] Compare C. Christensen, Biologiske Arbejder, Nov. 1911.

two of the pairs of genera discussed above, viz. *Struthiopteris* and *Onoclea*—*Peranema* and *Diacalpe*—*Woodsia* and *Hypoderris*—*Cystopteris* and *Acrophorus*—were related in the sense of being successive steps in a simple phyletic line. That is to say, no one of these groups can be held as the actual ancestors of another. It has been remarked above that none of these Ferns is really primitive. All show indications of a middle position in the general scale of the Filicales. The source from which they spread has without doubt been related to that of the Cyatheaceae, as shown by habit, by anatomy, by sorus, and in some cases by the details of the gametophyte. But in view of the anatomical and soral characters combined, it is difficult to relate any of them to any definite genus of the Cyatheaceae as we see them now. These Ferns with basal sori appear collectively to form a brush of separate minor lines of descent, related one to another by common origin, but separately specialized according to their habitat. The Cyatheaceae are essentially the intertropical forest representatives, showing increasing specialization in soral protection, in accordance with their dendroid habit. *Struthiopteris* and *Onoclea* are forms with still more special protection of the sori by incurving leaf-margins, in relation to their northern and mountain habitat. Similar circumstances have probably influenced *Peranema* and *Diacalpe*, and led to specialization of their sori along a distinct line of their own. *Woodsia* is a boreal dwarf type, and *Hypoderris* a type of tropical shade. *Cystopteris* is a world-wide type, but with its head-quarters in the temperate zones of both hemispheres. It is specially marked by its hardihood and resistance to alpine extremes. Most of the smaller genera are deciduous according to season, a character clearly indicating their specialization for life under marked seasonal change. This is in contrast to their larger and more successful correlatives, the intertropical Cyatheaceae, which maintain their constant tuft of leaves.

The Cyatheaceae are clearly the most successful, as measured by size, as well as by number of species. They were probably also the earliest type. The smaller genera show by their paucity of species, and in some cases by their limited spread, that they have met their external circumstances with only partial success. But indirectly in relation to two of them great phyletic developments have probably arisen. In near connexion with *Cystopteris* (and probably also in some relation with *Peranema* and *Diacalpe*), the great sequence of the Aspidieae has come into existence. Similarly, in relation to *Struthiopteris* has arisen the successful sequence of *Lomaria*, *Woodwardia*, and *Doodya*. Here it must suffice merely to suggest these lines of further progress, and the full discussion of them must be left over for a future occasion. The object here is to indicate that the Cyatheoid type, with gradate sorus and basal indusium, was not a blind or final evolutionary line, though very likely the genera of Cyatheaceae, as we see them to-day, were the ultimate exponents of it. Modifications, and especially the introduction of the mixed sorus, seem to have given a new phyletic impetus to some of the derivative forms, which has eventuated in the great Aspidioid sequence.

Returning lastly to the criteria above cited, we find the following progressions illustrated in our series:

(i) The dichotomous branching is frequent in the Gleicheniaceae, but becomes rarer in the higher types. The creeping axis of the earlier becomes in certain later types ascending or erect.

(ii) The peculiarities of the original Gleicheniaceous type of leaf are shown in reminiscent details in the Cyatheaceae, but lost elsewhere.

(iii) There is a progression from primitive hairs to scales, the former being characteristic of the lower, while all the higher terms of the series show the latter.

(iv) The vascular system, starting as a protostele in the *Mertensia* section of *Gleichenia*, becomes solenostelic in *G. pectinata* and *Lophosoria*, but is dictyostelic in all the rest. Special accessory strands occur in the Cyatheaceae. The foliar trace is correspondingly broken up into separate strands in the higher terms of the series.

(v) The sorus is superficial in all these Ferns, and is not distal on its supporting vein (the case of *Acrophorus* being left in doubt). It shows progression from a simple type in *Gleichenia* and *Lophosoria* to the gradate in Cyatheaceae, *Struthiopteris*, *Onoclea*, *Woodsia*, and *Cystopteris*. Finally, it becomes mixed in *Hypoderris*, *Peranema*, and *Diacalpe*, a condition leading probably to that of the Aspidieae.

(vi) The sporangia are large in the Gleicheniaceae, few in number, with large spore-output and median dehiscence. The size and spore-output fall in *G. pectinata*. In *Lophosoria* it is already of the number 64, and the dehiscence is lateral. In the Cyatheaceae the spore-number may fall to even lower figures, with oblique annulus and lateral dehiscence. But finally the annulus becomes almost vertical, and interrupted at the stalk in all the highest terms of the series.

(vii) The antheridium shows reduction in number of the spermatozoids which is roughly parallel with the reduction in the spore-number in the higher terms of the series. This goes with structural simplification, especially seen in the final absence of division in the cap-cell in the most advanced types.

It is believed that the Ferns here treated constitute a true phylum, though they probably include a brush of minor phyletic lines. This view is based primarily upon the constancy in the superficial position of the rounded sorus, borne not distally upon a vein, and the basal insertion of the indusium where present; also upon the characters of the sporangia themselves. A substantial parallelism of progression has been traced within the phylum in respect of the characters of external form, of dermal appendages, of anatomy, of soral construction, and of detail of the sporangia, together with certain gametophytic features. The constancy of the parallelism in features functionally so distinct is held to give just ground for drawing the phyletic conclusions sketched above. Further, it has been shown that they are in accord with physiological probability, and that the fossil record, so far as the known facts bear upon the question, supports the phyletic story thus reconstructed.

Accordingly it is held that in this phylum, which is believed to be quite distinct from that of the Ferns with originally marginal sori, a progression has been traced which extends from primitive forms showing the simple sorus, the protostelic axis, and undivided leaf-trace, to those with a mixed sorus, a dictyostelic axis, and highly divided leaf-trace; while in a middle position lie examples with a gradate sorus, and showing various conditions of solenostely in the axis, and of subdivision of the extended meristele of the petiole. Starting with the ancient Gleicheniaceous type, we have arrived at the Aspidieae as relatively modern representatives of the phylum, while the Cyatheaceae and their smaller correlatives take a middle position. No attempt has, however, been made here to trace critically the ultimate phyletic derivatives of the Aspidieae. This must be left over for further investigation.

The relationships thus recognized have been more or less clearly indicated by various systematic writers. What has been attempted here has been to widen the scientific basis upon which such conclusions ought to be founded. There is accordingly no striking change in nomenclature, beyond the upholding of *Lophosoria* as a substantive genus, quite distinct from *Alsophila*, but related to it. The conclusions arrived at should, however, have the effect of recommending a grouping of the families involved in their probable phyletic sequence, thus: Gleicheniaceae—Cyatheaceae (with minor groups, e. g. Woodsieae, &c.)—Aspidieae.

DESCRIPTION OF FIGURES IN PLATES XXX–XXXVI.

Illustrating Professor Bower's paper on *Lophosoria* and its relation to other Ferns.

PLATE XXX.

Fig. A. Hairs of peculiar form, from the base of the leaf of *Gleichenia pectinata.* Each hair is seated on a massive emergence, from which it is easily detached, the emergence remaining as a hard peg-like projection. × 50.

Fig. B. Part of petiole of *Hemitelia horrida,* bearing prickles, or peg-like emergences, and upon the tip of each is balanced a flattened scale, corresponding to the branched hairs in *G. pectinata.* Slightly enlarged.

Fig. C. Pinnule of *Gleichenia linearis*, showing venation and mature sori. Some of the sporangia are shown to have dehisced. Enlarged.

Fig. D. A single sorus of *Gleichenia linearis* on a larger scale, showing the disposition of the sporangia, several of which have opened by a median slit. Enlarged.

Fig. E. Pinnule of *Gleichenia pectinata*, showing the venation and mature sori. Some of the sporangia are shown to have dehisced. Enlarged.

PLATE XXXI.

Fig. F. A single sorus of *Gleichenia pectinata*, showing the sporangia so closely packed as to be flattened against one another. One has already dehisced. Note the inverted position of one of the outer sporangia on the right-hand side. Enlarged.

Fig. G. Upright stock of *Lophosoria*, after removal of the hairy covering. The erect appendages are all foliar, of which those inserted lower down are abortive, while those higher up are developed. From the base of several of them, but not all, arise the horizontal, solenostelic runners. Note the emergences on the bases of the fully developed leaves. From drawing by Mr. Thompson. Half natural size.

Fig. H. Pinnule of *Lophosoria pruinata*, showing venation and sori. Enlarged.

Fig. I. A single sorus of *Lophosoria*, showing the small number of sporangia, with their regular orientation. Each sporangium has its annulus apparently complete, the stomium being lateral, and therefore out of sight. Enlarged.

PLATE XXXII.

Fig. K. Dissection of a large stock of *Lophosoria pruinata*, showing the vascular solenostele exposed, by the removal of all superficial tissues. It is clear that the foliar gaps do not overlap, and transverse sections anywhere in the internode will show the complete solenostele. From drawing by Mr. Thompson. Natural size.

Fig. L. Dissection of a smaller shoot of *Lophosoria pruinata*, showing the vascular system exposed. The internodes are shorter than in Fig. K. Note the vascular supply to the runners arising horizontally from the bases of many of the leaf-traces. From a drawing by Mr. Thompson. Natural size.

Fig. M. Double-headed specimen of *Cyathea dealbata* in the Glasgow Botanic Garden. It is probably a dichotomy.

PLATE XXXIII.

Fig. N. Plant of *Hemitelia setosa* in Edinburgh Botanic Garden, showing numerous 'aphlebioid' pinnae. From a photograph prepared by the direction of Professor Balfour.

Fig. O. Plant of *Alsophila* sp. in Glasgow Botanic Garden, showing basal pinnae, comparable with the 'aphlebiae' of *Hemitelia*.

PLATE XXXIV.

Fig. 1. Very young sorus of *Gleichenia pectinata*, seen in vertical section, before the sporangia have been initiated. × 400.

Fig. 2. A similar sorus, rather older. × 400.

Fig. 3. A similar section of an older sorus, on which the sporangia have already been initiated. × 400.

Fig. 4. A similar section, showing some difference of age of the sporangia borne upon the same sorus. × 400.

Fig. 5. A young sporangium of *Gleichenia pectinata*, showing a typical segmentation of the relatively massive stalk. × 400.

Fig. 6. Section cut through a sporangium so as to traverse the sporogenous tissue in a plane transverse to the axis of the sporangium. The number of the spore-mother-cells is fifteen. × 400.

Fig. 7. A similar section cut in the median plane of the sporangium, and traversing twenty spore-mother-cells. × 400.

Fig. 8. A similar section, showing the spore-mother-cells separated and rounded off. × 400.

Fig. 9. A large stem of *Lophosoria* cut transversely, and represented natural size. A leaf-trace, subdivided into three strands, has recently been given off, leaving an open foliar gap, while preparation is being made for another opposite the mark ×.

Fig. 10. Transverse section of a runner of *Lophosoria*, showing a leaf-trace being given off from the solenostele. Note that the separation of the two margins is not simultaneous. Slightly enlarged.

Fig. 11. (i–v.) A series of transverse sections through a runner of *Lophosoria*. (i) is the lowest, and the series illustrates the successive stages of separation of a leaf-trace from the solenostele. In (v) the ring is again completely closed. Note that the leaf-traces in (i) and (iv) have their margins united to form a ring: this has only been observed in the traces of arrested leaves. Slightly enlarged.

Fig. 12. (i–iii.) Successive sections from below upwards transversely through an axis of *Lophosoria*, showing the relation of the vascular supply of a runner to that of the axis and leaf. *b* indicates the runner itself. × 4.

Fig. 13. (i–iii.) Tangential sections through the base of insertion of a runner upon the leaf-base to which it is related. (i) shows the runner itself, with its solenostele; (ii) shows the solenostele now connected with the leaf-trace, which being cut tangentially appears as a broad sheet of vascular tissue. Slightly enlarged.

Fig. 13 *bis*. *a*, *b*. Transverse sections of the petiole of *Lophosoria*. *a* is from a point near the base, and shows the trace divided into three strands, while opposite the gap between them there is on either side an interruption of the superficial sclerenchyma for ventilation purposes. *b* was taken at a point higher up, and the trace is here a single continuous strand. × 4.

PLATE XXXV.

Fig. 14. (i–vii.) Series of transverse sections of the rachis of *Lophosoria*, successively from below upwards, showing the origin of the pinna-trace. × 4.

Fig. 15. (i–vii.) Series of transverse sections of the rachis of *Gleichenia linearis*, for comparison with Fig. 14, showing the origin of the pinna-trace. × 4.

Fig. 16. Hairs from the sorus of *Lophosoria*, which are unbranched, and not obviously glandular. × 100.

Fig. 17. Transverse section of the stalk of a sporangium, showing its relatively massive character. × 200.

Fig. 18. Sporangium of *Lophosoria* presenting its 'peripheral' face, and showing that the annulus is uninterrupted and the dehiscence lateral, while the area surrounded by the annulus is very large. × 150.

Fig. 19. A similar sporangium presenting its 'central' face. × 150.

Fig. 20. A similar sporangium seen in lateral view, presenting the side which bears the stomium. The lower face, as seen in the drawing, would be opposite the surface of the leaf. × 150.

Fig. 21. Section vertically through a very young sorus of *Lophosoria*, taken so as to include the margin of the pinnule. × 400.

Fig. 22. Section of a similar sorus in a direction at right angles to that of Fig. 21. × 400.

Fig. 23. Transverse section of a similar sorus. The cells marked × will produce sporangia. × 400.

Fig. 24. Vertical section of a rather older sorus, in which the sporangia and hairs are becoming apparent. × 400.

Figs. 25–7. Longitudinal sections of young sporangia of *Lophosoria*, showing details of their segmentation, and the hairs which accompany them. × 400.

Fig. 28. Vertical section through a young sorus of *Lophosoria*, so as to follow the course of the vascular strand which supplies the receptacle. Two sporangia are traversed, and many of the simple hairs. × 200.

Fig. 29. A similar sorus cut transversely to the course of the vascular strand, showing three sporangia and the hairs as before. × 200.

Fig. 30. Vertical section through a sporangium of medium age. × 200.

Fig. 31. Transverse section through a sporangium of like age, showing the usual number of spore-mother-cells. × 200.

Fig. 32. A similar section, showing the unusual number of nine. × 200.

Fig. 33. Vertical section through an almost mature sorus of *Lophosoria*. The vascular supply into the receptacle is shown, and four sporangia, together with numerous hairs. The spores are still in tetrads. Note that the orientation of the outer two sporangia, as shown by the position of the

large cells of the annulus, is according to the type for *Gleichenia*, but that the inner sporangia do not maintain that regularity. × 100.

Fig. 34. The very characteristic spores of *Lophosoria*, and their germination. × 250.

PLATE XXXVI.

Fig. 35. A young prothallus of *Lophosoria*, which has just begun to form antheridia. × 66.

Fig. 36. The venation of the fertile pinna of *Struthiopteris orientalis*; the dots on the veins indicate the receptacles. Enlarged.

Fig. 37. Vertical section of a sorus of *Struthiopteris orientalis*, from a plant grown in the Glasgow Botanic Garden. It shows the indusium covering the basipetal sorus, and itself overlapped by the leaf-margin. Note that the vascular strand extends beyond the insertion of the receptacle. × 160.

Fig. 37 *bis*. Another example, showing clearly the basipetal sequence of the sporangia. × 160.

Fig. 38. *a*, *b*. Vertical sections of the sorus of *Cystopteris fragilis*. *a* cuts the leaf-lobe transversely; *b* cuts it longitudinally, so as to include the leaf-margin. The first sporangium appears in a median position, terminal on the convex receptacle. × 250.

Fig. 39. Section of an older stage cut as in Fig. 38 *a*, or as indicated by the line crossing Fig. 40 vertically. The median sporangia, which are the oldest, have been cut though their stalks only; the lateral ones, being shorter, have been cut through their heads. The indusium forms an efficient covering. × 250.

Fig. 40. Section of a sorus of *Cystopteris fragilis* corresponding to that of Fig. 38, but older. It shows clearly the basipetal succession of the sporangia, but only on one side of the receptacle. × 250.

Fig. 41. Vertical section through a sorus of *Woodsia obtusa*, showing the vascular supply into the receptacle, the basal indusium, and the basipetal succession of the sporangia. × 160.

Fig. 42. Very young sorus of *Woodsia hyperborea*, seen in surface view. *Sp.* indicates the sporangia. *i.i.* indicate the beginnings of the indusium. From a drawing kindly lent by Professor von Goebel.

Fig. 43. *a*, *b*, *c*. Transverse sections of the stalks of sporangia of *Hypoderris*, showing the differences which exist in complexity of the stalk, which shows sometimes three, sometimes two, or one cell in section. × 250.

Fig. 44. *a*, *b*, *c*. Three types of sporangia, showing different segmentations, which tally with what is seen in Fig. 43. × 250.

Figs. 45 and 46. Vertical sections of young sori of *Nephrodium Filix-mas*, cut in a direction corresponding to that of the figure (38, *b*) of *Cystopteris fragilis*. The similarity of structure is obvious. × 250.

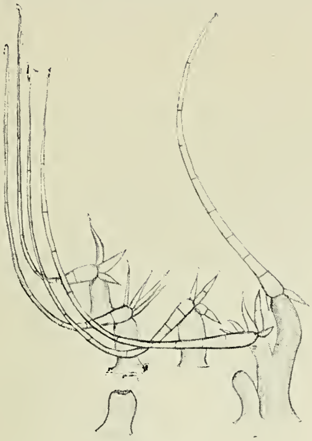


FIG. A

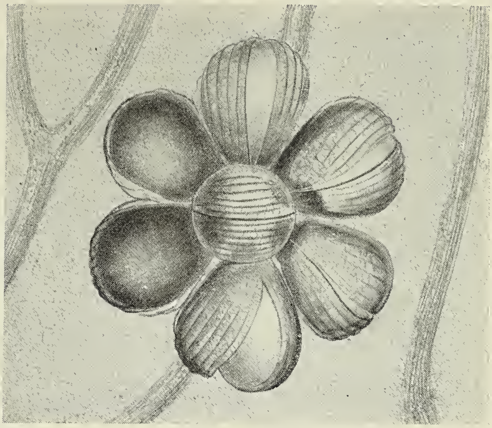


FIG. D

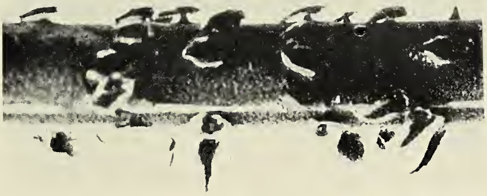


FIG. B

FIG. C

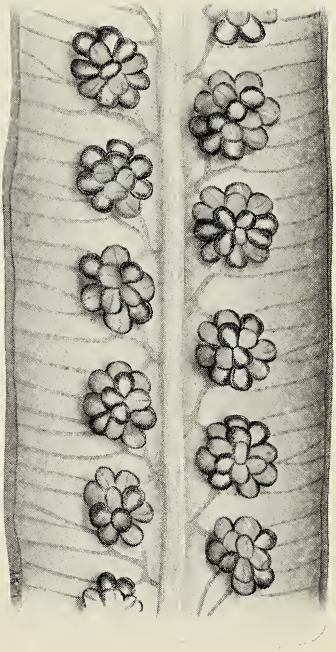


FIG. E

BOWER—LOPHOSORIA

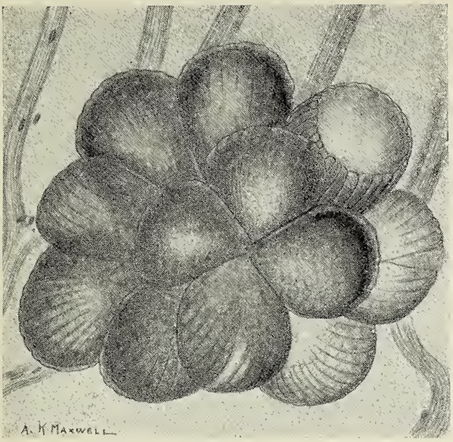


FIG. F

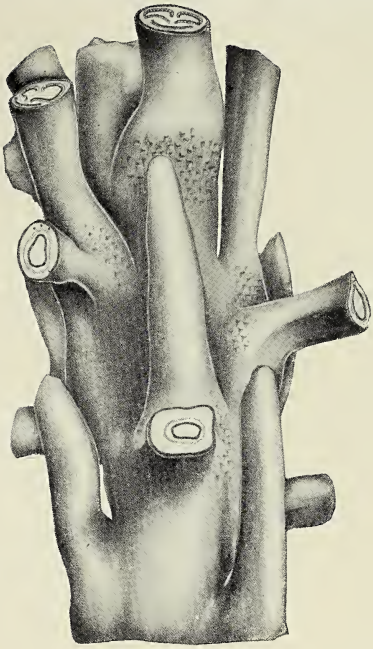


FIG. G

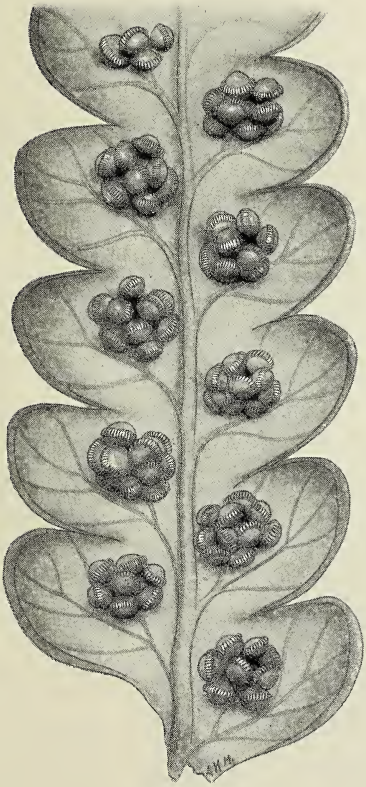


FIG. H

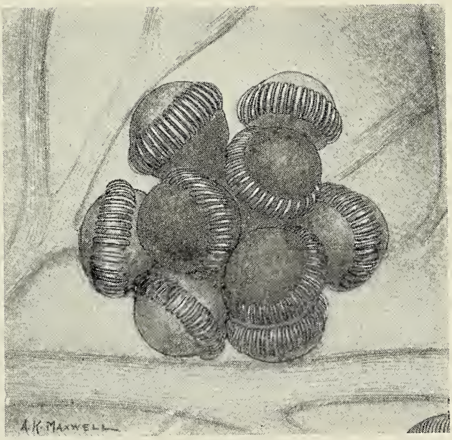


FIG. I

BOWER—LOPHOSORIA

Fig. K

Fig. L

Fig. M

BOWER—LOPHOSORIA

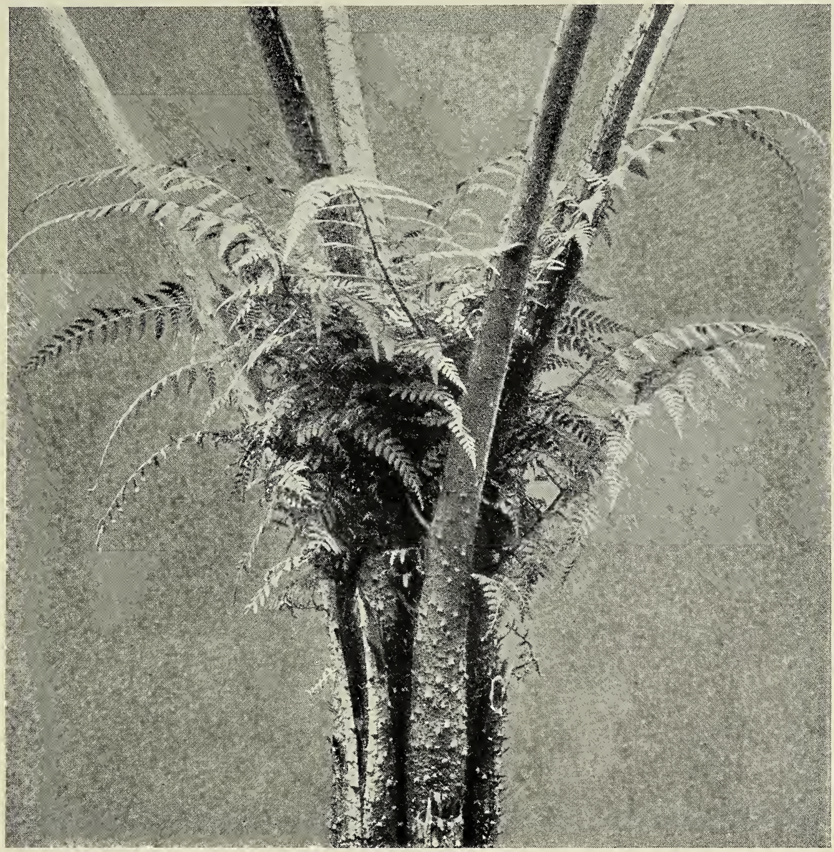


Fig. N

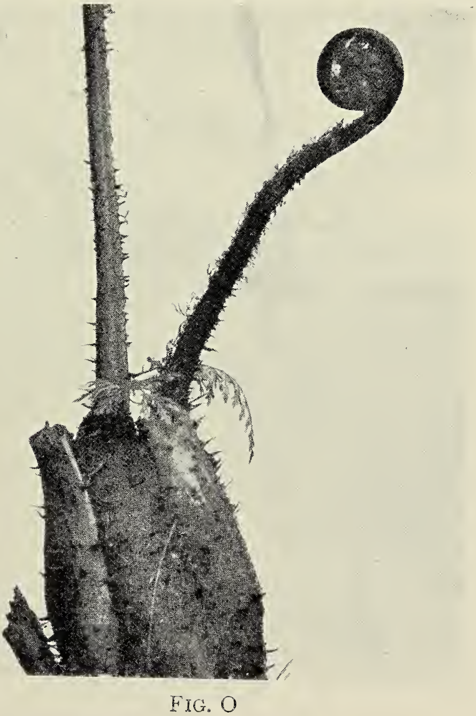
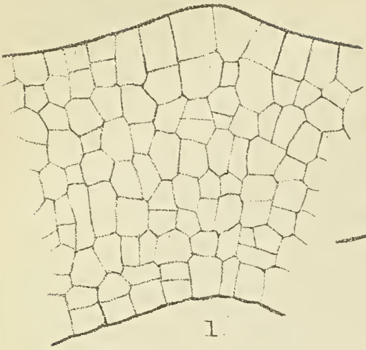


Fig. O

BOWER—LOPHOSORIA

Annals of Botany.



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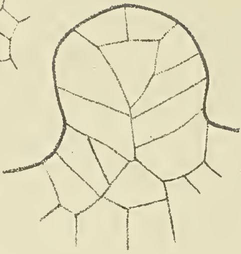

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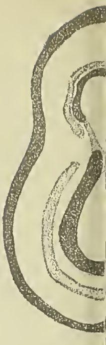

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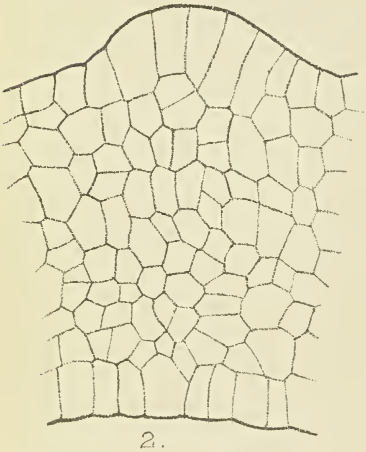

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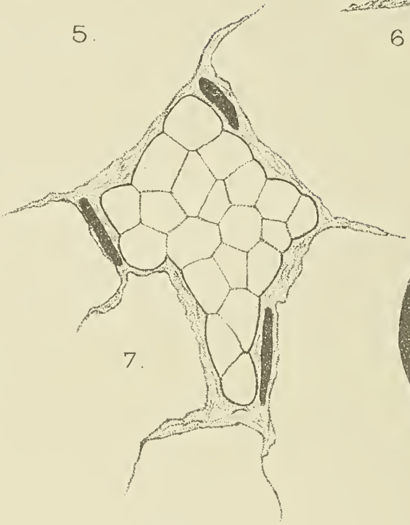

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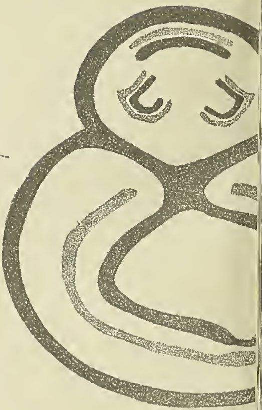

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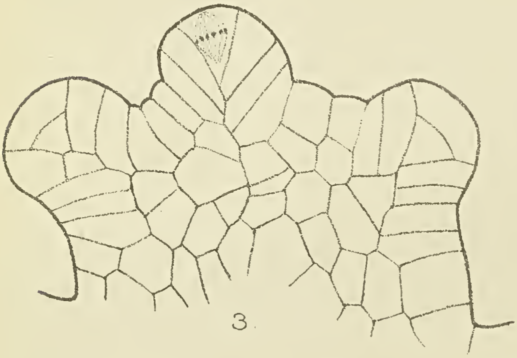

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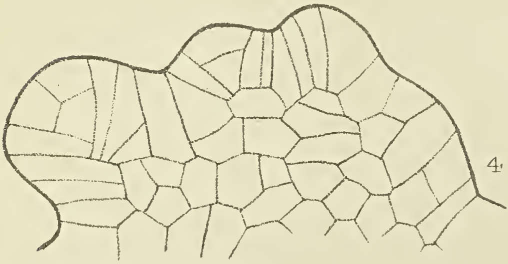

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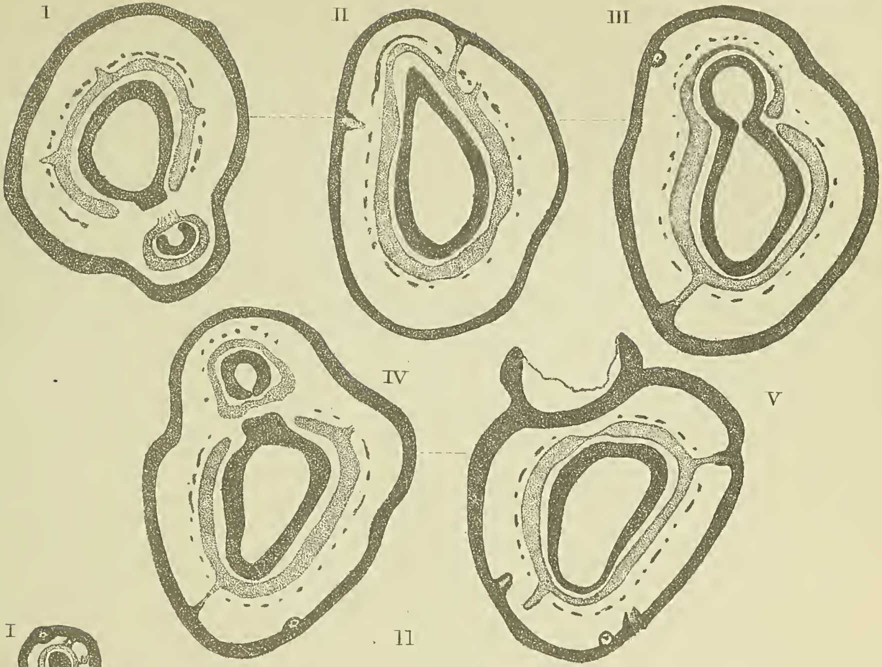
BOWER — LOPHOSORIA AND OTHER FERNS.



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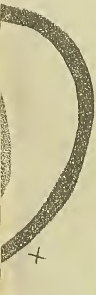

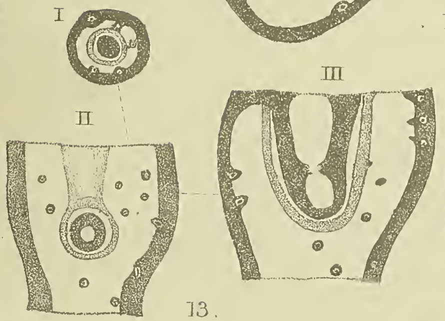

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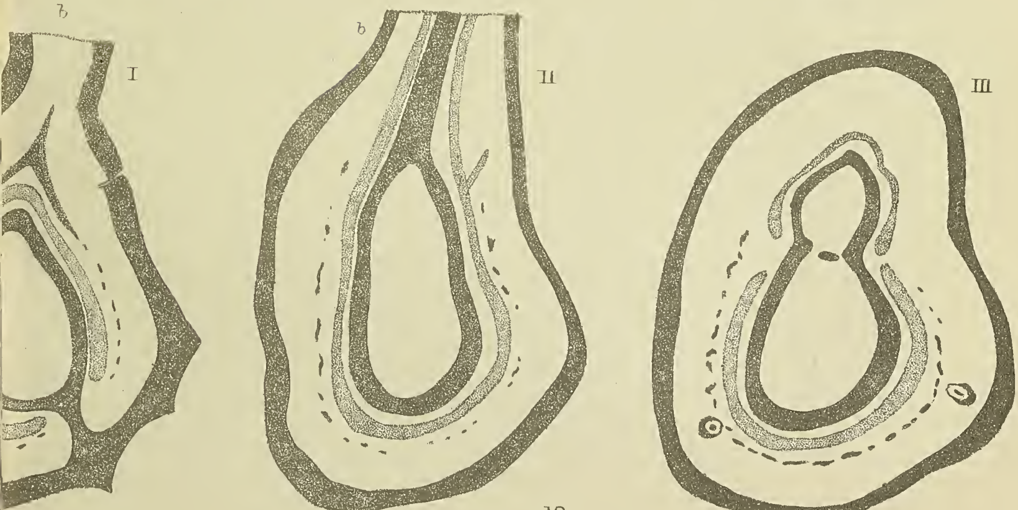
13. *bis*



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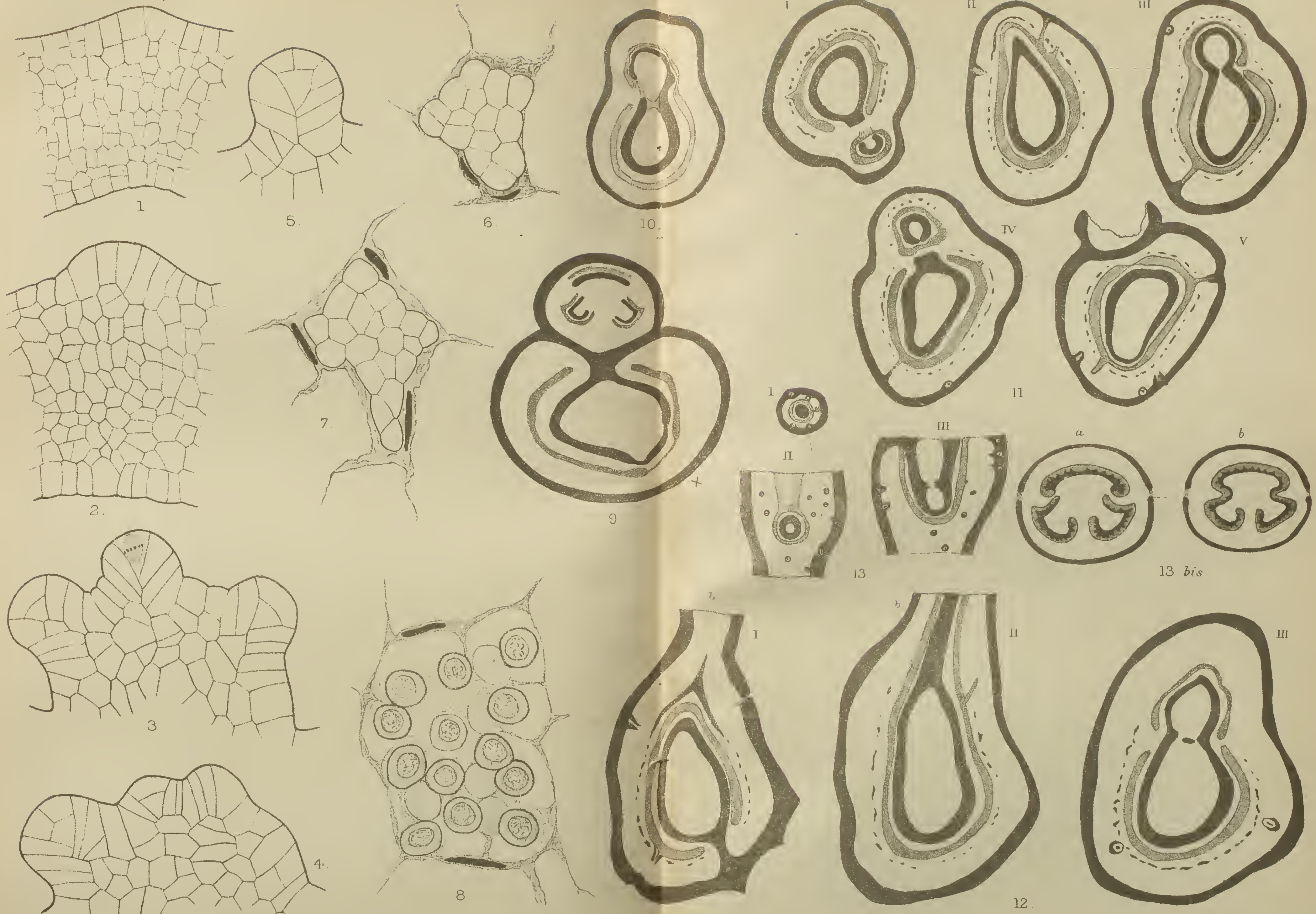

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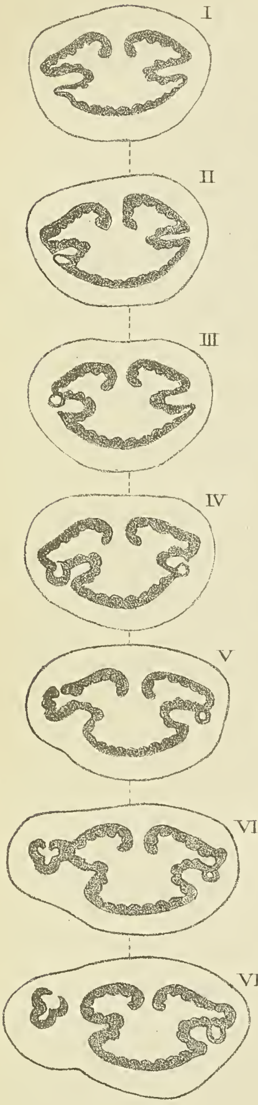
BOWER — LOPHOSORIA AND OTHER FERNS.



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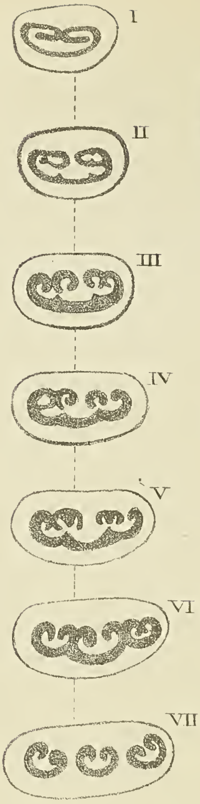

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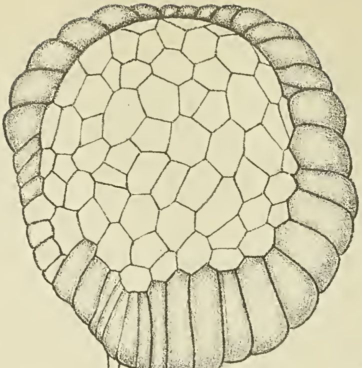

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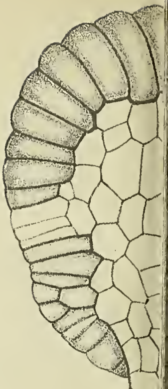

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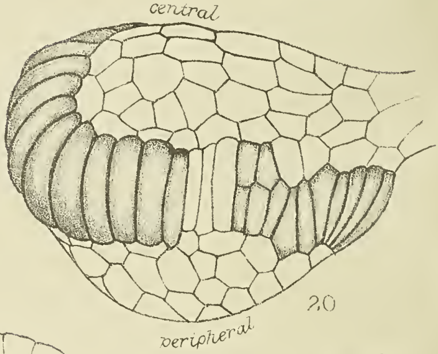

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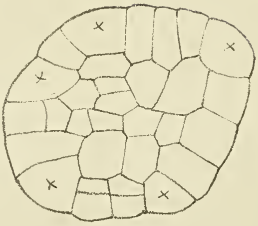

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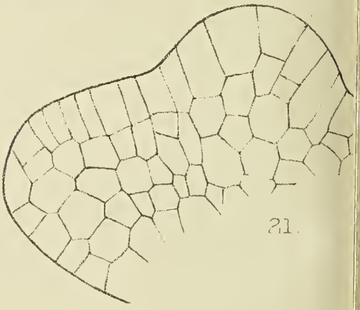

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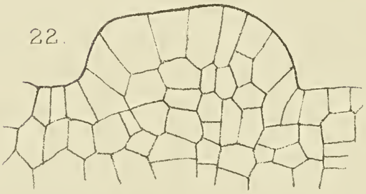

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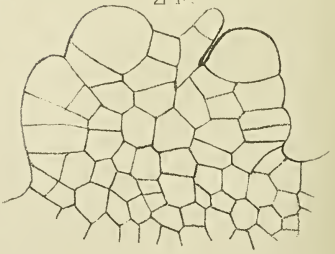

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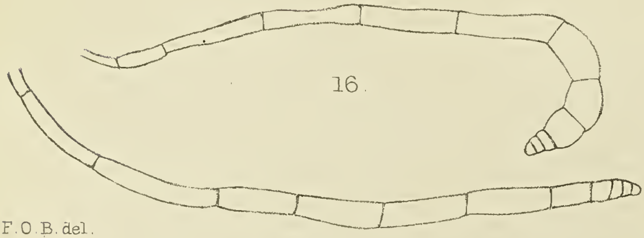

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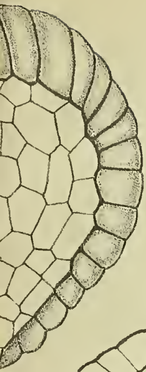
BOWER—LOPHOSORIA AND OTHER FERNS.



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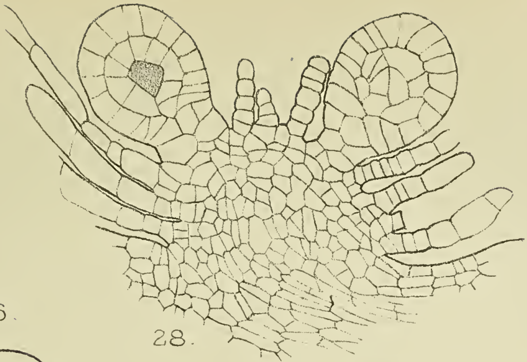

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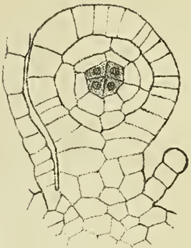

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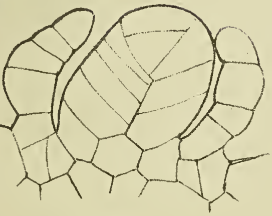

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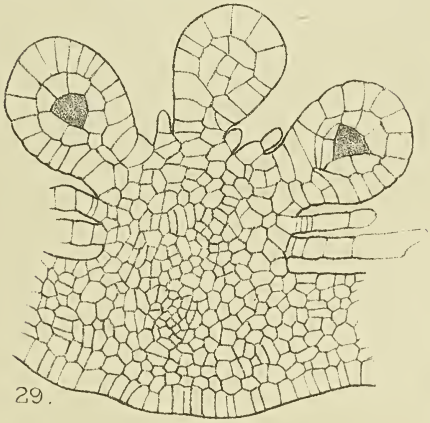

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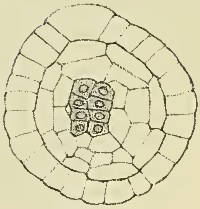

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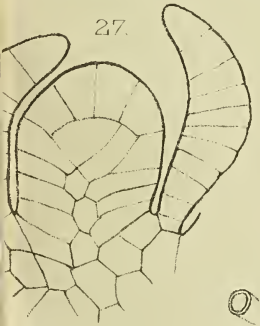

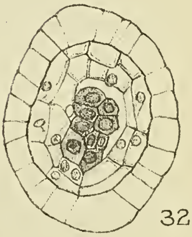

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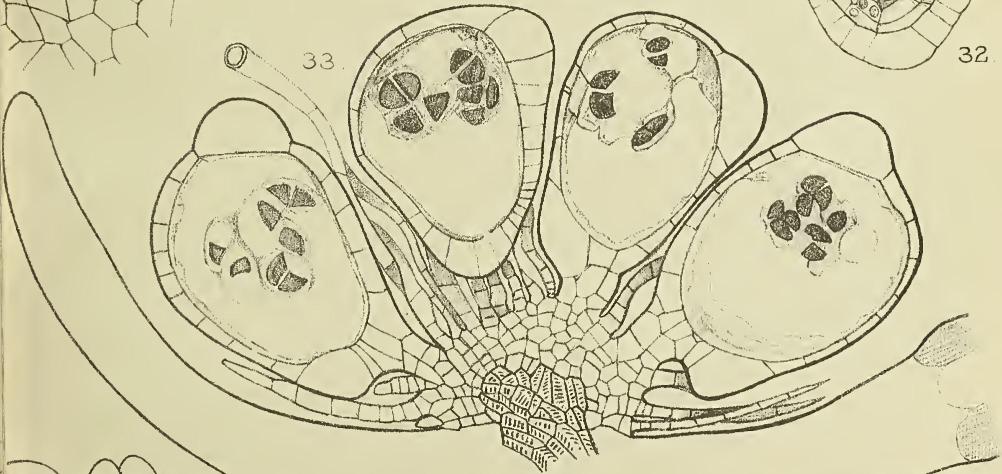

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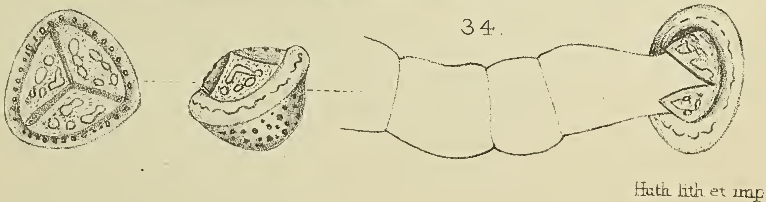

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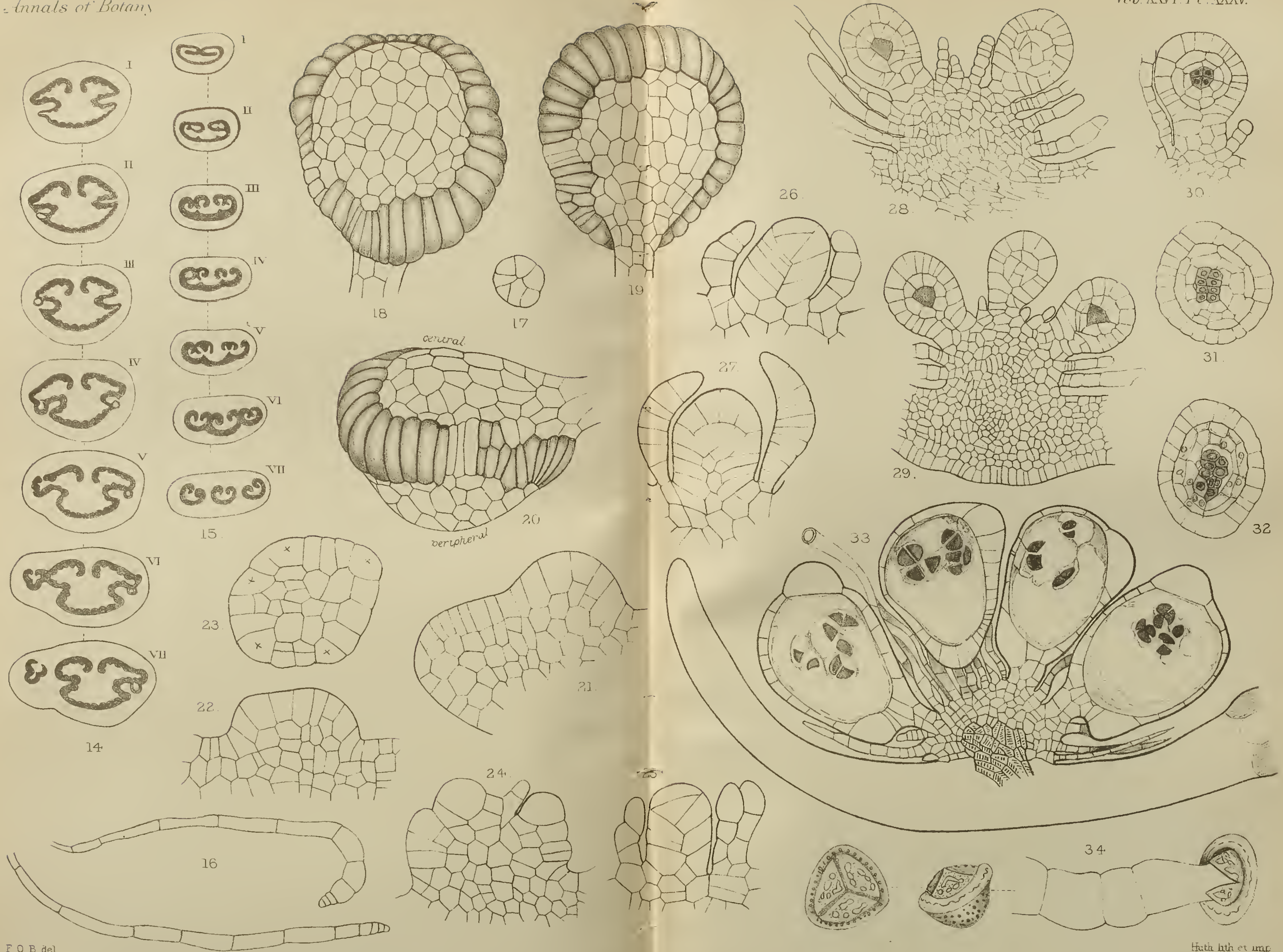

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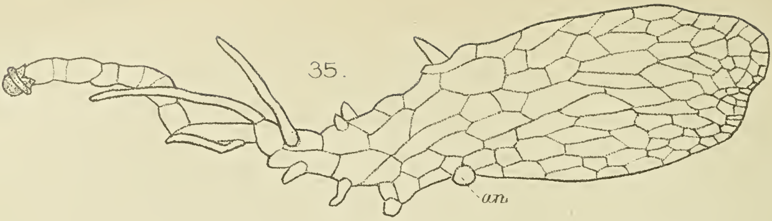

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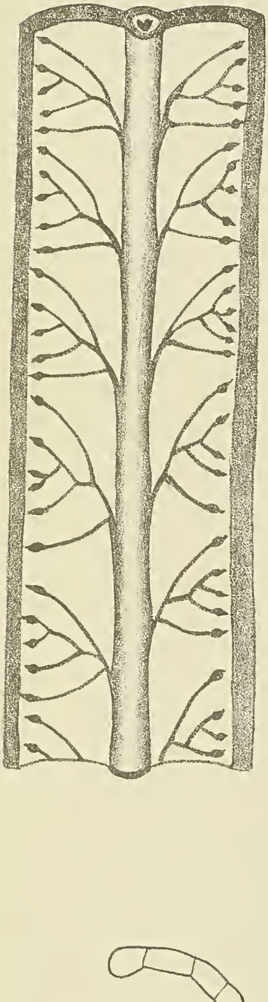

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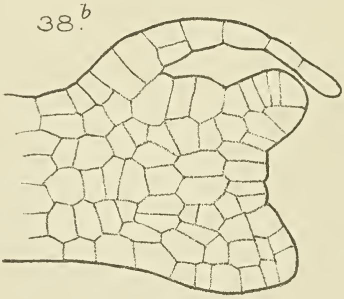

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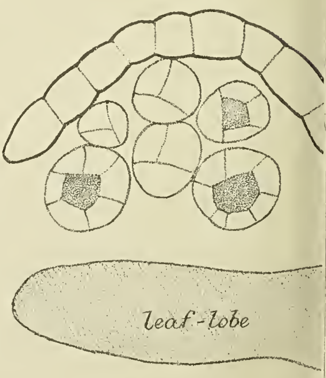

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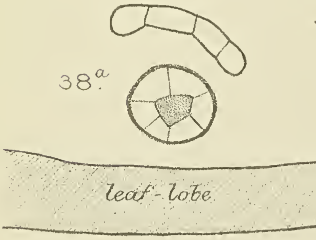

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BOWER——LOPHOSORIA AND OTHER FERNS.

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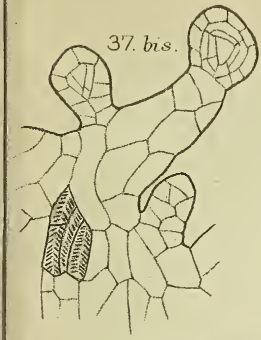

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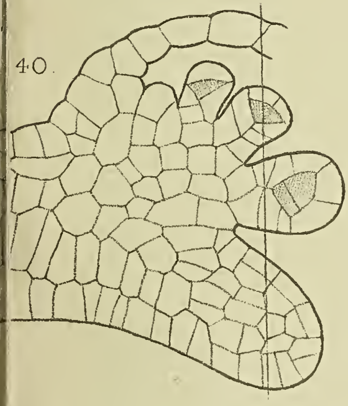

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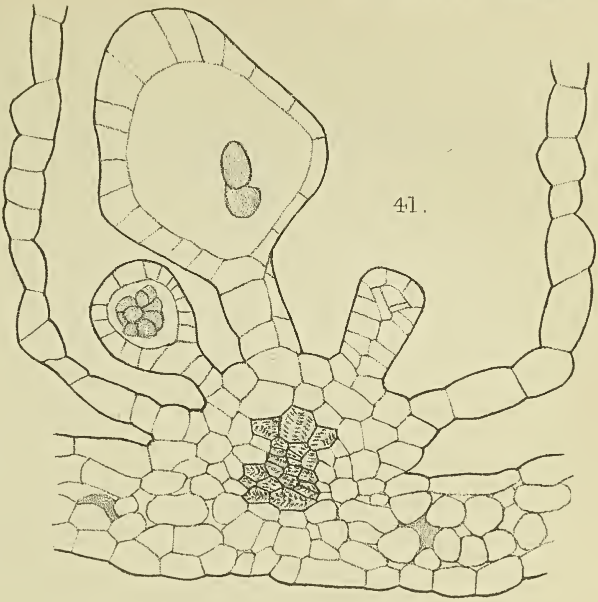

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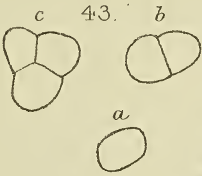

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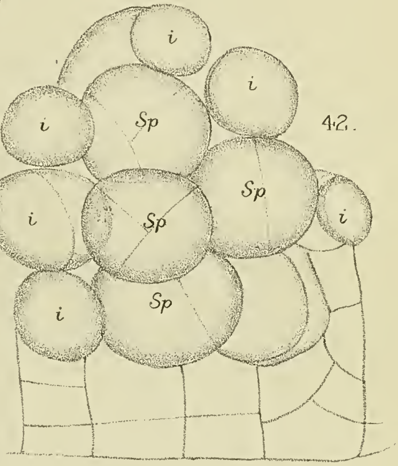

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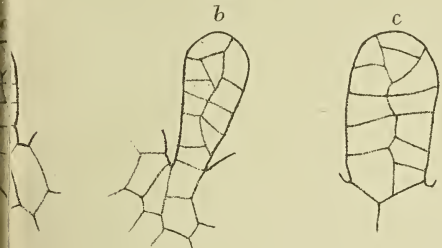

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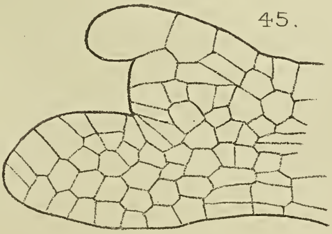

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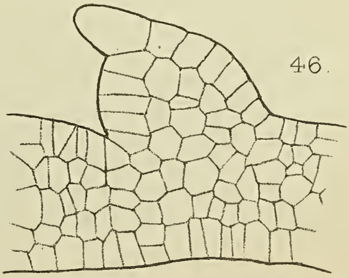

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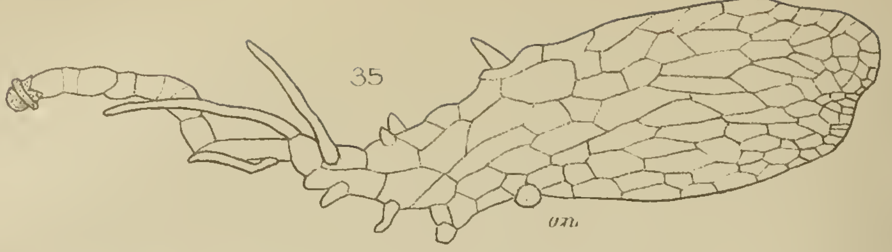

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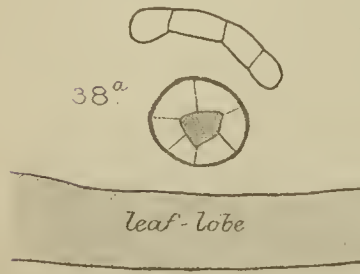

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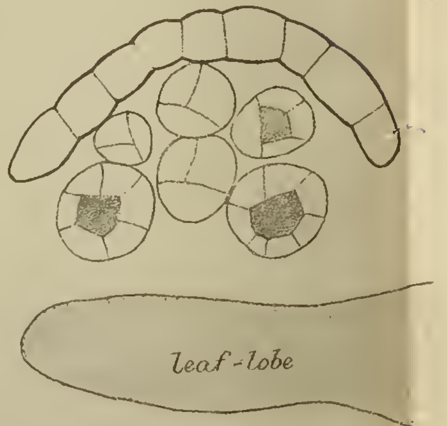

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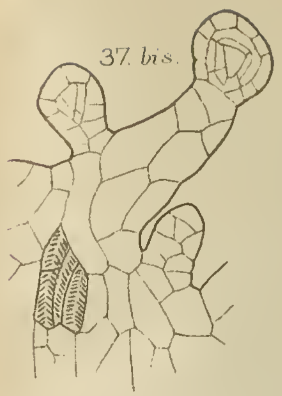

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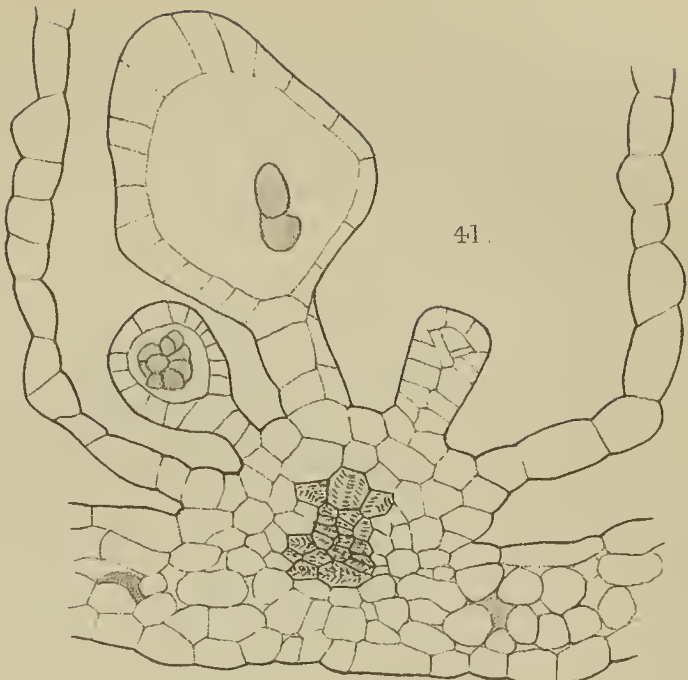

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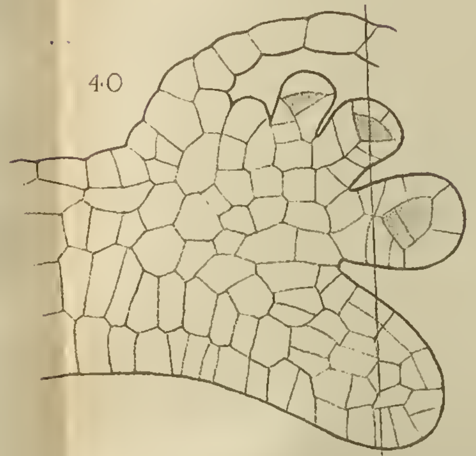

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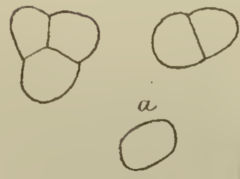

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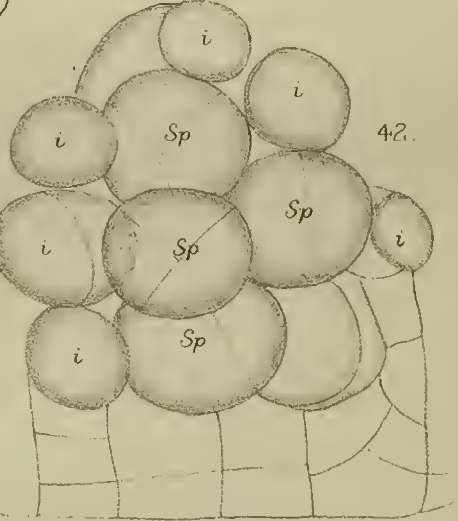

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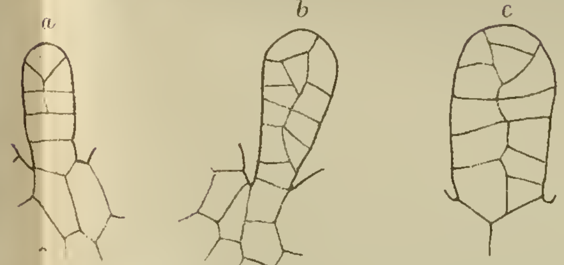

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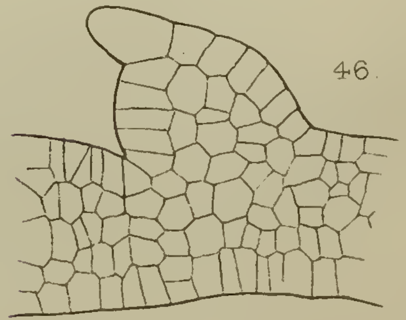

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BOWER—LOPHOSORIA AND OTHER FERNS.

Huth, lith et imp.

The Cytology of Laboulbenia chaetophora and L. Gyrinidarum.

BY

J. H. FAULL, B.A., Ph.D.,

University of Toronto.

With Plates XXXVII–XL.

THE Laboulbeniales possess many fundamentally important morphological features that cannot be properly interpreted until their underlying cytological phenomena have been elucidated. This is especially true of their organs of reproduction, including both gametangia and spore-sacs. Thus the latter have been rightly denominated asci by some botanists, but in the absence of any knowledge of their cytology—which recent investigations have shown to be essential to a definition of the ascus—others have advanced different views with regard to their nature, and correspondingly different speculations as to the taxonomic position of the group. By way of illustration it is needless to go farther afield than Engler's 'Syllabus' ('07), in which they are classified as Laboulbeniomycetes, a class of equal rank with the Ascomycetes and the Basidiomycetes. The gametangia have played an even more prominent part in these speculations. This is particularly true of the female organ, whose striking resemblance to that of the red seaweeds has won for it the name of 'procarp', and given rise to the theory that the Ascomycetes are an offshoot of the Florideae with the Laboulbeniales as the connecting link. But every biologist nowadays realizes the unreliability of schemes of phylogeny, the sole capital of which is external morphology. The true homologies of the gametangia are quite as likely to be expressed by their cellular and nuclear phenomena. Hence, a knowledge of the latter is almost certainly needed before a satisfactory estimate can be made of any phylogenetic hypothesis based on the morphology of the organs of reproduction.

The sexual organs have also been of interest from another standpoint. Because they are well marked and apparently functional they have been cited as proof that the Ascomycetes have not parted with their sexuality. As a matter of fact, it has never been shown whether they are or are not sexually functional, or just how they may function—that remains to be determined by following patiently the fate of their nuclei. It is likewise

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noteworthy that, within the limits of the order, bisexual and unisexual species exist, and that, in connexion with the latter, heterospory and dimorphism have arisen, but concerning the associated cytological phenomena we know nothing. These and other matters provide abundant cause and stimulus for investigation. Since up to the present no contributions, apart from two brief articles by the writer (Faull, '05, '11), have been made on their cytology, several forms have been placed under investigation in the hope of discovering an answer to some of the problems that await solution. Some of the results obtained are presented here and some will be published in other memoirs. In this connexion, I wish to express my gratitude to Professor Roland Thaxter, who has most generously placed the rich stores of his intimate knowledge of this group at my disposal, and who has kindly examined some of my preparations and extended many other courtesies.

MATERIAL AND METHODS.

Laboulbenia chaetophora occurs on the edges of the elytra and the free tip of the abdomen of several species of the genus *Gyrinus*, the familiar 'whirligigs' common on ponds and along the quieter margins of streams and lakes. With it is frequently associated *Laboulbenia Gyrinidarum*, though in the two rather remotely separated regions from which the material for the investigations described in this paper was collected, the former species was abundant and the latter rare in one locality, while in the other the conditions were reversed.

L. chaetophora seems to prefer the free tip of the abdomen, on which it often grows in tufts of from two to ten or more individuals, and *L. Gyrinidarum* the margins of the elytra, which are not uncommonly fringed with a growth of the plants of this species.

L. chaetophora is the larger form of the two, sometimes attaining a length approximating a millimetre and a width one-fifth as great, a perithecium up to 400 μ long, and a receptacle 525 μ or more. The smaller form rarely reaches half a millimetre in entire length and 160 μ breadth. There are other well-marked distinctions, all of which are noted by Thaxter in the second volume of his monograph.

Of the available species of *Laboulbenia*, *L. chaetophora* presents the advantages of size, and mode and place of occurrence on the insect, though both lend themselves rather well to the manipulations about to be described. The material was collected in the neighbourhood of Guelph and Ottawa, Canada. While most of it was of the larger species, it was impracticable if not impossible to separate the species, and especially to distinguish the younger stages of the two forms from one another. However, they are so nearly alike and so closely allied that chances of error are practically negligible.

The most serious difficulties in the way of technique, apart from those of obtaining suitable and adequate material, have been the securing of proper infiltration of the reagents used, and the sectioning of the embedded portions of the host. The chitinous external wall of the fungus impedes the ingress of fixatives and often prevents it. Hot fixatives were sometimes employed, and in some instances gave excellent results, but by far the best were obtained by making an incision in each plant in the manner to be next described.

Preparatory to fixing, portions of the insect bearing the fungus were dissected off with scalpel or scissors, as little as possible of the integument of the insect being taken. Immediately after detaching such a piece, it was laid in a drop of the fixing fluid on a slide, and with the aid of a dissecting microscope and a pair of chisel-pointed needles the ends of the perithecia were snipped off. For this purpose needles with the finest points obtainable were mounted in match-stick handles and the points ground down to a sloping chisel edge. The material was then at once transferred to a phial filled with the fixing reagent. In the case of young plants the application of such technique was impracticable, and they were fixed without snipping, with the result that not infrequently fixation was not good. Snipped and unsnipped mature perithecia were sectioned by way of comparison. The former were invariably better preserved, the latter usually so badly as to be unusable. Several reagents were employed, but Flemming's weaker solution proved to be most satisfactory and was finally exclusively employed. As for stains, there is little to choose between Flemming's triple and Haidenhain's iron haematoxylin, the choice possibly resting with the former.

In order to overcome the obstacles offered in sectioning the host, some attempts were made at detaching the plants, usually during the embedding process, and handling them individually. With the larger subjects this can be done successfully, and by improving the methods possibly with all. But on account of their minuteness there is bound to be more or less loss in such a procedure, and as this is a serious consideration where material is none too abundant to begin with, this method was not very extensively employed.

By embedding in paraffin with a melting point of from 57° to 60° C., and cutting with a hard knife, sections from 3 to 5 microns can be obtained, though the chitin of the host almost invariably fractures before the knife. In order to soften this chitin, experiments were made by immersing the material in a solution of eau de Javelle after it had been hardened. This method was useless, however, because, while it dissolved the chitin, it also rendered the plants quite colourless and caused them to loosen from their points of attachment.

CELL-WALLS AND GENERAL ENVELOPE.

The entire plant is enclosed by a thin, homogeneous, extremely tough, and impermeable continuous membrane or general envelope which is independent of the cells of the thallus. According to Thaxter it is the persistent gelatinous envelope which is first formed around the ripe spores just before they come to maturity and while still within the perithecium. Its impervious qualities are obviously of advantage, in that they protect the plant from the danger of desiccation.

This membrane is so thin that if embedded in hard paraffin it offers no serious resistance to the microtome knife. However, it very often checks or cracks and curls from the cut surface just as does a thin sheet of celluloid or hard rubber. It is often a serious hindrance to the fixing of sections to the slide, partly because it does not adhere well to glass, and because of its tendency to break and curl, thus disturbing the rest of the section during the various processes to which it is subjected in the course of staining and mounting.

The wall immediately underlying the general envelope is remarkably thick, and exhibits a curious differentiation, expressed both by structural peculiarities and characteristic reactions to staining media. Figs. 9 and 10 represent its morphological characters, from which it will be seen that it is laminated, and that the outer lamina is of different consistency and structure from the inner. The number of layers may vary, but usually from two to four or five can be counted.

The inner ones consist of a compact, non-granular substance, perfectly homogeneous and structureless, except for occasional radially arranged streaks, and with Flemming's triple or Haidenhain's iron haematoxylin take a light yellow stain or none at all. The streaks mentioned are darker in colour than the matrix in which they occur, since they vary from a smoky to a purplish tint. They have their bases or origin in the outer lamina, radiate more or less regularly inwards, at times branching, and frequently terminate at the inner boundary of a layer. Here they often expand into an irregular disc such as is illustrated in Pl. XXXVII, Fig. 9.

The outermost layer is of very different structure and staining capacity from the inner ones, and in the course of its development undergoes a very remarkable transformation. In the ripe spore nothing of peculiar interest is evident, but at an early stage in the history of the plantlet, after the walls have thickened, but while the substance of their component laminae is yet compact and non-granular, the peripheral layer soon shows its pronounced affinity for stains. The contrast to the inner layers is very marked, though not so much so as later, as at this stage the latter also show a tendency to take up stains. Later the contrast is sharp and complete, heightened so much the more by the circumstance that it adheres to the general envelope and often bears no apparent relationship to the protoplasts (Fig. 9). At a comparatively early stage the differentiation in structure referred to above becomes evident. At first it is little more than a mottling. The changes in structure, however, vary in different parts of the plant. In some regions there is a more or less uniform granulation of the lamella, in others the appearance of a radially arranged system of deeply stained rays grouped in plates. Reference to Figs. 9 and 14 will make clear what is meant. Viewed in section these plates look like dark, compactly striated bars of varying width, while viewed from the surface they resemble a honeycomb or net. With increasing age the plates disintegrate into granules, until finally the lighter areas, the meshes of the net, composed of the apparently almost unmodified wall substance, stand out as anticlinally oriented strands. In these changes it sometimes happens that one or more of the subjoined layers may become involved, but only secondarily so.

The strands which originate in this curious way are of very common occurrence in species of the genus *Laboulbenia*, and are of very characteristic appearance. Figs. 10 and 12 represent the fully formed strands. By light reflected from their surfaces they appear quite black in stained preparations, but by transmitted light are light-coloured and bright, the latter quality being due to their comparatively high index of refraction. Fig. 11 represents a portion of the general envelope that has been pulled away by the knife, and to which several of them are attached. The surface of such a portion of the plant viewed by transmitted light appears pitted or porous (Fig. 12). Indeed, Istvanffi ('95) described this punctate appearance, but interpreted the punctations as 'pore canals'. Thaxter rightly associated them with these strands or fibrillae, to the existence of which he was the first to call attention. Their formation, as has been seen above, is due to a more or less complete disintegration of the outer laminae, and in particular of the outermost. But as to the cause of this one can only conjecture. Instead of a granulation or degeneration of portions of the substance of the wall, as just described, in some instances there may be nothing more than a shredding, which is either an artifact or due to an unwonted stretching of an inelastic membrane. But shredding is exceptional, and cannot be offered as the cause of the origin of the ordinary type of fibril or the curious differentiation of the wall so early expressed in the life of the plant.

This account of the general envelope and external cell-walls does not apply to those of the trichogyne, the appendages, and the inner cells of the perithecium, all of which remain comparatively thin. Because of this fact the fixation of the appendages is usually good. In passing it may be pointed out that the appendages, in this species at least, appear to be capable of regeneration to a marked degree. New branches frequently spring from injured ones to replace the portions that may have been destroyed. The terminal cells in particular are long and thin walled, and are covered by a filmy colourless 'chitinous' envelope (Pl. XXXVII, Fig. 2). It is noteworthy, too, that the protoplasm and nuclei of all the cells of the appendages, even in older plants, are apparently well nourished, and that they never show indications of decay. This is in contrast with the larger cells of the receptacle. The protoplasm in the latter often becomes very scanty and loosely reticulate, and the nuclei frequently have every appearance of being in a state of decadence, as is manifested by the fact that they sooner or later lose their sharp outlines, their contents show little or no structure, and sometimes an irregular, densely staining nucleolus accompanied by a greater or smaller mass of equally densely staining débris is all that remains of them. This disorganization is rarely, if at all, seen in the appendages.

The septa in the receptacle are resolvable into several layers, of which the middle lamella stains purplish and the others yellowish. The former is continuous with the darkly stainable layer in contact with the general envelope (Figs. 8 and 9). On it the others are superposed, the younger being less compact and more hyaline than the older. The septa separating daughters of the same mother-cell are single pitted (Fig. 7), so that there is a continuous protoplasmic tract from the base of the plant to the tips of the appendages.

The septa of the appendages are constructed like those of the receptacle, but are rather thicker than might be expected after what has been said of their peripheral walls (Fig. 1). Their pits, too, are surprisingly large. In *L. chaetophora* and *L. Gyrinidarum* the septa are black as seen from the edge, but sections and face views show that the blackening is superficial and mainly restricted to the portion of the general envelope covering the rim of the septum. The septum stains reddish for a short distance out from the pit, then there is a wider yellowish peripheral portion, and then the black rim (Fig. 2, *a*).

PROTOPLASMIC BRIDGES.

Possibly the most interesting features of the cell-walls are the pits and protoplasmic bridges. They are beautifully demonstrated and figured by Thaxter ('96) in Part I of his monograph, and it is strange that no reference is made to his descriptions by A. Meyer ('02), Kienitz-Gerlöff ('02), Strasburger ('01), and other botanists who have examined various Fungi to discover whether or not they possessed these structures.

De Bary ('84) appears to have been the first to note the existence of pits in the partition walls of the Fungi. He pointed out that they often possessed simple pits, and that those in *Dactylium macrosporum* were strikingly like those of the Florideae, such, for example, as in *Callithamnion*. The first work of any consequence on the subject of protoplasmic bridges in Fungi was done by Wahrlich ('92), who published an account of extensive investigations on a large range of forms. The extent of his observations may be judged from the fact that he drew his material from the following groups: Mucorini, Ustilagineae, Uredineae, Agaricineae, Polyporeae, Hynaceae, Tremeliineae, Clavariaceae, Hymenogastreae, Lycoperdaceae, Nidularieae, Saccharomycetes, Perisporiaceae, Hypocreaceae, Pezizeae, Helvellaceae, Tuberaceae, Parmeliaceae, and Cladoniaceae. In every instance Wahrlich found that the transverse septa were perforated by a single simple pore. In only one species, however, did he find protoplasmic bridges that closely resembled those of the Florideae, and of the *Dactylium* recorded by de Bary.

A. Meyer ('96) has also examined the Fungi from this standpoint quite independently of Wahrlich's publication, and later ('02) published an extended account of his researches on protoplasmic bridges and cell fusions in the Fungi, in which he fully corroborates the observations of Wahrlich. Baur ('98), Darbishire ('99), Woronin ('00), Strasburger ('01), and Kienitz-Gerlöff ('02), and others have made more or less extensive examinations of the protoplasmic bridges in various Fungi and lichens, and all noted their existence.

It is aside from the purpose of this paper, however, to review the literature on this subject, except in so far as it touches the Laboulbeniaceae, the only account of which is given by Thaxter ('96). Concerning protoplasmic bridges in the Laboulbeniaceae, Thaxter offers the following: 'The protoplasm of adjacent cells, the origin of which is the same, is connected by a strand of the same substance, which passes from one cell to the other through a well-marked perforation of the cell-wall, the connexion being demonstrated with great ease by treatment with potash and subsequent staining (Pl. III, Figs. 11–12; Pl. II, Figs. 16–18). In many instances, also, it may be seen in the living plant without the use of reagents. This protoplasmic connexion is found in all the cells, including those of the trichogyne, when this organ is multicellular. In many cases in which the cells had been separated by potash, and the connecting protoplasmic strand stretched between them, I have seen a slight enlargement like that indicated in Fig. 12, Pl. III, recalling the similar structure through which the strands of protoplasm pass in the Florideae. I have, however, been as yet unable to determine its exact nature.'

Judging from microtome sections of the forms described in this paper, the thickening to which he refers may be the middle lamella, together with the granular accumulations to be found on both sides of it. The rather violent treatment of gross material with potash, followed by maceration, might very well tear out a portion of the middle lamella along with the strands. In this connexion Kienitz-Gerlöff's ('02) 'Neue Studien über Plasmodesmen' are of interest, as they deal with the bridges of the Florideae.

He was able to verify the observations of Wahrlich and Meyer on the Fungi, but, surprisingly, was not able to satisfy himself as to the existence of uninterrupted protoplasmic bridges in the red seaweeds, a condition that seems so patent from a low-power examination of almost any filamentous member of the group. Indeed, he doubts the existence of bridges even in such a form as *Polysiphonia*: 'Gleichgültig, ob man die Pflanzen ohne oder nach mehrtägiger Behandlung mit H_2SO_4 oder ZnClJ untersucht, die beide so gut wie unwirksam sind, gleichgültig ob man mit Jod, Methylviolett oder Hämatoxylin färbt, so findet sich in den meisten Fällen, *auch an den günstigsten Objecten, wozu die Haare und einfädigen Zweige gehören, in den Tüpfeln zwischen den Zellen scheinbar eine deutliche Schliesshaut*, welcher beiderseits eine halblinsenförmige, dunklere Protoplasmamasse angelagert ist, oder es liegt in den Verbindungen ein stark lichtbrechender Körper, über dessen Natur ich mir nicht ganz klar geworden bin. Mitunter gelang es mir freilich, ihn durch Behandlung mit Eau de Javelle zu entfernen, was für seine protoplasmatische Natur sprechen würde, aber diese Flüssigkeit löst allmählich die Zellen überhaupt aus ihrem Zusammenhange. Manchmal ist dieser Körper auch deutlich doppelt conturirt und linsenförmig. Ab und zu scheint freilich eine ganz homogene Verbindung zu bestehen, die so oft beobachtete Existenz jenes Körpers hat mich jedoch äusserst misstrauisch gemacht. Andererseits ist es auch möglich, dass die Erscheinung auf denselben Ursachen beruht, wie die so oft beobachtete Bildung des Knöpfchens in den Plasmodesmen der höheren Pflanzen.'

My observations on gross material, stained and unstained, coincide with those of Thaxter, but after an examination of hundreds of microtome sections of *L. chaetophora* I am driven to nearly the same conclusion as that reached by Kienitz-Gerlöff for the Florideae.[1] The pits are easily found, and, as in the Florideae, never more than one in a septum; and so far as could be judged none of these is the result of fusions between cells such as Meyer has described in detail for other Fungi. But the middle lamella or 'Schliesshaut' stretches across the bottom of the pit, as shown in Pl. XXXVII, Figs. 7 and 20, and there is considerable doubt as to the extent to which it is perforated. In a few instances, but very few, I have found a coarse strand of protoplasm, differing in no way from the cytoplasm of the protoplasts, passing from cell to cell, but these are undoubtedly exceptional phenomena.

The pits in the thin walls of young cells and of the inner sheath of perithecial cells are shallow and inconspicuous, but in thick septa are deep, always extending in as far as the middle lamella (Fig. 7). The cytoplasm dips into the pits, and, in the case of the deep ones, forms a coarse strand interrupted only by the middle lamella. The cytoplasm occupying

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[1] Connolly's observations on *Rhabdonia globifera* ('11) are not in accord with those of Falkenberg ('01) and Kienitz-Gerlöff ('02), for he reports a large strand of cytoplasm connecting contiguous protoplasts. His material appears to have been treated with strong solutions on the slide.

the pit is usually clearer and more structureless than the rest of the cytoplasm. One of its most striking features, and one by means of which the position of the protoplasmic bridges can always be located, is the presence of rather coarse, deeply staining granules (a brilliant red with Flemming's triple stain), some of which are closely in contact with the 'Schliesshaut' (Fig. 7). These are probably the 'metachromatic' granules observed by Claussen in the mycelium of *Ascodesmis* and by McCubbin in *Helvella*.

In favourable preparations the middle lamella or 'Schliesshaut' can be seen to be perforated by a very fine pore, and in some instances there is the appearance of several minute perforations. But that there are no large openings to accommodate such strands as are obtained by the maceration of entire plants, or that permit of the uninterrupted continuity of the strands that occupy the pits, is absolutely certain. A possible exception is to be made in the case of the appendages, for as judged from end views of their septa, which are easily obtained in almost any section, each is occupied by a comparatively large open pore (Fig. 1). Such an angle of observation is not favourable for the determination of the presence or otherwise of the thin middle lamella, but an occasional lateral view also gives the same impression. The black ring around the septum interferes seriously with observations from the side. The absence of the red granules characteristic of the bridges in the body of the plant lends further support to the possibility that the septa of the appendages are coarsely perforated, and that the protoplasmic bridges here are coarse strands of cytoplasm.

That there is a more or less definite organization of the cytoplasm related to the pitting of the septa is frequently evident, but will be referred to in another connexion.

THE PROTOPLAST.

The protoplasts are typically monoenergid, each cell possessing a single relatively large nucleus. Occasionally in older plants the nucleus in some of the cells may undergo one or more mitotic divisions. This may take place in the cells of the receptacle, especially in the larger ones, and in the cells lining the perithecium, but never in the foot, appendages, or spores. Fig. 19 illustrates an example of a large cell from the receptacle in which as many as ten nuclei were counted, all of uniform size and organization. Where such a proliferation occurs, the number varies from two to ten. Amitosis has not been seen in any instance.

The somatic nuclei are characterized by a large nucleolus and discrete masses of chromatin. There is no reason for believing that any portion of the chromatin is held by the nucleolus, for it is readily distinguishable in the resting nuclei in well-fixed preparations. The chromatin is especially abundant in the nuclei of the appendages. The nucleolus and chromatin stain bright red and the linin network blue. The chromatin is sometimes in shapeless masses, but most frequently in longer or shorter threads or granules (Fig. 1). Whether or not there is a definite number I cannot say. Now and then only two chromatic elements can be counted; at other times four, while in other instances there seem to be more. Nor can I satisfy myself that they bear any relation to a central body, or, indeed, that a central body is a constant concomitant of the nucleus. It may be. Not seldom a granule that might be interpreted as such lies in contact with the nuclear membrane in the cells of the appendages, but as there are other red-staining granules in the cytoplasm it is difficult to say just what this particular one really is.

Mitotic figures in the hyphae of several Ascomycetes have been observed by a number of cytologists, excellent illustrations, for example, being given by Fraser ('08) for *Humaria*, and Schürhoff ('07) for *Penicillium*. Faull ('05) and Dangeard ('07) have noted them in the spores.

The cytoplasm is finely granular or reticulate, but usually in the larger cells it is of a coarse spongy or reticulate structure. Oil bodies in *L. chaetophora* and *L. Gyrinidarum* are rare, but abundant quite highly refractive granular inclusions are of common occurrence. They may be small or quite large, and often regularly curved. Various sizes are shown in Figs. 3–6. They appear to be formed in the meshes or cavities of the protoplasm. The nature of these bodies was not determined. They make their appearance as the plant approaches maturity and do not show any especial affinity for the stains that were employed, though taking up the safranin and gentian violet to a limited extent, in contrast to the cytoplasm, which has a greater affinity for the orange G. Thaxter ('96) records an interesting fact in this connexion, namely, 'that the contents of the living cells consist of rather dense granular protoplasm, in which, as a rule, certain highly refractive spherical oily masses are conspicuous. In some instances these masses are few in number, one or more of them being often very large (Pl. V, Figs. 4 and 5), while again they may be more numerous and uniform in size, completely filling the cells, as in the case of *Laboulbenia Harpali*, *L. Philonthi*, and many others.' Whether or not the bodies I have described are to be identified as the smaller ones detected by Thaxter I cannot say, but it seems not improbable that they are the same.

There is still another feature to be mentioned, to which reference has already been made, that is, the presence of protoplasmic strands running out from the pits in the septa and from the nuclei. These phenomena have been repeatedly observed. Fig. 20 illustrates a case in point. The strands passing out from a pit in one of the cells intersect those coming from the pit in the opposite septum. The strands are slightly darker, as if of denser substance than the rest of the cytoplasm, but at the points of intersection are several brightly red-staining granules, such as are to be found in the bottoms of the pits. In another cell the 'fibrils' are shown passing around the nucleus on their way across. It was thought at first that possibly these strands of cytoplasm connected the nuclei of contiguous cells in some way, such for instance as Strasburger found to be the case with pairs of nuclei in the same cell in *Penicillium* (Strasburger ('02), Schürhoff ('07) *contra*), but Fig. 20 lends no support to such a view. It is true, nevertheless, that cytoplasmic fibrils can in some instances be traced from the pits to the nucleus, and that in degenerating cells they appear to be the most persistent parts of the cytoplasm.

That the nucleus bears a very evident relationship to the organization of the surrounding protoplasm at times has been dealt with in some detail by the writer in an earlier paper (Faull, '05). *Hydnobolites* and *Sordaria* furnish indubitable evidence of this contention.

ANTHERIDIA.

Careful and repeated search has been made for antheridia, but no organs resembling either the exogenous or the endogenous types have been discovered in either *L. chaetophora* or *L. Gyrinidarum.* So far as our knowledge goes these species afford examples of forms that have lost their antheridia. Belonging, as they do, to a genus characterized by conspicuous flask-shaped simple endogenous antheridia, it is not likely that they can have been overlooked, though that is not outside the range of possibilities. The cytology of both types of antheridia has been studied in other forms, and an account of the phenomena observed will be very shortly published.

PROCARP.

The procarp has its origin as a uninucleate terminal cell of a lateral branch of the receptacle (Pl. XXXVIII, Fig. 21). The two basal cells of the branch represented in Fig. 21 are daughters of the obliquely septated 'primordial cell of the perithecium', and give rise to the perithecium proper.

The course of development of the perithecium has been very carefully worked out by Thaxter, and so need not be recapitulated in detail here. It may be stated, however, that transverse sections wholly verify his statements that the number of rows in both the outer and inner walls is four, and that the rows of the two walls alternate with one another (Figs. 33–6). The inner is of later origin than the outer; in fact, does not appear until the latter is nearly complete and the procarp fully formed. Both are clearly outgrowths of the basal cells of the perithecium. They have been repeatedly seen in longitudinal sections in all stages of development, and their protoplasmic connexions with the basal cells demonstrated (Fig. 25).

The mature procarp consists of three parts, the carpogonium, the trichophoric cell, and the trichogyne. The trichogyne is very highly developed in *L. chaetophora* and *L. Gyrinidarum.* It is an elaborately branched, frequently septated structure consisting of uninucleate cells (Figs. 30, 31). It, too, has been figured and described by Thaxter, and little further can be added.

The possible functions of trichogynes that appear no longer to serve as receptive organs of spermatia have been recently fully discussed by Vuillemin ('07) and Brooks ('10), but so far as I know no experimental evidence has been adduced in confirmation of any of them.

The sequence of cell-formation in the carpogenic cell is strictly comparable to that described by Thaxter for *L. elongata.* The trichophoric cell undergoes no further division, though its nucleus as described below may play a very significant rôle. The carpogenic cell divides into three superposed cells, the superior and inferior supporting cells (*s.s.* and *i.s.*) and the ascogonium (*am.*) which lies between them. The ascogonium may again divide transversely, the lower half constituting a secondary supporting cell, while the upper usually divides by an oblique partition into the ascogenic cells (*asc.*) from which the asci (*as.*) bud out. In some plants this division does not take place, so that there may be but one ascogenic cell. Two, however, is the characteristic number, and never more than two have been found in the species here described. Two vertical rows of asci now bud out from each of the ascogenic cells. An account of their further development will be given in a subsequent section, and the cytology of the procarp only dealt with here.

The carpogenic cell is at first uninucleate, a relatively large nucleus with a large nucleolus occupying the centre of the cell (Fig. 22). The trichophoric cell is also uninucleate (Fig. 22). The single, centrally placed nucleus of the carpogenic cell now apparently divides; at all events it is succeeded by a pair of smaller nuclei (Fig. 24). I have not observed its actual division, but the nucleus represented in Fig. 23 appears to be in an early prophase stage. The nucleus of the trichophoric cell very soon moves down next to the carpogonium, as shown in Fig. 25, and undergoes a homotypic mitosis. The central bodies of the spindle are large and disc-like, but it is difficult to make sure of the number of chromosomes. At this stage, or even earlier, the partition separating the two cells disappears. Thus in Fig. 26 there is the appearance of a long single cell in which lie four nuclei, A, B, C, D, enumerating from the bottom up. A and B presumably are daughters of the carpogenic nucleus, C and D of the trichophoric. The lower end of this cell with nucleus A is eventually cut off as the inferior supporting cell, and the upper end with D as a 'restored' trichophoric cell. But it was impossible to determine the exact sequence of septa-formation.

The middle pair, B and C, divide after a short period of growth, and their division is immediately followed by the separation of the superior supporting cell, which receives two of the four daughters, the other two remaining in what is now the ascogonium (Fig. 27). The two nuclei left in the ascogonium again divide, and a secondary inferior supporting cell is sometimes cut off which likewise appears to be characteristically binucleate (Fig. 28). The remnant of the ascogonium is still binucleate, and it may at once begin to bud off asci, or, as usually happens, first divide into two binucleate ascogenic cells (Fig. 28).

So far no nuclear fusions, nor any indications of such, have been observed.

By this time the perithecium has grown beyond the upper end of the receptacle, over which it curves as shown in Fig. 32. The supporting cells are still intact, but they are beginning to show signs of breaking down. This soon happens, and then the ascogenic cells with their asci come to lie free in the cavity of the perithecium, where they are sustained by the cells lining the perithecium. The latter are thin walled and well nourished. The upper cells lining the perithecium and the canal cells of the neck are crushed by the increasing mass of asci and spores soon after the perithecium has attained its maximum size, and eventually the same fate befalls those in the base of the venter.

A general survey of the Laboulbeniales reveals the fact that the procarp is characterized throughout by the constant occurrence of two cells, the carpogonium and the trichophoric cell. There is always a trichogyne, but it is extremely variable. The trichophoric cell is of especial interest since it is not represented in the Florideae or the Uredineae, and, if represented, is not determinable in any other Ascomycetes. Whether it is to be looked upon as a part of the trichogyne or as a part of a two-celled 'scolecite', its history and functions call for closer scrutiny. In the apogamous species investigated in this paper its nucleus joins forces with that of the carpogonium, a phenomenon quite comparable, without any thought of implying homology, to the initial stages of fertilization in certain Uredineae and possibly in such sac-fungi as *Ascobolus.*

Harper ('96) found that the cells of the scolecite of *Ascobolus* are primarily uninucleate, and that subsequently there is a migration of nuclei from cell to cell through perforations in the transverse septa of the scolecite. Welsford ('07) has fully corroborated his observations. Welsford states that 'the transverse walls of the scolecite are perforated medianly by large circular pores. Pores could not be identified in the earliest stages of development, and it seems possible that they may be of secondary formation. The nuclei with the cytoplasm of the several cells pass through the pores till they reach the ascogenous cell, where they fuse in pairs.' Though the history of individual migrating nuclei was not followed, the phenomena described are suggestive of what takes place in *Laboulbenia chaetophora.*

THE ASCOGENIC CELLS.

The ascogenic cells are binucleate at all stages. The rather large nuclei divide simultaneously prior to the formation of each ascus (Fig. 37). This is a conjugate division: one of the daughters of each of the mother nuclei passes into the young ascus and the other remains in the ascogenic cell. The pair left in the ascogenic cell immediately begin to increase in size, and with the advent of the next ascus undergo another division. The divisions of the nuclei are homotypic, and, as has been seen to be the case elsewhere, the central bodies are conspicuous and the chromosomes four in number. In Fig. 39 a metaphase stage is represented in which the chromosomes are especially large and somewhat scattered, so that the count could be made with certainty.

The protoplasm of the ascogenic cells, as of the procarp, is dense and very finely granular, and exhibits a greater capacity for stain than the cells of the perithecium and receptacle, so that they always stand out clearly differentiated from the rest of the thallus.

The ascogenic cells of the Laboulbeniaceae have seemed to be unique among the Ascomycetes in respect to the limited and constant number for each species, and in respect to their capacity for giving origin to an unlimited number of asci. The number of ascogenic cells in *Amorphomyces* and two or three other genera is one, in *Laboulbenia* and several others two, in *Stigmatomyces* four; in *Haplomyces* there are eight, and in *Polyascomyces* there are thirty-two or more. This fixity of number is paralleled, however, by such genera as *Sphaerotheca*, in which there is a single ascogenic cell in every fruiting body, and as to the unlimited capacity of the ascogenic cells for ascus production I have found examples not at all uncommon in practically every order. The cytological details in these examples correspond to those in *Laboulbenia* in that a series of conjugate divisions takes place in the ascogenic cell in relation to a corresponding budding off of asci. Brefeld ('74) long since cited what has often appeared exceptional in the case of *Penicillium*, but theoretically at least his claims may be regarded as perfectly valid. The investigations of McCubbin ('10) and Brown ('10, '11) have shown the same thing expressed in another way for *Helvella elastica*, *Leotia*, and *Lachnea*, namely, that the nuclei of a potential ascogenic cell may undergo many conjugate divisions, and eventually give rise to many asci.

The common underlying principle is that of conjugate divisions, which is undoubtedly a character of the Ascomycetes. In *Laboulbenia* the apparatus involved is extremely simple, since the ascogenous cells correspond to the entire system of ascogenous hyphae and part of the female apparatus in most other groups. They are characterized by similar series of conjugate divisions, but undergo no considerable growth, no branching, and no septation.

THE ASCUS.

Thaxter was the first to discover that the spores of the Laboulbeniales are invariably borne in sacs, and constantly to the number of four, or in a few species of eight. On these grounds he has unequivocally maintained their ascigerous nature. It has only remained to examine the spore-sacs cytologically (Faull, '06, '11) to prove the correctness of his view, and to prove beyond question that they possess all the essential features of asci.

They begin as basipetally arranged folds or outgrowths of the ascogenous cells. They are never septated off from the latter, but their bases, which at first are very broad, gradually contract until finally the cytoplasmic connexion is broken. In *Laboulbenia chaetophora* the walls of the older asci are not always dissolved nor are the asci always completely sloughed off from the mother-cell (Pl. XXXIX, Fig. 41), though both are common phenomena and are said by Thaxter to be characteristic for the group.

At first small, the ascus grows to a relatively large size, during which time the definitive nucleus also increases enormously in bulk. The cytoplasm is finely granular and fairly uniform throughout. When mature there is a slight vacuolation in both the upper and the lower parts of the ascus (Fig. 51), and sometimes a few insignificant granular inclusions make their appearance.

Two nuclei which we may with good reason assume to be lineal descendants of the pair that occupied the carpogonium in its later stages of development enter the young ascus and fuse with one another. Their chromatin is ordinarily distributed irregularly throughout the nuclear cavity in the form of a thread, an arrangement which persists for some time after fusion (Figs. 43–5). Eventually a synaptic contraction sets in (Fig. 46) and the threads are matted to one side of the nucleus, though their connexion with the nucleolus is probably maintained.

The synaptic stage is succeeded by one in which the threads are again loosely arranged, but this time much more regularly and with a more or less obvious relation to the nucleolus and an appearance of being paired. These features are clearly illustrated in Figs. 47 and 48. It will be seen, too, that there is a more or less regular system of achromatic threads radiating out from the nucleolus and traversing the entire nuclear cavity.

The next stage is characterized by a very marked diminution in the apparent amount of chromatin, and by what may possibly be interpreted as a second contraction. Figs. 49 and 49 *a* represent the same nucleus from two consecutive sections, and are designed to show the entire amount and disposition of the chromatin. It will be observed that the volume has diminished, and that the threads are thicker and more irregularly arranged.

Up to this time a central body has not been conspicuous, though probably present as indicated in Fig. 44. But now it can be found without great difficulty, and as shown in Fig. 50 the chromatin threads are apparently connected with it. Such a phenomenon was first discovered by Harper ('05) in *Phyllactinia*. It will again be noted that the polarity of the nucleus with respect to the nucleolus is still maintained, in which phenomenon the linin threads already described may play some part, since they appear to converge at the side opposite the nucleolus and there stand in connexion with the central body. This stage (Fig. 50) is immediately prior to the first mitosis, and constitutes a part of its prophase. The number of chromatic threads is clearly four, and these are identical with the four chromosomes to be seen on the spindle.

During all this time the nucleolus has been a very conspicuous organ. Each of the initial pair of nuclei contributed a large nucleolus, and these fused to form that of the definitive nucleus. Its growth throughout is steady and remarkable. In the later stages it becomes quite vacuolate and at times even somewhat honeycombed. During mitosis it steadily diminishes in size, but a portion of it still remains almost up to the time the daughter nuclei begin to form (Pls. XXXIX and XL, Figs. 42–54).

The first spindle is in many respects a very interesting structure. It is clearly intranuclear and is terminated at both ends by an extranuclear disc-shaped central body, evidently the products of division of the pre-existing one already noted, from which stream out long astral rays. There are four elongated and curved chromosomes of unusual size for a fungus (Figs. 51–2). The achromatic portion of the spindle consists of two sets of fibrils, such as I have described in *Hydnobolites*, &c. (Faull, '05)—a central system of many rather closely packed threads, and an outer mantle system. The mantle rays bear the chromosomes and appear to be responsible for their transport to the opposite ends of the spindle. After the chromosomes have reached the poles of the spindle the central fibrils experience a great elongation, carrying the daughter chromatic elements far apart (Pl. XL, Fig. 54). In some instances, not figured, the remains of this central spindle have been observed subsequent to the stage represented in Fig. 54, the fibres no longer separable, the whole thing resembling a coarse sharply defined dark cord, and irregularly wavy or crooked as though it had lost its former tension and was incapable of contraction. The daughter chromosome masses had probably moved slightly towards one another after the central spindle had fulfilled its function. This wide separation of the daughters of the definitive nucleus is a characteristic phenomenon of the first mitosis in every order of the sac-fungi.

The chromosomes appear to divide transversely (Pl. XXXIX, Fig. 52) and, as they approach the poles of the spindle, show a tendency to split (Fig. 53). They are not homogeneous, for as seen in cross-section they do not stain uniformly. The centre may be hollow, at all events it is much lighter than the periphery. Fig. 53 presents some interesting features and is interpreted as follows: The chromosome at the extreme right is dividing transversely, the large one in the centre is still entire, the two pieces at the left are segments of the third chromosome, the uppermost is a daughter chromosome splitting, and portions of its sister, also longitudinally split, are on their way to the opposite pole.

The asters in the anaphase stage are even more distinct than earlier. A vacuole gradually forms around each of the chromosome masses, and the daughter nuclei very soon stand out clearly defined.

The second mitosis follows very swiftly on the heels of the first and is essentially different in many details. There is no preliminary synapsis stage, the spindle is sharper pointed, the central bodies are smaller, the chromosomes split longitudinally (Pl. XL, Fig. 55), the spindles are not differentiated as is the first, and there is no subsequent elongation. As a result of this the daughter nuclei lie side by side. Fig. 57 represents a tetranucleate stage of the ascus, showing the two pairs of sister nuclei.

The four nuclei now pass through a brief resting period, during which the chromatin is in the form of threads, and possibly in connexion with the persistent central bodies (Fig. 57).

During the prophase of the last mitosis the relation of the chromatin to the central bodies is more evident (Fig. 58). Presumably the latter divide, constituting the poles of the spindle on which four chromosomes, corresponding to the four threads of the prophase, take up a position in the equatorial plane (Fig. 59). It was not possible to determine just how the chromosomes divide, but they do divide, and there is no reduction in their number (Figs. 60, 61). Nor was it possible to detect a differentiation in the spindle, which, whatever its constitution, is extensible, since the daughter nuclei are carried far apart (Fig. 62). The astral rays are long and tenuous. They are represented in Fig. 62 just before the spores are delimited, and again in Figs. 63 and 64 after this process is completed. That they do not fuse laterally and do not form a part of the limiting membrane is perfectly obvious, even were such a phenomenon as lateral fusion physically possible.

The spores are delimited progressively, beginning always at the central body. Relative to the process nothing more can be added than has already been described by the author and corroborated by Fraser and Brooks ('09) and Brown ('11), and in part by Dangeard ('07). Faull ('05): The spores are delimited 'by the differentiation of a limiting layer of hyaline or finely granular layer of protoplasm that begins adjacent to the centrosome and continues progressively until completed at the opposite pole.' Possibly there is a cleavage in this layer resulting in the plasma membrane of the spore and a corresponding membrane lining the cavity in which the spore lies. 'The astral rays never appear to fuse, as is stated by Harper to be the case in *Erysiphe* and *Lachnea*.' Fraser and Brooks ('09). These authors describe 'an outward flow of some substance which emanates from the centrosome' in *Ascobolus* and *Lachnea*. 'It has already been suggested', they add, 'that this substance is not improbably an enzyme.' This layer is the seat of a cleavage resulting in the two membranes mentioned above. 'We have, however, obtained no evidence of a lateral fusion of astral rays to form a membrane such as Harper describes, but we hold rather that the rays indicate rather the direction of flow of altered substance from the centrosome.' Berlese ('99) first suggested the possible directive action of the astral rays in laying down the limiting plasmatic spore membrane. 'Il cinoplasma determina poi la formazione dello strato parietale plasmatica dell' ascospora, ed individualizza quindi questa il sen al trofoplasma.' Dangeard ('07) could not differentiate the astral rays in his preparations of *Pyronema*, but he did detect a homogeneous sheet of material that issued from the central body and gradually delimited the spore. 'Il y aurait peut-être lieu de modifier légèrement la description classique donnée par Harper du mode de formation des ascospores dans l'asque et du rôle des filaments de l'aster ; ici nous n'avons pas vu de filaments, mais seulement cette substance homogène chromatique qui enveloppe le noyau progressivement.' Brown ('11) affirms that the astral rays of *Lachnea* do not fuse to form the limiting membrane of the spore, and that they constitute no part of it. He also finds a membrane lining the cavity in the epiplasm in which the spore lies. Evidence from these independent sources indicates that Harper's interpretation of the part played by the astral rays of the last mitosis was incorrect. Moreover, from a physical standpoint Harper has affirmed an impossibility, as these long astral rays swinging down and out separate farther from one another (I refer to adjacent rays on the same side of the central body) rather than come into contact. Were they all laid side by side and welded into a ribbon they are so few and so tenuous that they would form a very small portion of the entire protoplasmic membrane of the spore. There is unanimity on one fact in this connexion described by Harper, namely, that the limiting membrane is formed progressively, but this feature is not more important than the determination of the origin of the membrane, as an exact knowledge of the latter must influence our conception of the homologies and phylogeny of the ascus itself.

After the spores are delimited in *Laboulbenia*, the flask-shaped nucleus continues to elongate until the neck is extremely attenuated (Fig. 64). In fact, the venter almost comes into contact with the opposite end of the young spore. The astral rays are observable up to this time, and in older stages lie much farther inward towards the nucleus and away from the limiting membrane than in the earlier stages, or for that matter in any other asci I have yet seen. They have been found here even more or less twisted about the attenuated neck of the nucleus (Fig. 64). These phenomena in themselves, even were lateral fusion of the longitudinal ribs of a globe physically possible, which is certainly not the case, are sufficient evidence that they do not fuse to form a protoplasmic membrane. The cavities in which the spores lie also appear to be lined by a protoplasmic membrane. The central body finally loosens its connexion at the older pole of the spore and takes up its position on the surface of the now spherical nucleus (Figs. 65 and 69).

The long axes of the spindles of the third mitosis lie in the long axis of the ascus, consequently the asters are directed towards the upper and lower ends of the ascus respectively. Since there is no relative change of position during spore-delimitation it is possible to state with almost absolute certainty that the lower daughter nucleus of each spindle functions as a spore-former. That they lie approximately side by side in two pairs is merely a natural consequence of the disposition of the four nuclei of the second generation (Figs. 57 and 63). The upper daughter nuclei, on the other hand, initiate no spores, and pass at once to the upper end of the ascus (Fig. 65), where they eventually disintegrate.

SPORES.

From the first the spores lie with their long axes parallel to the long axis of the ascus. Almost spherical at the outset, they become broadly elliptical, then continuously more narrowly elliptical until their normal mature form is attained (Figs. 63–6). During the earlier stages of growth the nucleus remains at the upper or younger pole of the spore. At the same time chromatin is distinctly aggregated in proximity to the central body, simulating in a more or less striking manner the phenomenon of synapsis. Later the nucleus moves down well past the middle of the spore and there undergoes an ordinary homotypic mitosis, the chromosomes numbering four (Fig. 70). The spindle elongates somewhat and a thin septum is quickly formed between the daughter nuclei, dividing the spore into two cells. Whether or not a simple plate is formed progressively from the periphery of the spore inward I cannot say. Fibrils, possibly the spindle threads, may be seen passing from the nucleus to the young septum, and often a small plug lies at the centre of the plate, such as is represented in Fig. 67. Presumably there is a protoplasmic bridge, but only special refined methods would bring it out. The spore now completes its growth, and in addition to the ordinary wall, finally clothes itself with a gelatinous sheath which is strongly developed at the upper end, the future basal cell of the plantlet into which the spore will grow should it find favourable lodgement on a suitable host.

DISCUSSION.

One immediate result of these investigations has been the confirmation of Professor Thaxter's classification of the Laboulbeniales as true Ascomycetes. This recognition is based on the characters of the spore sac, for the only positive diagnostic feature that distinguishes the Ascomycetes from other higher fungi is the ascus. That organ has been submitted to a very searching and successful examination during the past few years, so that our conceptions now concerning it when it is placed in comparison with the phycomycetous sporangium, the floridean tetrasporangium, or any other spore-bearing cell are no longer hazy. Every research has testified to the constancy of its nuclear heritage, and to the sureness with which certain characteristic cytological changes recur in the course of its development, all of which in combination suffice to distinguish it from every other spore-bearing organ known in the organic kingdom. Thus the unfailing inheritance of a pair of nuclei, the regularity of their fusion in the young ascus, the minutiae of the successive three mitoses, and the details of spore-delimitation by a remarkable method of free cell-formation are uniform throughout the group and without a parallel in any other. Precisely the same phenomena characterize the spore sac of the Laboulbeniales, in fact so exceptionless as to throw no new light on the origin of the ascus. This faithful cytological similarity, therefore, affords conclusive proof that the Laboulbeniales can no longer be counted among the doubtful Ascomycetes, or that the Laboulbeniomycetes of the Engler system can be maintained as a class, or that they can rank other than as an order or suborder of the sac-fungi. Unquestionably, in accordance with prevailing definitions, the Laboulbeniales, Thaxter, are rightly regarded as Ascomycetes and as a suborder of the Pyrenomycetes.

Studies on the cytology of the Laboulbeniales have still another and very special interest in that they promise material additions to our knowledge of the sexuality of the Ascomycetes. This is a fascinating subject on its own account, and has also a wide bearing on the difficult problem of the phylogeny of the sac-fungi.

There is a strong tendency at the present time to regard the Ascomycetes in point of sexuality as unique among plants and animals, for it is commonly believed that their sexual phenomena comprise two successive nuclear fusions, one in the female gametangium or its substitute, and the other in the ascus. This, if true, would involve a quadrupling of the chromosomes in the fully fertilized egg and a subsequent double reduction. There is likewise a tendency to deny the existence of normal series of conjugate nuclear divisions in the Ascomycetes, though the subject has not been more than superficially investigated. Turning to the species described in this paper it will be seen that the observations noted do not bear out either of these views. The fusion of the nuclei in the young ascus is the only nuclear fusion found in the life cycle ; there is but one meiosis ; and conjugate divisions play an important part.

Relative to the problem of the sexuality of the Ascomycetes it is becoming increasingly obvious that the phenomenon of conjugate divisions must be given serious consideration. The regularity of their occurrence among the Laboulbeniales is certain, and there is a growing body of evidence pointing to the generality of the phenomenon throughout all but one or two other subdivisions of the sac-fungi. That this feature has either been inherited from an earlier stock, or that it has been evolved within the Ascomycetes themselves, is self-evident. In either case, it would not be wholly unexpected to find occasional reversions to the more primitive procedure of a fusion of gamete nuclei directly following the conjugation of sexual cells. Such fusion nuclei would be potentially capable of at once forming spore-nuclei. It is understandable that sometimes, as Claussen asserts is true in *Pyronema*, there might be nothing more than pseudo-fusions in the gametangium, the actual fusions taking place normally in the asci. Such reversions would explain the recorded instances of fusion in the female gametangium, and the fallacy of assuming—what has never been demonstrated—that the sexual act comprises two successive nuclear fusions. The same tendency would also account for pseudo-fusions. In view of the need of research on the nature and extent of conjugate divisions in the Ascomycetes, especially because of the light an exact knowledge of this feature will throw on the problems of their sexuality and phylogeny, I venture to review the field and to explain the grounds taken above at greater length.

Cytological investigations on the higher Fungi in recent years have uncovered the interesting and universal phenomenon of a fusion of two nuclei in the spore mother-cell. An even more remarkable fact has been established for all but the Ascomycetes in this connexion, namely, that the fusing nuclei belong to two distinct lineal series the members of which have lived side by side in pairs, dividing conjointly without fusion for many generations.

Dangeard ('93), Sappin-Trouffy ('93 and '96), Blackman ('04), Christman ('05 and '07), Blackman and Fraser, ('06), and Olive ('08) have cleared the ground in the case of the Rusts. They have shown that a binucleate phase begins with the migration of a nucleus from one cell into another at a definite stage in the life-history, and that the progeny of these initial pairs accompany one another uncombined up to the teleutospores, where fusion ensues, followed at once by a reduction division. These events indicate that a curious type of sexuality has been evolved in the Rusts, one characterized by a long period of vegetative activity between the initial and the final phases of the sexual act.

Maire ('00–'02), Harper ('02), and Nichols ('04) have investigated a number of species of Autobasidiomycetes and have found that the cells of the lamellae of the Agaricaceae, and of the entire fruiting body and even part of the mycelium of some other forms, are binucleate, though so far no one has determined where or how this condition arises. Fruitful investigation is rendered difficult in most forms because the cells which might be expected to contain two nuclei are ordinarily multinucleate and the number of nuclei is not at all constant. Maire asserts the belief that where more than two occur in such cells the increased number is due to an amitotic multiplication of an original pair. The nuclear phenomena in the basidium have been studied by Wager ('93), Dangeard ('93), Maire ('02), and Fries ('11), and have been found to closely correspond to those in the teleutospore. Thus there is reason to believe that the same type of sexuality is expressed by the Autobasidiomycetes as by the Rusts.

The problems presented by the Ascomycetes have appeared at first sight less complicated in some respects than in the Basidiomycetes, owing to the existence among them of more or less conventional and in some instances apparently functional sexual organs, and the failure to recognize conjugate nuclei. A closer examination has revealed the fact, however, that a nuclear fusion takes place in the ascus just as it does in the basidium, and that the sexual organs exhibit a more perplexing multiplicity of forms than in any other group of plants. Several botanists have reported, too, that they have seen gamete nuclei fusing in the oogonium or its equivalent, and there is increasing evidence of the occurrence of characteristic conjugate nuclei. Indeed, the ensemble of facts has appeared so difficult of reconciliation that so far no view has been advanced that has not called into question the actuality of the existence of some one of them.

Harper ('96, '05), seconded by Blackman and Fraser ('06), Fraser ('08), and Claussen ('05), has contended that there are two successive nuclear fusions within the limits of a single life cycle, one in the female gametangium, the other in the ascus. They have actually seen both—at least, so they have interpreted what they have seen—but, granting the correctness of their interpretation, it is pertinent to insist that their claim that both occur in the same life cycle is a pure assumption. If their contention be correct, then, according to prevailing views regarding chromosomes in conjugating nuclei, there must be a quadrupling of the chromosomes with the second fusion, and in consequence a compensatory double reduction. Harper ('05) has consistently accepted this apparently inevitable theoretical deduction, and locates the site of the two reductions in the ascus. He considers that the double reduction is effected by the three mitoses in the ascus—an alluring hypothesis because of the obviousness of one meiosis and the universality of the three mitoses. Unfortunately—and here is the crux in this theory, the one direct proof that would establish beyond cavil the correctness of Harper's assumption of two nuclear fusions—he could find no indications of a second meiosis. The claim that there is such is merely the theoretical deducing of one assumption from another. Fraser ('08) and her co-workers, it is true, state that they have detected in certain forms indications of the theoretical second reduction, but Guilliermond ('11) has examined some of the same species and has seen nothing of them. Guilliermond, Dangeard, Maire, Brooks, the author, Claussen, as also Harper, have found no second reduction in the many forms examined by them, and the search has been by no means perfunctory.

Further, it may be added that though the generality of the occurrence of eight spores in the ascus is striking it is not exceptionless, and is no more remarkable and perhaps no more significant than the regularity with which the Laboulbeniales produce two-celled spores, or the Fucaceae eight-nucleated oogonia, or the Angiosperms eight-nucleated embryo-sacs.

While Harper's interpretations have received very general acceptance, there have been dissenters. Most notable among them is Dangeard, who has maintained on the strength of his own researches that the primary sexual organs, except in *Eremascus* and *Dipodascus*, are no longer functional, that they have been abandoned, so to speak, and that the sexual phenomena have been transferred to the ascus, in which the only nuclear fusion in the life cycle takes place. The gamete nuclei, he affirms, must then come from vegetative cells of the thallus, presumably from the peripheral cells of the ascogenous hyphae, and may travel together for one or more generations, dividing conjointly before fusing in the ascus. It seems strange that Dangeard has never observed a fusion of antheridium and oogonium in some of the forms studied by him, for example, *Sphaerotheca*, *Erysiphe*, and *Pyronema*, in view of the joint testimony of several seasoned botanists that such exist. Having failed to do so, however, he has unequivocally challenged the reliability of the observations of Harper, Claussen, *et al.*, in respect to their assertion that they have observed these phenomena.

Maire ('05) has followed Dangeard's lead, and deems from what he has observed in *Galactinia* (cp. also Guilliermond ('05) on *Acetabula leucomelas*) that conjugate nuclei, or in his own terminology *synkarions*, are characteristic of the Ascomycetes, and hence that there is a more evident parallelism or homology throughout the Eumycetes than has been hitherto recognized.

Finally, Claussen ('07), after examining *Pyronema*, has burned his bridges behind him, and in a paper that bids fair to become a classic has championed an hypothesis similar to Maire's and Dangeard's, except that he locates the origin of the gamete nuclei that finally fuse in the ascus in the gametangia. He has observed migrations of nuclei from the antheridium into the oogonium and a pairing up of the nuclei in the latter, followed by what he takes to be conjugate divisions. What have been designated nuclear fusions in the oogonium he construes to be pseudo-fusions, for he finds a less intimate association between the pairing nuclei after they have entered the ascogenous hyphae. He is thoroughly satisfied in his own mind that all of the reported nuclear fusions in the gametangia (Harper ('96, '05), Claussen ('05), Fraser ('08), &c.) have been misinterpretations of what were really pseudo-fusions—an explanation that lacks demonstration and hence open to some objections.

A perusal of the literature of the last decade or more on this subject cannot but occasion amazement at the enormous amount of energy expended in the effort to determine whether or not nuclei fuse in the female gametangium, and equally so at the meagre positive results that have been attained. Very probably some actual fusions have been observed, but the number is small, and if the theory of synkarions holds they may well be regarded as reversionary. On the other hand, the question of conjugate divisions has not been in the minds of most investigators, and so the subject has not been approached with anything like the same vigour from this angle. In spite of that, part of the ground in that direction has been cleared, and with promise that further effort will lead to a final solution.

The first step in this connexion has long since been taken, for it has been proved again and again that the nuclei fusing in the ascus are not sister nuclei, but that they are daughters of the mother nuclei that undergo a simultaneous division. In other words, they afford an instance of conjugate division recurring with unfailing certainty at a definite stage in the life cycle, and which every one admits. Maire ('05) and Guilliermond ('05) have likewise shown that both pairs of this division remain together in *Galactinia* and *Acetabula*, and that both are presumably capable of fusion. Some attempts to trace the ancestral lines of these nuclei farther back have been made, but as yet, with the exception of Claussen's studies on *Pyronema*, any certain knowledge is fragmentary. Massee ('05) has pointed out that binucleate cells are characteristic of thalli of various Ascomycetes. McCubbin ('10) in a much more definite manner has shown that in *Helvella elastica* there may be several conjugate divisions prior to fusion. Brown ('10, '11) reports the same features in *Leotia* and *Lachnea*. My own observations indicate that not only is this true of *Helvella*, but it is demonstrable for a number of forms belonging to the Exoascaceae, Helvellineae, Pezizineae, Plectascineae, and Pyrenomycetineae, including the Hypocreales, Sphaeriales, and Laboulbeniales. The repeated proliferation of hooks and the fusion of terminal and antepenultimate cells as observed by McCubbin, and almost simultaneously by Brown, is not an abnormality, as Carruthers ('11) would have it, but is an exceedingly common phenomenon in the Helvellineae and Pezizineae. Nor are conjugate nuclei restricted to the penultimate cells. Often, especially in the Plectascineae and Pyrenomycetineae, but also in some of the Discomycetes, they occur most abundantly in terminal cells, from which any number of asci may spring, or from other cells which may or may not be definitely located.

In view of these data I am thoroughly convinced that any investigations on the sexuality of the Ascomycetes and any phylogenetic scheme drawn up for the group must take cognizance of the phenomenon of conjugate nuclei or synkarions. It is admittedly manifest in the peripheral cells of the ascogenous hyphae throughout the class, except in a few forms that are marked by great simplicity. Indeed, it ranks here next in the regularity of its occurrence to the intricate phenomena in the ascus itself, and there is increasing evidence that the nuclear divisions in the ascogenous hyphae are of this type. Once having arisen, it is readily understandable how such a variation so favourable to increased sporogenous productivity from a single pair of gametes would persist and develop in one or several directions. The main difficulty arises in discovering just where the beginning was made. At some stage in the evolution of the group the principle must have been adopted. That is self-evident, for, regardless of differences of opinion as to its use, every one admits that this principle is utilized by all so-called typical sac-fungi. But where are these ancestors to be sought?

Many have held that the Laboulbeniales are the living representatives of the first sac-fungi and that they had their beginnings in the red seaweeds. But this phenomenon of conjugate divisions only adds another specialization to the several of which no hint is given by the Florideae, and we know of no connecting links. It is true that our phylogenetic conceptions of the Ascomycetes are in such a plastic state that it is not inconceivable that delay in the fusion of gamete nuclei might have originated in some family of the Florideae, especially as there are good reasons for believing that this phenomenon has originated independently more than once in the Thallophyta. But in the present state of our knowledge it would seem a more reasonable working hypothesis from many standpoints to look to the less specialized sac-fungi for the progenitors of the group. In them the gametes copulate and at once produce a single ascus. In *Eremascus*, for example, there is a fusion of the gamete nuclei followed by the immediate formation of an eight-spored ascus (Stoppel, ('07)). By a division of these nuclei before fusion two asci would have been possible, and by repeated divisions, several; and by the elaboration of a system of *synkarion carriers* or ascogenous hyphae, an unlimited number.

It is, of course, to be borne in mind that, starting with the primitive forms, whatever they may have been, in which the phenomenon originated, several parallel lines have probably been worked out in which advantage of this method of multiplying the reproductive powers has been retained and elaborated. At the same time there would have been other changes of importance, such, for example, as an evolution of the sexual organs in more than one subgroup, resulting in heterogamous and apogamous conditions of varying complexity. In evidence, every one will admit that the sexual organs are among the most striking diagnostic features of such an assemblage as the Laboulbeniales. Simultaneously or otherwise, types of fruiting bodies would have been elaborated, especially with regard to protective and spore-disseminating adaptations. Obviously, whatever our conceptions of their race history may be, there will be agreement in believing that not one character alone but several have been involved in the evolution of the various subdivisions of the Ascomycetes, though there may be differences of opinion as to which, in certain instances, most importance is to be attached.

SUMMARY.

1. The cell-walls are laminated. The layer just below the general chitinous envelope is frequently differentiated into a fibrillar system by what appears to be a process of localized degeneration.

2. Single pits occupy septa separating cells of common origin. The protoplasmic bridges are typically very tenuous.

3. The protoplasts are monoenergid. In older cells the nucleus may repeatedly divide. Up to ten nuclei have been counted in a single cell of the receptacle.

4. No indications of antheridia in *L. chaetophora* or *L. Gyrinidarum* were found.

5. The procarp has its origin as a uninucleate terminal cell of a branch of the receptacle. The procarp consists of a uninucleate carpogonium, a uninucleate trichophoric cell, and a branched and septated trichogyne, each cell of which is monoenergid.

6. After the procarp is mature the carpogonium and trichophoric cell become continuous. Meanwhile the nucleus of the carpogonium is succeeded by two, which are apparently daughters of the carpogonial nucleus, and almost simultaneously the trichophoric nucleus undergoes division. Later a uninucleate trichophoric cell and a uninucleate inferior supporting cell are septated off from the now four-nucleated fusion cell. After further nuclear divisions a binucleate superior supporting cell and sometimes a binucleate inferior supporting cell are cut off. The binucleate ascogonium now begins to bud off asci or divides into two ascogenic cells, each of which contains a pair of nuclei. Up to this stage no nuclear fusions have been observed.

7. The nuclei of an ascogenic cell divide conjointly, a daughter of each passing into a young ascus. This process is repeated at the birth of every ascus. The pair entering the ascus soon fuse.

8. The fusion nucleus divides meiotically after a period of growth. The number of chromosomes is the same as in other mitoses.

9. There are two other mitoses prior to spore formation, and both are homotypic.

10. The spores are delimited by the method characteristic for ordinary sac-fungi. The astral rays do not fuse laterally to form the primary protoplasmic spore membrane.

11. Four only of the eight nuclei, the lower on each spindle, are functional in spore formation, the others soon degenerate.

12. The main theoretical conclusions reached are as follows:—

(1) the Laboulbeniales are true Ascomycetes;
(2) it is probable that the only nuclear fusion in the life cycle takes place in the ascus;
(3) conjugate divisions of nuclei constitute a significant phase in the sexual phenomena of the sac-fungi.

UNIVERSITY OF TORONTO,
October 23, 1911.

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DESCRIPTION OF FIGURES IN PLATES XXXVII–XL.

Illustrating Professor Faull's paper on the Cytology of *Laboulbenia*.

PLATE XXXVII.

Fig. 1. Part of appendages of a mature plant of *Laboulbenia chaetophora*. All of the septa except the two uppermost are represented in section. The two in black illustrate the septa as they ordinarily appear, because of the fact that the wall at the periphery of the septum is opaque black in nature and so obscures the inner details. × 2,700.

Fig. 2. The apical cell of an appendage. × 2,700.

Fig. 2 *a*. An ordinary septum from an appendage as seen in face view. The black line represents the peripheral black chitinous wall substance, and the opening in the centre the single 'pore' or 'pit'. × 2,700.

Figs. 3–6. Portions of cells from various parts of the main body of the mature plant, showing the structure of the protoplasm with granular inclusions as it appears in sections. Fig. 3 is from one of the lower and larger cells. × 2,000.

Fig. 7. The ordinary type of pit in the main body of plant. Hyaline cytoplasm and intensely staining granules occupy the depressions. The middle lamella is very finely perforated, if at all. × 1,350.

Fig. 8. A very exceptional instance of a wide open pore from the lower part of receptacle. × 1,350.

Fig. 9. A section of outer wall of a young plant, showing the chitinous covering, the fibrous layer in course of differentiation (the unstippled bars are the unchanged wall substance that later constitutes the fibrils), and the inner laminae streaked here and there by fine dark threads. × 2,700.

Fig. 10. A later stage, showing the fibrils. × 2,700.

Fig. 11. A part of the chitin torn away by the knife and to which several fibrils are attached. × 2,700.

Fig. 12. A bit of the outer wall, comprising the chitinous covering and the fibrous layer, shown in three dimensions. The porous appearance of the chitinous covering is due to underlying transparent fibrils. × 2,700.

Fig. 13. The surface of the fibrous layer immediately in contact with the chitin. × 1,800.

Fig. 14. A bit of the fibrous layer in an early stage of differentiation shown in three dimensions. The portions not stippled are unmodified parts of the layer which later give rise to the fibrils. × 1,800.

Fig. 15. From uninucleate stage of spore; the nucleus in division. × 2,700.

Figs. 16–18. Stages immediately following Fig. 15. In Fig. 18 the septum is complete. × 2,700.

Fig. 19. A multinucleate cell from the receptacle of a mature and probably old plant. × 900.

Fig. 20. A portion of thallus, showing pits in walls and cytoplasmic fibrils. × 1,800.

PLATE XXXVIII.

Fig. 21. Part of young thallus. Uppermost cell is the initial of the procarp. The cells just below give rise to wall of perithecium. × 600.

Fig. 22. Section through a young plant. The outer wall of perithecium is complete; one cell

of the inner wall, which is just beginning to form, is shown at the left. The carpogonium and trichophoric cell of the procarp occupy the axis of the perithecium. × 600.

Fig. 23. A slightly older stage. The inner wall of perithecium is now well on the way. × 600.

Fig. 24. A still older stage, although the inner wall of perithecium is not as far developed as in Fig. 23. The nucleus of the carpogonium appears to have divided into two, and the septum between the carpogonium and trichophoric cell is no longer visible. × 1,350.

Fig. 25. A later stage, in which the trichophoric nucleus is seen in the act of dividing. × 600.

Fig. 26. A later stage. × 600.

Fig. 27. An older procarp. The cells in order from the top down are, trichophoric cell, superior supporting cell, carpogonium, and inferior supporting cell. × 450.

Fig. 28. From the bottom up are the inferior supporting cell, secondary inferior supporting cell, ascogenic cells, superior supporting cell, and trichophoric cell. (Drawn from two sections.) × 600.

Fig. 29. In the axis are to be seen an ascogenic cell, secondary inferior supporting cell, and the remains of inferior supporting cell. × 600.

Fig. 30. Upper end of a young procarp, at the stage represented in Fig. 22, showing trichophoric cell and trichogyne. × 450.

Fig. 31. A combination of Figs. 22 and 30, with the trichogyne restored from other sections of same series, and hence in part conventional. × 450.

Fig. 32. An older stage than in Fig. 28. The young asci have begun to grow out. In the axis, from the upper part down, are the trichophoric cell, superior supporting cell, two asci, two ascogenic cells, secondary inferior supporting cell, and inferior supporting cell. Partly conventional. × 600.

Figs. 33–6 are transverse views of the same young fruiting organ taken at successively lower levels, and designed to show the position of the procarp and the relation of the two perithecial walls to one another. × 450.

Fig. 37. An ascogenic cell in which the nuclei are dividing, and an ascus in which the nuclei have not yet fused. × 2,700.

Fig. 38. An ascogenic cell and four of its asci. × 1,800.

Fig. 39. Nucleus of a spore in metaphase stage. × 2,700.

Fig. 40. Photograph of anal plate of host with two attached *Laboulbenia chaetophora.*

PLATE XXXIX.

Fig. 41. Two ascogenous cells with asci, as seen in a single section. The collapsed walls of asci from which the spores have been shed appear in the middle. × 1,350.

Fig. 42. Two young asci. × 1,800.

Fig. 43. A young ascus. The nuclei have not yet fused. × 2,700.

Fig. 44. A young ascus, the nuclei fusing. × 2,700.

Fig. 45. An ascus with young fusion nucleus. × 2,700.

Fig. 46. Part of ascus, showing fusion nucleus in synapsis. × 2,700.

Fig. 47. Fusion nucleus with what appear to be paired chromatin threads. × 2,700.

Fig. 48. A similar, but somewhat later stage. × 2,700.

Figs. 49 and 49 *a*. The same nucleus from two sections, showing the secondarily contracted chromatin. × 2,700.

Fig. 50. The fusion nucleus with its four chromosomes standing in relation to the central body, but as yet no spindle has been formed. × 2,700.

Fig. 51. The first spindle with central and mantle fibres and three of the four chromosomes. × 1,800.

Fig. 52. The first spindle with two chromosomes, the right of which at least appears to have broken into two parts. × 2,700.

Fig. 53. The first spindle, anaphase stage. × 2,700.

PLATE XL.

Fig. 54. The first spindle, a later stage. Only the central fibres have persisted. There are indications of vacuolar cavities marking the beginnings of the daughter nuclei. × 1,800.

Fig. 55. One of the two daughter nuclei in division. × 2,700.

Fig. 56. A rather earlier stage in mitosis of the nuclei shown in Fig. 55. × 2,700.

Fig. 57. The four-nucleate stage of the ascus. The sister nuclei are paired. × 1,800.

Fig. 58. A prophase stage of the third mitosis. × 2,700.

Fig. 59. A later period in the prophase. × 2,700.

Fig. 60. Equatorial plate stage in third mitosis. × 1,800.

Fig. 61. A later period of same mitosis. × 2,700.

Fig. 62. The daughter nuclei of the last mitosis about to form. Four of the eight shown—the two spindles have disappeared. Counting from the top the first and third are sisters. × 1,800.

Fig. 63. The young spores with nuclei still attached to one pole. Two of the four non-functional nuclei in upper part of ascus. × 1,800.

Fig. 64. Two young spores—a later stage than Fig. 63. The rays from the central body have floated in towards nucleus. × 1,800.

Fig. 65. A later stage. The necks of nuclei have been retracted and nuclei are now spherical. × 1,350.

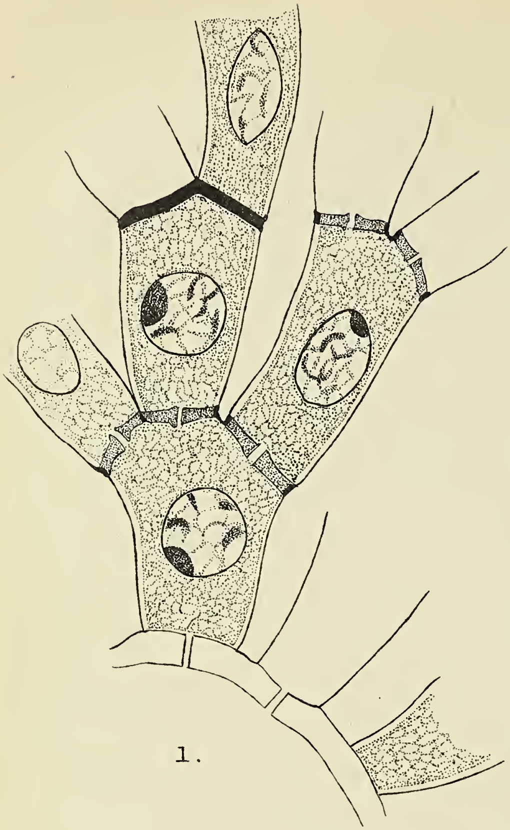
Fig. 66. A still later stage, showing the elongating spores and the still persistent non-functional nuclei. × 1,350.

Fig. 67. A single mature spore. × 900.

Figs. 68 and 69. The resting primary nucleus of spore. × 2,700.

Fig. 70. The primary spore nucleus in mitosis. × 2,700.

Annals of Botany,



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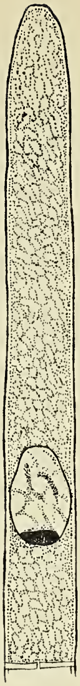

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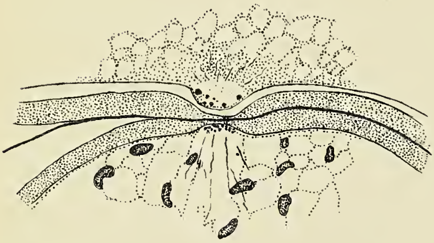

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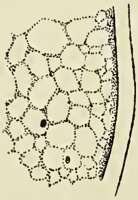

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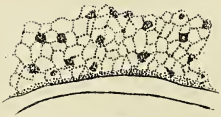

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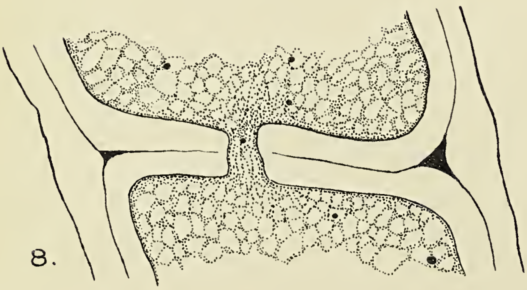

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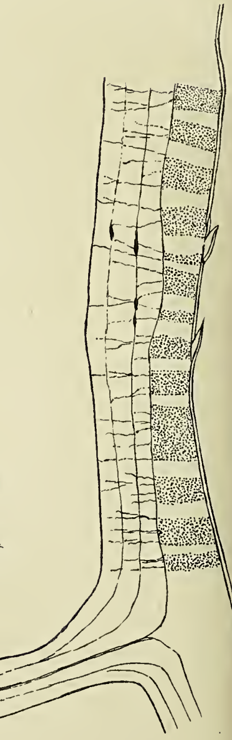

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FAULL — LABOULBENIA

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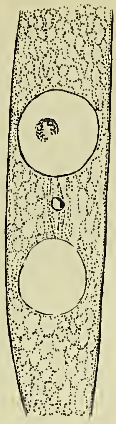

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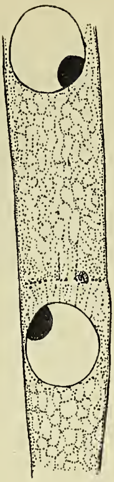

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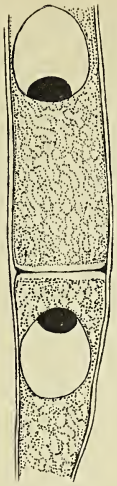

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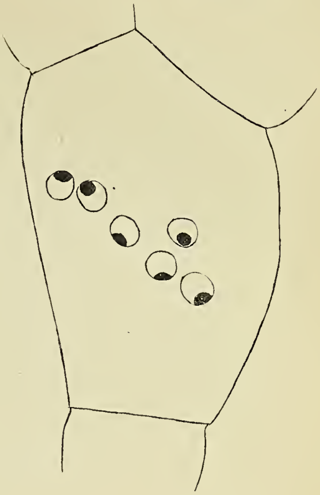

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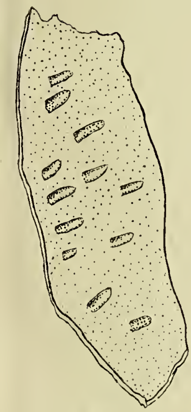

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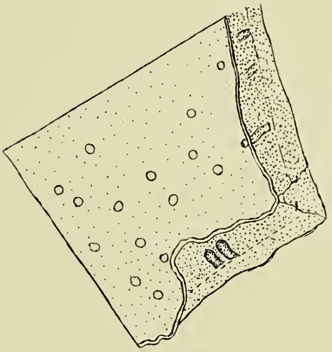

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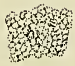

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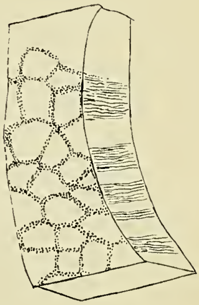

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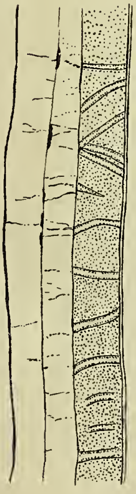

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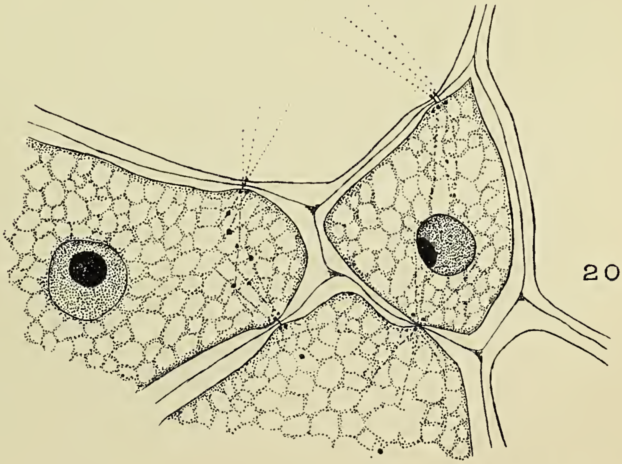

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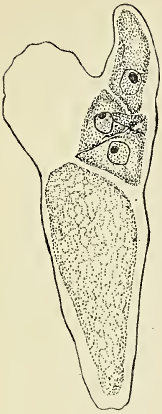
Huth, lith et imp.



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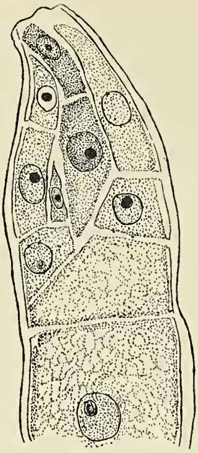

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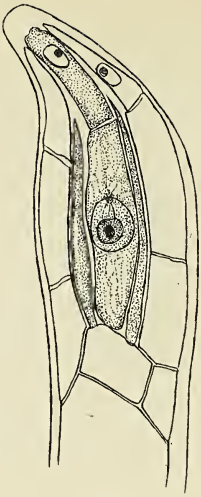

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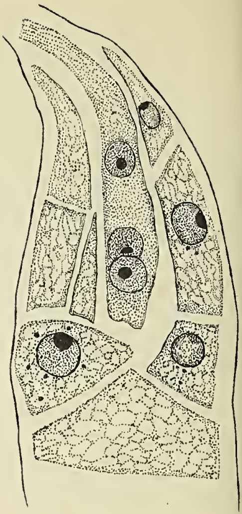

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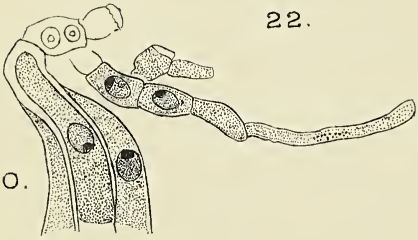

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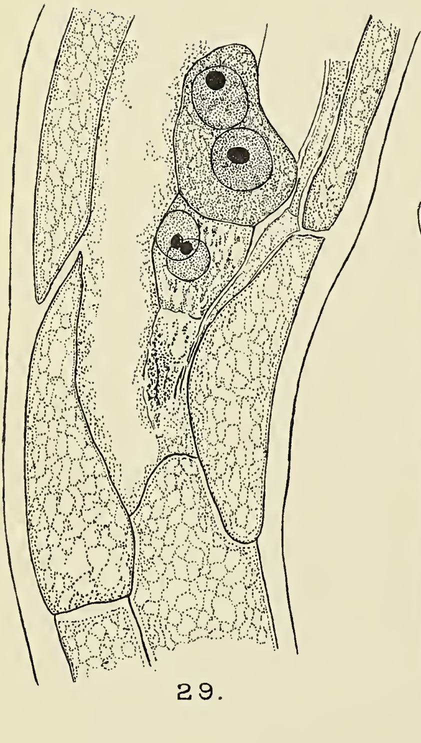

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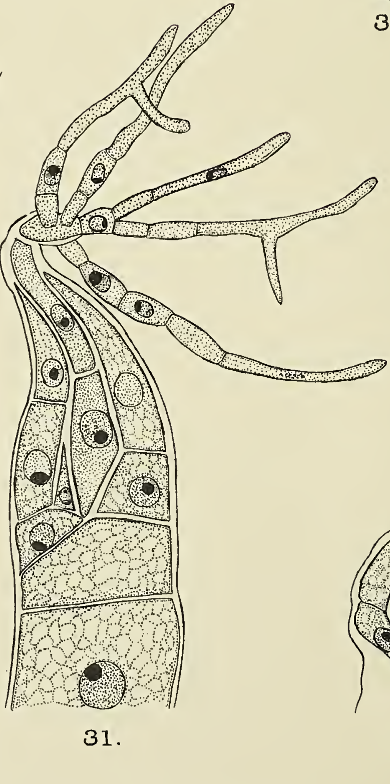

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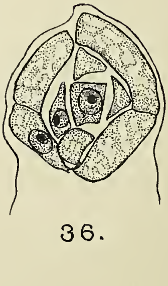

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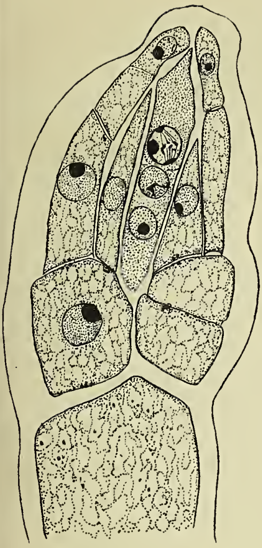
FAULL — LABOULBENIA



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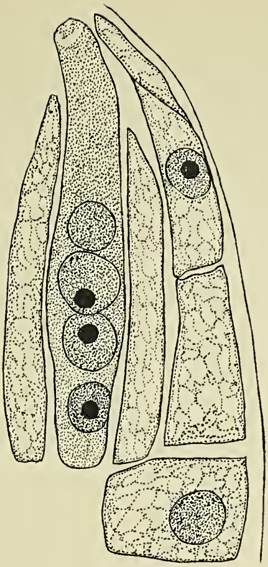

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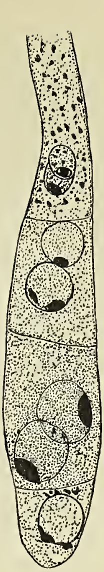

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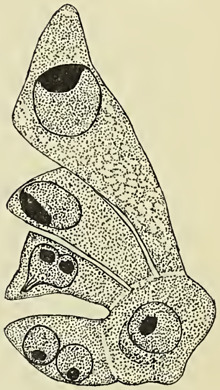

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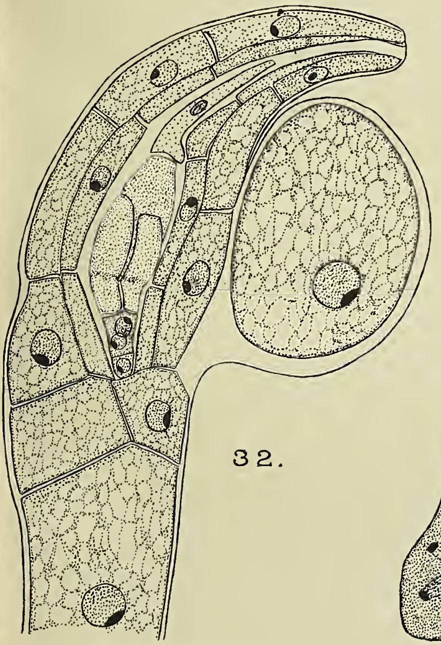

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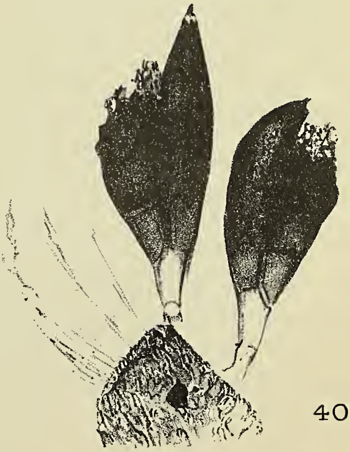


40.

Huth, lith. et imp.

The Cytology of Primula kewensis and of other related Primula Hybrids.

BY

L. DIGBY.

With Plates XLI–XLIV and two Figures in the Text.

At no time has the great problem of heredity, which involves such far-reaching results, been more widely discussed or more diligently studied than at the present day. It is now realized that the possibility of an ultimate solution must entail the study of genetics combined with that of microscopical investigation, for it is within the cells that the factors reside which determine the morphological and physical character of the individual.

Much work has been done on the cytology of hybrids, both on the animal and on the vegetable side, and in many cases it has been possible to compare the nuclei of the parents with those of the offspring. The appearance and general character of the cytoplasm have been observed, the number and form of the chromosomes and their method of pairing in the bivalent combination have been described. After consideration of these cytological facts, conflicting hypotheses have been put forward in explanation of the causes of their frequently sterile condition. At present many of the detailed results remain as uncoordinated data, though it may be confidently anticipated that they will, in the future, be united into an intelligible scheme. It is in the hope of adding another item to these communications that this piece of work has been undertaken. Having regard to the curious horticultural history of the hybrid *Primula kewensis*, it seemed that a record of its cytology might be of interest. The attempt has been repaid, as the nuclear phases are wonderfully clear and the numbers of the chromosomes so low as practically to eliminate all chance of error. Moreover, the origin of the bivalent chromosome is suggestively easy of interpretation, and may throw some light on this much-vexed question.

METHODS.

The buds have been capricious as regards fixation, and unless it has been good, the nuclei take the stain diffusely. In those cases where fixing and staining have been successful, nothing can exceed the clear definition of the nuclear phases. Sharpness of detail is characteristic of this series of Primulas.

[Annals of Botany, Vol. XXVI. No. CII. April, 1912.]

The buds have been fixed in strong Flemming, Hermann, alcohol and acetic, strong and medium chromo-acetic, and Merkel. Fixations have been made between 11 a.m. and 12.30 p.m. on bright warm days. Under these conditions plentiful nuclear divisions have been obtained.

It is found that the nuclei of many of the buds are in the state of rest which intervenes between the last premeiotic and the first meiotic division. This stage must be one of long duration, for during its period the whole bud grows considerably. There is again a resting stage during synapsis, for it is not unusual to find all the five anthers of a bud with their pollen mother-nuclei in close synapsis. From the coming out of synapsis to the completion of the homotype division the sequence of the nuclear phases is extraordinarily rapid. Often, in the microscope field of a transverse section of a bud, the nuclei of the youngest loculus will be in ‘hollow spireme’, whilst those of the oldest loculus will have already passed through the two meiotic divisions and be in the tetrad stage. The two outer loculi of an anther are always in advance of the inner two, and one loculus in each respective pair is slightly ahead of the other in the progress of nuclear division. Moreover, there is a great difference in the stages between the pollen mother-nuclei near the top of a given anther and those near the base of the same anther, so that by following the sequence of the sections of an anther cut transversely a perfect gradation can be obtained.

A variety of stains has been used, including Heidenhain's iron-alum haematoxylin with a counter stain; Flemming's triple; Breinl; methylene blue and eosin; gentian violet and orange G, &c.

HORTICULTURAL HISTORY.

It is well known that certain Primulas are not difficult to cross artificially, and occasionally accidental hybrids have arisen, and to this class belongs *Primula kewensis* (13 and 14). *P. floribunda* and *P. verticillata* were grown in quantities at Kew, and when in flower were constantly placed near to one another. In 1899 Mr. F. Garrett, the foreman, noticed in a pan of presumably pure *P. floribunda* seedlings one which was stronger in growth and different in foliage to the others. When it flowered it was evident that it was a hybrid, for it possessed the combined characters of *P. floribunda* and *P. verticillata*. It inherited from *P. floribunda* its continuous flowering nature, the colour of its flowers, and the shape of its leaves, and from *P. verticillata* its size, the length of its leaf-stalks, its foliaceous bracts, the character of its flowers, and the traces of mealiness on the corolla tube, on the upper part of the calyx, and on the leaves. The feature of mealiness is a most pronounced characteristic of *P. verticillata*. This hybrid plant, *Primula kewensis* (type form), was exhibited at the Meeting of the Royal Horticultural Society on February 27, 1900, and was awarded a First Class Certificate.

During the summer of 1900, in order to verify the supposition that *P. floribunda* and *P. verticillata* were in fact the parents of *P. kewensis*, the cross was successfully achieved artificially, using *P. floribunda* as the seed parent and *P. verticillata* as the pollen parent. A proportion of the resulting plants were true *P. kewensis* (29). The hybrid was sterile; all its flowers were thrum-eyed, and hence the stock could only be increased by cuttings or division.

In 1901 most of the sterile stock of *P. kewensis* passed into the hands of Messrs. Veitch and Sons of Chelsea. There it continued to bear only thrum-eyed flowers, until, about 1905, a single pin-eyed flower was noticed. This flower was fertilized with the pollen of a thrum-eyed flower and good seeds were set. These germinated, and the resultant plants bore both pin-eyed and thrum-eyed flowers and were fertile. Thus the whole present-day stock of the seedling or fertile *P. kewensis* has originated from the single pin-eyed flower on the type or sterile *P. kewensis.* Messrs. Veitch have since produced by selection, not by cross-breeding, a varietal form, which they have named *P. kewensis farinosa.* This resembles the fertile *P. kewensis*, except that it possesses the pronounced mealiness of the leaves, calyx, and corolla so characteristic of *P. verticillata.*

The cross between *P. floribunda* and *P. verticillata* has again been repeated at Kew by Mr. J. Coutts, the foreman, in 1910 (29), but this time the offspring resembles the seed parent *P. floribunda*, and not a single plant of *P. kewensis* has appeared. On the other hand, *P. verticillata* crossed with *P. floribunda isabellina* has given rise to plants resembling *P. kewensis farinosa*, and, as will be observed, the interest is further enhanced by the fact that the number of chromosomes in this hybrid coincides with that of the plants of *P. kewensis farinosa* which were obtained by selection from *P. kewensis* (seedling form).

Such is the horticultural history of *P. kewensis.* It is now necessary to compare the chromosome numbers in the various generations.

Chromosome Numbers in the Primula Generations.

Both parents, *P. floribunda* and *P. verticillata*, have 18 for their diploid and 9 for their haploid number of chromosomes. These numbers, as might be expected, are repeated in the hybrid *P. kewensis* (type).

The fertile (seedling) *P. kewensis* has, however, by some means doubled the number of chromosomes, and possesses 36 for its diploid and 18 for its haploid number. These numbers are also retained in the varietal form *P. kewensis farinosa*, and again reappear in the *P. kewensis farinosa* which has arisen at Kew (29) as the result of crossing *P. verticillata* with *P. floribunda isabellina*, the pale form of *P. floribunda.*

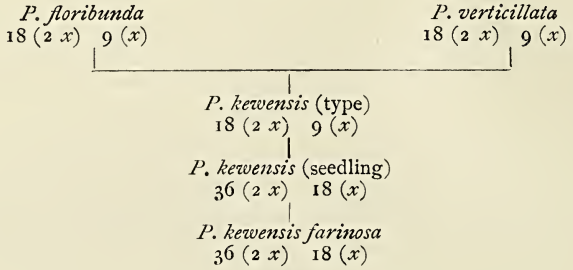
Material of the fertile *P. kewensis* has been obtained from several sources, and in every case the doubled number of chromosomes has been substantiated.

A similar example of the sudden duplication in the number of chromosomes has been found amongst the *Oenothera* mutants (31, 17, and 20). *O. gigas*, which is known to have originated from *O. Lamarckiana*, has 14 haploid chromosomes, whilst *O. Lamarckiana* has only 7 haploid chromosomes.

Another unlooked-for result has been obtained from crossing *P. floribunda isabellina* with *P. kewensis* (seedling form) (29). This cross resembles that of *P. floribunda isabellina* crossed with *P. kewensis* (type) in external characters as well as in the number of the chromosomes. Both hybrids have the typical *P. floribunda* number, 18 diploid and 9 haploid. In the case of *P. floribunda isabellina* × *P. kewensis* (type) this result would be expected, but in the case of *P. floribunda isabellina* × *P. kewensis* (seedling), where one parent has twice as many chromosomes as the other, there must have been some reducing process at work whereby the sum of $9x + 18x = 18\,2x$. An analogous reduction in the number of chromosomes in the hybrid, one of whose parents possesses a higher number than the other, occurs in the classical instance of *Drosera obovata* (33 and 34) and in a hybrid *Oenothera*.

According to Geerts (20), when *O. lata* with its 7 haploid chromosomes is crossed with *O. gigas* with its 14 haploid chromosomes, at the first meiotic division the 7 homologous chromosomes pair and the 7 superfluous chromosomes disintegrate. It may be that some such explanation may account for the elimination of the chromosomes in the *Primula* cross.

TABLE OF PRIMULA CROSSES WITH THEIR CHROMOSOME NUMBERS.



1910.

SEED PARENT.	POLLEN PARENT.	RESULT.	CHROMOSOMES.	
P. floribunda	*P. verticillata*	*P. floribunda*	18 (2 *x*)	9 (*x*)
P. floribunda isabellina	*P. kewensis* (type)	*P. floribunda isabellina*	18 (2 *x*)	9 (*x*)
P. floribunda isabellina	*P. kewensis* (seedling)	*P. floribunda isabellina*	18 (2 *x*)	9 (*x*)
P. verticillata	*P. floribunda*	*P. verticillata*	18 (2 *x*)	9 (*x*)
P. verticillata	*P. floribunda isabellina*	*P. kewensis farinosa*	36 (2 *x*)	? 18 (*x*)[1]
P. kewensis (type)	*P. floribunda isabellina*	No seed.		

[1] The (*x*) number of chromosomes has not been counted, the most advanced pollen mother-nuclei being only in synapsis.

My thanks are due to the authorities at Kew, through whose courtesy it has been possible to obtain buds of the original type form of *P. kewensis*, and of all the crosses made by the foreman, Mr. Coutts, during the year 1910.

Messrs. Veitch have kindly supplied material from their stock of seedling *P. kewensis* and of *P. kewensis farinosa*.

It is proposed to begin the account of the cytology of this series of Primulas with a description of the premeiotic divisions. The seedling *P. kewensis* has been selected for this examination on account of the larger size of its nuclei as compared with those of the other forms. This will be followed by a review of the meiotic phase of the seed parent, *P. floribunda*. Subsequently that of the pollen parent, *P. verticillata*; of the sterile and of the fertile *P. kewensis*; of *P. kewensis farinosa*, and of the various crosses of 1910 will be dealt with in succession, but only the points of difference in the meiotic phase of these as compared with that of the selected type *P. floribunda* will be noticed. Then it is proposed to consider briefly the mode of union of homologous lengths of spireme in the bivalent combination as exhibited in these Primulas, with regard to the 'parasynaptic' and 'telosynaptic' views. Finally, to survey the cytological work that has been done on hybrids, with special reference to the Oenotheras, which have so many features in common with the Primulas.

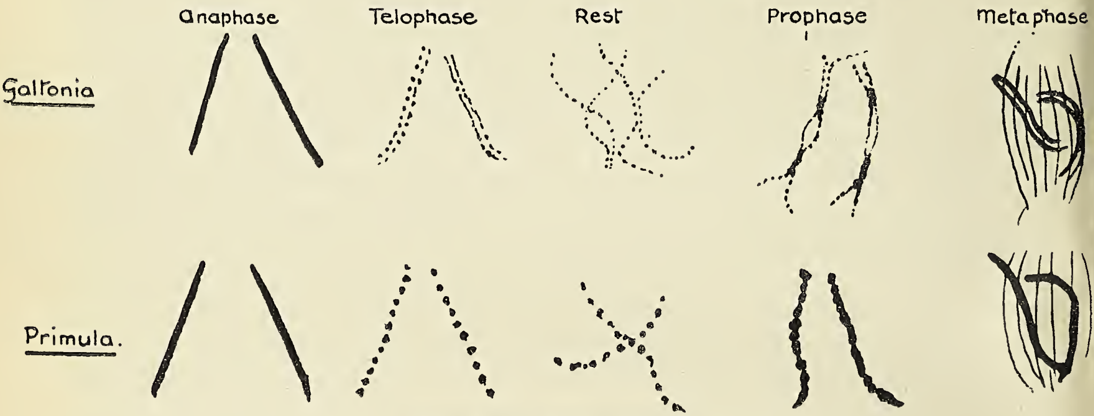
PREMEIOTIC DIVISIONS.

It has already been stated that there is a long rest between the premeiotic and meiotic divisions of these Primulas. Hence, unlike *Galtonia* (9) and other forms, where the one series of divisions passes imperceptibly into the other, in *Primula* there is no possibility of tracing the transition between the two. *Primula* therefore is not illuminating as regards the origin of the parallel threads and paired chromatin masses in the heterotype prophases.

In a telophase of one of the premeiotic or of the somatic divisions, no diagrammatic alveolization of the chromosomes is to be found, resulting in the formation of two parallel threads (9 and 21), phenomena which are so beautifully displayed by the nuclei of some plants. In the Primulas the substance of each entire chromosome breaks up, for the most part transversely, into rounded granular-like portions (Pl. XLI, Fig. 1). Thus these granules are bits of *whole* somatic chromosomes, not bits of the *longitudinal halves* of somatic chromosomes. In *Primula* the granules may fragment into smaller and smaller particles, but in this fragmentation it would of course be impossible to say whether the division were longitudinal or transverse. In a late telophase the nucleus is traversed by a very fine arrangement of threads, and on these threads chromatin granules of various sizes are carried (Fig. 2).

When the nucleus enters upon the prophase, the granules trend together like beads on a string (Fig. 3). At first they take the stain lightly, but as they increase in volume and in definity they become more and more chromatic. Gradually the limits of the beaded individual chromosomes become discernible, and fine threads are seen connecting the beads of one chromosome to those of another (Fig. 4). Then the beaded appearance becomes less and less marked, until finally it blends into the homogeneous chromosome with a smooth outline (Figs. 5 and 6). The chromosomes go on to the spindle, and the neat compact equatorial plates are most characteristic. It is only when on the equatorial plate that the longitudinal fission in the substance of each chromosome becomes discernible.

Thus the different manner in which the formation of the mature somatic chromosome is accomplished in the Primulas as compared with



TEXT-FIG. 1. Somatic division.

such forms as *Allium* (21), *Galtonia* (9), &c., is simply due to the fact that in the Primulas the longitudinal halves of the portions of segmenting chromosomes during telophase tend to remain together, whereas in *Allium*, &c., the longitudinal halves of the portions of segmenting chromosomes during telophase tend to separate (Text-fig. 1). In this latter case the separation of the two halves results in two parallel threads, each of which is a portion of the longitudinal half of a somatic chromosome. During the ensuing prophase these longitudinal halves come together again to form the whole somatic chromosome, but often this union is incomplete, leaving a space (longitudinal fission) between the halves which will take effect and separate the two daughter chromosomes when they are arranged on the equatorial plate. On the other hand, in the Primulas, the homogeneous, unsplit chromosome of the telophase breaks across transversely into portions, which therefore represent pieces of whole somatic chromosomes. During the ensuing prophase these whole pieces come together end to end, remaining homogeneous, and do not display any inherent longitudinal fission until the chromosomes are about to proceed to the poles.

This tendency of the longitudinal halves of the univalent chromosomes to adhere to one another is also characteristic of the heterotype divisions, for in the early hollow spireme stage the univalent strands are homogeneous, and it is scarcely ever possible to detect any longitudinal fission in their substance.

During the somatic prophases the nucleolus buds constantly. This budding is carried on to a still greater extent during the heterotype prophase.

MEIOTIC PHASE OF P. FLORIBUNDA.

There is a long-drawn-out rest between the last premeiotic and the prophase of the first meiotic division. At the last premeiotic telophase the whole chromosome breaks up transversely into rounded portions (Fig. 1). During the rest, these granules for the most part disappear; perhaps they are absorbed by the large nucleolus. The nucleolus occupies the centre of the nucleus and usually stains very deeply, but sometimes appears as a plasmosome. The nucleolus, whether it be chromatic or not in staining reaction, buds freely (Figs. 7 and 8). Apparently it acts as the store-house for chromatin, which it deburses by exuding droplets of its substance into the nuclear cavity. The small buds whilst still attached to the nucleolus are more faintly staining, but as they separate they become chromatic and take a very bright stain, and pass to the periphery of the nucleus. Very delicate connexions join these round chromatic bodies to one another and individual bodies to the nucleolus. When the bodies arrive at the periphery they break up into smaller granules which as a rule stain less brightly. These bodies might be considered as representing prochromosomes, but without doubt they are derived from the nucleolus, and they are irregular in size, in number, and in shape. The cytoplasm round the nucleus, at this stage, is very dense.

A precisely similar resting stage has been described by Gates (15) and by Davis (5) in the Oenotheras.

As the nucleolus continues to bud, the chromatin in the nucleus shows a decided increase, and this is accompanied by growth of the nucleus (Fig. 9), which may be said now to have entered upon the prophase. At the same time the fine connexions form more definite strands (Fig. 10), and the rounded beads of chromatin arrange themselves on the threads (Fig. 11). Sometimes several beads amalgamate to form an irregular mass, the earliest indication of the preparation for the coming synapsis (Fig. 12). In places the linin threads, bearing the chromatin beads, are seen to run parallel to one another and sometimes to join (Figs. 10, 11, 12).

It is impossible to determine the significance of the parallel threads present in the heterotype prophase, owing to the undecipherable character of the nuclear phases that immediately precede and succeed it. During the rest which intervenes between the telophase of the last archesporial division and the prophase of the heterotype, the chromosomes resolve themselves so completely that all trace of their identity is lost to view, and the arrangement of their units passes beyond the limit of interpretation. Again, during synapsis, the course of events is completely veiled by the dense tangle of the spireme. The parallel threads may either represent the pairing of whole univalent lengths of chromosome, according to Grégoire's view (22), and in that case each thread would be homologous with a length of univalent beaded spireme of the premeiotic prophases, or they may represent the coming together of the halves of univalent lengths of chromosomes preparatory to the condensation for the whole somatic (univalent) chromosome. In *Galtonia* the homology between the parallel threads of the last premeiotic division (the result of the alveolization of each chromosome) and the parallel threads of the heterotype prophase can be traced through a continuous and progressive series. In both nuclear phases the parallel threads represent the longitudinal halves of the univalent chromosomes. During the telophase the process of the breaking up of the chromosomes and the distribution of their substance throughout the nucleus entail a *separation* of the longitudinal halves, whilst during the prophase the gradual evolution of the chromosomes involves an *approach* and an increasingly closer association of the longitudinal halves. It is believed that the same interpretation of the parallelisms found in the heterotype prophases may hold good for *Primula*, but for the reasons already given the surmise cannot be directly verified. Moreover, in *Primula*, as in other forms, it seems probable that the considerable thickening of strands may be due to the premature pairing of whole univalent chromosomes, a pairing which, in *P. floribunda*, is most clearly shown in the post-synaptic stages. When it is remembered that the completion of a mature bivalent chromosome entails not only the condensation of the two split halves of each univalent chromosome, but also the union of the two univalent chromosomes to form one bivalent combination, it does not seem irrational to believe that preparations for both may be proceeding simultaneously in the presynaptic stages.

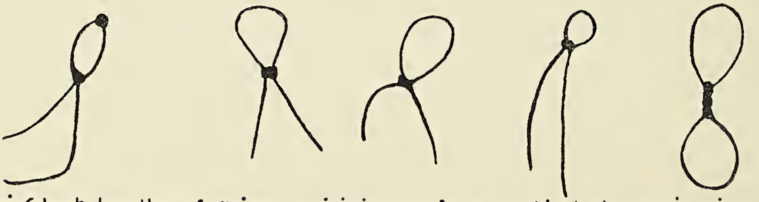
The chromatic contents of the nucleus increase and concentrate into groups (Fig. 13), and diffuse throughout the linin. Gradually the whole masses together at one side of the nucleus. Parallelisms in the escaping portions can often be seen; and faint strands of linin may extend to the periphery of the nucleus as loops, or apparently as free ends. At complete synapsis the chromatin mass has a very granular appearance, and is contracted into an extremely small space (Fig. 14). *Primula* does not conform to Lawson's view (30) that there is no chromatin contraction during synapsis. The nucleolus is generally to be seen projecting from the synaptic knot, and is for the most part cytoplasmic in staining reaction, and once more buds freely. Droplets of nucleolar material may adhere to the chromatic knot (Fig. 14). Such droplets have been recently figured by Tischler (40) in *Musa sapientum*, var. *Kladi*, and he suggests that they may be of an excretory nature. They were also frequently seen in the synaptic figures of *Galtonia* (8 and 9).

As the synaptic knot unravels it loses its granular appearance and becomes more thread-like (Fig. 15). Occasional rounded chromatin masses are associated with the more definite lengths of spireme, and globules of faintly staining nucleolar material may be suspended with the chromatic substance (Fig. 15). The nucleolus is often pushed out first, and lies caught up by the loops in the clear space of the nuclear cavity. The appearances displayed by the loosening spireme are very various. For the most part it emerges in the form of loops, which stain homogeneously and show no longitudinal fission in their substance (Fig. 17). The looped spireme which comes out of synapsis and constitutes the familiar 'open spireme' stage is (for the most part) univalent in nature. It is, therefore, homologous with the univalent spireme of the somatic prophases. In the somatic prophases the univalent spireme segments directly into the independent univalent chromosomes; in the heterotype prophases, subsequent to the 'open spireme' stage, a pairing of univalent lengths of spireme takes place, resulting in a temporary association of two univalent chromosomes, forming the typical bivalent heterotype chromosome. Sometimes lengths of thick spireme, with bifurcating ends, emerge (Fig. 16). In some cases it seems obvious that the univalent lengths of spireme have already joined side by side—have, in fact, completed the approximation sometimes prepared for in the heterotype prophase. Especially in superficial sections of loosening knots, parallel threads arranged in a ladder-like way, united by bands of chromatin, are constantly to be found (Fig. 18). Notwithstanding the fact that some of the spireme, as it emerges from synapsis, may be bivalent in nature, yet the greater part is univalent, and it is only later that the definite arrangement of the individual univalent lengths into pairs to form the bivalent combinations takes place. Thus it seems probable that the rearrangement during synapsis has been chiefly concerned in the *formation* of the univalent lengths of spireme, and has not participated to any great extent in the *pairing* of the homologous univalent lengths of spireme. The looped nature of the spireme becomes more and more accentuated, and the already approximated portions tend to separate and to open out (Fig. 19). At the places of anastomosis there is always a chromatic swelling (Figs. 19 and 20).

During this time the nucleolus continues to bud freely. The buds become chromatic, and for a time remain tangled in the spireme. Sometimes more than one nucleolus is present.

At this and subsequent stages the nucleolus may stain either cytoplasmically or chromatically; its reaction does not depend on the fixative, for contrary results may be obtained in buds killed in the same manner.

When the coming out of synapsis is completed, and the nucleus has returned to the centre of the cytoplasm, the beautiful looping of the *continuous spireme* is most striking (Fig. 20); by this time the chromatic aggregations have been absorbed. Although there is always a certain amount of anastamosis, it is often possible to focus the loops passing freely and separately within and without one another. If the spireme be both *continuous* and *univalent*, then the future univalent chromosomes must necessarily be arranged *end to end* in the spireme. This supposition is supported by subsequent phenomena leading to the realization of the heterotype chromosomes. The loops show no longitudinal fission in their substance; they are homogeneous, smooth surfaced, and faintly staining. The earliest indication of the association in pairs of homologous lengths of univalent spireme is the withdrawal of the spireme from the periphery of the nucleus, which at the same time becomes arranged in fewer and more distinct loops (Fig. 21).



Univalent lengths of Spireme joining to form the bivalent combinations

TEXT-FIG. 2.

The sides of the loops tend to approach one another and finally to join in places (Fig. 22). At the points of union there is always a chromatic swelling (Figs. 22 and 23). Sometimes the two sides of the same loop approximate, sometimes it appears as if the side of one loop crossed over and associated itself with the side of another. The nipping in of the loops at the places of contact and the otherwise somewhat wide divergence of their sides produce a variety of figures as shown in the diagram (Text-fig. 2).

This is the beginning of the union of univalent strands to form bivalent combinations. As will be shown, each side of one of these loops becomes eventually a univalent chromosome. By degrees the sides of the loops come to lie closer together, and are concentrated into more definite figures (Fig. 24). Then each bivalent combination, the future heterotype chromosome, begins to dissociate itself from its fellows, and for the first time free ends are to be seen (Fig. 24). A still closer approximation of the univalent sides continues, forming thick bivalent strands (Fig. 25). Sometimes this approximation takes the form of a ladder-like union (Fig. 24), as was described in the loosening of the synaptic knot (Fig. 18). There is a great variety, even in a single nucleus (Fig. 26), in the appearance of the chromatic segments at this stage, owing to the degree of progress exhibited in the process of the combination of the univalent strands. Some of the thick bivalent portions may open out into univalent loops ; in others, the sides of the loops may be still apart and only united at intervals, whilst in others the loop may be curved like a ring, the future ring-shaped chromosome. Although, perhaps, in some cases the univalent homologous segments of a bivalent combination may never be intimately joined together, yet, as a rule, at this stage, most of the univalent segments are united side by side in pairs forming thick strands (Figs. 26 and 27), which always reveal their double character.

There is no definite second contraction in *P. floribunda* ; it seems to be represented by the close approximation of univalent segments in each separate bivalent combination (Figs. 26 and 27). This fact materially simplifies the difficulties as regards the evolution of the bivalent chromosome, for so frequently the massing of the segments in the second contraction hides the sequence of events. In *P. floribunda* it is often possible to identify the limits of the nine bivalent chromosomes (Fig. 27) at this stage.

Having accomplished the necessary lateral approximation, the two univalent sides then proceed to split apart (Fig. 28). At first this splitting may be restricted to certain lengths, leaving places where the two are still in contact (Figs. 28, 29, and 30), thus repeating the figures of their first association, but very rapidly the splitting extends until the two univalent chromosomes are only attached at one end, or at both ends when a ring-shaped chromosome results (Fig. 31). At the place where the two chromosomes are in contact there is constantly a chromatic swelling (Fig. 31) like that which has been described at the points of union of the loops of the spireme. As the split separates the chromosomes, the chromatin of each becomes concentrated (Fig. 31).

The sides of the bivalent chromosomes thicken considerably and their staining power intensifies. They are typical and beautifully shaped heterotype chromosomes, and there are usually three or four rings amongst them. Radiations from four centres appear in the cytoplasm (Fig. 32), and these extend to the nucleus, which by this time has lost its limiting membrane. Sometimes the four apices of the spindle can be detected in the microscopic field. The quadripolar spindle may persist for some time, but eventually it becomes bipolar. The chromosomes, which by this time have considerably contracted, attach themselves to the spindle. The spindle tapers to a point at either end and the fibres are coarse and distinct. Sometimes, in badly fixed material, where there has been much contraction, the spindle remains entire, showing that it is of so tough a consistency as to be untouched by violent chemical influences. The rigid character of the spindle radiations has been described by Farmer (10, p. 475) in *Fossombronia.* Often a laggard chromosome caps one of the spindle poles (Fig. 33), and then, belated, joins the other chromosomes on the plate. The equatorial plate is very neat, the chromosomes, dumb-bell or kite shaped, being arranged in perfect symmetry. A transverse section of this stage shows the nine chromosomes (Fig. 34). Gregory (24) has found the reduced number of chromosomes in *P. sinensis* to be twelve.

The nucleolus disappears as the chromosomes go on to the spindle. The separation of the univalent chromosomes and their retreat to the poles, to which they are drawn by stout spindle fibres, is very clearly demonstrated. The chromosomes are round or oval in shape, they stain deeply, and show no sign of longitudinal fission (Pl. XLII, Fig. 35). Arrived at the poles, they at first, in the usual manner, mass together and lose their individuality. Then they separate, fine threads join the segments one to the other, a nucleolus makes its appearance, and the nucleus becomes bounded by a limiting membrane. Cytoplasmic connexions *passthrough the cell walls* and join diasters of adjacent cells to one another (Fig. 36). These connexions may be observed at earlier stages, but they are the more striking at the diaster, when the cytoplasm, contracted from the cell-walls, is mainly collected round the spindle. The fact that these cytoplasmic strands pierce the cell-walls gives a possible explanation for the transit of the chromatin bodies from one cell to another (8). Gates (15) has figured similar protoplasmic connexions between the pollen mother-cells of *Oenothera rubrinervis*.

To return to the telophase. The stages leading from the heterotype to the homotype division are extremely quickly passed through, in fact sometimes the chromosomes appear at once to reconstitute themselves. The more or less homogeneous masses of chromatin, representing the individual chromosomes, split longitudinally (Fig. 37), their sides become beaded (Fig. 38), and fine threads join the various portions to one another. The nearest approach to a resting stage that has been found is a nucleus containing the skeleton of each chromosome clearly outlined by granules, and amongst them some indeterminate groups of granules (Fig. 38).

The prophases of the homotype are indistinguishable from the telophases of the heterotype division, and the only means of recognizing the one from the other is that, by the time that the chromosomes are reconstituting themselves, there is no trace left of the spindle fibres of the heterotype division. The chromosomes are re-formed by the concentration of the granules. They show complete longitudinal fission, the two halves being in the same relative position to one another as the limbs of a V or of an X (Fig. 39). The figures are markedly tetrad-like in character (Fig. 40). Sometimes the fully formed chromosomes are composed of concentrated chromatin, sometimes they are beaded. Spindle fibres appear, and the chromosomes attach themselves irregularly (Fig. 40), but they finally collect at the equator, where they form a neat circle of dyads (Fig. 41). One spindle is generally at right angles to the other, but occasionally they may both lie in the same plane. In a transverse section of an equatorial plate of the homotype division, the chromosomes are seen to be arranged in a square of three (Fig. 41). The spindles taper bluntly. As in the heterotype anaphase, the chromosomes, on arriving at the poles, form themselves into a compact mass, and then separate out once more, and a nuclear wall forms (Fig. 42). The chromosomes then proceed to break up into granules. In the resting tetrad nuclei these granules are scattered in the nucleus, and are joined together by fine connexions. The cytoplasm between the nuclei constricts, and each of the four nuclei, surrounded by its cytoplasm, becomes an independent cell. At the late telophase of the homotype division the protoplasmic threads perforating the cell-walls and joining tetrad groups to one another may still persist. The pollen-grain is tetrahedral in shape.

P. VERTICILLATA.

P. verticillata is a stronger and larger form than *P. floribunda*; the pollen mother-nuclei in both plants are almost identical in size, but the pollen-grains of *P. verticillata* are considerably the larger of the two.

The resting, presynaptic, and synaptic stages are similar to those already described in *P. floribunda.* From the synaptic stages onward there is, however, a decided modification on the simple process adopted by *P. floribunda* in the formation of its bivalent chromosomes, a process which can be clearly followed throughout its course, and is not marked by the confusion of the second contraction. *P. verticillata*, on the other hand, has a well-marked second contraction from which the bivalent chromosomes emerge. This is but another example of a feature which these Primulas share with the Oenotheras. Gates found a well-marked second contraction in *Oenothera rubrinervis* (15), whilst Davis saw none in *O. biennis* (5).

During the early hollow spireme stage of the heterotype division, tracts of spireme trend together, forming a flat, deeply staining, band-like chromatic mass (Fig. 43). From this mass strands pass to the periphery of the nucleus. These strands are irregular in their distribution, vary in thickness, and anastomose freely with one another. The usual chromatic swellings are found at the places of anastomosis. At a slightly later stage the chromatic mass has further increased in bulk and is lumpy and thick in appearance, and from it radiate a few irregular loops of spireme (Fig. 44). Gradually, though the central chromatic concentration remains undecipherable, the escaping portions of spireme are seen to have a more definite arrangement and significance (Fig. 45). These may be in the form of loops, the univalent sides united at the apex, where there is the familiar chromatic swelling; or free bivalent ends may project showing the intimate side by side union of univalents; or loops of univalent spireme may be seen, anastomosing freely, but showing no approximation of homologous lengths. It is difficult to get a good fixation of this stage. In these preparations there has always been a considerable amount of contraction which is displayed by the crinkled edges of the cytoplasm. Gradually, as the second contraction becomes sorted out, the protruding portions of spireme thicken considerably, and clearly show their bivalent nature. Sometimes in the same nucleus (Fig. 46) some of the chromosomes may be almost fully formed, whilst the outline of others may be but indicated by the union of univalent spireme lengths, recalling the figures in the realization of the bivalent segments in *P. floribunda.* As the bivalent chromosomes emerge from the second contraction, they are often in the shape of rings joined together like the links of a chain (Fig. 47). Several rings may go to form one bivalent chromosome, for a large loop of spireme may become constricted in one or more places, resulting in a spectacle-shaped or chain-like chromosome.

When the nine bivalent chromosomes have been evolved out of the confusion of the second contraction they tend to segregate into two groups, and often the chromosomes in these groups become so pressed together as to lose their visible individuality (Fig. 48). Sometimes the chromosomes split into their univalent halves after they have issued from the second contraction. In that case the usual deeply staining, unsplit portions can be sharply distinguished from the symmetrically arranged, lightly staining, newly split univalent halves (Fig. 50). Under favourable conditions, but very rarely, the longitudinal fission in the but lately split limb can be seen (Fig. 49). The univalent chromosomes remain attached at one end, condense, and thicken considerably, and adopt the typical heterotype form (Fig. 51). There are always several large ring chromosomes. A transverse section of an equatorial plate shows the nine diagrammatic chromosomes (Fig. 52). The spindle is at first quadripolar, then becomes bipolar, and the univalent chromosomes proceed to the poles in the normal way. At the late telophase of the first meiotic division, or the early prophase of the second meiotic division, the nine longitudinally split chromosomes are beautifully clear (Fig. 53).

There is nothing further to add about the homotype division. It has already been mentioned that the pollen-grains of *P. verticillata* are larger than those of *P. floribunda.*

P. KEWENSIS (type).

The stages prior to the coming out of synapsis are like those of *P. floribunda* and *P. verticillata.*

In the formation of the bivalent segments, *P. kewensis* (type) adopts a method intermediate between the simple pairing of univalent strands characteristic of *P. floribunda* and the well-marked second contraction of *P. verticillata.* Although *P. kewensis* has no definite second contraction, there are small, isolated, thickened, chromatic areas to be found, formed by the massing, in places, of several univalent segments.

P. kewensis (type) resembles *P. verticillata* in the tendency of the bivalent chromosomes, after their evolution, to segregate into two groups, and also in the formation of chains.

As the spireme comes out of synapsis, it is much looped, and these loops intersect one another; at the junction there is the usual chromatic swelling (Fig. 54). By the time that the nucleus has returned to the centre of the cell, portions of chromatin of one nucleus may be protruded into the adjoining cell, at the same time retaining a connexion with the mother nucleus (Fig. 55). This phenomenon has not been seen in the other species of *Primula* examined, and recalls a similar condition figured by Gregory (23) in the Sterile Pea. These chromatic protrusions when found in *Primula* are not nearly so striking as the 'bodies' which are thrown off in *Galtonia* (8), and moreover suggest possible nuclear degeneration.

After the univalent loops have arranged themselves throughout the nuclear cavity, the spireme is as a whole much more beaded in character than is that of either of the parents. The beaded lengths of spireme arrange themselves in squares and in parallel lines, and in some places they unite to form the bivalent combination (Fig. 56). A single nucleus may exhibit very varied contents, for the same nucleus may have portions of its spireme in the beaded condition, whilst other portions may be homogeneous and pair as in *P. floribunda* (Fig. 57). Where the rows of beads, or the lengths of strands come together, there is always a decided thickening. A ladder-like condensation of univalent spiremes is of very common occurrence (Fig. 58). Strands may coalesce in one or more places, and create an undecipherable mass which may be described as an attempt to form a second contraction (Fig. 58).

In *P. floribunda* there is a stage when all, or nearly all, of the univalent segments are joined in their homologous pairs prior to the final splitting apart. On the other hand, in *P. kewensis* (sterile), some of the univalent lengths may be in the act of pairing, whilst others may already be splitting apart, and it is possible that in some cases a close union of the univalent members of each pair may never be achieved. Gates (15) has described the attraction for pairing between homologous chromosomes as being very weak in the mutant *O. rubrinervis*.

All stages in the splitting apart of the joined univalent segments may be seen (Fig. 59). These then concentrate and thicken, until the forms of the future bivalent chromosomes are recognizable (Fig. 60). When the bivalent chromosomes are individualized they tend, as in *P. verticillata*, to mass together into two groups (Fig. 61). When they again distribute themselves throughout the nucleus chains of rings are constantly to be seen (Fig. 62), and, as in *P. verticillata*, it is evident that several rings go to form one bivalent chromosome.

Then the univalent limbs of each bivalent chromosome diverge widely, and together they fill up the greater part of the nuclear cavity (Fig. 63). This is followed by a shortening and thickening of the chromosomes (Fig. 64), the spindle makes its appearance and the chromosomes attach themselves to the fibres (Fig. 66). A transverse section of an equatorial plate shows the typical nine chromosomes (Fig. 65).

There is nothing further to add about the heterotype division; this is followed by the homotype resulting in the formation of tetrads (Pl. XLIII, Fig. 67). It is only when the tetrad nuclei begin to separate from one another that some of the cells atrophy. A relatively small proportion of the pollen-grains appear to be normal (Fig. 68), but there are always depauperate ones amongst them (Fig. 69). Some of the anthers have a far higher percentage of healthy pollen-grains than have others.

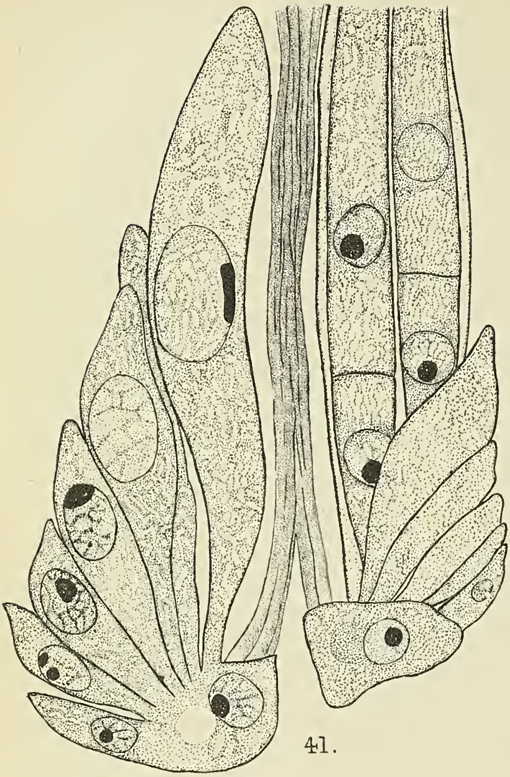
In order to ascertain that apogamy is not responsible for the unaccountable doubling in the numbers of the chromosomes the embryo-sacs of both *P. kewensis* (sterile) and *P. kewensis* (fertile) have been examined. In both cases everthing is regular. In *P. kewensis* (sterile) 18 chromosomes (Fig. 70) are found in the nuclei of the nucellus, and 9 in the nuclei of the embryo-sac mother-cell (Fig. 71).

P. kewensis (seedling form).

The striking and all-prevailing difference between *P. kewensis* (fertile or seedling) and those Primulas to which it is related is the fact that it has doubled its chromosomes. By some means, probably at the act of fertilization of the single pin flower, by pollen of the thrum flower, borne on the sterile stock, the 4 (x) number of chromosomes has been obtained, and this 4 (x) number is characteristic of all its subsequent generations. Moreover, *P. kewensis* with the 4 (x) number is fertile, whilst *P. kewensis* with the 2 (x) number is sterile. *P. kewensis* (fertile) has therefore 36 diploid (Pl. XLI, Fig. 6) and 18 haploid chromosomes (Pl. XLIII, Fig. 96). This double number is continued in the variety *P. kewensis farinosa* (Pl. XLIV, Figs. 107 and 108).

A most interesting recurrence of the doubling of the chromosomes has reappeared in the *Primula* crosses made at Kew in 1910 (29). The result of *P. verticillata* crossed with *P. floribunda isabellina* is a hybrid, which not only resembles *P. kewensis farinosa* in its external characters, but also with regard to its nuclei, for it possesses the number of chromosomes characteristic of the fertile *P. kewensis*, namely 36 (2 x) (Fig. 108) chromosomes, that is to say it has duplicated the parental number. Thus the variety *P. kewensis farinosa* has arisen as the direct result of a cross, and indirectly as the result of a cross which has passed through an intervening sterile period. In both cases the number of chromosomes has been doubled.

The phenomenon of the sudden duplication of a chromosome number



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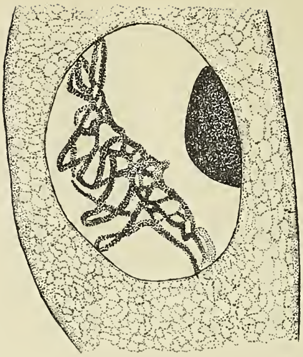

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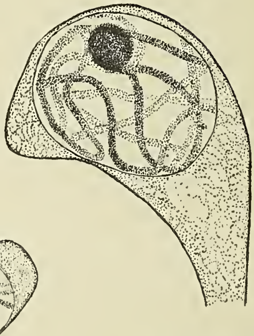

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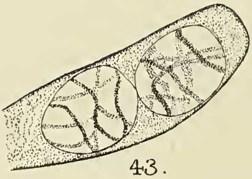

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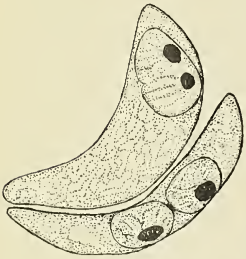

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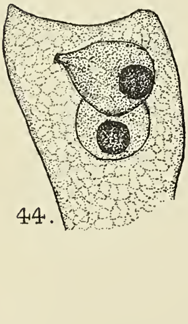

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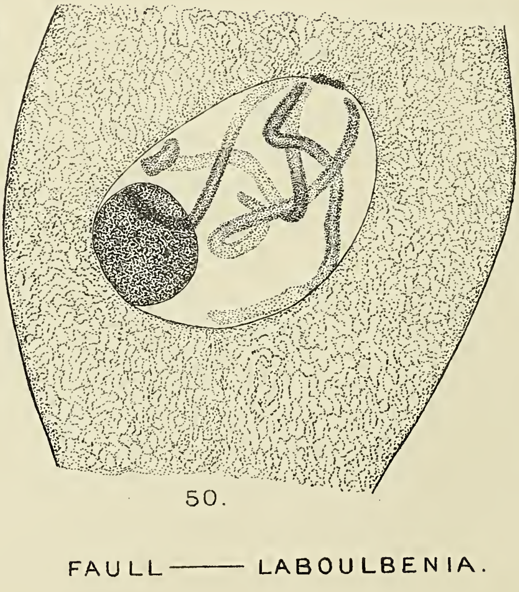

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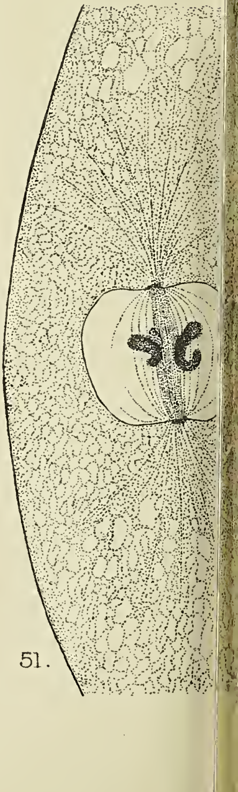
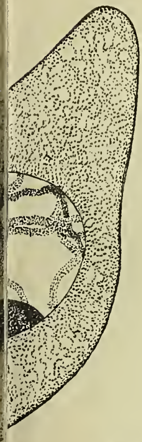
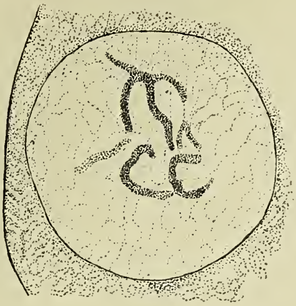


51.

FAULL —— LABOULBENIA.





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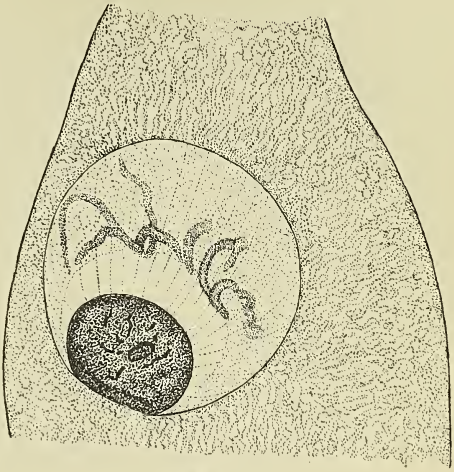

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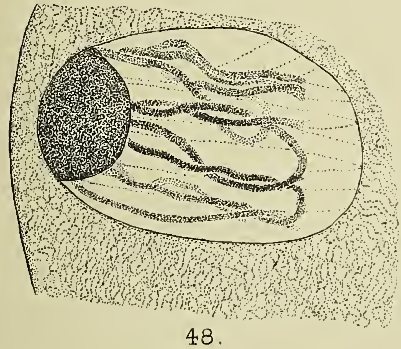

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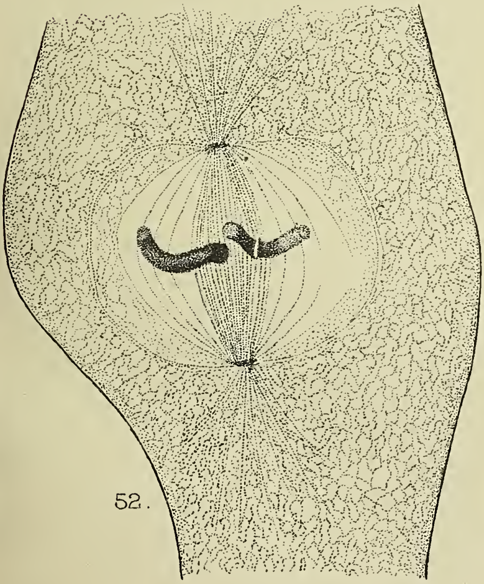

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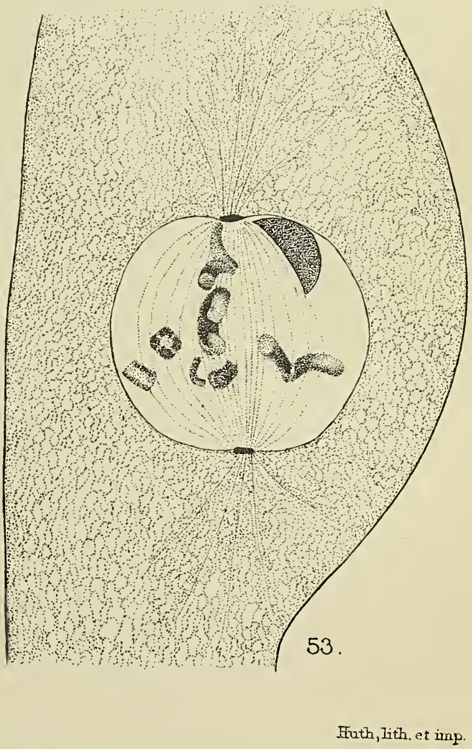

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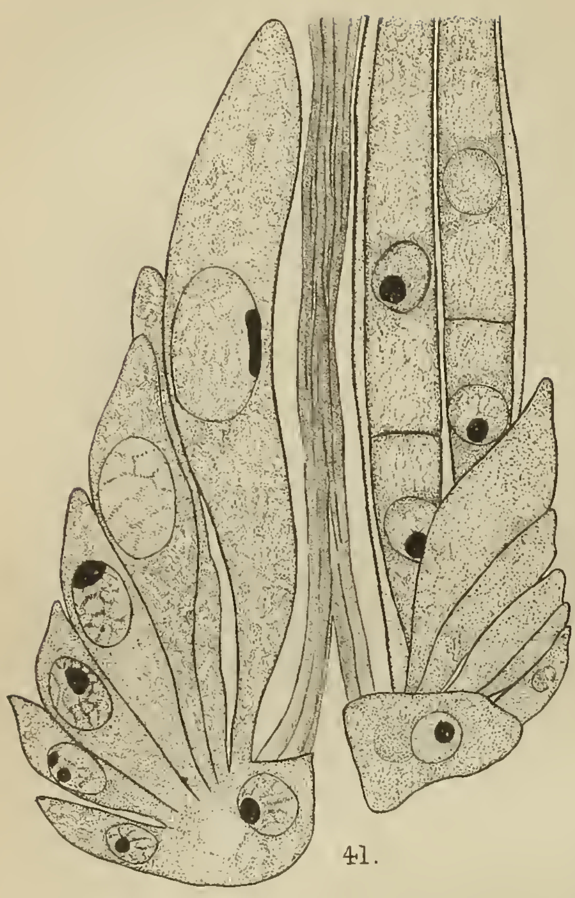

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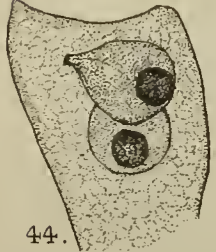

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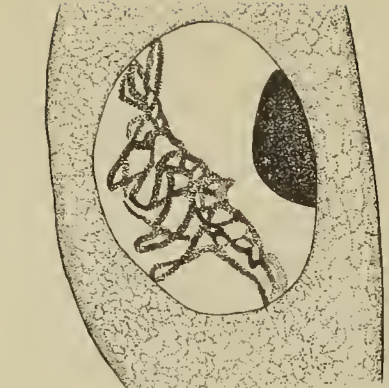

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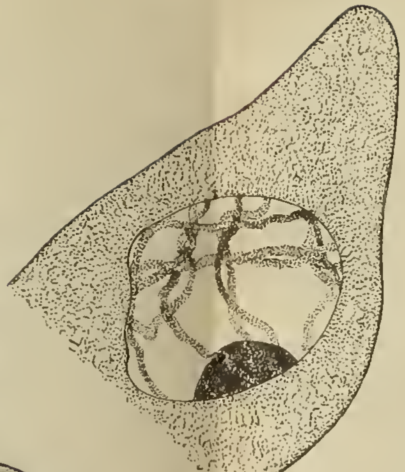

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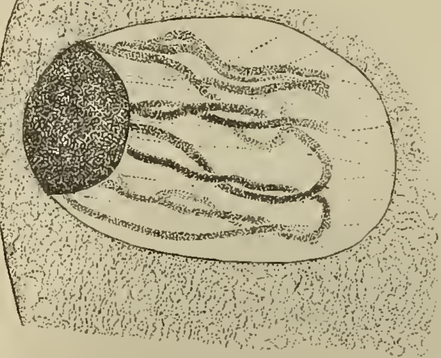

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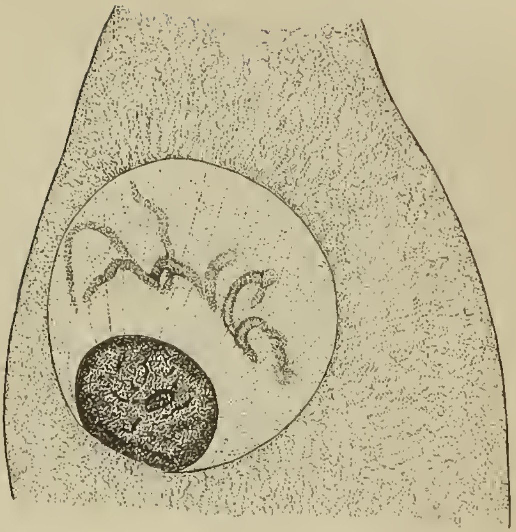

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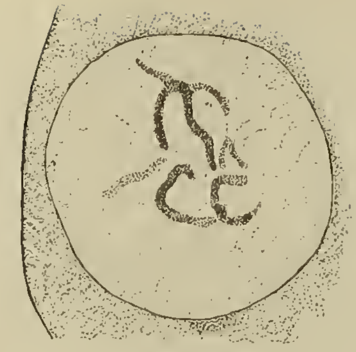

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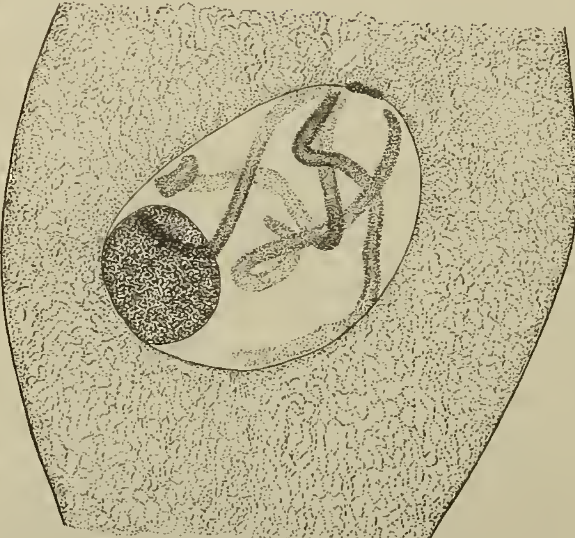

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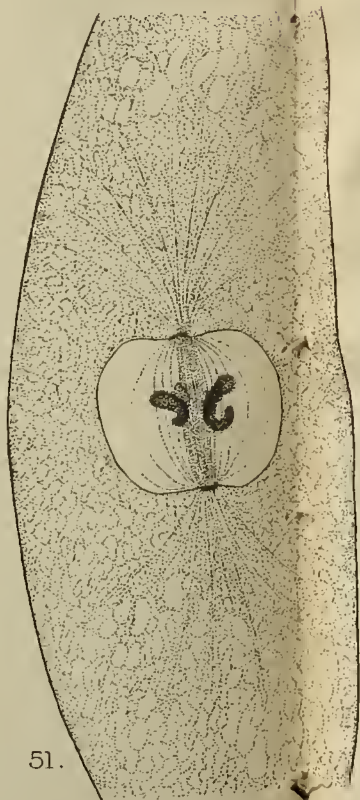

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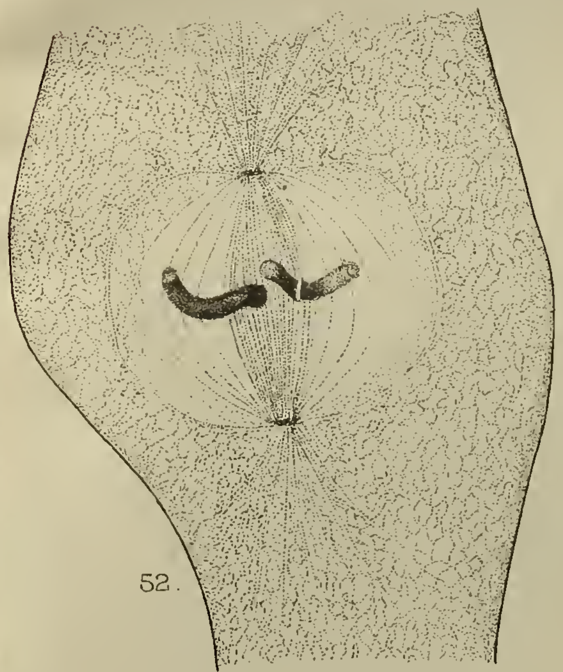

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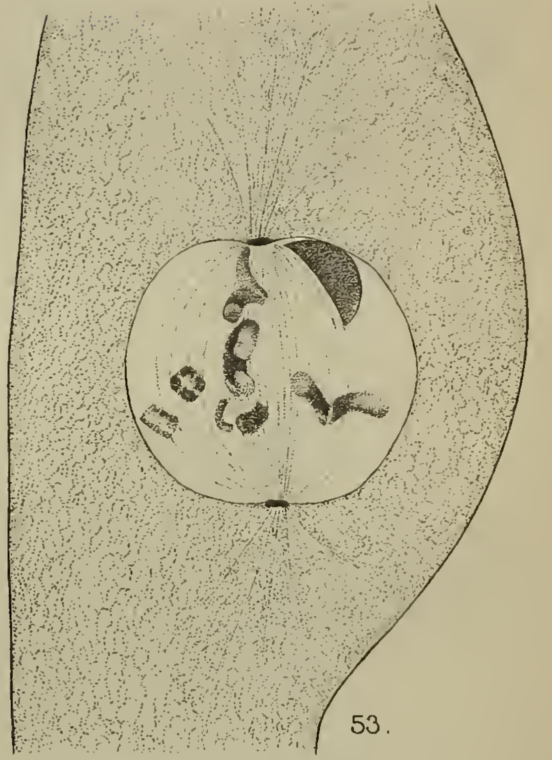

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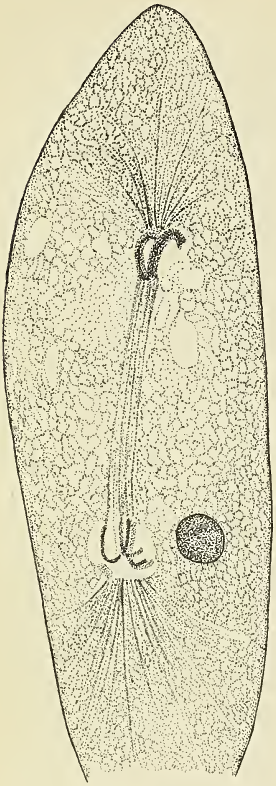
Huth, lith. et imp.

FAULL —— LABOULBENIA.



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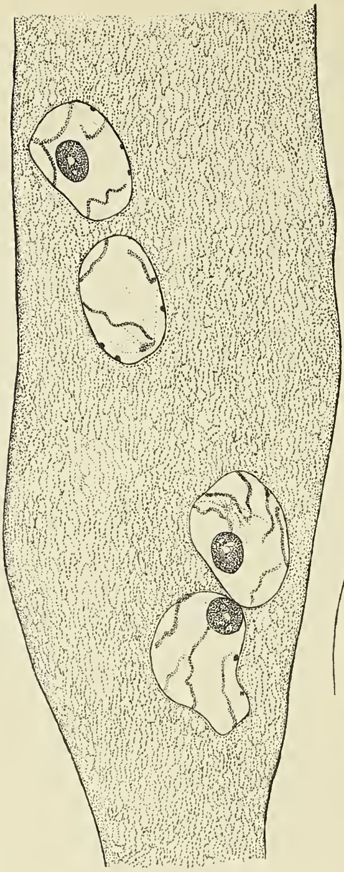

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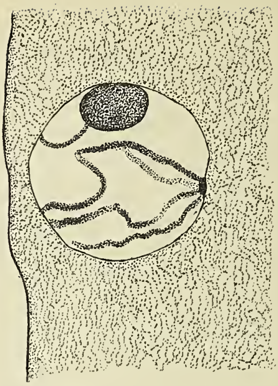

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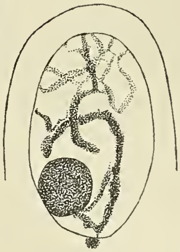

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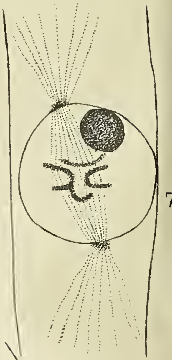

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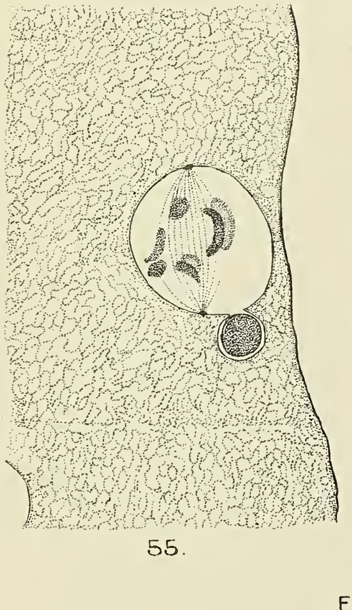

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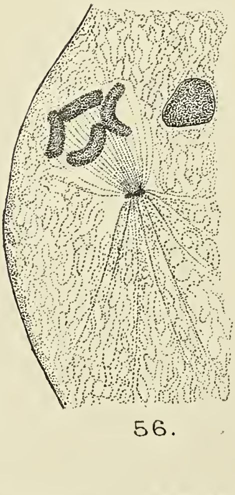

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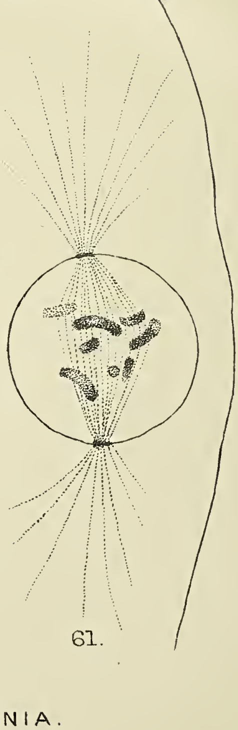

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FAULL —— LABOULBENIA.

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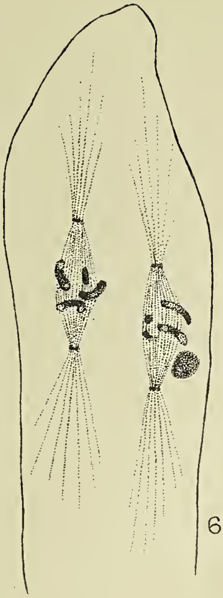

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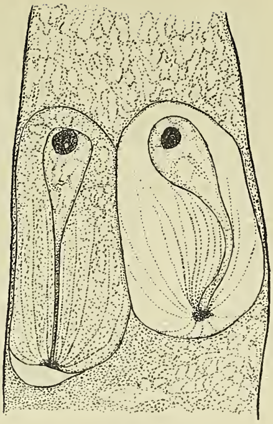

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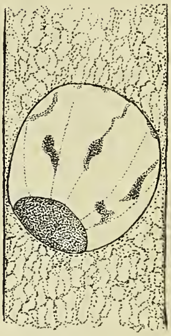

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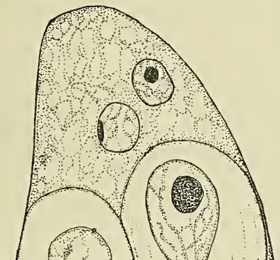

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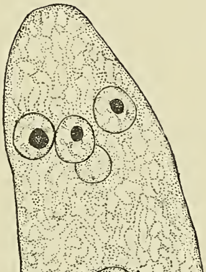

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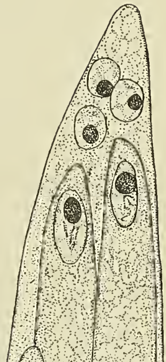

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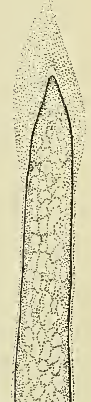

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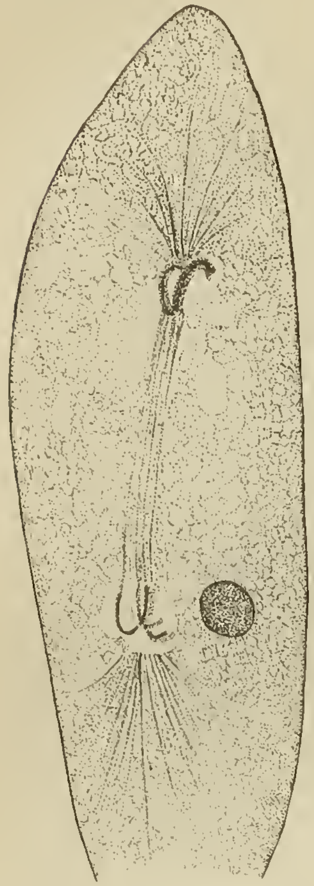

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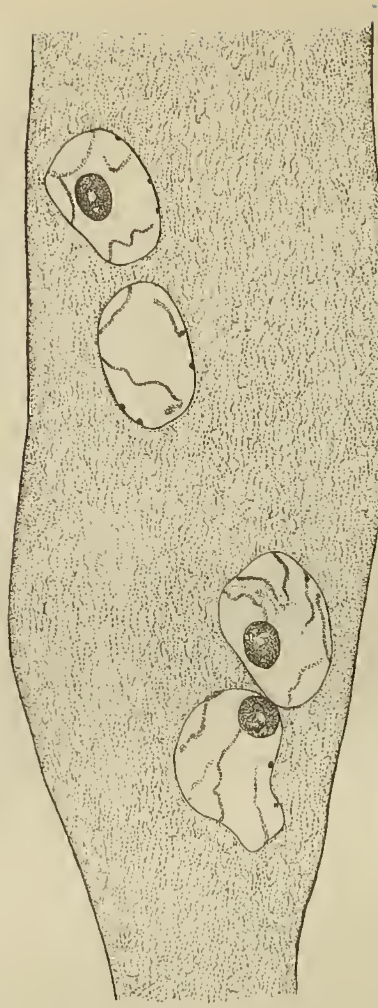

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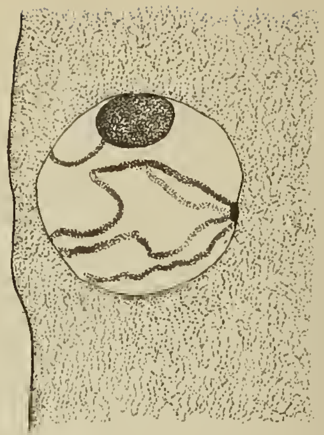

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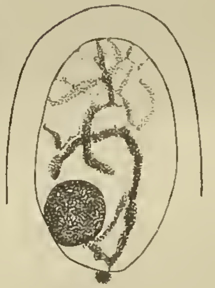

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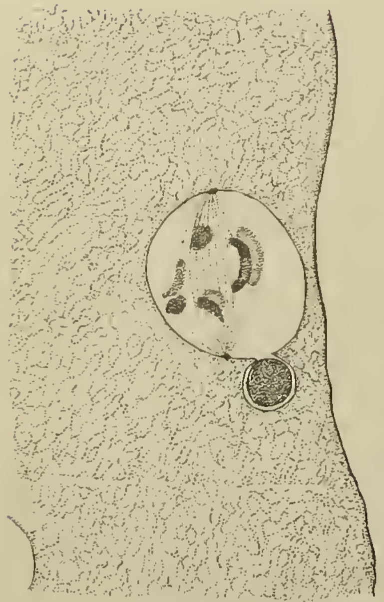

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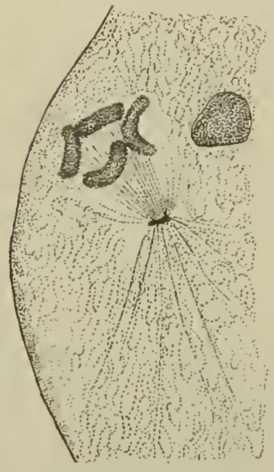

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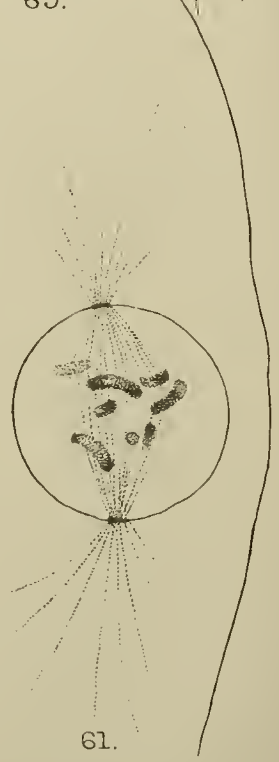

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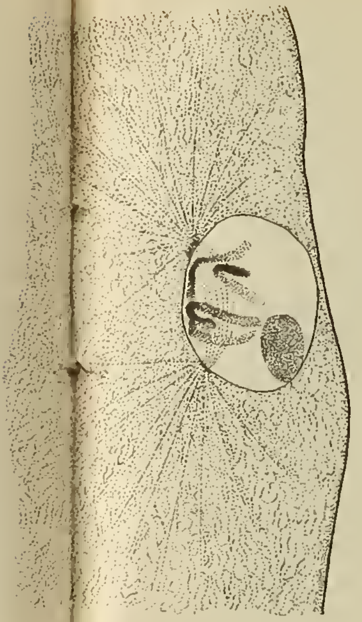

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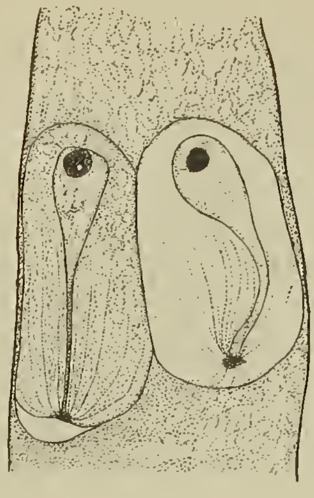

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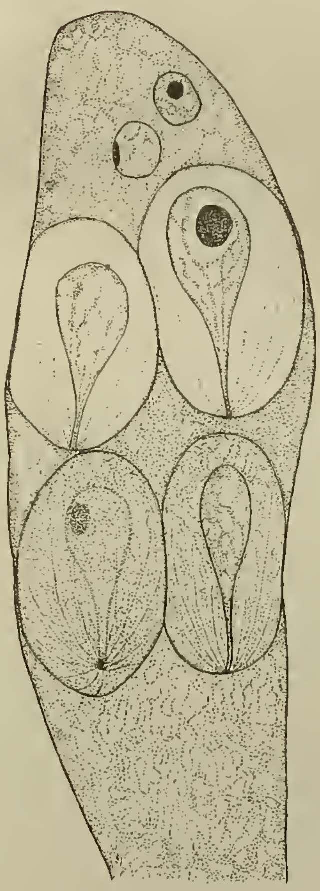

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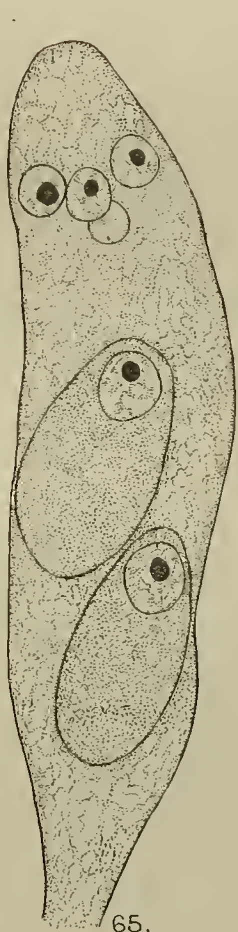

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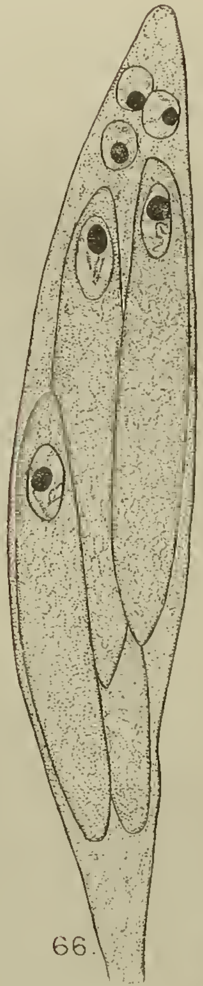

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FAULL —— LABOULBENIA.

Huth, lith. et imp.

has its parallel in the Oenotheras. The parent type, *O. Lamarckiana*, possesses 14 ($2x$) and 7 (x) chromosomes; its mutant, *O. gigas*, possesses 28 ($2x$) and 14 (x) chromosomes. This fact was first observed by Lutz (31), who counted 28 or 29 chromosomes in the roots. The numbers 14 ($2x$) and 7 (x) seem to be characteristic of this series of *Oenothera*; they are found in *O. lata*, in *O. nanella*, in the mutant *O. rubrinervis*, and in the hybrid arising from the cross *O. lata* × *O. Lamarckiana*.

O. gigas appeared first in de Vries's experimental garden in 1895 amongst a crop of *O. Lamarckiana* which was known to have bred true for three previous generations (7). It arose without any intermediate stage, and the plants, grown from self-fertilized seeds, were with one exception pure *gigas*. Thus a new elementary species can 'appear without any obvious cause in a single individual and be absolutely constant from the very first' (7). *O. gigas* reappeared in 1898 from the seeds of a plant of *O. sublinearis*, which had itself arisen from the *Lamarckiana* family; and again in 1899 from a cross between *O. lata* and *O. hirtella*. Thus, like *P. kewensis* (seedling form), *O. gigas* has originated from more than one source. Gates (17) has worked out the cytology of the pollen mother-cells of *O. gigas* in detail, and he believes that the doubling of the chromosome number is to be regarded merely as a duplication of the set of chromosomes present in *O. Lamarckiana*. 'There is no evidence that any new unit characters have been added, or that anything really new has come into the germ plasm.' Whereas in *P. kewensis* the acquirement of the double number of chromosomes is apparently associated with the change from a sterile to a fertile condition.

P. kewensis (fertile) makes no attempt to form a second contraction, and in that respect resembles its parent *P. floribunda*. Another cytological characteristic peculiar to *P. kewensis* (fertile) is the temporary joining together of two of the bivalent chromosomes in the first meiotic division, resulting in a large quadrivalent chromosome, an association which is maintained until the univalent chromosomes, after having collected at the equatorial plate, separate off to the poles of the spindle. Further, *P. kewensis* (fertile) has a remarkable diakinesis in which the chromosomes are much contracted, a stage which has never been seen in the other Primulas. Lastly, the nuclei of the pollen mother-cells of *P. kewensis* (seedling) are considerably larger than are those of its parents. Thus it affords another example of the size of the nucleus in relation to the number of its contained chromosomes.

A rest occurs between the last premeiotic and the first meiotic division as in the other Primulas. During this and the subsequent stages the nucleolus buds most vigorously (Pl. XLIII, Fig. 72). There is a close synaptic knot, which leaves a large nuclear space. The buds at first stain faintly, but they gradually become chromatic in their staining reaction.

As the spireme comes out of synapsis the nucleolus continues to bud, with the result that many large rounded chromatin masses are caught up in the loops of the spireme (Fig. 73). In course of time these seem to become absorbed (Fig. 74), for they disappear, leaving only a few isolated beads, which are generally situated at the points of intersection of the loops (Fig. 74). The somewhat beaded character of the spireme recalls that of the sterile *P. kewensis* (Fig. 75). The beads are joined together by fine threads, and these tend to run in pairs, and where they meet there is always a chromatic swelling (Fig. 75). Even at this early stage in the pairing of homologous univalent spireme lengths it is possible to distinguish the skeleton of the future bivalent chromosomes. Gradually the paired portions of univalent spireme severally dissociate themselves from the others. At first these parallel threads have paired chromatin masses arranged at intervals along their length (Fig. 75), but as concentration proceeds less and less of the thread is visible, the chromatin becoming more diffuse (Fig. 76); as the sides of the chromosomes concentrate they become beaded (Figs. 77 and 78).

When it is possible to identify the limits of the individual bivalent chromosomes, it is apparent that two are joined together end to end (Figs. 79 and 80), forming as a rule a large looped figure. The univalent sides of each bivalent chromosome thicken, and often one or two chromosomes remain attached to the nucleolus (Fig. 81). Gradually the chromosomes become typically heterotype in shape (Fig. 82), and the large quadrivalent chromosome is most striking (Fig. 83). Then the heterotype chromosomes separate from one another, they become increasingly beaded (Fig. 84), and the fine threads joining them are tightly stretched. They stain less deeply and contract considerably (Figs. 85 and 86); the large chromosome often takes the shape of a ring (Fig. 86). After passing through this extremely beaded phase the chromosomes gradually lose their granular appearance and become slightly more condensed (Fig. 87), more homogeneous, and rounded in outline (Fig. 88). There is still further contraction of the chromosomes, each univalent chromosome of the bivalent combination becoming oval or rounded in shape (Fig. 89), the large chromosome appearing as a tetrad (Figs. 89, 90, and 91). The tetrad may be in the form of a square, or of a closed or partially opened ring, but in every case its fourfold character is evident (Fig. 91). The bivalent chromosomes are placed widely apart, and they are still connected to one another by fine threads. The nucleolus has by this time fragmented, and rounded globules of faintly staining nucleolar material may be seen adhering to the chromosomes (Fig. 92). Such is the peculiar and characteristic diakinesis of *P. kewensis* (fertile), the preparation for which is so prolonged.

Several counts have been made of the chromosomes at this stage. Very rarely 18 bivalent chromosomes all of the same size are to be found;

the greater number show 17 bivalent chromosomes together with 1 quadrivalent chromosome, a few show 16 bivalent and 1 quadrivalent, and one case was found of 15 bivalent and 2 quadrivalent. Some of the discrepancies may be accounted for by the fact that the nuclei appeared in two sections and had to be pieced together, but the trend of the results seem to prove conclusively that the large chromosome represents the joining together of two bivalent chromosomes, and this statement is further corroborated by the subsequent behaviour of the large chromosome on the equatorial plate.

It is interesting to note that Rosenberg (33) in the hybrid *Drosera obovata* found that the chromosomes in the homotype division sometimes appeared as tetrads, and remarks 'dass wir in dem Vorkommen der Vierergruppen im zweiten Teilungsschritt einen Ausdruck der Hybridität sehen können' (p. 116).

Strasburger (35) in *Melandrium rubrum* describes one chromosome in the heterotype division which is much larger than the others; it reappears in the homotype division, and is present also in the embryo-sac mother-cell.

Agar (1) noted a conspicuously larger pair of chromosomes in *Lepidosiren paradoxa*, which are seen in the spermatogonial divisions, and which can be identified throughout the two maturation divisions.

In both *Melandrium* and *Lepidosiren* the large bivalent chromosome results from the association of two proportionately larger univalent chromosomes, and not from the temporary union of two bivalent chromosomes as in *P. kewensis* (fertile).

The spindle of *P. kewensis* (fertile), like that of the other Primulas, is quadripolar at its origin (Fig. 93). When the chromosomes have completed their equatorial arrangement, the quadrivalent chromosome is easily recognized (Figs. 94 and 95). Sometimes before the other chromosomes have separated, the quadrivalent chromosome may have prematurely divided into its four univalent units, though the members of each pair may continue to be joined one to the other (Fig. 94); in other cases the quadrivalent chromosome may not divide until the other chromosomes are about to pass to the poles (Fig. 95). The chromosomes have been counted several times in the polar views of equatorial plates. In several cases the number has been found to be 18 (Fig. 96), but 17, 16, 15, and even 14 chromosomes have been counted. Possibly this inaccuracy may be due to the position and relative degree of separation of the quadrivalent chromosome. This suggestion is supported by the fact that polar views of anaphases and polar views of asters (Pl. XLIV, Fig. 99) almost invariably show 18 chromosomes.

As they proceed to the poles (Pl. XLIII, Fig. 97) the univalent chromosomes which composed the quadrivalent combination cannot be distinguished from the others (Pl. XLIV, Fig. 98). The anaphase and telophase have been studied in detail, and there is no reappearance of the large chromosome. The chromosomes of the late anaphase at first mass together (Fig. 100), then as they protrude and stretch to the periphery of the nucleus a limiting nuclear membrane appears (Fig. 101); gradually each chromosome lies independently, as a rounded mass of homogeneous chromatin, in the nuclear cavity (Fig. 102). In a polar view of this stage 18 chromosomes have again been counted. A definite cloudy band, indicating the position of the future cell-wall, stretches across the equator of the fast disappearing spindle (Fig. 102). The next stage shows the longitudinal fission of the chromosomes preparatory for the homotype division (Fig. 103); again 18 chromosomes can be counted. There is nothing remarkable about the homotype division; it is normal in every way.

The pollen-grains are larger than those of *P. floribunda*, and approximate in size to those of *P. verticillata*.

The embryo-sac has been examined, and the embryo-sac mother-cell nuclei show 18 bivalent chromosomes (Fig. 106). There is no indication of the adherence of two bivalent chromosomes; apparently this peculiarity is only characteristic of the heterotype division of the pollen mother-nuclei. At diakinesis (Fig. 104), in the profile (Fig. 105), and in the polar (Fig. 106) views of equatorial plates all the bivalent chromosomes are of the same size and are independent of one another.

P. kewensis farinosa.

It has already been mentioned that *P. kewensis farinosa* has arisen from two sources: in the one case as a variety of the fertile *P. kewensis*, in the other as the direct result of a cross between *P. verticillata* and *P. floribunda isabellina* (29), and both have doubled the number of the parental chromosomes.

The meiotic phase of *P. kewensis farinosa* obtained by Messrs. Veitch from selection of *P. kewensis* (seedling) has only been studied with the view of ascertaining the number of the chromosomes, and these without any doubt are 36 and 18 (Fig. 107).

The buds procured from the *P. kewensis farinosa* hybrid at Kew were young and had not as yet entered upon meiosis, but the somatic and archesporial divisions showed clearly that their chromosomes numbered 36 (Fig. 108).

Therefore *O. gigas*, whether it arises as a mutant or as a hybrid, and *P. kewensis farinosa*, whether it arises indirectly or directly as a hybrid, have both in each case doubled the parental number of chromosomes.

P. verticillata × P. floribunda.

The result of the cross between *P. verticillata* and *P. floribunda* (29) is a hybrid which repeats the number of the parental chromosomes, 18 ($2x$) and 9 (x), and resembles the seed parent, *P. verticillata*.

P. floribunda isabellina × P. kewensis (type).

The hybrid derived from this cross resembles the seed parent, *P. floribunda isabellina*, and possesses the 18 ($2x$) and 9 (x) chromosomes.

The reciprocal cross, *P. kewensis* (type) × *P. floribunda isabellina*, set no good seed.

P. floribunda isabellina × P. kewensis (seedling form).

An unexpected result is obtained from this cross. The hybrid resembles that of the preceding cross, *P. floribunda isabellina* crossed with the sterile *P. kewensis*, in external features as well as in the number of chromosomes, which are accordingly 18 ($2x$) and 9 (x), notwithstanding the fact that one of the parents possesses the doubled number, 36 ($2x$) and 18 (x).

It is difficult to imagine by what regulating process this reduction in the number of chromosomes has been achieved; that is to say, how 18 (x) uniting with 9 (x) can give the result of 18 ($2x$). Once more the Oenotheras show an analogy, and offer a possible explanation for this phenomenon. *O. lata*, which has 14 ($2x$) and 7 (x) chromosomes, crossed with *O. gigas*, which has 28 ($2x$) and 14 (x) chromosomes, gives rise to a hybrid which has 21 ($2x$) chromosomes. In the heterotype prophase the chromosomes appear unpaired in their $2x$ numbers. Subsequently, as in the *Drosera* hybrid (34), Geerts (20) finds that the homologous chromosomes derived from either parent pair with one another and that the supernumerary chromosomes remain unpaired. In the *Oenothera* hybrid, therefore, 7 bivalent and 7 univalent chromosomes go on to the spindle. The 7 bivalent chromosomes separate normally, whilst the 7 univalent ones disintegrate. Some of the fragments may eventually reach the poles, but many may be left on the spindle and never enter the reconstructing nuclei. Geerts obtains the same results in the hybrid resulting from the cross between *O. gigas* and *O. Lamarckiana* and in the reciprocal cross of *O. Lamarckiana* × *O. gigas*. In the polar views of the asters of the heterotype and homotype divisions of both these hybrids there are 7 distinct and perfect chromosomes to be seen, and amongst them many fragments. In the second generation of the cross between *O. gigas* and *O. Lamarckiana* the number of $2x$ chromosomes has reverted to the original 14. Thus 'wenn die Chromosomen wirklich die Träger der erblichen Eigenschaften sind, genügen also 7 Chromosomen der *O. gigas* für die Vertretung aller erblichen *gigas*-Eigenschaften in diesen Hybriden' (p. 163).

It may be that the hybrid *Primula* makes use of some similar means, perhaps at the first segmentation division, whereby it eliminates its superfluous chromosomes.

Further interesting results have been recorded from crossing *O. gigas* with *O. lata* and *O. Lamarckiana*. De Vries (6) has found that when

O. lata is crossed with *O. gigas* the F. I. generation shows 50 per cent. of a type intermediate between *O. lata* and *O. gigas*, and 50 per cent. intermediate between *O. lamarckiana* and *O gigas*. Lutz (32) experimented on crossing *O. lata* with *O. gigas*, and from a study of forty hybrid plants was able to divide them into three classes, both in respect to their external characters and to their number of chromosomes: (1) *lata* type with 15 (2 x) chromosomes; (2) *gigas* type with 30 (2 x) chromosomes; and (3) intermediate type with 22 or 23 (2 x) chromosomes.

GENERAL COMPARISONS.

It is proposed to limit this brief discussion to the manner of union of the lengths of spireme in the formation of the bivalent chromosome; to the cytological detail of the nuclei of hybrids, especially with regard to the number of their chromosomes; and to the fertile or sterile character of those hybrids.

Firstly, as regards the origin of the bivalent chromosome. A mass of controversial literature has accumulated round certain disputed points, especially concerning the significance of the parallel threads in the presynaptic stages and whether the union of the univalent spiremes in the formation of the bivalent chromosome may be described as telosynaptic (11) or parasynaptic (22), and consequently whether the fission in the spireme separates two whole univalent chromosomes or whether it is the premature fission in the substance of the univalent chromosome which will take effect in the homotype division.

This series of *Primula* does not throw any light on the meaning of the parallelisms in the heterotype prophases. During the long rest between the last premeiotic and first meiotic divisions all sequence of events is lost, so that it is impossible to trace any relationship between the parallel threads of the heterotype prophases and the chromosomes of the preceding mitosis. Neither can any relationship be established between the parallel threads of the heterotype prophases and the parallel lengths of post-synaptic spireme, for during synapsis all proceedings are hopelessly obscured. Therefore whether each thread represents the longitudinal half of a portion of a univalent chromosome, and the pairing is the preparation for the condensation to form the whole univalent chromosome, or whether, according to the view held by Grégoire (22), each thread represents a portion of a whole univalent chromosome, and the parallelism is the premature pairing of homologous chromosomes, is a problem which cannot be solved by the study of *Primula* alone. Perhaps both arrangements may be proceeding simultaneously in the presynaptic prophases. Moreover, the interpretation of the parallelisms does not materially affect the question as to the ultimate telosynaptic or parasynaptic origin of the bivalent chromosome, for it is the post-synaptic stages that elucidate this point.

As the spireme comes out of synapsis it exhibits a certain amount of approximation between its lengths, but by the time that the open spireme stage is reached the spireme is thrown into loops, the sides of which are widely separated, the only places of union between them being the points of intersection. Gradually the sides of portions of the looped spireme approach one another, and thus indicate, in the *still continuous spireme*, that association which will eventually result in the formation of the respective future bivalent chromosomes. The lateral association between the strands becomes increasingly more intimate. The spireme then segments. When the necessary blending of the two is accomplished, then the bivalent segment splits into its two univalent chromosomes. These retain their connexion during diakinesis and finally separate on the spindle of the first meiotic division.

Thus *Primula* clearly illustrates the views held by Farmer and Moore (11) regarding the telosynaptic arrangement of the univalent chromosomes in the spireme. It is evident that in the looping of the continuous spireme the future univalent chromosomes, which separate at the heterotype mitosis and are distributed between the two daughter nuclei, are arranged end to end, for each side of a loop becomes in process of time a univalent chromosome. As the loops fold over and the sides approximate there is secondarily a greater or a less degree of parasynapsis between them before their ultimate separation.

This telosynaptic origin of the bivalent chromosome is wonderfully clearly shown in *Primula*, both on account of the sharp definition of the spireme and also owing to the fact that in two of the Primulas examined there is no second contraction, so that the whole process of association, fusion, and splitting apart of the univalent spiremes can be traced in perfect sequence.

The univalent spireme of *Primula* is of even thickness and its outline so definite that the places where it forms a bivalent association are most obvious. This gives it a great advantage over some other forms in which the spireme is apparently very viscous, which makes it impossible to decide whether the strands are of the bivalent or of the univalent order, for they may be stretched into a fine thread or contracted into a broad ragged band.

Besides being regular in thickness, the spireme of *Primula* is almost invariably homogeneous. It has been shown that the spireme is univalent in nature, so that in the rare cases when fission in its substance has been seen it must be the premature appearance of the fission which will divide the univalent chromosomes at the homotype division.

Secondly, as regards the character of the nuclei of hybrids. As a rule hybrids are sterile, or partially so, but it is not a feature peculiar to them, as it has frequently been shown that pure races may be also largely sterile. Geerts (19) found that 50 per cent. of the pollen-grains of *O. Lamarckiana* were abortive. In a hybrid cotton plant obtained by crossing *Gossypium Barbadense* with *G. herbaceum* Cannon (2) observed that in the material collected in November and December most of the male cells were normal, whilst in the material collected in the spring amitosis occurred. Jenčič (26) has worked out the ratio of the sterile to the fertile pollen-grains in many hybrids. In the hybrid *Primula austriaca*, a cross between *P. pannonica* and *P. acaulis*, he found that between 50 and 60 per cent. of the pollen-grains were sterile, and in the hybrid *P. venusta*, a cross between *P. carniolica* and *P. Auricula*, that 42 per cent. were sterile.

It is generally accepted that the 2 x number of chromosomes in the hybrid is equivalent to the sum of the x number of parental chromosomes, and in most cases this statement can be corroborated. One of the most convincing examples is to be found in the eggs of *Ascaris*. Herla (25) discovered that some *Ascaris* eggs possessed three chromosomes, of which two were larger than the third, and that the small chromosome was always isolated from the others. He concluded that it must have arisen from the *univalens* parent, but he was unable to verify the hybrid parentage. Zoja (41), later, definitely proved that the eggs with three chromosomes were the result of a cross between *Ascaris megalocephala bivalens* and *A. megalocephala univalens*. He confirmed Herla's supposition that the small chromosome was the paternal one, and hence concluded that 'la cromatina paterna e la materna restano indipendenti nel nucleo delle cellule embrionali'.

Again, the investigation of Rosenberg (33 and 34) on the hybrid *Drosera obovata* furnishes another convincing example of the fact that the hybrid possesses the sum of the x numbers of the parental chromosomes. In the hybrid the 10 (x) chromosomes obtained from the parent *D. rotundifolia* pair with 10 of the (x) number of the chromosomes of the parent *D. longifolia*; the remaining 10 chromosomes of *D. longifolia* remain unpaired. The ten bivalent chromosomes behave normally, but it seems to be a matter of chance to which pole the unpaired chromosomes repair, both in the heterotype and in the homotype divisions. Consequently the resulting tetrads possess a variable number of chromosomes. The pollen, as might be expected, is usually sterile. After several attempts Rosenberg has successfully crossed *D. obovata* with *D. longifolia* (34), and the offspring has about 37 chromosomes.

The recent work of Geerts (20) on the hybrid resulting from the cross between *O. lata* and *O. gigas*, and his interpretation of the union and behaviour of the parental chromosomes, are in direct accordance with the phenomena exhibited by the *Drosera* hybrid. On the other hand, Gates (16) has come to different conclusions as regards the behaviour of the chromosomes in the hybrid *O. lata* × *O. gigas*. He agrees with Geerts that, in the heterotype prophase of the hybrid, the chromosomes appear in their $2x$ number, namely 21. No pairing of homologous chromosomes ensues, but 10 move off to one pole and 11 to the other. 'The 10–11 segregation of chromosomes in the formation of the germ cells of this hybrid shows that there is not here a pairing and separation of homologous chromosomes of maternal and paternal origin, but that the segregation tends to be into two numerically equal groups' (p. 195).

The hybrid fern *Polypodium Schneideri* (12) furnishes another example of an inequality in the number of the parental chromosomes, one parent having a far higher number than the other. The parent *P. aureum* has 35 (x) chromosomes, the other parent, *P. vulgare*, var. *elegantissimum*, has about 90 (x) chromosomes, and the number of chromosomes appearing at meiosis in the hybrid *P. Schneideri* varies from 95 to 125. This figure can only be considered as an approximate estimate, as the chromosomes are so irregular and numerous, whilst at diakinesis some are apparently bivalent, and others univalent, that it is impossible to make an accurate count. The peculiar amitosis which is of such common occurrence in hybrids, and which may occasionally be found in pure strains, is frequently met with in *P. Schneideri*, dividing the pollen mother-nuclei, first into two, and then into four. The spores of this hybrid fern are always abortive.

The above enumerated hybrids have parents possessing unequal numbers of chromosomes. There are other cases in which the parents possess the same number as in the case of *P. kewensis* (type). It has been shown that this hybrid, derived directly from *P. floribunda* crossed with *P. verticillata*, is sterile. On the other hand, Cannon (3) crossed two peas, both of which had 14 ($2x$) and 7 (x) chromosomes, and the hybrid which repeated the same number was fertile.

In certain 'race hybrids' of *Lathyrus odoratus*, Gregory (23) found that abortion took place at various stages in the course of the meiotic division. The chromosomes were more ragged than were those of the parents and showed a deficiency of chromatin. Sterility in this case is a recessive character and is generally confined to those plants which have light-coloured leaf axils, that being a recessive character.

Finally, there are those cases of hybrids in which the chromosomes of the parents are too numerous, and the spindles of the hybrids too irregular to afford accurate data as to the relative numbers of the chromosomes. Such is the case with the hybrid *Syringa rothomagensis*, a cross between *S. vulgaris* and *S. persica*. Juel (28) was unable to count the chromosomes, but he believes that there are more in the hybrid than in the parents. He finds very irregular spindles, and also the curious modification of amitosis. 'Bei so vielen Arten von Unregelmässigkeiten bei der Tetradentheilung kann es nicht Wunder nehmen, dass *S. rothomagensis* in Bezug auf den Blütenstaub fast steril ist' (p. 647). Tischler (39) has confirmed Juel's investigation of this hybrid, which he calls *S. chinensis.* Tischler (36) found in the hybrid *Cytisus Adami*, a cross between *C. Laburnum* and *C. purpureus*, most irregular divisions resulting in an unequal distribution of chromosomes to the poles. Again, in the hybrid *Bryonia* (37), a cross between *B. alba* and *B. dioica*, he has shown that the presynaptic and synaptic stages are normal, but that the chromosomes go on to the spindle irregularly, while some remain behind in the cytoplasm. The hybrid is always sterile. In the hybrid *Ribes intermedium*, a cross between *R. sanguineum* and *R. nigrum* (38), the meiotic phase is normal and 10 to 15 per cent. of the pollen-grains are well developed.

The fact that a hybrid may be either sterile or fertile does not therefore appear to depend on the pairing of an equal number of parental homologous chromosomes, nor can the irregularity of the spindle figures be called a determinant of hybridity. It is true that irregular figures and the peculiar amitosis must entail an unequal partition of chromatin amongst the tetrads, and hence may render them functionless, but these abnormalities are not confined to hybrids, but may also occur in pure breeds. Juel (27) has emphasized this fact in the nuclei of *Hemerocallis fulva.*

Tischler and others believe that the arrangement of the chromosomes cannot be taken into account with regard to the cause of sterility, but that the sterile condition is more probably due to the poverty of the cytoplasm and to the alteration in the constitution of the idioplasm.

Most cytologists have arrived at the conclusion that, in the blending of the male and female germ cells, physiological and chemical changes are induced, which fundamentally disturb the metabolism of the cells of the resultant offspring. The abnormal nuclear phases, the anomalous behaviour of the chromosomes, and the feeble condition of the cytoplasm afford visible expressions of the influence of unrevealed agencies which effect these intrinsic changes.

In conclusion, it is of interest to emphasize the points of similarity between this series of Primulas and the varieties of *Oenothera.* Briefly the character of the heterotype prophases, the presence in some forms and the absence in others of the second contraction, the longitudinal fission in the chromosomes throughout the interkinesis of the two meiotic divisions, the cytoplasmic connexions between the pollen mother-cells, are all features which the Primulas possess in common with the Oenotheras.

Moreover, both in the Oenotheras and in the Primulas a form has arisen from different sources, which has suddenly doubled the number of the chromosomes characteristic of the type. From the *Oenothera* evidence it seems probable that this duplication has been brought about by longitudinal fission, creating what is believed to be a double set of homologous chromosomes, for the duplication of the chromosomes has not been accompanied by the acquirement of any new characters. On the other hand, in the case of *P. kewensis*, the duplication is accompanied by the acquirement of the character of fertility.

Further, both in the Oenotheras and in the Primulas, an example of a decrease in the chromosomes is found, whereby the duplicated number is reduced to the typical number. In the Oenotheras this reduction has been shown to be achieved by the actual disintegration of the supernumary chromosomes. It may be that in the hybrid produced by crossing *P. floribunda isabellina* with *P. kewensis* (seedling form) a similar elimination of chromosomes has taken place in the first segmentation division after fertilization.

SUMMARY.

1. The parents, *P. floribunda* and *P. verticillata* of the sterile hybrid *P. kewensis* have both 18 ($2x$) and 9 (x) chromosomes.

This number is repeated in the sterile hybrid *P. kewensis*.

P. kewensis (seedling form), which originated from *P. kewensis* (sterile) by the fertilization of a pin flower with pollen of a thrum flower (both on the sterile stock), has doubled the typical number of chromosomes and possesses 36 ($2x$) and 18 (x).

This number is repeated in the variety of the seedling form, *P. kewensis farinosa*, and reappears in the *P. kewensis farinosa*, which resulted from crossing *P. verticillata* with *P. floribunda isabellina.*

P. floribunda isabellina crossed with *P. kewensis* (seedling form) results in a hybrid which has reduced its chromosomes to the typical number of 18 ($2x$) and 9 (x).

2. The prophases of the premeiotic divisions show a stringing together of homogeneous beads of chromatin, which gradually condense to form the chromosomes. Longitudinal fission in the substance of the chromosomes is seldom seen until the chromosomes are arranged on the spindle.

3. The long rest between the last premeiotic and the first meiotic division makes it impossible to trace the homology of the parallel threads of the heterotype prophases.

4. In *P. floribunda* and in *P. kewensis* (seedling), there is no second contraction. Hence the sequence of events leading to the formation of the bivalent chromosomes can be traced without intermittence, namely, the looping of the *continuous univalent spireme*; the approximation and association of the univalent sides of the loops; and the final splitting apart of the approximated sides to form the univalent chromosomes of the bivalent combination.

5. This series illustrates clearly the telosynaptic arrangement of the *univalent* chromosomes in the *continuous univalent spireme* of the hollow spireme stage of the heterotype division, and the secondary parasynaptic union of homologous univalent segments to form the future bivalent heterotype chromosomes.

6. In *P. verticillata* there is a typical second contraction.

In *P. kewensis* (type) the spireme segments tend to amalgamate in parts, but they never form a definite second contraction.

7. In *P. kewensis* (seedling form) two of the bivalent chromosomes join together in the heterotype prophase of the pollen mother-nuclei, forming a large quadrivalent chromosome. This union is maintained until its univalent portions separate on the spindle.

In the homotype division all the chromosomes behave normally. There is also no connexion between two of the chromosomes in the first meiotic division of the embryo-sac mother-cell.

8. *P. kewensis* (seedling form) has a most typical diakinesis. The chromosomes are extremely contracted and are widely separated from one another, but are connected together by fine tightly stretched threads. The ordinary bivalent chromosomes resemble dyads, the quadrivalent chromosome a tetrad.

9. Protrusions of chromatin from a nucleus into the adjacent cell are sometimes found in the 'open spireme' stage of *P. kewensis* (type).

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EXPLANATION OF PLATES XLI–XLIV.

Illustrating Miss Digby's paper on the Cytology of hybrid Primulas.

All the figures were drawn with the camera lucida under a 2 mm. apochr. *Hom.* imm. Zeiss, N.A. 1·40, with comp. oc. 18. × 2,250.

Figs. 1–6. *P. kewensis* (seedling form). Premeiotic divisions.
Figs. 7–42. *P. floribunda.* Meiosis.
Figs. 43–53. *P. verticillata.* First meiotic division.
Figs. 54–71. *P. kewensis* (type). Meiosis.
Figs. 72–106. *P. kewensis* (seedling form). Meiosis.
Figs. 107 and 108. *P. kewensis farinosa.*

PLATE XLI.

Fig. 1. *P. kewensis* (seedling form). Premeiotic divisions. Telophase.

Fig. 2. Late telophase or resting stage.

Fig. 3. Early prophase showing the stringing together of beads of chromatin.

Fig. 4. Concentration of beads to form the chromosomes.

Fig. 5. The chromosomes gradually become homogeneous.

Fig. 6. Fully developed chromosomes.

Fig. 7. *P. floribunda.* Pollen mother-cell. Resting stage between the premeiotic and first meiotic division. The nucleolus buds freely.

Fig. 8. Complete resting stage. The nucleus appears empty except for a few large rounded chromatic masses, and some smaller chromatin granules.

Fig. 9. Early prophase. The nuclear contents have increased owing to the continuous nucleolar budding.

Fig. 10. The nucleus is considerably larger, and the chromatin granules are more definitely arranged on linin strands.

Fig. 11. The linin strands tend to run parallel to one another.

Fig. 12. The earliest stage in the massing of the granules preparatory to synapsis.

Fig. 13. Further concentration of the granules into groups.

Fig. 14. Synapsis.

Fig. 15. Loosening of the synaptic knot.

Fig. 16. Lengths of spireme come out of synapsis showing bifurcated ends.

Fig. 17. Spireme emerging from synapsis in the form of loops.

Fig. 18. Superficial section of loosening synaptic knot, showing ladder-like association of lengths of spireme.

Fig. 19. Opening out of the loops of spireme.

Fig. 20. Hollow spireme.

Fig. 21. The loops of the hollow spireme withdraw from the periphery of the nucleus, the sides of each loop tend to approach one another and to join in places.

Fig. 22. Closer association of the sides of each loop. Note the chromatic swelling at the points of union.

Fig. 23. The associated sides of each loop form more definite figures.

Fig. 24. Each bivalent segment dissociates itself from its neighbours.

Fig. 25. Maximum degree of concentration of the univalent strands in the bivalent combination.

Fig. 26. Nucleus showing some of the univalent strands approximated to form thick bivalent segments, whilst others still form the sides of open loops, united at intervals.

Fig. 27. The limits of the nine bivalent segments (chromosomes) can now be identified.

Fig. 28. The splitting apart of the two univalent chromosomes.

Fig. 29. Later stage in the separation of the univalent chromosomes.

Fig. 30. Shows the chromatic swelling where the two univalent chromosomes are still in contact.

Fig. 31. The univalent sides have diverged and concentrated to form the typical heterotype chromosomes.

Fig. 32. Origin of the quadripolar spindle.

Fig. 33. The chromosomes arrange themselves on the equatorial plate. Note the laggard chromosome.

Fig. 34. Polar view of the equatorial plate of the first meiotic division, showing the nine chromosomes.

PLATE XLII.

Fig. 35. Chromosomes separating and passing to the poles.

Fig. 36. Telophase of the first meiotic division. Protoplasmic connexions streaming through the cell-walls join spindles of adjacent cells to one another.

Fig. 37. Late telophase. The chromosomes split longitudinally and their sides are beaded.

Fig. 38. Resting stage. The skeleton of each chromosome is outlined in beads.

Fig. 39. Reconstitution of chromosomes.

Fig. 40. Longitudinally split chromosomes going on to the spindle of the second meiotic division.

Fig. 41. Homotype spindle showing the nine chromosomes.

Fig. 42. Telophase of second meiotic division.

Fig. 43. *P. verticillata.* Pollen mother-cell. Spireme coming out of synapsis; tracts coalesce, forming a band-like chromatic mass, the beginning of the second contraction.

Fig. 44. The mass has increased in size.

Fig. 45. Second contraction from which radiate irregular loops of spireme.

Fig. 46. Sorting out of the second contraction. The protruding portions show all stages in the association of univalent strands.

Fig. 47. The chromosomes may come out of second contraction in the form of chains of rings.

Fig. 48. The chromosomes tend to segregate into two groups.

Fig. 49. Shows longitudinal fission in the substance of the newly separated, univalent chromosomes.

Fig. 50. The splitting apart of the univalent chromosomes.

Fig. 51. Condensation of the univalent chromosomes to form the typical heterotype chromosomes.

Fig. 52. Polar view of an equatorial plate of the first meiotic division.

Fig. 53. Interkinesis between the first and second meiotic divisions.

Fig. 54. *P. kewensis* (type). Pollen mother-cell. Hollow spireme, showing the association of the sides of the loops.

Fig. 55. Protrusion of chromatin from a nucleus into the adjacent cell.

Fig. 56. Arrangement of beaded strands of spireme into squares and parallel lines.

Fig. 57. Concentration of these associations to form the bivalent segments.

Fig. 58. Further concentration of univalent strands into ladder-like associations. The strands coalesce in places.

Fig. 59. Splitting apart of the univalent segments of each bivalent combination.

Fig. 60. Further stage in the splitting apart of the univalent chromosomes, and their subsequent thickening.

Fig. 61. The thickened ring-shaped chromosomes tend to collect together into one or two groups.

Fig. 62. As they redistribute themselves throughout the nucleus they often appear as chains of loops.

Fig. 63. The opening out of the rings to form the bivalent chromosomes.

Fig. 64. Typical heterotype chromosomes.

Fig. 65. Polar view of an equatorial plate of the first meiotic division, showing the nine chromosomes.

Fig. 66. Separation of the univalent chromosomes on the spindle.

PLATE XLIII.

Fig. 67. Tetrad.

Fig. 68. Pollen-grain apparently normal.

Fig. 69. Pollen-grain depauperate.

Fig. 70. Nucellus. Prophase showing eighteen chromosomes.

Fig. 71. Embryo-sac mother-cell. Prophase of first meiotic division, showing nine chromosomes.

Fig. 72. *P. kewensis* (seedling form). Synapsis. Note budding of the nucleolus.

Fig. 73. Hollow spireme; large round masses of chromatin lie within the meshes.

Fig. 74. The chromatin masses become absorbed, and an association between univalent portions of spireme begins to be seen.

Fig. 75. The thin strands of spireme, carrying beads of chromatin, lie parallel to one another and fuse in places.

Fig. 76. The paired univalent segments form definite bivalent combinations, the future bivalent chromosomes.

Fig. 77. The univalent segments become beaded.

Fig. 78. The limits of the beaded chromosomes can now be distinctly seen.

Fig. 79. Two of the bivalent chromosomes may be joined together.

Fig. 80. The chromosomes tend to lose their beaded character. Note two bivalent chromosomes are joined together.

Fig. 81. The univalent chromosomes thicken, and become more homogeneously chromatic.

Fig. 82. Slightly later stage in which the chromosomes are more definite, some being still beaded whilst others are concentrated.

Fig. 83. The univalent chromosomes begin to contract. The quadrivalent chromosome is most apparent.

Fig. 84. The contracted chromosomes once more become beaded.

Fig. 85. Contraction proceeds, resulting in an extremely beaded condition of the chromosomes.

Fig. 86. Same stage as Fig. 85. The large chromosome is to be seen.

Fig. 87. The chromatin of the beads diffuses throughout the linin, and the chromosomes increase in size.

Fig. 88. The chromosomes become more homogeneous.

Fig. 89. Typical diakinesis. The bivalent chromosomes resemble dyads, the quadrivalent chromosome a tetrad.

Fig. 90. Same stage as Fig. 89.

Fig. 91. Some shapes adopted by the quadrivalent chromosome.

Fig. 92. Droplets of nucleolar material attached to the chromosomes.

Fig. 93. Chromosomes going on the spindle. Note the quadrivalent chromosome.

Fig. 94. Equatorial plate of the first meiotic division. The quadrivalent chromosome is separating into its univalent segments.

Fig. 95. The bivalent chromosomes are beginning to separate into their univalent parts. The quadrivalent chromosome is still a ring.

Fig. 96. Polar view of an equatorial plate of the first meiotic division, showing eighteen chromosomes.

Fig. 97. Passing of the univalent chromosome to the poles. Note the separation of the chromosomes of the quadrivalent union.

PLATE XLIV.

Fig. 98. The chromosomes approach the poles.

Fig. 99. Polar view of an aster of the first meiotic division, showing the eighteen chromosomes.

Fig. 100. Early telophase.

Fig. 101. The chromosomes begin to separate and a nuclear limiting membrane appears.

Fig. 102. Later telophase. The chromosomes are all distinct and separate.

Fig. 103. Interkinesis between the first and second meiotic divisions, showing the split chromosomes.

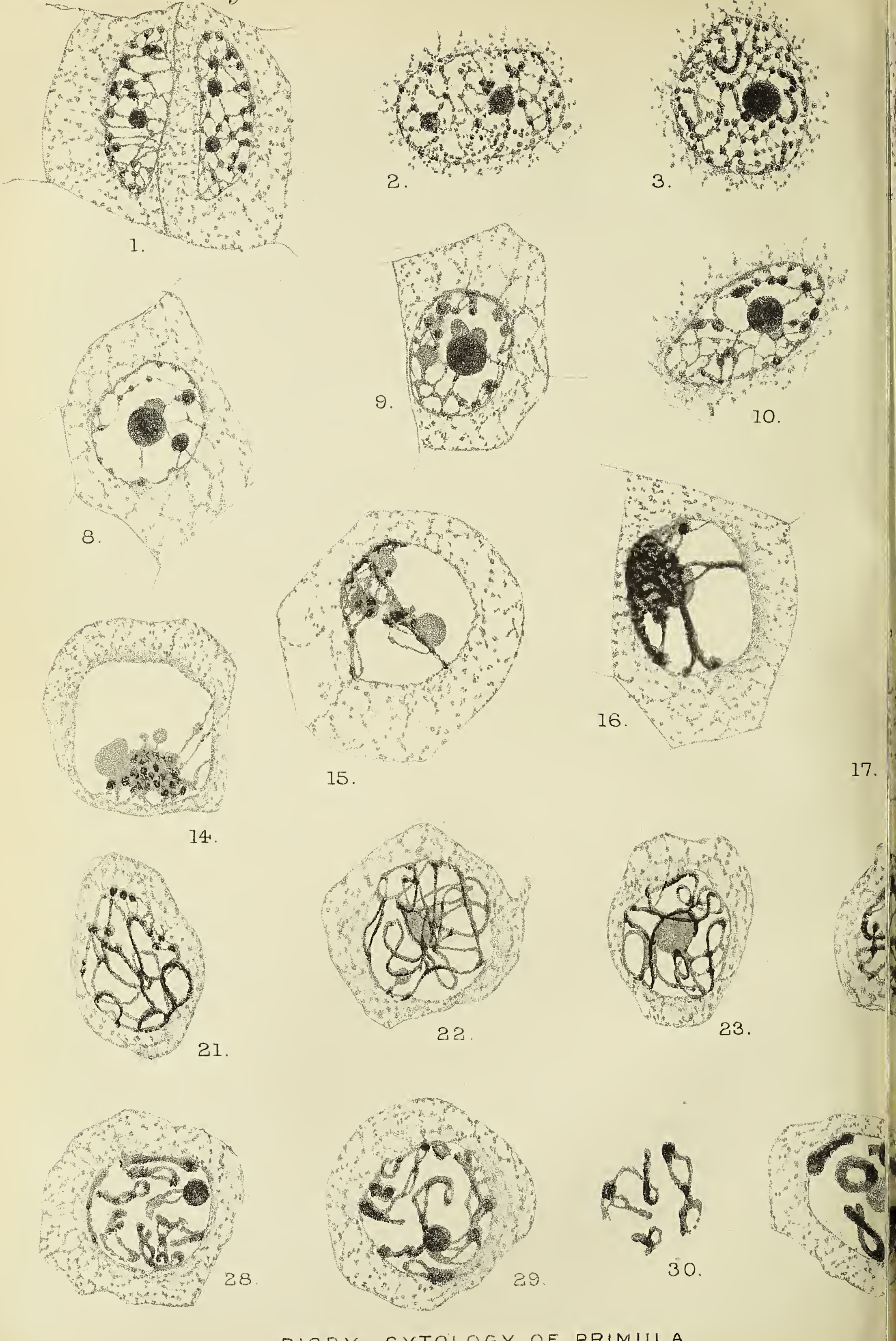
Fig. 104. Embryo-sac mother-cell. Prophase of the first meiotic division. The chromosomes are all separate from one another. Note seventeen chromosomes and one in the next section.

Fig. 105. Embryo-sac mother-cell. Equatorial plate (in two consecutive sections). All the chromosomes are of the same size, and there is no quadrivalent combination.

Fig. 106. Embryo-sac mother-cell. Polar view of equatorial plate, showing the eighteen chromosomes.

Fig. 107. *P. kewensis farinosa* (varietal origin). First meiotic division. Polar view of aster, showing eighteen haploid chromosomes.

Fig. 108. *P. kewensis farinosa* (hybrid origin). Premeiotic prophase, showing thirty-six diploid chromosomes.



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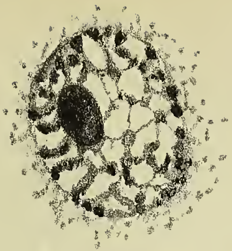

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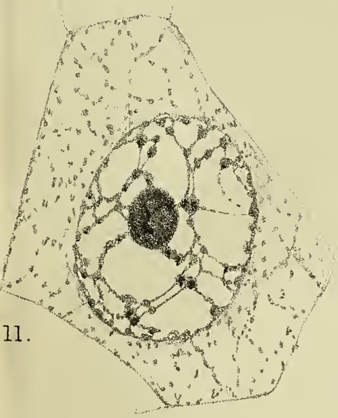

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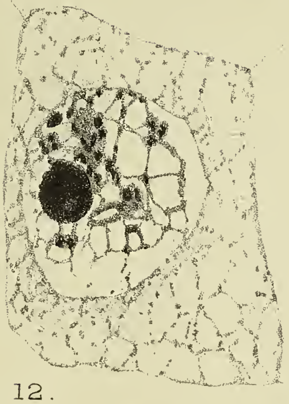

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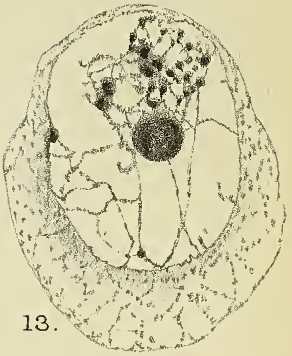

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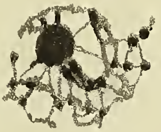

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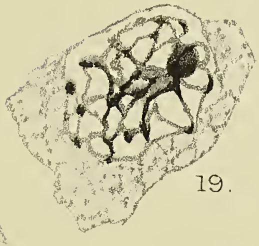

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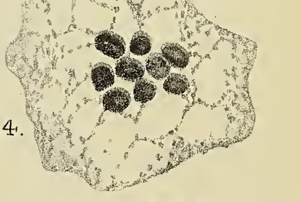

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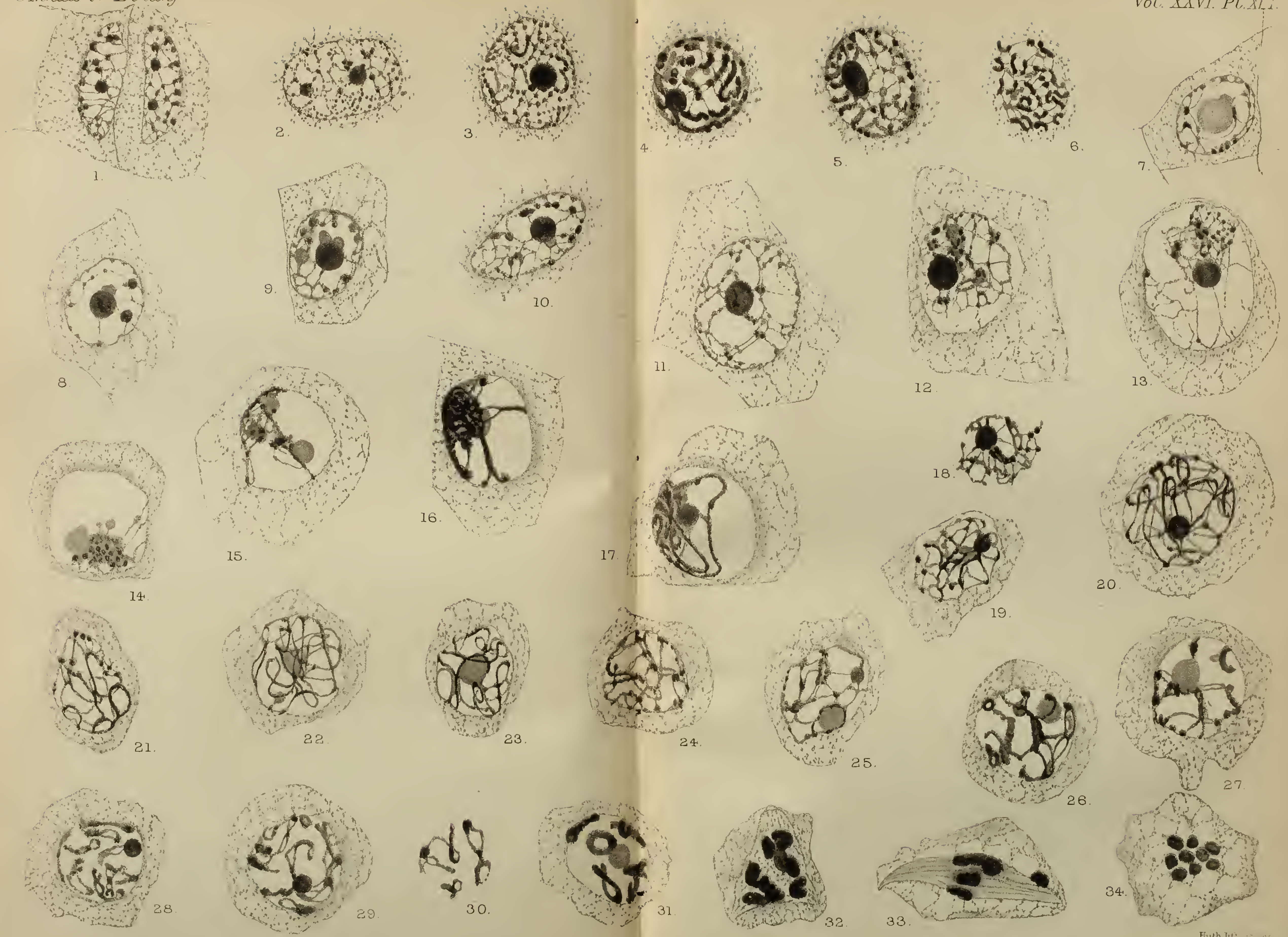

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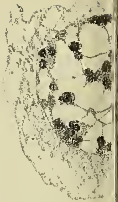


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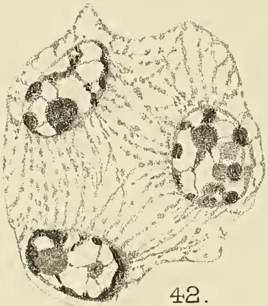
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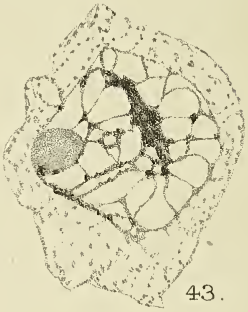
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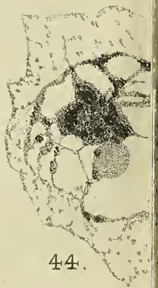
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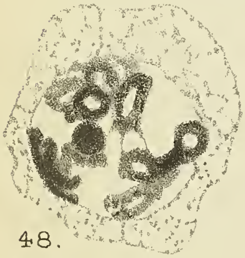
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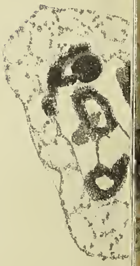

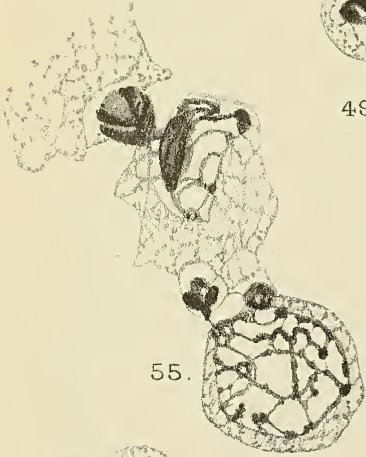
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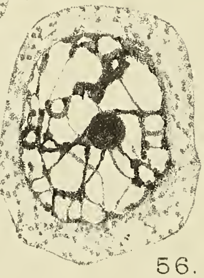
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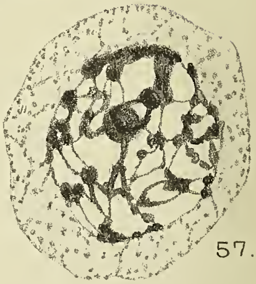
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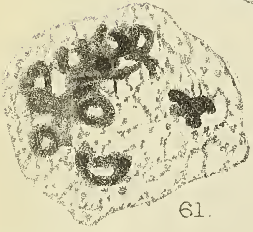
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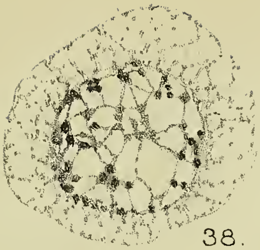

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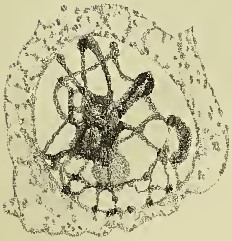

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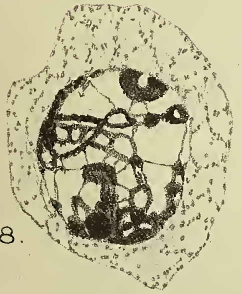

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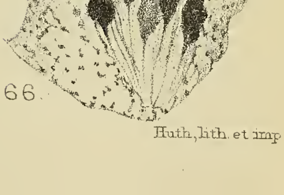

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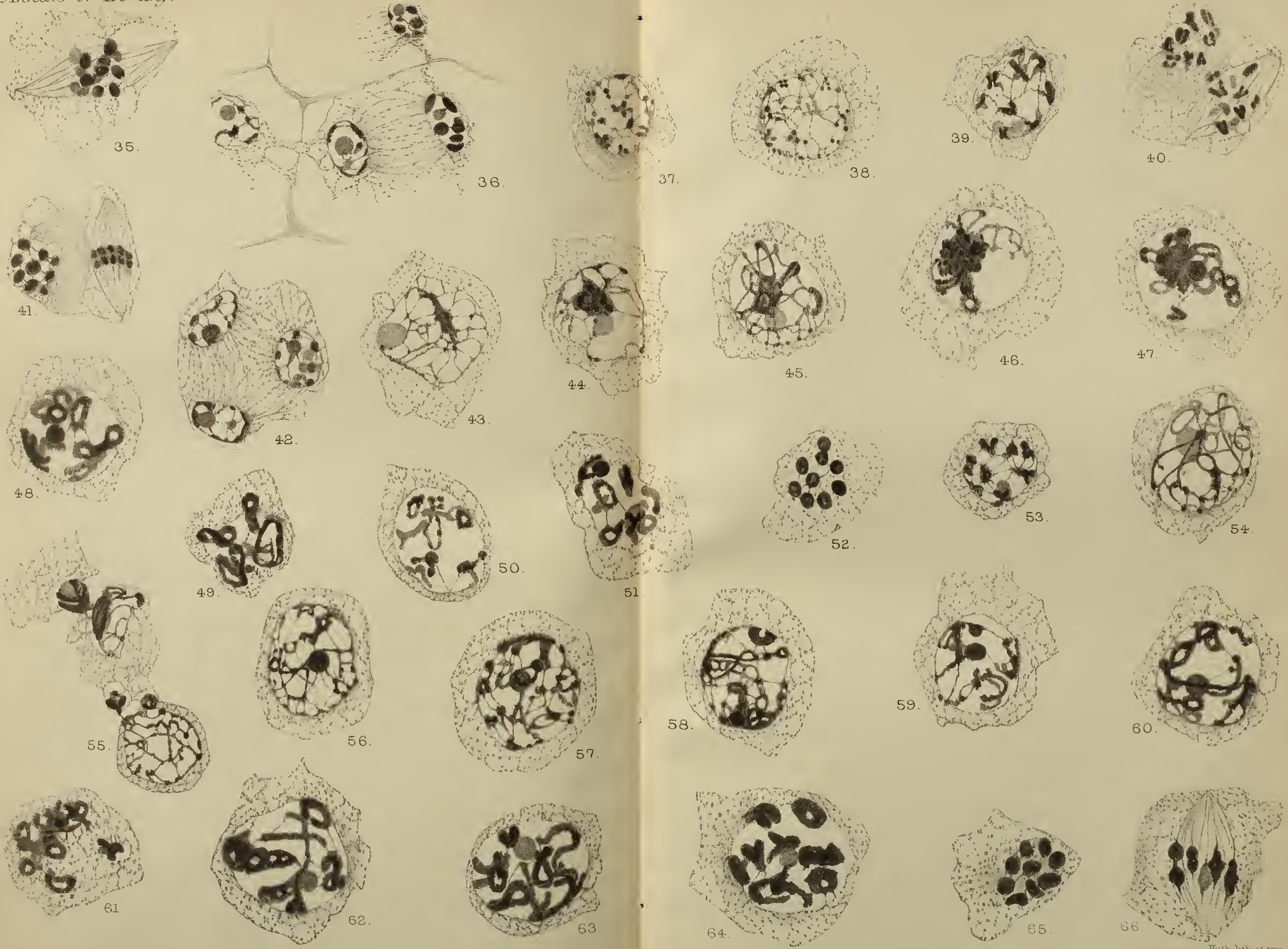

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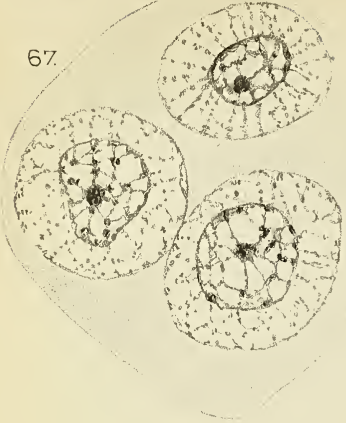

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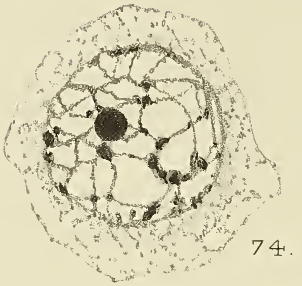

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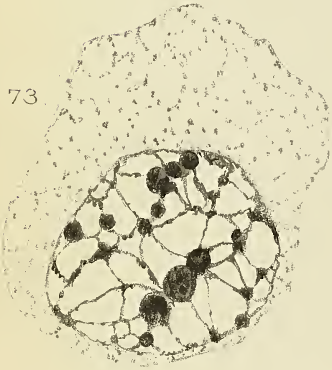

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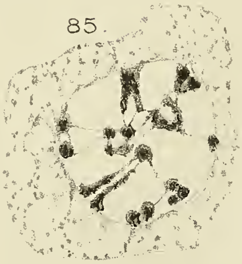

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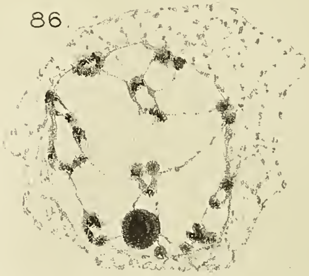

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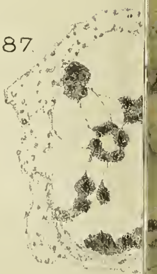

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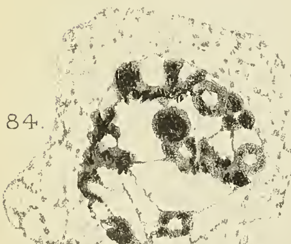

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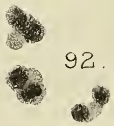

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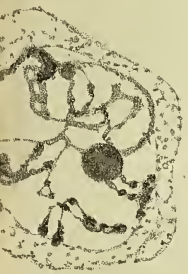


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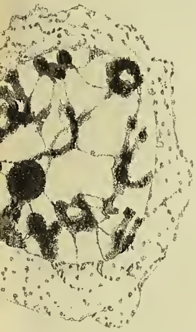


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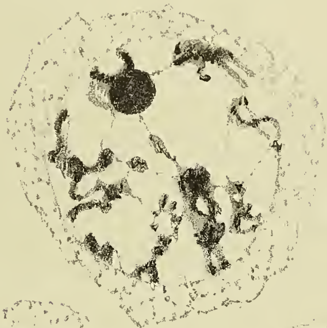
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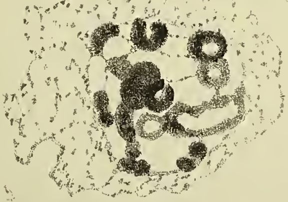
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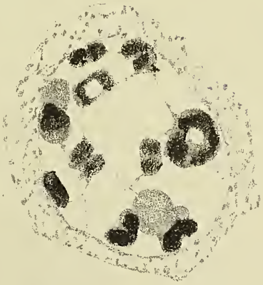
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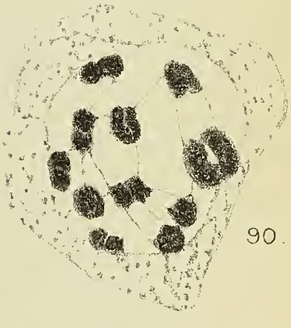
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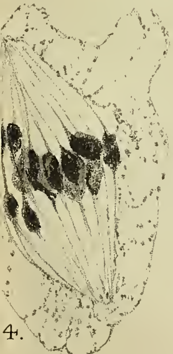
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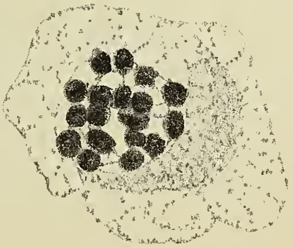
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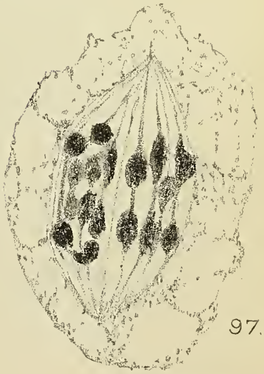
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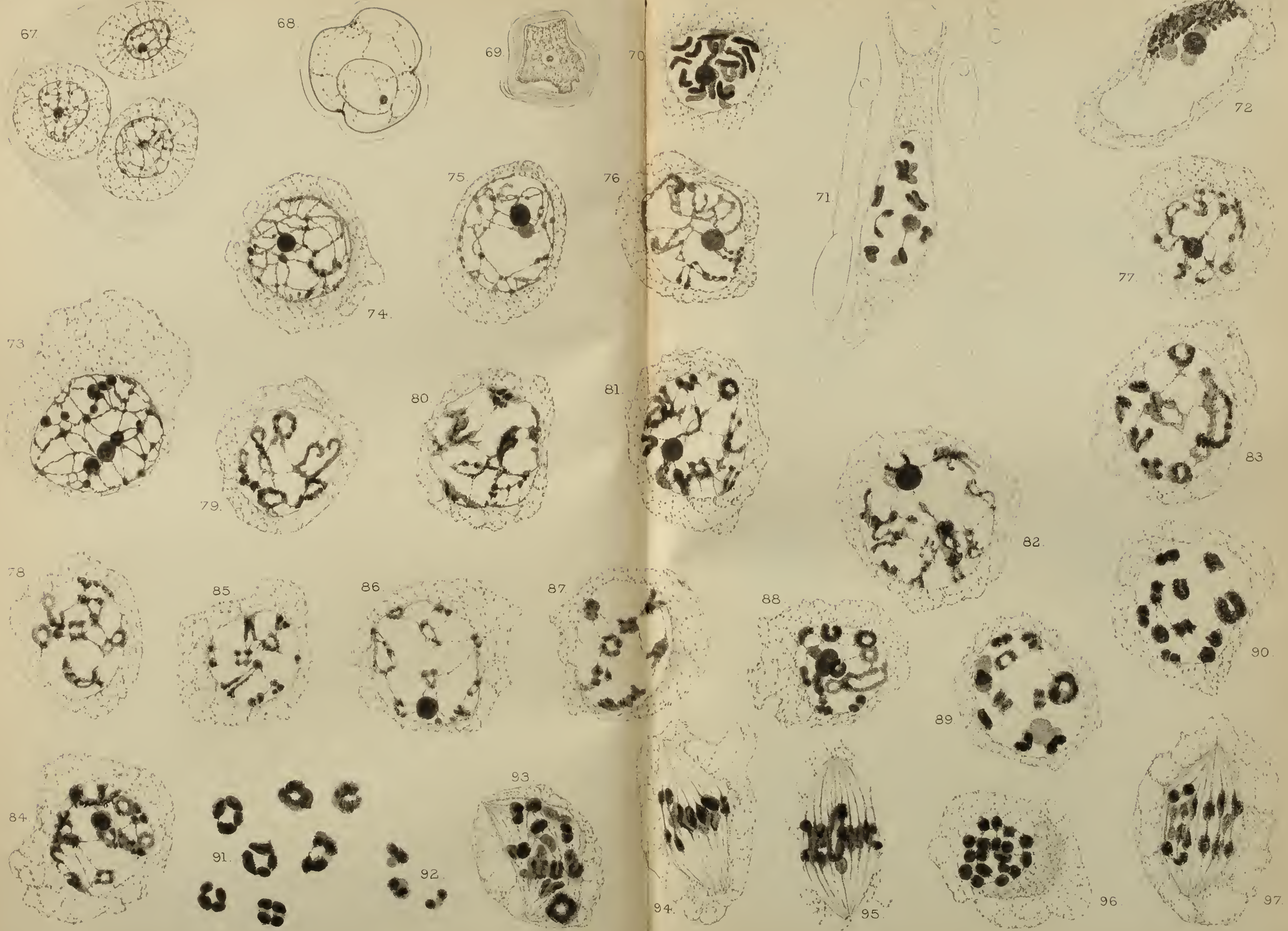

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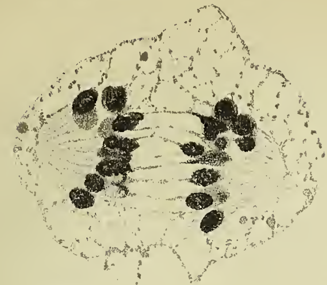

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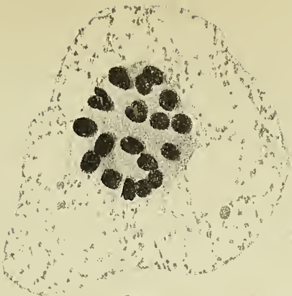

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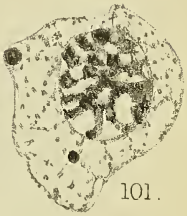

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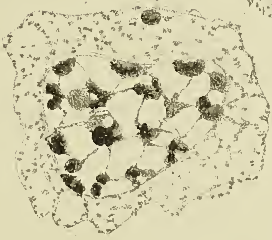

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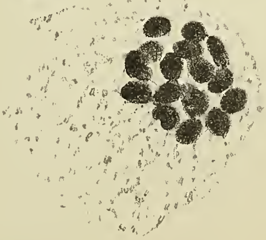

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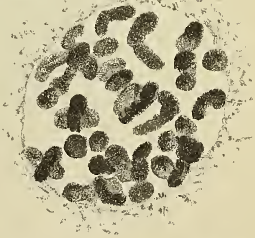

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DIGBY—CYTOLOGY OF PRIMULA.

Some Points in the Morphology and Physiology of Fasciated Seedlings.

BY

T. REED, A.R.C.Sc.

Senior Demonstrator of Biology, Guy's Hospital Medical School (University of London).

With nine Figures in the Text.

A FASCIATION is a form of abnormality in which the organ concerned becomes flattened or banded. This may be brought about in two ways, (1) by the fusion of organs which are ordinarily distinct, or (2) by the lateral expansion of an organ at its growing point.

The study of abnormalities in general has always occupied a more or less prominent place in botanical science, but the point of view from which they have been regarded has largely been that of the 'curious'. The two following quotations show in a striking manner the attitude of the botanists in the middle of the nineteenth century and the beginning of the twentieth. M. T. Masters (13), in his 'Vegetable Teratology', is describing the attitude of the botanists of the day, and says of malformations, 'They have been too long looked upon as monsters to be shunned, *lawless* deviations from the ordinary rule and unworthy of the attention of botanists.' Hugo de Vries (21) in 1905 says, 'Monstrosities are often considered as accidents, and rightly so from the morphological point of view, but *physiology excludes all accidentality.* Some internal hereditary quality is present though often latent, and the observed abnormalities are to be regarded as responses of this innate tendency to external conditions. Monstrosities should always be studied by physiologists from this point of view.'

Looked at from this point of view abnormalities become invested with a much greater importance.

The normal development of an individual is the result of two factors, the inherent tendencies of the organism and the external influences to which it is subjected. An abnormal form is one which deviates to any considerable extent from the normal or 'usual' form, as a result of the disturbance of one or other of these factors. If, however, the organism

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deviates from the 'usual' course of development because of its being subjected to *new* external influences, then the term abnormal should cease to have any meaning *so far as that individual* alone is concerned, for obviously if it has been caused to deviate from the usual path of development because of certain specific new conditions, then that altered form is merely a *new* form which fits in with new conditions; that is to say, it is *normal* in so far as it is an *individual* which has developed as a result of inherent tendencies and external conditions, but abnormal when considered in relation to the 'type form'.

Within recent years many experimental facts have been obtained which go to show the truth of de Vries's suggestion.

It has been shown by G. Klebs (10), amongst others, that an alteration in the nutrient medium of an organism may have an important effect upon the form or phase of life of the individual concerned, and A. F. Blakeslee (1) has shown that it is possible to cultivate distinct physiological races by varying the conditions of culture. Similarly with the variations in *Oenothera Lamarckiana*, which de Vries discovered near Amsterdam in 1886. In its native soil the form had proved fairly constant, but in the potato field at Hilversum, where it met with *new* conditions, it soon began to assert its power to vary by producing 'abnormal' forms in the shape of fasciations and pitcher leaves, annual and triennial forms in place of biennials, and numerous other forms.

While altered conditions of culture may only result in a temporary 'variation' of the organism, it is important to notice that the individual can apparently be made to change in some degree.

It does not seem to be a far cry from these altered forms to those recently obtained by D. T. MacDougal (12). This observer injected solutions of sugar, zinc sulphate, &c., into the ovaries of flowers of *Oenothera biennis* and *Raimannia odorata*, and from the ovules of these plants he obtained seeds which on germination gave rise to individuals which deviated to a considerable extent from the normal parental type. These observations are particularly interesting in that not only were the individuals very different from the parental type, but also in that the derived forms have bred true over a period of five years. In other words, it would appear that we have here an 'abnormality' induced by a specific alteration of conditions of growth, i. e. an induced mutation. The 'derivative' form also breeds true to type when placed in the habitat of its parental type.

In nature it is not always easy or possible to analyse all the factors which may be operating on the individual, but it seems probable that there must be factors the effects of which, either singly or collectively, may be to induce modifications in the organism. What the actual mechanism is would be difficult to determine. Is it a definite chemical action of the disturbing substance on the constitution of the plant body, or is it an alteration in the physical environment which 'permits' the variability of the organism to assert itself anew?

Abnormalities in the form of fasciations are of fairly frequent occurrence, and many examples have been described. C. Linnaeus (11) speaks of fasciations in his 'Philosophia Botanica'. He discusses their nature, and concludes that fasciated stems are due to the formation of an unusual number of growing points which become coherent, so that a whole bundle of shoots emerge as one. He says, 'cum plures caules connascuntur, ut unus ex plurimis instar fasciae evadet et compressus.'

At the beginning of the nineteenth century A. P. de Candolle (2) began to study malformations, but principally from the morphological point of view.

T. A. Knight (8, 1822), in a letter to the Secretary of the Horticultural Society, gives us some interesting facts concerning the production of fasciation in the Cockscomb (*Celosia*), perhaps one of the most widely known fasciations. He found that by applying larger quantities of manure he could produce larger and flatter specimens of the flower stalk of *Celosia.* By manuring the soil with 'one part of unfermented horse-dung fresh from the stable and without litter, one part of burnt turf, one part of decayed leaves, and two parts of turf, the latter in lumps about half an inch in diameter', and by applying 'liquid manure of steeped pigeon-dung in water', he could produce fasciated flower stalks eighteen inches wide. This fasciation is interesting in that the fasciated stem has now become normal and its inheritance is absolute. It is further instructive in that it is a definite example of a fasciation that has been induced by altered external conditions, the external condition which promotes fasciation being the superabundant nourishment.

A. Moquin-Tandon (15) in 1841 published his 'Éléments de tératologie végétale', wherein he discusses the significance to be attached to malformations. In this work he makes the interesting observation that the seeds of a fasciated *Circium* reproduced the same condition in the seedlings.

W. Hinks (7) in 1853 described instances of fasciation in *Primula vulgaris*, *Hieracium aureum*, *Ranunculus bulbosus*, *Bunium flexuosum*, *Cotoneaster microphylla*, &c. He states 'that these malformations are due to the operation of causes or principles, the ordinary operation of which produces the normal structure of the species'. He evidently recognized that 'extraordinary structures' were the result of 'extraordinary influences'. He, however, goes on to say that 'fasciated stems seem to be best explained on the principle of adherence of a number of buds which have arisen in close proximity owing to the presence of *superabundant nourishment*, especially if accompanied by check or injury'. He also combats the argument that fasciated stems are due to the dilation of a single stem, as had been supposed by many people.

M. T. Masters (13) published his 'Vegetable Teratology' in 1869, and there gives a host of examples of plants showing various forms of malformation. He recognizes that malformations are to be regarded as irregularities differing from variations mainly in their wider deviation from the customary structure and in their more obvious dependence on external conditions, and by their smaller liability to be transmitted by inheritance. He further goes on to say 'that it is even possible that malformations, especially when they acquire a permanent nature and become capable of reproducing themselves by seed, may be the starting-point of new species, as they assuredly are of new races, and between races and species he would be a bold man who would undertake to draw a hard and fast line'. Thus even as early as 1869 Masters thought it not improbable that mutations, caused by external influences, might give rise to new species.

Coming to more recent times, A. Braun, Celakowsky, Worsdell (23), Goebel (4, 5), Vöchting (18), and de Vries (19, 20, 21, 22) have given us many interesting observations on malformations.

K. Goebel (4, 5) states that it was mainly owing to the study of malformations that the idea of the foliar nature of the stamen came to be recognized. 'It is rather to the causes which condition the deviation from the normal development that we ought to pay most attention.' If by altering the external conditions an alteration can be induced in the organism, then we are in a fair way towards throwing some light on the great question of organic form. He gives examples of fasciations in *Taraxacum officinale* and mentions that it is only well-nourished specimens which show this phenomenon.

H. de Vries (19, 20, 21, 22) describes several examples of fasciated stems in *Taraxacum officinale*, *Thrincia hirta*, *Picris hieracioides*, and *Crepis biennis*. The latter gives off about 30 per cent. to 40 per cent. of heritable forms. He states that weak seedlings are not favourable examples for obtaining malformations, but strongly nourished plants should be taken. In speaking of stems which show biastrepsis and fasciation, de Vries (19) also states that 'most monstrous plants require much manure; manure can hardly be given in excess; the richer the soil the better the proportion of twisted stems'.

A. D. Knox (9) describes instances of fasciation in *Oenothera biennis* and *O. cruciata*. She describes the anatomy of the fasciated stems and gives an account of the degree of heritability of the malformation. The causes of fasciation in these cases are said to be two, (1) injury to the growing point by insects, and (2) superabundant nourishment. She concludes that 'given similar conditions of culture the factors involved in the production of fasciations are the specific mode of attack of insects, the character of the plant, and the rapidity of development. The second of the three appears to be of most importance.' A point worthy of special notice is that the fasciations usually just precede the date of flowering, i. e. at a time when the carbohydrates of the plant will be very abundant.

Molliard (14) describes cases of fasciation in which he found the apices of the plant injured by insects.

J. Peyritsch (17) found fasciations which he thought were due to fungal attacks.

T. Petch (16) states that fasciations are frequently found in *Hevea Brasiliensis*. The trees affected are principally young trees. He thought that in some instances fasciation was due to attack of insects and Fungi, but in the majority of cases no definite cause could be stated.

R. H. Compton (3) describes the anatomy of a 'ring fasciation' in the Mummy Pea, *Pisum umbellatum* (Miller). This form also breeds true, its peculiar character behaving as a simple recessive in crosses with the normal type.

A review of the historical evidence leads us to conclude that fasciations are due to at least two causes. Firstly, the presence of superabundant nourishment and suitable conditions for rapid development; and, secondly, the sudden arrest of the activity of growing points due to insect and fungal attacks. It would appear, though, that a third factor ought to be added to account for the many cases of fasciation which appear for no apparent reason, but which might very easily be caused by mechanical injury to the growing point.

In any case, it now appears to be fairly certain that whatever the inducing factor may be there must be present within the plant a considerable quantity of elaborated sap to supply the demands of increased meristematic activity, and it may well be that this is the essentially determining cause in all.

In the present investigations seedling plants of Leguminoseae were chiefly used, but others were introduced for purposes of comparison.

The seeds were germinated in various soils, and when the plumular shoots had developed a length of about an inch or so the apical bud was cut away and the seedling allowed to continue its growth.

Two types of seedling were used, the epigeal and the hypogeal types.

A. HYPOGEAL.

I. PHASEOLUS MULTIFLORUS.

The plants were grown in both soil and sawdust. In about two days the radicle had penetrated the testa and grown down into the substratum. Two or three days later the epicotyl and plumular leaves had appeared above the soil and attained a length of about an inch. The main portion of the epicotyl was removed and the seedlings allowed to continue their growth. As a consequence of the arrested growth at the stem, apex shoots were developed in the cotyledonary axils (Fig. 1, *b*). The

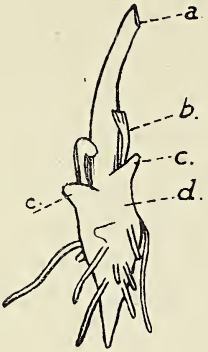

FIG. 1.

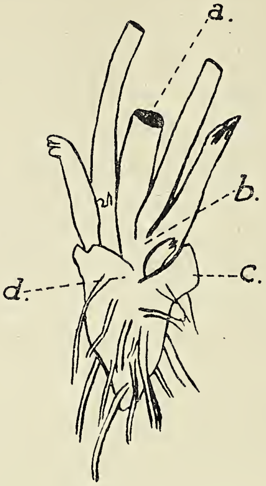

FIG. 2.

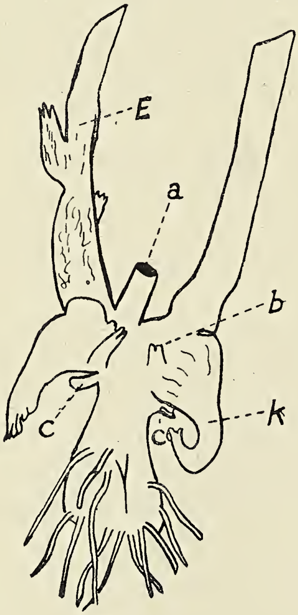

FIG. 3.

FIG. 4.

FIGS. 1–4. *a*, cut end of plumular shoot. *b*, axillary shoots. *c*, cotyledonary petiole. *d*, hypocotyl. *E*, fasciated shoot. E_1, the reverse side of *E*. *k*, twisted and fasciated shoot.

buds from which these shoots are developed normally remain dormant. Many of these shoots, instead of following their normal course of development, were found to show very pronounced fasciations and biastrepsis

(i. e. twisting). If the further development of these shoots is also inhibited by the removal of their apical buds, more fasciated and twisted shoots are produced at their bases until quite a tangle of such shoots is formed in the cotyledonary axil. Such seedlings are shown in Figs. 2 and 3, *b*. If the seedling is now permitted to continue its growth it does so for a short time only, and sooner or later all further growth ceases and the plant dies.

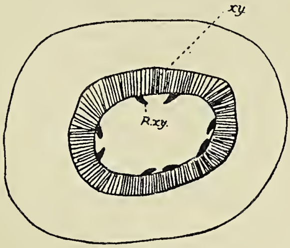

Fig. 5.

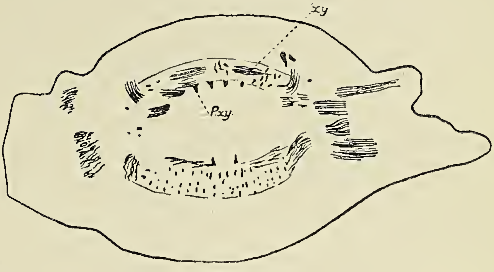

Fig. 6.

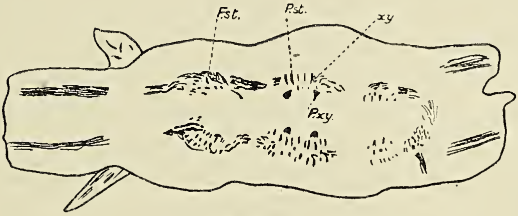

Fig. 7.

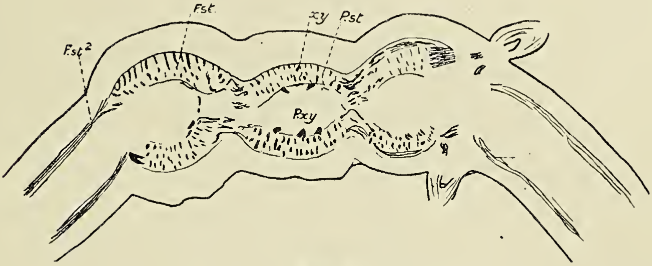

Fig. 8.

Figs. 5–8. *xy*, secondary xylem. *P.xy.*, protoxylem. *P.st.*, plumular stele. *F.st.*, *F.st.*², first and second fasciated shoot steles.

In addition to the development of the axillary fasciated shoots, the hypocotyl also develops enormously and presents a fasciated appearance (Fig. 2, *d*).

The leaves which are laid down on the fasciated shoots also remain small and in many cases do not even unfold from the bud (Fig. 4, *f*).

The development of the root system appears to be quite normal.

The power of the plant to respond to various stimuli also appears to be interfered with. The shoots twist and curl in all directions, with an apparent disregard for all heliotropic and geotropic stimuli (Fig. 3, *k*).

Anatomy of fasciated shoots. As is well known, four bundles pass into the hypocotyl from each cotyledon. In passing down the hypocotyl these become orientated, so that a tetrarch stele is formed in the root.

Secondary thickening commences very early, so that in a young seedling an almost complete vascular ring is found near the top of the hypocotyl (Fig. 5). From this ring is given off the vascular supply to the fasciated shoots. To the right and left of this central stele lateral steles are given off which pass out into the fasciated shoots. It has been pointed out above that the hypocotyl has also become fasciated, and this fasciation is also reflected in its anatomical structure. These lateral steles do not immediately become separated from the central stele, but they run parallel with it for some little distance, so that at this level one gets a polystelic structure consisting of three parallel steles (Figs. 6 and 7, *F.st.*). This condition of the stele has been frequently described by Worsdell and others for other plants.

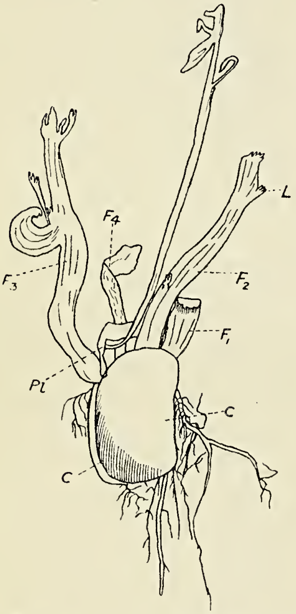

Fig. 9. *Pl,* arrested main shoot. F_1, F_2, F_3, F_4, fasciated shoots. *L*, reduced leaves. *C*, cotyledons.

Before these steles are separated from the parent stem they proceed to give off other lateral steles which pass out into the second fasciated shoots. These steles behave similarly to the first pair of laterals and remain attached to the parent stem, so that a polystelic structure consisting of five steles is shown (Fig. 8, *F.st.*^{2}). Sooner or later, however, the fasciation breaks up and the steles pass out into the separate fasciated shoots.

The anatomical features of the individual fasciated shoots call for no detailed description. The normally cylindrical stele becomes laterally attenuated and follows more or less completely the configuration of the malformed shoot. No pronounced abnormal structures were seen in the stele of these shoots, although it is not uncommon to find that the whole arrangement of the stele may be considerably interfered with. On the whole one finds perhaps fewer lignified elements in such vascular tissue, but this may be correlated with the comparatively rapid growth of the shoot and its generally etiolated appearance, and the fact that its subsequent development is also arrested, so that the elements remain in a more or less immature condition.

A case which calls for special mention is one in which the fasciation appeared 'spontaneously'; that is to say, the fasciation was not artificially induced by amputation of the growing point. Although I was not able to determine with absolute certainty the immediate factor which brought about the fasciation, a careful examination of the seedling showed that the development of the main shoot had been interfered with and its growth checked. This check may have been due to mechanical injury of the growing point, or the apical bud may have been attacked by a fungus or insects, &c. As a result of the check the axillary shoots developed and became strongly fasciated. This seedling is figured in Fig. 9.

VICIA FABA.

Seedlings of this plant were also grown and treated in a similar manner to those of *P. multiflorus*. Fasciations were obtained, but they were not nearly so pronounced or numerous as in the case of *P. multiflorus*.

The same general features were observed in the hypocotyledonary anatomy, but never more than three vascular rings were met with.

PISUM SATIVUM.

Seedlings of this plant were similarly treated, but here again, while fasciations were produced, they were few in number.

It will be noticed that the three types of seedling above studied are all of the hypogeal type and all show fasciations to a greater or lesser degree. It was therefore thought desirable to test the power for producing fasciation in epigeal seedlings.

B. EPIGEAL TYPES.

Phaseolus vulgaris (French Bean), *P. vulgaris* (var.?) (Butter Bean), *Lupinus Douglassii*, *Ricinus communis*, and *Cucurbita Pepo* were chosen because of the fleshy nature of their cotyledons or because of the large amount of reserve food available to the seedlings.

The seedlings were grown in pots along with the types already studied, and also grown separately in pots with the other types as controls grown near to them. They were subjected to treatment similar to that which the previously studied types had undergone. Although the cotyledonary shoots developed in many instances, in no case was a fasciation produced.

Is this power of the hypogeal type of seedling to produce fasciations an accidental quality which is not possessed by the epigeal type, or is it that the food reserves of the two types of seedling differ in quality or quantity?

It has frequently been stated by other observers that an abundant supply of food material is necessary for the production of fasciations.

Analyses were made of the seeds of *P. multiflorus*, *P. vulgaris*, and *Vicia Faba*. For these analyses I am greatly indebted to Mr. T. J. Ward, of the Chemical Department of St. Mary's Hospital Medical School, Paddington.

In each case the testa was removed from the seed before the analysis was made.

The results of the analyses were as follow:—

Constituents.	*P. multiflorus.*	*Vicia Faba.*	*P. vulgaris.*
Moisture	14·44	13·305	13·46
Benzine extract. (Oils, fats, resins, &c.)	2·72	2·11	1·98
Alcohol extract. (Tannin, glucosides, and some sugars)	(11·04)	(4·51)	(29·99)
Soluble carbohydrates, as dextrose	10·155	16·004	9·81
Starch	46·35	37·296	43·01
Cellulose	2·31	2·09	2·15
Proteids	21·64	27·83	21·96
Diastatic value as wt. of starch converted by 100 grm. of bean	(3·5)	(1·85)	(3·33)
Calcium (as oxalate)	0·196	0·123	0·216
	97·811	98·758	92·586

Each sample contains dextrine, glucosides, albumins, globulins, albuminoses, and substances which on hydrolysis yield pentoses.

The total percentages are in all cases below one hundred, and this is due to the fact that the alcohol extracts are not included in the total percentages. The extracts contain a considerable quantity of a resinous substance which was not identified with certainty owing to the methods for the determination of glucosides and tannins being unsatisfactory. *P. vulgaris*, however, contains a larger quantity of this resinous substance than either of the other two. This result is important, for it is stated by Jost that tannin would inhibit the action of diastase. This point, however, will be referred to again later.

The differences in the amounts of carbohydrates are not considerable, but the epigeal type (*P. vulgaris*) contains the smaller quantity, the combined amounts of soluble carbohydrates, starch, and cellulose being:—

P. multiflorus.	*Vicia Faba.*	*P. vulgaris.*
58·815	55·390	54·97

In regard to their proteids *P. vulgaris* contains a smaller amount as compared with *V. Faba*, but a slightly larger quantity as compared with *P. multiflorus*.

The 'diastatic value' was estimated as follows: A *cold*-water extract was made from the bean and so it contained no starch, as was shown by testing before making an estimation. Definite amounts of the extract were added to a dilute solution of pure starch. The tubes were immersed in a water bath at 40° C. for one hour and then cooled and tested, after making sure no starch remained unconverted. The experiments were carried out by gaslight, but as the tubes were immersed from five to six inches in water they were removed from the influence of light.

The smallest value is given for *V. Faba* and the greatest for *P. multiflorus*.

P. vulgaris contains a greater quantity of calcium oxalate than either of the other two.

The analyses show that the epigeal type contains less carbohydrates, about the same amount of proteid, but more resinous matter. Apart from the resinous substances the three seeds do not differ to any considerable extent quantitatively. It does not follow, however, that this ‘ready manure’ is equally available for use by the two types of seedling respectively. In the one case the food reserves are not normally exposed to the action of light, whilst in the other they are carried up into the light. It is quite possible that this latter fact is of importance, for J. R. Green (6) states that light has an inhibitory effect upon the action of diastase and so the starch may not be as available to the seedlings of the epigeal type as to those of the hypogeal type. The presence of a greater quantity of glucosides in the epigeal type may also interfere with the conversion of starch into soluble carbohydrate, for according to Jost this also has an inhibitory effect upon diastatic activity.

That there is a difference between the two types of food reserve and general organization of the developmental cycle is shown by the degree of dependence which each type of plant has on its reserves.

Young seedlings of *P. multiflorus* and *V. faba* were allowed to grow until the first pair of plumular leaves was unfolded. The cotyledons were then completely severed from the seedling and the plant permitted to continue its development. Growth is continued for a very short period only; the leaves expanded a little more, but sooner or later the mutilated seedlings died. During the same interval (Oct. 11—Nov. 3) the normal control plants grew about four feet in height.

A second batch of seedlings was then taken and allowed to grow until the first pair of plumular leaves was fully expanded; their cotyledons were then removed. In this case growth in length of the stem continued for a little while, but no new leaves were unfolded, the plant on the whole showing an etiolated appearance. More advanced stages of germination were taken, and it was found by this series of experiments that these plants were dependent upon their cotyledonary food reserves until they had expanded their third or fourth pair of leaves.

A similar set of experiments was made with the French Bean and Butter-bean. In these cases the cutting away of the cotyledonary food supply appeared in no way to affect the subsequent growth of the plants, the mutilated ones being indistinguishable from the controls.

From this series of experiments it would appear that, although the reserve food materials of the cotyledons may be very similar in the first instance, their immediate and relative availability to the two types of seedling is very different. The fact that the cotyledons are hypogeal in the one case suggests that the reserve food stored within them is sufficient to give the plant a good start in its development, such, at any rate, that it does not bring them above ground to assist in the processes of assimilation. On the other hand, the epigeal type brings its cotyledons into the service of the plant by enabling them to assist in assimilation. On the whole, then, it seems that the hypogeal type has an immediate and readily available stock of food, on which it draws largely when germination begins. In the case of the epigeal type of seedling it may well be that the cotyledonary food reserves are early used up in the growth of the cotyledons and the fairly long hypocotyl, and as a consequence the seedling has from the early stages acquired the habit of foraging for itself.

Then again, since the cotyledons of the epigeal type are carried into the light and become green, it is quite possible that the materials produced by photosynthesis become mixed up with the reserves, which in this way may be rendered less directly available for the plant.

With a view of testing this suggestion, seedlings were grown in the dark room and the plumular buds amputated as in previous experiments; controls were also kept under the same conditions. Fasciations were only obtained in those plants which show them under the other experimental condition. The seedlings of *P. multiflorus* produced good fasciations, but the epigeal types developed their cotyledonary shoots without showing any trace of fasciation. A second set of experiments was also made in which *P. multiflorus* seeds were germinated in the ordinary way and the plumules amputated as before. The cotyledons were then uncovered and the testa removed, so that the cotyledons were fully exposed to the light. The exposure of the cotyledons, however, had no apparent effect on the plants' power to fasciate, for fasciations appeared in considerable numbers.

It would appear, then, that light is not the only determining factor, for the epigeal type does not fasciate when its cotyledons are removed from the influence of light, nor does the hypogeal type cease to fasciate when its cotyledons are brought under the influence of light.

Further experiments were made in which seedlings of the epigeal type were given a plentiful supply of nitrogenous manures. Although the unmutilated plants were very vigorous in their growth the mutilated plants did not produce any fasciated structures.

SUMMARY.

1. The plants used in these experiments may be grouped in two classes: (1) Hypogeal types—*Phaseolus multiflorus*, *Vicia Faba*, and *Pisum sativum*; (2) Epigeal types—*Phaseolus vulgaris*, *Phaseolus vulgaris*, var.?, *Lupinus Douglassii*, *Ricinus communis*, and *Cucurbita Pepo*.

2. Fasciations were induced in the former class but not in the latter.

3. The hypocotyl of hypogeal seedlings may be fasciated as a result of the adherence of a number of fasciated shoots. The vascular anatomy of such a structure is polystelic.

4. The food reserves in the two types of seedling may be similar, chemically, but the availability of that food is different for the two types of plants.

5. Hypogeal seedlings are remarkably dependent on their cotyledonary food reserves for their early development as contrasted with seedlings of the epigeal type.

My thanks are due to Prof. J. B. Farmer for suggesting that the work should be undertaken and for much valuable help and criticism during the earlier stages of the investigations; to Mr. W. Hales, of the Chelsea Physic Garden, where most of the experimental work was done, and also for much valuable advice on the culture of seedlings; to Mr. T. J. Ward, of St. Mary's Hospital Medical School, for the analyses of some of the seeds; and to Mr. E. Lee for much friendly criticism during the preparation of the paper.

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The Attaching Discs of the Ulvaceae.

BY

E. MARION DELF.

With Plate XLV and three Figures in the Text.

THE thallus in the Ulvaceae consists of a somewhat delicate green frond, terminating below in a narrow and very often short stalk or stipe, which is attached to stones, wood, or, less often, to other Algae by means of a tough disc-like expansion.

This attaching organ was described by Thuret[1] in 1878 as a small disc formed by the interwoven tubular prolongations of the cells of the thallus. Any cell of the thallus was said to have the power of forming the tubes, with the possible exception of those cells which become zoosporangia. Just above the disc the thallus is distinctly thicker than higher up, and Thuret noticed that in some cases the tubes were woven together in dense strands or bundles, several such strands occurring side by side in one disc.

Some material of *Ulva latissima* (Harvey), preserved in formalin, was accidentally obtained growing upon a fragment of the frond of some other seaweed of the *Furcellaria* type. Hand sections showed that the tubular prolongations of the cells (or disc filaments, as I shall call them) had in some places displaced the small peripheral cells of the supporting thallus, in a way which suggested either a parasitic or a saprophytic habit. Later on, in fresh material from Rottingdean, a frond of *Ulva* was found attached to part of a thallus of the *Polysiphonia* type of structure, and here the disc filaments had actually penetrated into the cells of the host, just as is done by the intracellular hyphae of some parasitic Fungi. Other *Ulva* plants were collected which were attached to small chalky stones, and the discs of these were detached, carefully scraped, and washed free from grit as far as possible; they were then fixed in weak Flemming solution and embedded in the usual way. The microtome sections obtained from this and also from the parasitic material were stained with Kleinenberg's haematoxylin and eosine. Hand sections and preparations of teased-out hyphae were also examined.

A vertical section through the disc of a thallus of *Ulva* at its point of attachment to the supporting seaweed showed the tubular prolongations of

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[1] Thuret: Études algologiques, 1878.

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the superficial cells passing obliquely down to the disc, where they spread out and appear to become closely interwoven with one another. When they reach the host, the tips of the filaments swell up and become closely appressed to the surface, often appearing to form a kind of pseudo-parenchymatous investment; this is shown diagrammatically in Text-fig. 1.

TEXT-FIG. 1. Longitudinal section through attaching disc of *Ulva* : outline, and cells of host, drawn with camera lucida; filaments of the disc shown much simplified, and somewhat diagrammatically. × 100.

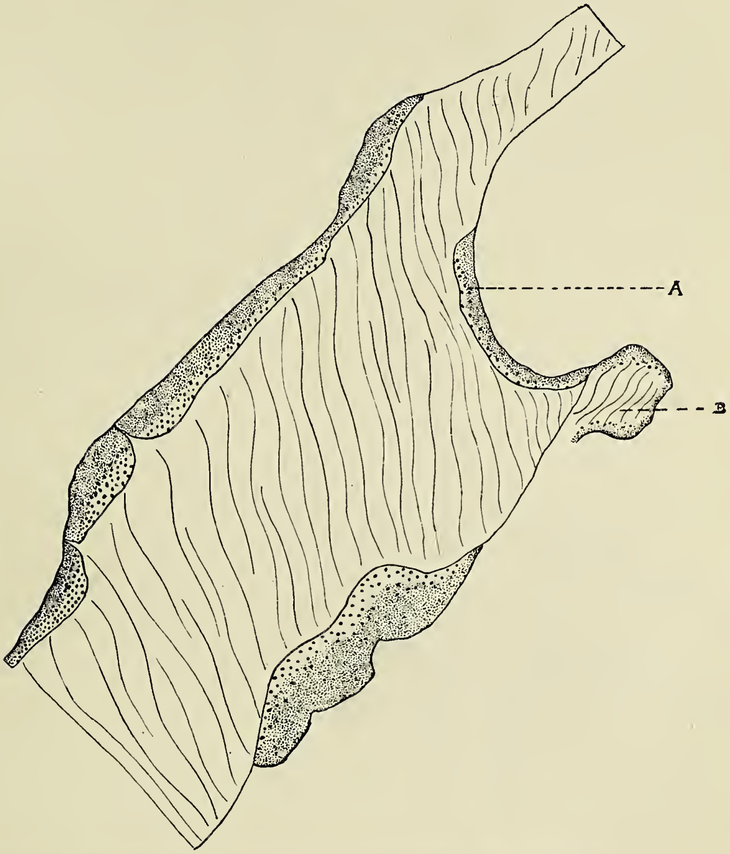
Microtome sections through the discs of fronds which had been attached to stones showed that wherever the filaments had reached the exterior their tips had become swollen and multinucleate; often definite cell-divisions appeared to have taken place, two or three successive segments having been cut off from the swollen part of the filaments (Pl. XLV, Figs. 5, 6). These larger segments were often surrounded, especially on the outside, by the cut ends of many minute filaments. Some of these had their origin in peripheral cells of the thallus, as seen in Pl. XLV, Fig. 8, at *a*; others appeared to have reached the periphery from within, and to have found no room for expansion at the end of their course; it is possible that some may also have formed by the branching of larger filaments, such as that shown in Pl. XLV, Fig. 1. The longitudinal section of such a disc is seen in Text-fig. 2, and a small part taken from the region A is shown in detail in Pl. XLV, Fig. 7.

The region marked B is part of the disc of another frond, formed, as it appears, from an outgrowth of the disc filaments, but this capacity for forming new fronds from the tissue of the disc was seen better in a hand section, which is represented diagrammatically in Text-fig. 3.

On teasing out the filaments from thick sections, it was found that they pursue a somewhat sinuous course through the lower part of the thallus, often bending on themselves, and the pointed awl-shaped tip suggests an active boring movement, as though the penetration of the tangled filaments were against distinct resistance (Pl. XLV, Figs. 2, 3). In some cases the filaments appeared to have branched, as in Pl. XLV, Figs. 1, 4, but these were very difficult to isolate, owing to the delicate nature of the tubes. In one case, however, no fewer than ten branches had been given off (Pl. XLV,

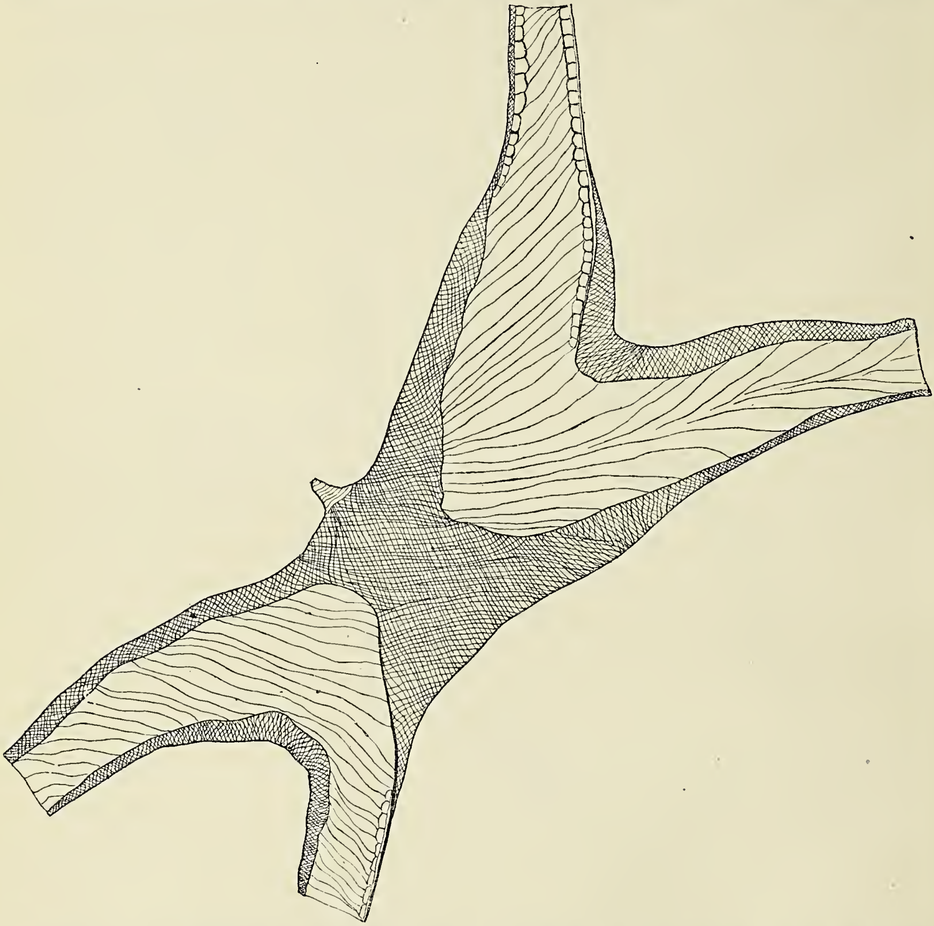
Fig. 1). Thuret states that the tubular filaments may reach a length of 6–10 mm., but those measured by me were never more than 3 mm. in length.

The superficial cells of the thallus giving rise to the filaments seem to be always multinucleate. In their upper region from three to five nuclei have usually been observed (Pl. XLV, Fig. 8). The tubes are exceedingly narrow, and are smaller when running on the outside than when running in the interior of the thallus (Pl. XLV, Fig. 8, at *a*); in either case minute nuclei occur at intervals down these tubes, and from two to five nuclei are commonly found crowded together in the tips of the tubes. These apices vary very much in size; they are usually narrow and pointed, with small nuclei, until the periphery of some part of the disc is approached, when they increase greatly in size, the nuclei become larger and more numerous,



TEXT-FIG. 2. Diagram of longitudinal section through disc of *Ulva latissima*. The oblique lines show the general direction in which the filaments run. The dotted regions show the false tissue formed by the large and small filaments at the periphery of the disc: the region around A is that from which Pl. XLV, Fig. 7 is drawn in detail. At B is the lower part of a frond formed from an outgrowth of the disc.

and two or three successive segments with usually more than one nucleus are cut off (Pl. XLV, Figs. 5, 6). It is probable that the small filaments bore their way into crevices and become firmly attached there, whilst the larger multinucleate segments give rise to the new thalli. All the filaments appeared to have been full of living contents at the time of fixing (early spring), so that it is probable that, whilst the thallus of this species is annual, the discs are perennial.



TEXT-FIG. 3. Diagram of longitudinal section through disc of *Ulva latissima*, which has given rise to four fronds. The lines show the general direction of the filaments; the cross-hatched region is part of the periphery of the disc which has not been cut in the median plane.

The nuclei vary somewhat in size, but always appear as small refractive bodies, with a single nucleolus. Two nuclei may often be seen closely associated, but no case of an actually dividing nucleus has been observed.

Stained microtome sections through discs of *Ulva*, which were attached to a thallus of the *Polysiphonia* type, leave no doubt as to the parasitic or saprophytic nature of the fronds. The host plant had been completely encircled by the developing disc, and the thick walls of the pericentral cells were pierced and riddled with the small pointed ends of the filaments, which usually contained more than one nucleus. In many of the cells the tips of the filaments had evidently expanded, forming a delicately vacuolated lining of cytoplasm almost filling the cell, and containing numerous and characteristic embedded nuclei (Pl. XLV, Figs. 9, 10). At the periphery of the host may be seen the appressed tips of large and small filaments, just as in the disc already described; both these kinds of filaments were found in the process of boring their way through the solid wall, and this is probably achieved, as in the Fungi, by the secretion of some cytohydrolithic ferment.

It is impossible for me to say whether the host plant was living at the time of fixing, but I have myself often observed both *Ulva latissima* and *Ulva lactuca* growing attached to the frond of some other living seaweed, and it seems probable, therefore, that this plant is at least a facultative parasite. Since, however, it is known that both species of *Ulva* grow luxuriantly in the presence of the nitrogenous products of decay, it is probable that the saprophytic habit could also be readily adopted.

THEORETICAL CONSIDERATIONS.

It is well known that the different species of *Ulva* which commonly occur on our coasts are especially abundant in the brackish water of estuarine localities, and also on the open shore in the neighbourhood of towns. In some cases the fronds grow so luxuriantly as to be cast up in masses by the tide, and the offensive smell of the sulphuretted hydrogen which is formed as these plants decay is sometimes a serious nuisance to the inhabitants. Before, however, this can be effectively remedied, much more must be known of the life-history and habits of the plant, as has already been shown in detail by Mr. A. D. Cotton in his suggestive paper on the subject.[1]

From the above account it appears that there is probably a very effective means of reproduction in the formation of these adhesive discs, which are undoubtedly very tenacious of life, can probably live through the winter independently of the thallus (which is described by Harvey and others as annual), and can bear new fronds by the upgrowth of the filamentous tubes of the disc. It would therefore be necessary to adopt some measure to destroy the vitality of these in order to check their spread effectively.

From the point of view of affinity, the multinucleate character of the filaments of the disc in *Ulva* serves to emphasize the isolation of the whole group of Ulvales, and to remove it from any near relationship with the Ulothricales. On the other hand, the simple segmentation of the tips of the filaments has no resemblance to the method of wall formation in

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[1] Cotton, A. D.: On the Growth of *Ulva latissima* in Water polluted by Sewage. Kew Bulletin, 1910.

a typically coenocytic group such as the Siphonales. The method of formation of the disc, indeed, resembles far more closely that found in the simplest red seaweeds, such as *Bangia* and *Porphyra*, but whether this is a case of true affinity, or of parallel development in widely different groups, must be decided only on the result of further research.

The apparently parasitic penetration of the disc filaments into the tissue of some other Algae, and the way in which they appear to entirely take possession of the cells of the host, are, as far as I know, unparalleled at present amongst the Chlorophyceae.

PLATE XLV.

Illustrating Miss Delf's paper on the attaching discs of *Ulva*.

All the figures were drawn with a No. 3 eyepiece (Swift) and D. D. objective (Zeiss), excepting Figs. 7 and 8, which were drawn with a No. 4 compensating ocular (Zeiss) and $\frac{1}{12}$ apochromatic objective (Swift).

Fig. 1. Part of a single filament teased from just above the disc. × 510.

Fig. 2. The tip of a disc filament with two nuclei. × 510.

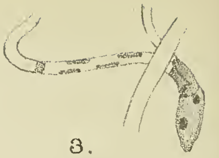
Figs. 3 and 4. Disc filaments from a teased preparation, showing irregular branching. × 510.

Figs. 5 and 6. Cell-divisions in filaments which had reached the periphery of a disc. × 510.

Fig. 7. Part of the periphery of a disc, showing large and small filaments. × 510.

Fig. 8. Part of the stipe in longitudinal section, showing filaments extending within the surface of the thallus and (*a*) forming a small-celled tissue externally. × 510.

Figs. 9 and 10. Small part of the periphery of the cross section of a seaweed resembling *Polysiphonia*, infested with filaments from an attached disc of *Ulva*. ($\frac{1}{12}$ apochromatic.)



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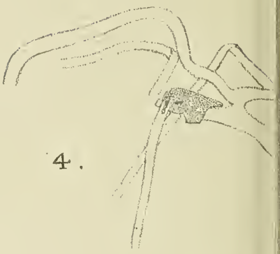

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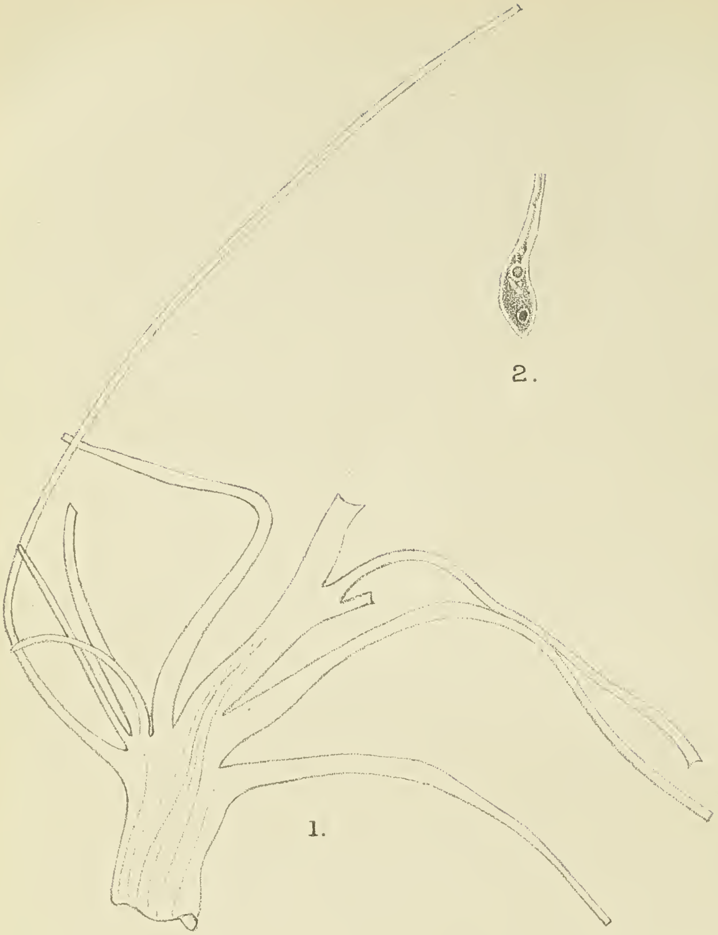

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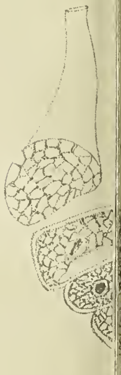

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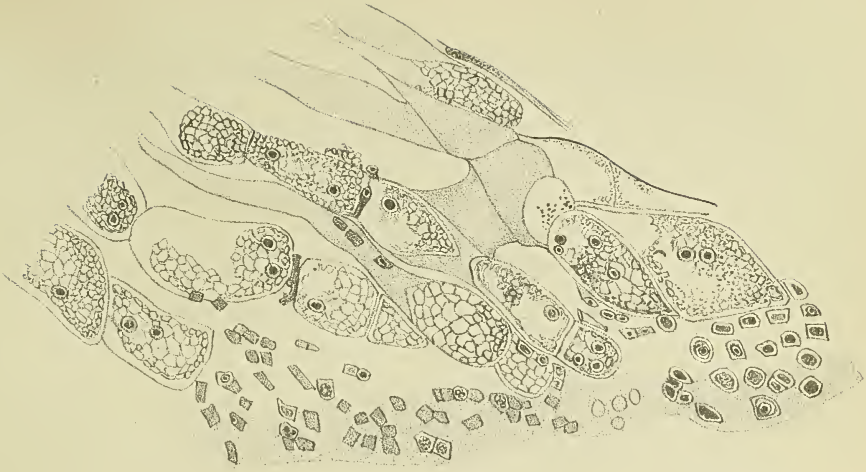
DELF — ULVA



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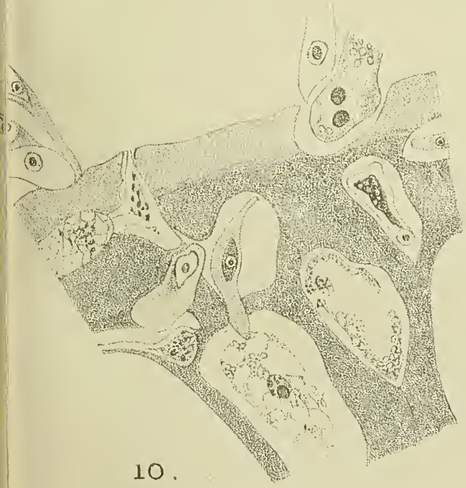

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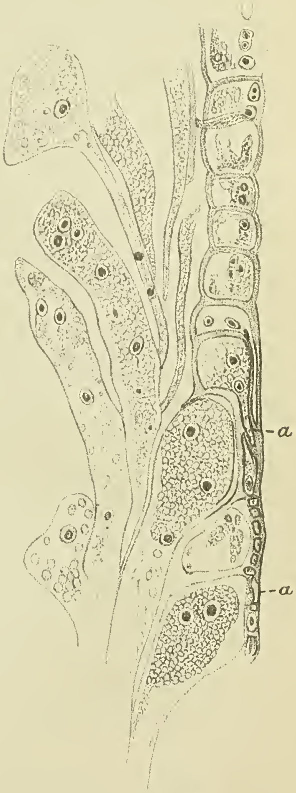

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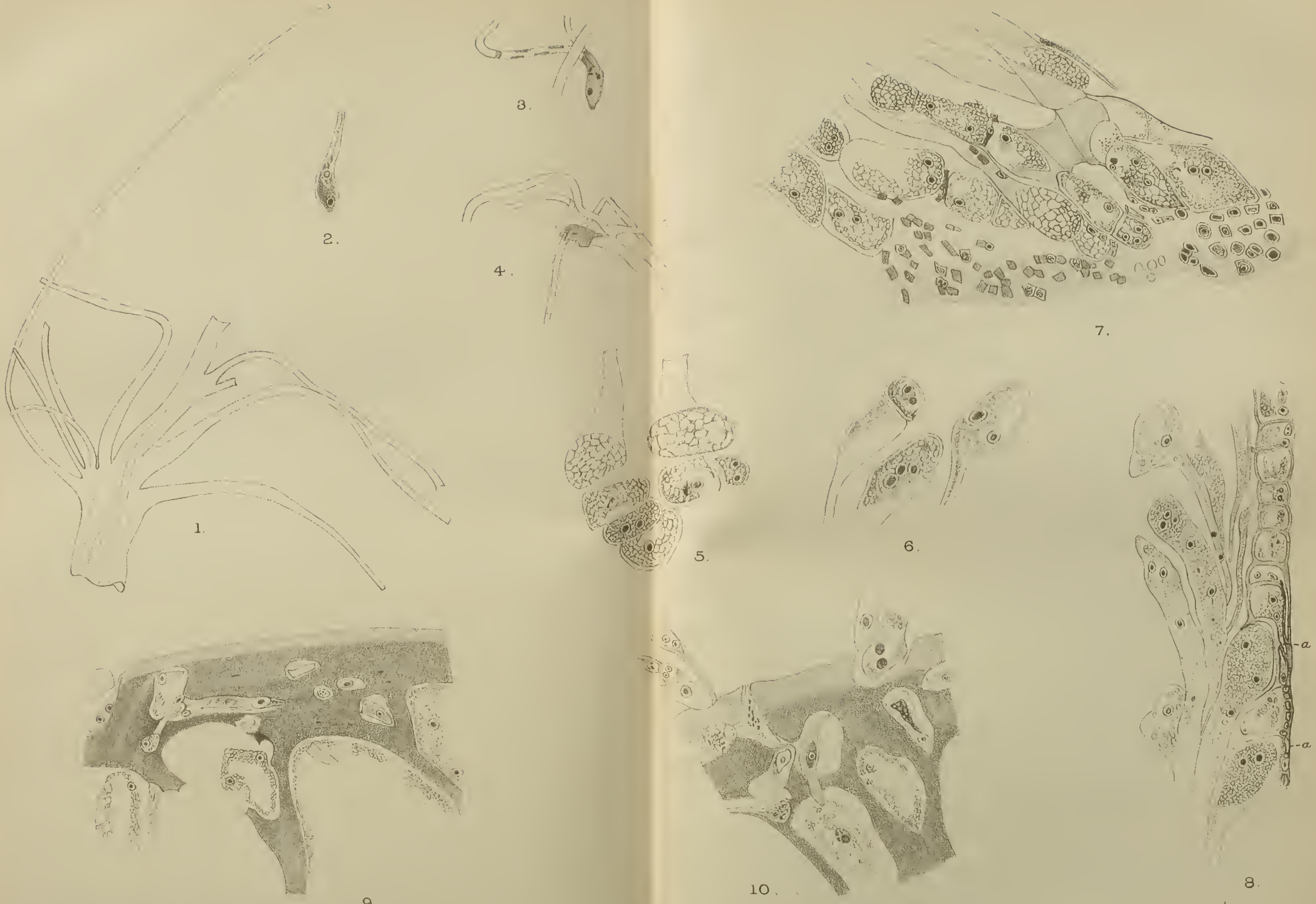
Huth, lith et imp.



DELF — ULVA

Huth, lith et imp.

Transpiration in Succulent Plants.

BY

E. MARION DELF.

With one Figure in the Text.

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INTRODUCTION.

SUCCULENT plants are largely characteristic of dry, sandy, or rocky regions, where the physical conditions seem to favour rapid transpiration, whilst the water supply is limited, or in some way precarious. They are, however, also found in very different situations, as, for example, the epiphytes of a typical tropical forest where the atmosphere is warm and damp. Other succulent plants inhabit maritime districts, especially the neighbourhood of salt-marshes, and others, such as many of the Crassulaceae, though commonly found in stony or rocky places, thrive equally well in ordinary soil, and may then be freely associated with mesophytes. We may thus distinguish, according to their habitat, four main classes of succulent plants, which may be briefly designated as (1) *Desert Plants*, (2) *Rock Plants*, (3) *Epiphytes*, (4) *Halophytes*.

We are naturally led to ask how far the succulent habit is to be regarded as an adaptation to environment in all these cases; and in the

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following pages this question will be discussed in its bearing on the problem of transpiration, firstly in relation to structural features, and secondly in relation to habitat.

I. TRANSPIRATION IN RELATION TO STRUCTURE.

Succulent plants have a number of anatomical features in common which are undoubtedly connected with the process of transpiration. We shall consider the character of (α) the transpiring surface, (β) the storage system, and (γ) the conducting system, from the physiological point of view.

(α) The Transpiring Surface.

In plants of extremely dry habitats there is often a notable reduction in leaf surface, the stem itself taking on the bulk of the work of assimilation and transpiration; there is therefore, presumably, a corresponding diminution in the total water loss, although not necessarily in the actual rate of transpiration, per unit area of surface. Examples of this are seen in the thorny almost leafless stems of desert species of *Euphorbia*, and the equally thorny swollen stems of *Opuntia*, *Echinocactus*, and some other genera of the Cactaceae. The same tendency to eliminate the leaf as a distinct organ is seen in the halophytic genera *Salicornia* and *Arthrocnemum*, where the leaves are reduced to mere fleshy lobes almost entirely adnate to the stem.

A large number of plants, however, have fleshy leaves or succulent stems without any such extreme reduction of surface, as, for example, the majority of epiphytic or halophytic plants; these are often termed semi-succulents.

It is commonly assumed that the percentage water content of a plant is in itself a criterion of its degree of succulence;[1] but I have reserved this phrase to indicate the water content per unit area of surface.[2] In this sense the degree of succulence of a plant is of some interest for problems of transpiration, and a number of determinations from my own experiments are given in Table I.

The method employed was that of finding the fresh weight of a leafy shoot, estimating the surface area, and drying at 100° C. until a constant weight is obtained. The greatest difficulty lay in the determination of the surface areas. For large-leaved forms the outline of the leaf was carefully traced on paper ruled in millimetre squares; when a number of leaves had to be traced in succession, they were kept in a damp chamber or floated on water until just before use, in order to minimize the shrinkage in surface due to loss of water in transpiration, which has been demonstrated to take place even before withering becomes perceptible.[3] In the case of small-leaved forms the procedure was varied to suit the particular type of leaf.

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[1] Cp. Aubert.

[2] Delf, E. M.: Transpiration and Behaviour of Stomata in Halophytes. Annals of Botany, 1911.

[3] Cp. Thoday, D.: Experimental Researches on Vegetable Assimilation and Respiration, V. Proc. Roy. Soc., B, vol. lxxxii, 1909.

In the case of *Suaeda* and *Salsola* the method employed was that described in my paper on ' Transpiration and Behaviour of Stomata in Halophytes ' ('Annals of Botany', xxv, p. 487); for *Salicornia* the micrometer screw method detailed in the same paper (p. 487); whilst the small leaves of *Mesembryanthemum crystallinum* were estimated both by the micrometer screw and celluloidin method,[1] and the mean between the two determinations (which usually differed by from 1–3 per cent.) taken as a fair approximation to the true surface area. In all cases the stem was included in the determinations both of areas and of dry weights.

The succulence of the leafy shoots of *Salsola Kali* and *Arenaria peploides* is probably somewhat underestimated, for the only material available had been sent by post from some distance, and although kept in water for some time before using were probably not fully turgid at the time of the experiment. The shoots of the remaining examples were obtained direct from healthy plants cultivated in the garden at the James Allen's Girls' School, Dulwich,[2] where there is an artificial salt-marsh, which is watered at intervals with a 1–2 per cent. solution of Tidman's sea-salt, and also an artificial sand-dune, and pebbly sea-shore, in a very flourishing condition. It was ascertained, by means of the thallium sulphate test, that the plants used had a very considerable salt content, but they probably show a somewhat less succulence than that of plants in their natural habitat, especially in the case of variable plants, such as *Aster Tripolium* and *Arenaria peploides*.[3]

TABLE I.

Water Content per sq. dm. in some typical Succulent and Mesophytic Plants.

	Plant.	*Water Content %.*	*Surface Area.*	*Water Content in grm. per sq. dm.* (*' Degree of succulence'.*)
I.	*Mesembryanthemum edule*	96·3	70·54	12·6
	" *crystallinum*	97·7	3·28	6·2
	Suaeda maritima	90·3	40·38	10·0
	Salsola Kali	86·6	17·16	6·9
	Salicornia annua	89·5	24·43	6·5
	Arenaria peploides	89·2	12·34	5·5
	Plantago maritima	95·2	84·76	4·8
II.	*Aster Tripolium*	91·5	92·18	3·7
	Sedum Sieboldii	93·6	26·87	3·4
	Atriplex portulacoides	88·9	54·14	3·3
	Cakile maritima	91·8	14·96	2·8
	Eryngium maritimum	85·0	90·72	2·5
	Glaucium	89·0	46·50	2·1
III.	*Statice Limonium*	81·2	141·78	1·7
	Saponaria officinalis	78·4	82·28	1·4
	Mercurialis annua	77·0	45·14	0·95

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[1] Delf, E. M.: Loc. cit., pp. 488, 489.

[2] By kind permission of the Head Mistress, Miss Howard; and of Miss L. J. Clarke, the senior Science Mistress, in whose laboratory these determinations were also made.

[3] Cp. Lesage, P.: Recherches expérimentales sur les modifications des feuilles chez les plantes maritimes. Revue Générale de Botanique, 1890.

The plants fall roughly into three groups which may be termed 'succulent', 'semi-succulent', and 'mesophytic', respectively. The plants belonging to the first group possess from 5 to 12 grm. water content to every square decimetre of surface; to this class belong, probably, the xerophytic succulent Cactaceae (*Opuntia*, &c.). The same degree of succulence may be attained by large and small leaved forms, such as *Mesembryanthemum edule* and *Suaeda maritima*; by adnation of the reduced leaf (as in *Salicornia*, and to a less extent in *Salsola*), or by the development of aqueous tissue in each separate leaf, as in *Arenaria peploides* or *Plantago maritima.* The plants belonging to the second group comprise most of our British halophytic plants, many rock plants, and would probably also include many subtropical or tropical epiphytes, and plants typical of the Mangrove swamps. These have from about 2 to 5 grm. water content per sq. dm. surface. The third group includes only typically mesophytic plants, such as *Saponaria* and *Mercurialis*, and perhaps also a few exceptionally thin-leaved halophytes like *Statice*, in which the aqueous tissue is limited to the region of the midrib, and in which the epidermis is provided with small excretory glands. *Frankenia* and *Tamarix*, two other somewhat thin-leaved halophytic genera, at least in some of their species, are also provided with epidermal glands, and it is possible that the excretion of salt which is said to take place through these glands may account for the absence of the succulent habit.

Whether reduced or not, the transpiring surface is protected by a definite tissue, the epidermis, which may be more or less cuticularized. Haberlandt[1] describes an experiment in which he demonstrates the protective power of the epidermis in succulent plants. Two pieces of an Aloe leaf were covered with wax, except for a definite area, equal in both cases. The epidermis was carefully stripped from the exposed surface of one piece, but was left intact on the other; both pieces were then exposed to the air for three hours. The pieces were then weighed and the stripped leaf was found to have lost 524 mg., whilst the uninjured piece had lost only 2 mg. In this case the epidermis has a well developed cuticle, but there is, no doubt, a considerable amount of protection derived from even a slightly cuticularized epidermis.

Many succulent xerophytes have a thick cuticle and relatively little water loss, as the work of both Schimper[2] and Volkens[3] shows. Holtermann also found some Cactaceae with thickly cuticularized epidermis in which the transpiration was almost imperceptible. In alpine plants, according to Leist and Bonnier,[4] the outer wall of the epidermis is more strongly

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[1] Haberlandt: Physiologische Anatomie, p. 96.

[2] Schimper, A. F. W.: Ueber Schutzmittel des Laubes gegen Transpiration, besonders in der Flora Javas. Monatsber. der Akad. Wiss. in Berlin, xl, 1890.

[3] Volkens, G.: Zur Flora der ägyptisch-arabischen Wüste, auf Grundlage anatomisch-physiologischer Forschungen, 1887.

[4] Leist and Bonnier: Quoted by Dr. A. Burgerstein, in 'Die Transpiration der Pflanzen', 1904.

cuticularized than that of the same species living on the plains; and here again the function of the cuticle appears to be primarily protective rather than mechanical, as Fleischer[1] believed. Wiegand[2] suggests that cuticularization is particularly characteristic of plants which are exposed at all times to the danger of too great transpiration; whereas a thinner cuticle with a covering of hairs is more often found in plants in which transpiration must be reduced at times, and yet must remain unhindered when the air is damp, and transpiration therefore rendered difficult.

Schimper claimed that a thickened cuticle, as well as other xerophilous adaptations, is to be found in most epiphytes,[3] in Mangrove plants,[4] and in the plants of the Solfataras,[5] or regions bordering a fumarole. He stated that in the first case the precarious water supply rendered protection against too rapid evaporation necessary; in the second case the presence of chlorides, and in the third case of sulphates in the soil, renders the process of absorption so difficult that the transpiration also must be diminished by means of xerophilous adaptations. According to the more recent observations of Holtermann,[6] however, this is by no means always the case. The epiphytic vegetation was found to occur, at least in Ceylon, mostly in damp tropical or subtropical forests; the plants found in such localities showed little or no cuticularization of the epidermis, and they were, as a matter of fact, found to be capable of very rapid transpiration; such are, for example, many species of *Ficus*, *Peperomia*, *Drymoglossum*, many Bromeliaceae, and some Orchidaceae. A few epiphytic orchids which were found growing in a dry atmosphere had a thickened cuticle, but these were distinctly exceptional. In *Sonneratia*, *Rhizophora*, and other plants of the Mangrove formations there was similarly but little development of cuticle, and, at times, a rapid rate of transpiration. In *Agapetes vulgaris* and *Rhododendron retusum*, the two most characteristic plants of the Solfataras of Ceylon, there was also absolutely no protection against excessive water loss.

Aubert[7] measured the rate of transpiration of a number of succulent and mesophytic plants and came to the following general conclusions:

1. With equal surfaces, succulent plants, such as many Crassulaceae and species of *Mesembryanthemum* which have a thin cuticle, transpire more freely than many mesophytes with a thick cuticle (*Hedera Helix*, *Picea excelsa*); with equal fresh weights, however, the succulent types transpire less than these.

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[1] Quoted by Dr. A. Burgerstein, loc. cit., p. 205.

[2] Wiegand: Relations of Hairy and Cutinised Coverings to Transpiration. Bot. Gaz., 1910.

[3] Schimper, A. F. W.: Die epiphytische Vegetation Amerikas, 1888.

[4] Schimper, A. F. W.: Die indomalayische Strand-Flora, 1891.

[5] Schimper, A. F. W.: Ueber Schutzmittel des Laubes gegen Transpiration, besonders in der Flora Javas, 1890.

[6] Holtermann, Dr. C.: Der Einfluss des Klimas auf den Bau der Pflanzengewebe, 1907, pp. 138, 61, 73.

[7] Aubert, E.: Turgescence et transpiration des plantes grasses. Ann. des Sci. Nat., Bot., 1892.

2. The Cactaceae transpire less than any plants examined, whether reckoned on equal surfaces or equal fresh weights.

The work of both Stahl[1] and Rosenberg[2] points to the fact that many European halophytes possess the power to transpire freely, as judged by the cobalt paper test. I have shown,[3] and have often observed, that in many of our British salt-marsh plants the transpiration may be much greater for equal transpiring surfaces than in a typical mesophytic plant; in others the value approaches, but rarely falls below, that of the typical mesophyte under similar conditions at the time of the experiment. The figures in Table II give some of these results; but since the observations were made of necessity at different times,[4] the transpiration is estimated per hour per square decimetre, relative to the loss of a water surface measured at the same time under the same experimental conditions, the latter value per hour per square decimetre being taken as 100 in each case; the variations in transpiration due to the purely physical effect of the environment are now eliminated, as Livingston[5] pointed out. Throughout the experiments the transpiration observed is that of detached leafy shoots with no water supply; the cut end was sealed immediately after being severed from the plant, and the fresh weight at once obtained; the shoot was then allowed to transpire, hung up in the air of the laboratory, away from any draught or direct sunlight, and the water loss during the first hour or two hours of withering determined by means of an accurate balance. The values obtained will be somewhat below those of the normal plant, since, in all the plants examined, the stomata close within the first half-hour of experiment, and also because the transpiration falls off as the water content of the whole plant falls below the normal.

TABLE II.

Relative Transpiration per hour per sq. dm.

Water	100
Salsola Kali	64·1
Mesembryanthemum edule	66·9, 47·6
Atriplex portulacoides . .	61·7
Salicornia	32
Sedum spurium	36·0
,, *Sieboldii*	49·6
Saponaria officinalis . .	27·6, 7·4
Vicia Faba	26·0
Aster Tripolium . . .	22·4

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[1] Stahl, E.: Einige Versuche über Transpiration und Assimilation. Bot. Zeit., 1894.

[2] Rosenberg, O.: Über die Transpiration der Halophyten. Öfvers. af Kongl. Vetenskaps-Akad. Förhandlingar, Stockholm, 1897.

[3] Delf, E. M.: Transpiration and Behaviour of Stomata in Halophytes. Annals of Botany, xxv.

[4] These figures are taken mostly from unpublished experiments made during the months of August and September, 1911.

[5] Livingston, B. E.: The Relation of Desert Plants to Soil Moisture and Evaporation.

A large number of succulent plants, therefore, show but little development of cuticle, and can endure a rapid rate of transpiration per unit area of surface without harm.

Many succulent plants, however, in addition to the possession of a more or less developed cuticle, have a coating of wax which acts as a further protection against water loss. Garreau[1] offered experimental evidence as to the protecting power of wax coverings in the case of *Centranthus ruber*, *Syringa vulgaris*, *Sedum verticillatum*, and some others, where the rate of transpiration was increased from 1·5 to 3 times by removing the wax. Frequently the wax not only coats the free surface of the epidermal cells, but also blocks the apertures of the stomata more or less completely. Dr. F. Darwin[2] has shown that, generally speaking, the stomata of a plant tend to become aggregated in the areas covered by the wax or bloom. Thus, if bloom be only found on one side of a leaf, the stomata are often either less numerous or altogether lacking on the side which is not thus protected. The association of stomata with a wax covering may, however, also be connected with the prevention of the blocking of the stomata by rain or heavy dew, since the maintenance of gaseous exchange is of great importance in the economy of the plant.

A wax covering is chiefly characteristic of plants inhabiting dry regions, as the observations of Schimper, Volkens, and others show. Holtermann[3] found that in Ceylon those epiphytes which lived in the warm damp air of the tropical forests were glabrous, whereas those which were found in exposed, sunny places were frequently coated with wax. Wiegand,[4] from his experiments with artificial surfaces, concluded that a wax covering would form an equally effective protection whether in still air or in wind.

Most halophytes which live in damp or marshy situations are glabrous, as, for example, *Rhizophora*, *Salicornia*, and *Aster Tripolium*. *Suaeda maritima* is an exception, for the small leaves are covered with a fine bloom, and it is often found in marshy places. *Psammophilous* halophytes are frequently covered with wax if sclerophyllous, or very slightly succulent, as in *Elymus* and *Eryngium maritimum*; but if succulent the leaves are commonly glabrous, as in *Cakile maritima*, which also has only a very slight development of cuticle.

Hairs, whether glandular or protective, are not common in succulent plants; they are, indeed, much more characteristic of sclerophyllous vegetation. We may, however, instance *Sempervivum arachnoideum*, a succulent alpine plant, the rosettes of which are clothed, and perhaps also protected by long loose hairs. Some species of *Avicennia* are hairy, and *Evolvulus*

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[1] Quoted by Dr. A. Burgerstein in Die Transpiration der Pflanzen, 1904, p. 39.

[2] Darwin, Dr. F.: Stomata and Bloom. Journ. Linn. Soc., 1887.

[3] Holtermann: Der Einfluss des Klimas auf den Bau der Pflanzengewebe, 1907.

[4] Wiegand: Relations of Hairy and Cutinised Coverings to Transpiration. Bot. Gaz., 1910.

alsinoides is a littoral plant from the drier coastal regions of Ceylon, which is stated by Holtermann to have a number of small, succulent, and yet hairy leaves. According to Wiegand[1] a hairy covering is a relatively much more effective protection against transpiration in windy than in still weather; whilst in damp air it yet allows of a considerable amount of transpiration.

Many species of *Atriplex* are covered with short, closely set hairs, which form an almost continuous sheet of large thin-walled cells covering the much smaller cells of the epidermis, except for narrow chinks above the stomata. According to Volkens these hairs readily give up their water in time of need to the cells below, and then their walls collapse, and the empty cells thus form a very complete protection for the whole transpiring surface; the hairs can probably also absorb water from rain or dew, or they may be replenished from within when the roots are able to absorb freely. Some species of *Mesembryanthemum*, as *M. crystallinum*, *Forskalii*, and *nodiflorum*, have a number of swollen papillae which, according to Hagen,[2] have a protective function. The papillae are thin walled and appear to be filled with a mucilaginous cell-sap, which does not easily allow of evaporation; we shall see that these papillae probably also function as absorbing hairs for rain and dew.

TABLE III.

Distribution of Stomata in Succulent and Mesophytic Plants.

	Plant.	*Habitat.*	*Stomata per sq. mm.* *Upper Surface.*	*Lower Surface.*	*Average.*
I.	*Mesembryanthemum edule*	dry, rocky	60	80	70
	Suaeda maritima . . .	damp, saline	63	63	63
	,, *fruticosa* . . .	sandy, saline	37	37	37
	Salsola Kali	,, ,,	(stem) 71	—	71
	Salicornia annua . . .	marshy, saline	,, 104	—	104
	Plantago maritima . .	,, ,,	143	231	187
	Arenaria peploides . . (Hunstanton)	marshy or sandy	49	80	65
	,, (cultivated) .		134	194	164
II.	*Aster Tripolium* . . .	marshy or dry, saline	94	87	90
	Sedum album	dry	94	87	90
	,, *Sieboldii*	,,	88	71	79
	Cakile maritima . . .	sandy, saline	127	163	145
	Eryngium maritimum .	,, ,,	243	149	196
	Glaucium maritima . .	stony, saline	117	88	102
III.	*Statice Limonium* . .	moist, saline	143	109	126
	Saponaria officinalis . .	moist	60	349	204
Czech's Figures.	*Populus nigra*	dry	Whether upper or lower surface unspecified		135
	,, *alba*	damp			315
	Brassica lyrata . . .	dry			400
	,, *palustris* . . .	damp or marshy			609
	Veronica Chamaedrys .	dry			175
	,, *Beccabunga* . .	aquatic or marshy			248

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[1] Wiegand: Relations of Hairy and Cutinised Coverings to Transpiration. Bot. Gaz., 1910.

[2] Burgerstein: Die Transpiration der Pflanzen, p. 224.

It was established by Czech,[1] and has been confirmed by Volkens and other observers, that, in general, the stomata of plants growing in wet or even damp situations are more numerous per unit area than are those of dry places. Thus *Veronica Chamaedrys* was found to have 175 stomata per sq. mm., whilst *Veronica Beccabunga* had 248. So far as I have been able to observe, this holds true also for succulent plants, those from dry regions, such as some species of *Mesembryanthemum* and *Sedum*, having considerably fewer than have those, such as *Salicornia*, from marshy places. Table III gives the distribution of the stomata of such plants as I have observed, and also a few of Czech's figures for comparison.

According to Burgerstein [2] the degree of opening of the stomata affects the amount of transpiration far more than the actual number present. Leitgeb [3] was the first to call attention to the power of the stomata to open and close, and regarded it as a means for regulating the amount of transpiration. This view has been widely accepted, but has been objected to recently by Lloyd,[4] who found that in *Fouquiera splendens* and in *Verbena ciliata* the 'rate of transpiration may undergo sudden and wide changes, without the accompaniment of a sufficient change in the dimensions of the stomata to account for them on the theory of stomatal regulation of transpiration.' [5] According to Lloyd the capacity for diffusion of the stomata of *Fouquiera* is well in excess of what would be required for the greatest observed transpiration rate, and he therefore concludes 'stomatal regulation of transpiration does not occur, though of course conservation of the contained water follows on complete closure of the stomata. It is open to doubt, however, if this condition ever obtains.' On the other hand, some experiments of Dr. F. Darwin, a preliminary account of which was given at the meeting of the British Association, 1910, and a further account of which is now in the press, lead to the conclusion that in many plants, if they can be observed by a sufficiently delicate method, stomatal movements are found to correspond closely with changes in the rate of transpiration caused by alteration in external conditions. It is clear, therefore, that the whole question of stomatal regulation requires further investigation.

So far as the power of movement of the guard cells in the stomata of succulent plants is concerned, we have but little direct evidence. In xerophytic succulents the stomata may be so much sunken or overlaid by cuticle that they seem to have little or no scope for movement. Stahl,[6] however, states that the stomata of xerophytes all possess the power to close, and they are, therefore, presumably capable of the reverse action.

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[1] Burgerstein: Die Transpiration der Pflanzen, p. 210.

[2] Burgerstein: ibid., p. 211.

[3] Leitgeb: Beiträge zur Physiologie der Spaltöffnungsapparate, 1886.

[4] Lloyd, F. E.: The Physiology of Stomata.

[5] Lloyd, F. E.: ibid., p. 137.

[6] Stahl: Einige Versuche über Transpiration und Assimilation, 1894.

In a paper recently published by Linsbauer[1] on the physiological anatomy of the epidermis of some Bromeliaceae, it is shown that the stomata are surrounded by at least two pairs of subsidiary cells, which are arranged as though to protect the guard cells from contraction when the neighbouring aqueous tissue collapses. In the case of *Quesnelia* the stomata were found to be completely closed, owing to the formation of a membrane across the outer respiratory cavity. In many of the Bromeliaceae the arrangements of the stomata and of the air passages in the leaf seemed to be such as to ensure the best ventilation with the minimum of transpiration.

In all the succulent plants which I have examined the stomata appear to be capable of movement, but I have frequently found the stomata of species of *Sedum* and of various fleshy halophytes in the closed condition, especially when the sun was on these plants and the weather dry. Stahl[2] asserted, chiefly from the results of experiments made on the transpiration of halophytes grown in an artificial salt-marsh, that the stomata of these plants are not capable of closing. Stahl used the cobalt paper test, relying chiefly on the time of coloration of the dried paper; but the careful observations of Rosenberg,[3] using the same method and confirmed by means of direct microscopic examination, show conclusively that this is not the case, at least when the plants are growing in their natural habitat. Rosenberg suggested that the inactivity of the stomata observed by Stahl was due to the culture conditions. However, in the artificial salt-marsh at Dulwich I have frequently observed that the stomata of halophytes possess the power of closing, and they appear to me to be especially sensitive to air of low humidity. The stomata of *Salicornia annua* and of *Aster Tripolium* also close in darkness.[4]

So far as I have seen, the stomata of succulent plants close during the earliest stages of withering, and this appears to me to be an adaptation to conserve the water supply as far as possible during time of need. Lloyd[5] states that in *Fouquiera splendens* and in *Verbena ciliata* 'the beginning of closure occurs somewhat later than initial wilting, and seems to be a result of water loss by the leaf as a whole. There is no adaptive closure, meaning by this a closure in anticipation of wilting.' On the other hand, Rosenberg[6] found that when he observed the transpiration of the leaves of some halophytes (as *A. triplex*, *Cakile maritima*, *Plantago maritima*) immediately after detaching from the parent plant, a distinct drop in the rate of trans-

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[1] Linsbauer: Zur physiologischen Anatomie der Epidermis und des Durchlüftungsgewebes der Bromeliaceae, 1911. Anz. Kais. Akad. Wiss. Wien, mathemat.-nat. Klasse, ix.

[2] Stahl, W.: Einige Versuche über Transpiration und Assimilation, 1894.

[3] Rosenberg, O.: Ueber die Transpiration der Halophyten. Öfvers. af Kongl. Vetenskaps-Akad Förhandlingar, Stockholm, 1897.

[4] Delf, E. M.: Transpiration and Behaviour of Stomata in Halophytes, 1911.

[5] Lloyd: The Physiology of Stomata, 1907.

[6] Rosenberg: Ueber die Transpiration der Halophyten, 1897.

piration was to be detected by means of the cobalt paper test; this may be fairly interpreted as due to closure of the stomata prior to wilting. Other plants were brought into the laboratory and there the test was repeated. No such slowing down of the transpiration was then observed immediately after detaching; and microscopic examination showed that the stomata on the whole plant had closed. I have myself ascertained by direct experiment that the stomata of *Mesembryanthemum edule* closed within fifteen minutes after detaching a healthy leaf on which the stomata were initially open; and in the case of *Aster Tripolium* and *Plantago maritima* the stomata closed in five to seven minutes after detaching a leaf. In neither of these cases could any sign of withering be detected until some time after the stomata had been observed to close. Moreover, in the case of *Mesembryanthemum edule*, the leaf is well supplied with centrally placed aqueous tissue. When such a leaf withers the cells of the aqueous tissue gradually collapse, beginning from the innermost and slowly extending to the more peripheral cells. All the cells of the aqueous tissue show signs of collapse before the palisade tissue appears to suffer at all from want of water; and the epidermis of a detached leaf only shows wrinkling after many hours' exposure to drought, because the actual volume of the leaf is slowly diminishing. It appears to me impossible, at least in this case, to regard the closure of the stomata within the first fifteen minutes after detaching as due to 'the loss of water in the leaf as a whole'; on the other hand, it seems probable that the closing of the stomata so promptly is, in this and probably also in many other cases, an adaptation which reduces the transpiration of the leaf to the least possible under the particular physical conditions experienced at any time.

How far the stomata of succulent plants are able to regulate transpiration is, however, a very difficult question. I am inclined to think that the limiting power of the stomata may be much greater than has been supposed. Even in the apparently closed condition of the guard cells some regulation of the size of the outer respiratory cavity may be demonstrated, as the following experiment with *Salicornia* shows.

Two healthy similar shoots of *Salicornia annua* were chosen, and the cut ends sealed with a wax mixture. Both shoots were placed under the same conditions, one being used to determine loss of water during transpiration, and the other to determine the condition of the stomata during the course of the experiment. It was observed that the stomata appeared closed even before the shoots were cut; the guard cells, however, showed a wide rift above the closed pore. The average dimensions of the stomata and the loss due to transpiration are shown in Table IV. At the end of the first hour the shoots were removed to a more shaded place, and this probably accounts mainly for the diminution in size of the rifts, since, owing to the abundant aqueous tissue, an exposure of three hours could hardly affect seriously the water content of the epidermal and guard cells. It will be seen that a change in dimensions of the rift of 20 per cent. was accompanied by a change of 28 per cent. in the rate of transpiration; it is suggested that the two processes are therefore closely connected.

TABLE IV.

Transpiration and Behaviour of Stomata in Salicornia annua.

Loss in Weight due to Transpiration.			*Average size of Stomata.*			
			Guard Cells.		*Rift.*	
Time.	*Light.*	*Water Loss.*	*Length.*	*Diameter.*	*Length.*	*Diameter.*
11.35 a.m.						
12.35 p.m.	11	0·0194 gm.	9·5	7·2	5·1	1·9
2.35 p.m.	50	0·0140 gm.	7·7	6·9	4·1	1·4
Diminution in rate of Transpiration =			Diminution per cent. =			
		28 %	6	7·7	20	20

As has already been stated, the stomata of xerophytic succulent plants are commonly depressed below the level of the epidermal cells. In many cases they are further protected by wax, as in *Euphorbia Tirukalli*,[1] where a ring-like wall of wax surrounds each stoma; by cuticular projections overarching the guard cells, as in the case of *Aristida ciliata* described by Volkens; or by subsidiary cells, as in the stomata of some of the Bromeliaceae recently described by Linsbauer, to which reference has already been made.

The stomata of epiphytes are, however, often not at all sunken or protected, as in the epiphytes described by Holtermann[2] from damp, shaded spots in the Ceylon forests. The stomata of *Rhizophora*, *Bruguiera*, *Aegeciras*, and other Mangroves were stated by Schimper[3] to be sunken, but in nearly every case examined by Holtermann this was not found to be the case. In many species of *Atriplex* the stomata lie below chinks in the parenchymatous covering of leaves, but are not at all depressed below the level of the epidermal cells. In all the species of *Sedum* and in all the succulent halophytes (excepting two species of *Suaeda*) which I have observed, the guard cells are not at all sunken.

In many cases, especially in the leaves of tropical epiphytes, the transpiring surface is supplied by aqueous tissue interposed between itself and the assimilating cells. The aqueous tissue consists of colourless, usually parenchymatous cells filled with a watery cell-sap, which is often readily given up, both in transpiration and in supplying the assimilating tissue. As the cells of the aqueous tissue are gradually depleted of their water, the thin walls collapse, and the collapsed cells form a more or less complete protection to the cells of the mesophyll. When a further water

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[1] Haberlandt, Dr. G.: Physiologische Pflanzen-Anatomie, 1896, p. 397.

[2] Holtermann, Dr. C.: Der Einfluss des Klimas auf den Bau der Pflanzengewebe, 1907.

[3] Schimper, A. F. W.: Die indomalayische Strandflora, 1891.

supply is available, whether from root absorption or from rain or dew, the cells expand again and become again water reservoirs. In the leaves of the epiphytic *Peperomia incana* the delicate cell-walls of the aqueous tissue are strengthened at the corners, where they abut on the palisade tissue, with a horn-like substance, so that a firm framework is interposed between the mesophyll cells and the aqueous tissue when the latter collapses in dearth of water.[1] Occasionally the aqueous tissue is formed of deep, narrow, palisade-like cells, as in *Carappa moluccensis* and *Rhizophora mucronata.* Very often the amount of aqueous tissue increases in volume as the leaf grows in age, and this is accomplished by an often considerable increase in the size of the individual cells. This increase in storage capacity as the leaf attains its full size is of obvious importance in the economy of the plant.

Peripheral aqueous tissue is very characteristic of the leaves of epiphytes and of Mangroves. According to Holtermann[2] many epiphytic species of *Ficus* and *Peperomia* show well-developed aqueous tissue, whereas terrestrial species of the same genera may be entirely lacking in this respect. If the plant is growing in a very exposed, sunny place, the aqueous tissue will be protected on the outside from excessive water loss, as in *Ficus tomentosa*, by the formation of cuticle and hairs; but in epiphytes of damp regions, and constantly in Mangroves and plants of the Solfataras, the aqueous tissue is little protected, and at certain times may show enormous loss of water in nature.

Volkens[3] has described various species of *Atriplex* with water-storing hairs, and also some desert species of *Mesembryanthemum*; to these reference has already been made.[4] There is no doubt, as Haberlandt insists,[5] that the presence of mucilage in the cell-sap of the aqueous tissue greatly increases its power of resistance to desiccation, even in otherwise little protected leaves, such as those of *Mesembryanthemum crystallinum.* The mucilage may be distributed throughout the whole of the aqueous tissue, as in *Agave*, or localized in special cells, as in *Rhizophora mucronata*; or it may be associated with storage tracheides, as in the leaves of *Loranthus europaeus.*

Most European succulent plants have an internal but no peripheral aqueous tissue; however, the colourless cells of the epidermis may themselves function to some extent as water reservoirs, as may be observed by actual experiment. For example, in one case observed by me, a leaf of *Aster Tripolium* was picked and at once placed in water. Owing, perhaps, to the dry air of the laboratory, both upper and lower epidermis when

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[1] Haberlandt, Dr. G.: Physiologische Pflanzenanatomie, 1896, p. 347.

[2] Holtermann, Dr. C.: Der Einfluss des Klimas auf den Bau der Pflanzengewebe, 1907.

[3] Volkens: Zur Flora der ägyptisch-arabischen Wüste, 1886.

[4] See p. 416 in present paper.

[5] Haberlandt, Dr. G.: Physiologische Pflanzenanatomie, 1896, p. 352.

examined next day showed much wrinkling and folding on the outside walls of all the cells except the guard cells, which were perfectly turgid, although all were closed or only narrowly open; the leaf itself did not appear at all limp to the eye or to have suffered from want of water. The whole leaf was then immersed in water, and in less than three hours had increased in weight by 13·7 per cent. The epidermis was again examined, and the wrinkling of the epidermal cells was much less apparent; about half the cells showed no wrinkling at all, and in the remaining cells the folds were clearly less numerous. Here, then, we have a case in which the epidermal cells may be partly depleted and filled again, without apparent injury, as in true aqueous tissue.

Glands are not found as a rule on succulent or semi-succulent leaves and stems. Volkens,[1] however, records the presence of glands on the somewhat fleshy leaves of *Cressa cretica*, *Reaumuria hirtella*, *Tamarix manifera*, and *Frankenia pulverulenta*, which appear to excrete salts, chiefly sodium chloride; these salts seem to have the power of condensing moisture, even from an apparently dry atmosphere, so that the leaves sometimes appear to be covered with moisture when no rain has fallen and, all around, the vegetation is dry. There is some difficulty in supposing with Volkens that the glandular tissue can so far alter in permeability as to first excrete a highly concentrated salt solution, and then absorb only pure water back again; but there appears to be no doubt that the layer of salt may be of value in preventing water loss, for these plants are found in some of the driest regions of the Egyptian-Arabian desert, and can withstand long and severe drought. If, however, the layer of salt be removed from the leaves of a cut shoot, the power of resistance to desiccation is strikingly diminished.

According to Rohlf[2] the same function is subserved by the glands in the leaves of some desert species of *Statice*; and Holtermann observed in the leaves of *Laguncularia racemosa*, *Acanthus ilicifolia*, and *Aegeciras racemosa* (all Mangrove plants) superficial glands which, when tested with thallium sulphate, showed an abundant salt content, and which sometimes even showed minute crystals of salt visible to the naked eye. Holtermann interprets the function of these glands as excretory, the salt being washed away by the dew, which falls heavily in those regions at night. The excretion of salt by these glands is used as an argument against Schimper's theory that salt accumulates in the leaves of halophytes to a harmful extent. Similar glands are found on the leaves of some European species of *Statice*, *Glaux*, and other halophytes; but these are scarcely succulent in habit, and little is known of the true function of the glands in these plants.

It is well known that many plants are capable of absorbing water over

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[1] Quoted by Dr. A. Burgerstein: Die Transpiration der Pflanzen, 1907.

[2] Quoted by Dr. A. Burgerstein: ibid.

their whole surface; but it has long been a matter of dispute how far this power is of importance to the plant in nature. The work of Wille[1] and others shows that the proportion of water thus absorbed, relative to that taken up by the roots, is mostly so small that the surface absorption can be of but little meaning biologically.

However, there is no doubt that such surface absorption may be of the utmost value; rootless epiphytes, indeed, depend wholly on this method for their water supply, and in such cases adaptive mechanisms for collection and storage of water are often found, as, for example, the spoon-shaped leaves of some Bromeliaceae and the absorbing hairs of *Usnea tillandsioides*.

Aerial roots form additional organs of absorption in many epiphytes, especially in the epiphytic Aroids and tropical orchids. These are fleshy, greenish roots which stand out from the surface of the plant and are covered with a parchment-like layer of aqueous tissue, several cells deep. The cells of the aqueous tissue are usually thin walled, isodiametric, and lined with a delicate spiral of cellulose; often the walls are perforated, so that when water reaches the surface it is sucked in rapidly by capillary attraction. It has even been suggested by Schimper and Goebel[2] that the aqueous tissue, or velamen, as it is here frequently termed, has the power to absorb water vapour and nutritive gaseous compounds, such as ammonia, from the air; a very probable suggestion, although not yet substantiated by actual facts.

A number of other plants, however, show a considerable power of surface absorption, the value of which has been perhaps under-estimated. The papillate hairs of certain species of *Mesembryanthemum*, to which reference has already been made, are of some interest in this connexion, for they appear to have a marked power of absorption in addition to their capacity for retaining and storing water. Some cuttings of *M. edule* and *M. crystallinum* had been received by me from Cornwall, where both plants grow freely in a semi-wild state. They had been packed loosely in a cardboard box and were obviously suffering from want of water when unpacked. *M. edule* is a glabrous species with somewhat thickened cuticle and slightly sunken stomata. *M. crystallinum* has papillate leaves with little or no cuticle, and small unprotected stomata, and it therefore seemed worth while to compare their power of surface absorption. A shoot of each was chosen with about the same number of healthy uninjured leaves; they were detached and weighed accurately after the cut end of each had been sealed up with an adhesive wax mixture. The shoots were then immersed in water, and dried and weighed at intervals. The results given in Table V show that *M. crystallinum* has an absorption from three to seven times as great as *M. edule*. Both these species live in dry, rocky places where there is a limited

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[1] Burgerstein, Dr. A.: Die Transpiration der Pflanzen, p. 232.

[2] Haberlandt, Dr. G.: Die physiologische Pflanzenanatomie, 1896, p. 201.

water supply, and *M. crystallinum* transpires, as I have observed, at about the same rate, relative to the amount of surface, as a typical mesophytic plant, such as *Saponaria*, so that there must often be a need of water. In the above experiment the shoot of *M. crystallinum* was flaccid at first, but appeared to be quite turgid after five hours in water, and therefore, since dew often falls heavily, and lasts for longer than five hours, this plant may well be benefited by the absorptive power of the hairs. In *M. edule*, however, the absorption is so slight that dew formation could hardly be of value except in checking transpiration for the time.

TABLE V.

Absorption of Water by Transpiring Surfaces in Mesembryanthemum.

	Mesembryanthemum edule.		*Mesembryanthemum crystallinum.*	
Time.	*Absorption % of Initial Weight.*	*Absorption % per hr.*	*Absorption % of Initial Weight.*	*Absorption % per hr.*
5 hrs.	0·47	0·09	1·7	0·34
23 hrs.	2·7	0·11	16	0·7
3 days	10·7	0·15	27	0·38

Holtermann [1] asserts that the Mangroves of Ceylon appear to have the power of absorbing rain and dew, since the leaves were always fresh and turgid in the early morning after a rainless but dewy night, following a hot day in which they had all perceptibly flagged. However, as Burgerstein [2] points out, this is not necessarily due to surface absorption of the dew ; it may simply be that the presence of the dew checks transpiration and so allows the water absorbed by the roots to accumulate within the plant until the tissues are again turgid. In those Mangroves and desert plants provided with salt glands the superficial absorption appears to be of real biologic importance, and is regarded by some as an adaptive mechanism for increasing the resistance of the plant to drought.

Burgerstein,[3] who has made an exhaustive study of the whole question of superficial absorption of water, came to the conclusion that any part of the plant, whether heavily or slightly cuticularized, and whether provided with stomata or not, was yet capable of water absorption to some extent. However that may be, the presence of cuticle is a distinctly retarding factor, and in the absence of hydathodes or glandular hairs the surface absorption of a much cuticularized surface is practically nil. The presence of wax or bloom which makes the leaf practically unwettable is also a character which prevents any appreciable surface absorption, and those succulent plants which have bloom are not those which can absorb freely. The hairs

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[1] Holtermann, Dr. C. : Der Einfluss des Klimas auf den Bau der Pflanzengewebe, 1907.

[2] Burgerstein, Dr. A. : Die Transpiration der Pflanzen, 1904.

[3] Burgerstein, Dr. A. : Übersicht der Untersuchungen über die Wasseraufnahme der Pflanzen durch die Oberfläche der Blätter, 1891.

which clothe the surface of many Chenopodiaceous plants may be absorbing, but this has not yet been experimentally demonstrated.

I [1] have suggested that species of *Saliconia* and *Aster* T*ripolium*, which are very sensitive to dry air and which are subject to a variety of conditions physically, may derive real benefit from their well-marked power of surface absorption. The same suggestion was made independently by Miss A. C. Halkett,[2] whose experiments bear this out to some extent.

(β) The Water-storing System.

We come now to the question of the system of water-storing tissues which are essentially characteristic of succulent plants. We have already seen that many epiphytes and mangroves have a superficial storage tissue, which perhaps also affords protection to the chloroplasts from the intense sunlight; many of these also have internal water-storing tissue, so that the assimilating cells are covered both above and below by aqueous tissue, as in many species of *Peperomia.* In fleshy leaves the water-storing tissue often gradates insensibly into spongy mesophyll cells with very scattered and pale chloroplasts; it is, however, sometimes localized in thick fleshy petioles. In stems the aqueous tissue is often located in the cortex, either completely encircled by the chlorophyll-containing cells, as in *Salicornia*, or interrupting the assimilating tissue in places, as in *Salsola*, where the stem shows streaks of transparent tissue between the chlorophyllous tissue; the epidermis above the transparent streaks has no stomata. Water is also stored in tubers or corms, as in epiphytic orchids, and in the species of *Sauromatum* popularly known as 'Monarch of the East'. In these cases the plant can live for long simply on this reserve, and in the last case the whole flowering spadix and the single large leaf are produced at the expense of the corm, which shrinks greatly as development proceeds.

In many cases the aqueous tissue contains a highly acid or highly saline cell-sap, which produces a high osmotic pressure internally. This may serve to facilitate surface absorption in plants, such as *Mesembryanthemum crystallinum* and *Salicornia*, but it certainly tends to retard evaporation, for water evaporates less readily from stronger than from weaker solutions; thus Aubert [3] found that in *Sedum dendroideum* the acid content increases as the young leaf becomes adult, and those leaves which had the maximum acid content had also the least transpiration. In many cases, in addition to the acid cell-sap, mucilage passages or cells occur, as in the Cactaceae, which are even more retentive of water; for example, with *Opuntia*, Aubert

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[1] Delf, E. M.: Transpiration and Stomatal Behaviour in Halophytes. New Phytologist, 1911.

[2] Halkett, A. C.: Some Experiments on Absorption by the Aerial Parts of Certain Salt-marsh Plants. New Phytologist, 1911.

[3] Aubert, E.: Turgescence et transpiration des plantes grasses. Annales des Sciences Nat., Bot., 1892.

found that the rate of transpiration increased steadily from the young shoot to the old, until the quantity of mucilage and gums began to become important. From that time the transpiration steadily fell off, until in the adult segments it was almost negligible.

Aubert's work further shows that the presence of organic acids is intimately connected with the succulence of the plant; thus a number of different species of *Sedum* arranged in order of their acid content per gramme fresh weight were found also to be arranged roughly in order of their fleshiness. This is intelligible when we remember that both organic acids and the sugars which are derived from them in metabolism are osmotically active substances, and would exert a high attraction for water.

In the Cactaceae the organic acid is mostly present as salts of malic acid; in the Crassulaceae mostly as free or combined isomalic acid, together with traces of tartaric acid, whilst the species of *Mesembryanthemum* examined gave abundant oxalates together with traces of mineral acids. The observations of Wolff[1] and others show that in halophytes malic acid is present in small amount, in addition to chlorides.

It has been suggested by Diels[2] that it is the presence of organic acids in halophytes which prevents the harmful accumulation of salts in the aqueous tissue of those types which do not possess secretory glands. The succulent habit is then regarded as connected with the formation of organic acids in restricted respiration, rather than with the biologic necessity of reducing transpiration, in order to avoid harmful accumulation of salts brought up by the ascending sap.

This theory was based upon a series of experiments, in which it was found that if a normal halophytic plant, such as *Cakile maritima* or *Salicornia*, was kept for some time in distilled water, or in a very dilute solution of chlorides, a gradual diminution in the percentage chloride content of the plant took place. Since the plants were, as far as possible, uninjured, the whole root system having been transferred to the solution, the inference was that the chlorides had in some way interacted with the organic acids and passed out from the plant in a form not thus far detected experimentally.

Unfortunately, all the calculations made were reckoned on equal initial fresh weights, no allowance having been made for changes in water content during the course of the experiment, owing to the effect of the transference to water or salts, or owing to changes in the physical conditions affecting the rate of transpiration. In a critical account of Diels's theory, Benecke[3] drew attention to these omissions, and further recorded some experiments made by himself, following Diels's method as nearly as possible, but ob-

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[1] Wolff: Quoted by Burgerstein, loc. cit.

[2] Diels: Die Entchlorung und der Stoffwechsel der Halophyten. Pringsheim's Jahrbücher, 1906.

[3] Benecke, W.: Ueber die Dielsche Lehre von der Entchlorung der Halophyten. Pringsheim's Jahrbücher, 1901.

serving the chloride content relative to the total ash content, as well as relative to the fresh weight. Benecke's figures show clearly that, although there is an apparent diminution of chloride content owing to the changes in water content of the plant, there is no change in the actual proportion of chloride present in the ash. The whole theory of diminution and disappearance of the chlorides, through the intervention of organic acids, thus stands at present unsubstantiated by facts.

Lesage [1] was the first to show clearly that it is the saline character of the habitat which appears to chiefly cause the succulent character of the leaves of halophytes. No less than ninety species found near the coast were examined, representing thirty-two families. Of these fifty-four had leaves thicker by the sea than when inland, twenty-seven were indifferent, and four were thicker inland than by the sea. Of the fifty-four which showed increase in fleshiness from the more saline position, some were oftener found inland than by the sea (and may be termed 'facultative' halophytes), such as *Solanum Dulcamara*, *Plantago Coronopus*, and *Ranunculus sceleratus*, and some were typical littoral forms, as *Beta maritima*, *Cakile maritima*, *Glaucium luteum*, and *Arenaria peploides* ; a few species, although littoral, were indifferent, as *Psamma arenaria*, *Suaeda maritima*, and *Triglochin maritimum*. With the exception of the few species mentioned, there was a distinctly greater fleshiness in the littoral than in the inland forms.

Most of these plants have no definite aqueous tissue, and their succulence is brought about by an increase in the number or size of the mesophyll cells. Thus the mesophyll cells of *Parietaria* appeared to increase in size, those of *Ranunculus sceleratus* increased in the number of layers, whilst *Beta maritima*, *Atriplex portulacoides*, and *Cakile maritima* showed increase in both number and size of the cells. Unfortunately, no mention is made of aqueous tissue as such, although the observation is made that in some cases the chloroplasts become paler and fewer in the plants grown in salty substrate. However, there is little doubt that some of these interior cells with little chlorophyll function as water-storing tissue, especially in plants with fleshy leaves, susceptible to salt content, such as *Salicornia*, *Cakile maritima*, and *Arenaria peploides*; there is little doubt, also, that this colourless water-storing tissue tends to increase in saline situations. Observations were supplemented by culture experiments with *Lepidium sativum* and other plants which could endure saline solutions, when it was found that, in general, the more concentrated the solution the more fleshy were the leaves produced.

Some experiments of Holtermann throw further light on the question of the effect of the salt content of the habitat in the Mangrove vegetation of Ceylon. A number of Mangroves (*Lumnitzera*, *Avicennia*, *Rhizophora*,

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[1] Lesage, P.: Recherches expérimentales sur les modifications des feuilles chez les plantes maritimes. Rev. Gén. de Botanique, 1890.

and others) were grown in ordinary soil in the Botanic Gardens at Peradenyia, but were watered with salt solutions of varying strength. After a time the culture plants were compared with the same plants growing in their natural habitat. In nearly every case the aqueous tissue was found to be less developed than the normal in those plants which had been watered with dilute solutions, and it was more strongly developed in those which had been watered with more concentrated solutions of salts. In one instance the leaves of a swamp plant of *Avicennia officinalis* had from four to five layers of aqueous tissue, whereas leaves from a plant cultivated at Peradenyia and watered with a 5 per cent. solution of potassium chloride showed from seven to eight layers. In the case of the flora of the Solfataras, observed by both Schimper and Holtermann, the excessive amount of sulphates in the soil appears to have the same effect in favouring the formation of aqueous tissue as the chlorides in the case of the Mangrove vegetation.

Not only are succulent plants provided with a reserve of water, but very often there is a marked conservation of the water supply, the younger leaves in time of drought being kept continually turgid at the expense of the older; this is probably true of all succulents with the exception of those which, like *Salicornia*, live in very moist habitats. It is also true, although to a much less extent, of a large number of mesophytic plants.

Meschayeff, in 1882, appears to have been the first to call attention to the oecological importance of this movement of water in plants; and subsequently numerous other observations of the same kind were made. Haberlandt hung up cut shoots of *Rhizophora mucronata* without any water supply. After one day the older and yellower leaves became withered, while the remaining leaves, except the youngest, were scarcely withered.

A more careful experiment is that of Burgerstein, who chose a pot plant of *Gasteria vittata*, a plant which has opposite pairs of fleshy leaves. The pot was allowed to stand with no water in a sunny place for six weeks, at the end of which time the earth was quite dry. Six leaves on one side were now cut off and weighed, the water content of each being found separately. The plant was now well watered, so that at the end of a month all the leaves seemed to have recovered their turgidity. The six remaining leaves were then detached and weighed, their water content being calculated as before. In comparing the water content of the full and the nearly depleted leaves, the amount of depletion was greatest at the lowest leaf and fell off regularly to the uppermost, as may be seen from the figures in Table VI.

The same gradual depletion of the old leaves at the expense of the young may be demonstrated more simply by observing the shrinkage in the dimensions of the different leaves on a shoot when allowed to wither. I have made such observations with species of *Mesembryanthemum* and of *Sedum*.

TABLE VI.

Water Content in successive pairs of Leaves of Gasteria vittata.

	Water Content % of turgid leaves.	*Water Content % of withering leaves.*	*Loss during drought.*
First Pair (lowest)	86·3	78·6	7·7
Second Pair	90·3	82·8	7·5
Third ,,	90·8	83·7	7·1
Fourth ,,	91·1	84·9	6·2
Fifth ,,	91·3	86·0	5·3
Sixth ,, (youngest)	93·4	91·8	1·6

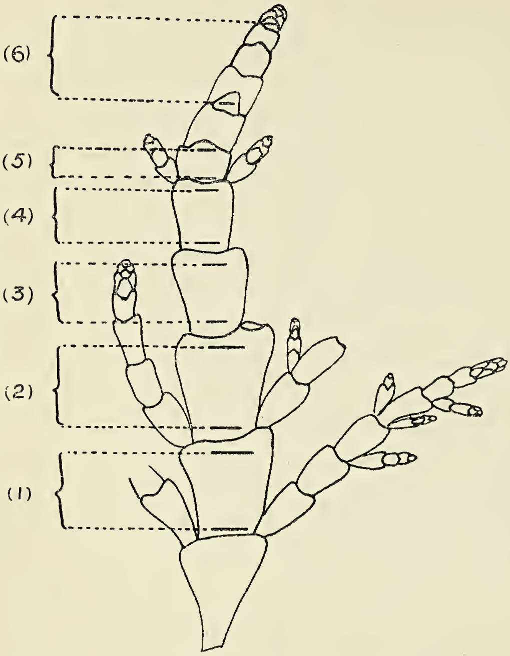
In the case of *Mesembryanthemum edule* a shoot was chosen which had three pairs of expanded leaves and one very small pair, the leaves of which were closely appressed to each other. One leaf of each of the three pairs of expanded leaves was marked with Indian ink along the length and breadth of both upper and lower surfaces. The cross-section of one of these leaves forms an isosceles triangle, so that the marks on the lower surface were made on one flank only. The measurements were made with an accurately divided rule and a lens magnifying ten times. The shrinkage observed calculated as percentages is recorded in Table VII.

TABLE VII.

Shrinkage in Surface of Mesembryanthemum edule when withering.

			1st day. %	*2nd day.* %	*3rd day.* %	*6th day.* %	*7th day.* %
Lowest Leaf.	Upper Surface	Length	4·6	0·3	2·4	(Shrinkage had obliterated marks.)	
		Breedth	3·4	3·6	1·9		
	Lower Surface	Length	7·4	0	0		
		Breadth	3·3	0	0		
Second Leaf.	Upper Surface	Length	0	1·1	0	5·6	2·7
		Breadth	1 9	0·9	0	12	2·2
	Lower Surface	Length	1·8	0·7	0·3	4·6	1·6
		Breadth	0	0	0	0	5
Third Leaf.	Upper Surface	Length	0	0	0	4·0	1·3
		Breadth	0	0	0	10	0
	Lower Surface	Length	0	0	0	(marks illegible)	
		Breadth	0	0	0		

It may be seen that the upper leaves were entirely, and the middle pair almost entirely, protected from loss in water content during three days' exposure to warm, dry air. After the third day the lowest leaves shrivelled rapidly, turning yellow, and finally becoming mere empty shrivelled bags; during this time the second pair of leaves first began to suffer seriously, and the third pair to a less extent. The innermost pairs of leaves grew to double their original size and remained perfectly turgid, as far as could be seen, throughout the experiment; they were, however, too inaccessible for accurate measurement.



Detached shoot of *Salicornia* (probably *S. ramosissima*).

Very similar results were obtained with different species of *Sedum*, but with *Salicornia* the behaviour is quite different. A shoot of *Salicornia* was marked as shown in the figure, and the distance between the marks observed as before with a lens magnifying ten times. Only the lengths were observed, for it is almost impossible to obtain an accurate estimate of the diameter of anything as soft as a flaccid *Salicornia* stem. In this plant, as will be seen from the figures in Table VIII, the younger parts suffered first from the loss in water, and later the older internodes also showed collapse, the whole plant having much less endurance in conditions of drought than *Mesembryanthemum*.

Table VIII.

Shrinkage in length of Shoots of Salicornia (probably S. ramosissima) during withering.

	Region.	*Loss % per 24 hours.*	
		1st day	*2nd day*
Main Shoot	(1) Lowest internode	0	0
	(2) Next above	10	0
Branch	(3) Lowest internode	0	10·2
	(4) Next above	3·6	6·7
	(5) ,, ,,	11·9	3·2
	(6) ,, ,,	11·9	3·2

Pringsheim,[1] in a paper dealing with this subject, records some experiments in which the attempt is made to analyse the water output of adult leaves which are both transpiring and supplying water to the growing parts.

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[1] Pringsheim, E.: Turgorregulation und Wasserbewegung in welkenden Pflanzen. Pringsheim's Jahrbücher für wiss. Bot., 1906.

In a typical experiment with *Sedum spectabile* six similar shoots were chosen, with as nearly as possible the same number of leaves. On June 20 the ten oldest leaves of three shoots were detached and weighed; no account was taken of the cut surface where the leaf had been attached, since this is unusually small in species of *Sedum*. The detached leaves were left to dry on blotting-paper, together with the remaining three shoots, the fresh weight of which was also found. On July 10 the six lowest leaves of the shoot were quite dry; the detached leaves were withered, but less so than those on the plant. The weight of each set of leaves was again taken, and the initial weight of the cut leaves was calculated in terms of an equal weight of the shoots with attached leaves. The results are shown in the following table, taken from Pringsheim's paper:

TABLE IX.

Leaves of Sedum spectabile, withering.

I. *Detached Leaves.*	*All* 10 *leaves.*	*Lowest* 6 *leaves.*	*Upper* 4 *leaves.*
a. Initial wt. June 20	7·92 grm.	4·29 grm.	3·71 grm.
b. Wt. July 10	5·23 ,,	2·74 ,,	2·49 ,,
c. Loss % fresh wt.	33·9 ,,	36·1 ,,	32·9 ,,
II. *Attached Leaves.*			
d. Wt. July 10	1·64 ,,	0·165 ,,	1·45 ,,
e. Loss % fresh wt.	79·5 ,,	96·3 ,,	60·9 ,,
f. Transport of water % fresh wt.	45·6 ,,	60·2 ,,	27 ,,
g. Transport % of Total Loss	57·1 ,,	62·5 ,,	46 ,,

From these figures Pringsheim concludes that the water loss of individual leaves on the plant is greater than when detached, and that the greater part of this water loss is due to displacement within the plant. Very similar results were obtained from experiments with the most varied type of plant, such as *Bryophyllum*, *Cotyledon*, *Mesembryanthemum*, *Tradescantia*, *Rhipsalis*, *Sambucus*, and *Vicia*.

Not less interesting are the observations made by Pringsheim on the osmotic conditions within the same plants, both normally and when withering. It was found that, with very few exceptions, the older leaves had a lower osmotic pressure than the upper; for example, in leaves of *Sedum spectabile*, the osmotic equivalent expressed in terms of a standard 10 per cent. solution of potassium nitrate was found to be—for leaves

5–5½	cm.	long (oldest)	18–20 per cent.
3	,,	,, (younger)	24–26 ,, ,,
2–2½	,,	,, ,,	30 ,, ,,
1–1½	,,	,, ,,	38 ,, ,,

The osmotic equivalent was thus greatest at the growing point, falling off quickly and remaining fairly constant for the adult leaves.

In a few plants no difference could be detected, as in *Rochea falcata*, where all the leaves are fleshy, and in *Phaseolus*, where the leaves are mesophytic, the youngest leaves had a lower osmotic equivalent than the older ones. In these exceptional cases, however, there appeared to be no water transport from the older leaves to the younger during withering.

Wiesner suggested that the water transport was due to the correlation between the young growing parts and the older leaves, the need of the actively growing parts forming a kind of mechanical check on the water loss of the older leaves by evaporation. Pringsheim remarks that this theory would not account for the fact that adult attached leaves transpire from the same fresh weight more strongly than similar detached leaves under the same conditions, and suggests that there is an internal regulating mechanism which adjusts the osmotic condition of the cell-sap throughout the plant, according to circumstances. In support of this theory, Pringsheim describes a number of experiments on the osmotic conditions of withering plants. Not only is there found to be what may be termed an 'osmotic gradient' in the normal plant, but this gradient is, in many cases, more or less maintained during withering; a few of Pringsheim's results are quoted in Table X.

TABLE X.

Osmotic Conditions in Withering Plants.

		Fresh. %	*After one week's withering.* %
Tradescantia fluminensis	Young parts	22–24	30–32
	Adult ,,	10–12	20
Sempervivum tectorum	Young ,,	28–30	34
	Adult ,,	20–22	30

However, the most striking example of turgor-regulation was seen in the case of storage organs for food reserves, such as tubers. In the potato the osmotic pressure of the growing shoot was always greater than that of the tuber itself, but fell off from day to day as the reserve was gradually depleted, and in this way only as much starch would be utilized as would be needed for growth. The mechanism of turgor-regulation as conceived by Pringsheim consists in the manufacture of osmotically active substances in the actively growing regions, thus setting up a diffusion stream upwards which, however, can last only as long as the turgidity of the cells concerned is not wholly lost.

It is probable that there may be some such regulatory mechanism within the plant, but this difficult question can hardly be regarded as wholly solved. As regards the question of the rate of transpiration of attached and detached leaves, whilst Pringsheim's figures seem indisputable for transpiration measurements reckoned on fresh weight, yet, in experiments which I have made, the loss per unit area of the detached leaves was undoubtedly greater than that of attached similar leaves taken at the same time, under the same conditions. My experiments, however, were generally for much shorter periods of time, lasting from one to eight hours instead of several days, as did Pringsheim's. In the case of *Mesembryanthemum edule* a shoot and an adult detached leaf, both sealed at the cut end, were weighed at intervals for three weeks. Throughout that time the loss in water per unit area of the detached adult leaf was much greater than that of the shoot with which it was compared. In the other cases examined by me, where the same is true, pairs of similar shoots were chosen and the transpiration per sq. dm. of a whole shoot compared with that of the lowest leaf of the control shoot detached from its stem. In every case loss from evaporation at the cut surface was prevented by sealing over with an adhesive wax mixture. In every case the transpiration of the detached leaf per unit area was greater than that of the attached ones.

Whatever be the explanation, however, of this conservation of water in the plant, there is no doubt that it may be of great oecological value, as Pringsheim points out, not only in enabling the plant to withstand long periods of drought without injury to the growing point, but also in providing a means of vegetative reproduction ; for in many cases the exhaustion of the water supply of older parts causes the death of the lower parts ; the apex then continues its growth, and often roots freely, and becomes independent in some more favourable spot. This is seen in many species of *Sedum*, *Mesembryanthemum*, *Crassula*, *Rhipsalis*, *Cassytha*, and many others. The ease with which adventitious roots form in these examples, however, varies greatly. In *Sedum* one or more adventitious rootlets regularly form, even in very dry air, just above the axil of the leaf, when each leaf is about half depleted. In *M. edule* roots are formed, but much less readily, and in *M. crystallinum* often not until many weeks have passed. In mesophytic plants the advantage is less obvious, for they have less power of storing water and often none of rooting from the apex ; but the greater transpiration of the younger, less protected leaves may be, at least in part, compensated by the water supply from the older ones.

(γ) **The Conducting System.**

There is no doubt that the means of water conduction in a plant stands in close relation to the rate of transpiration. In general, the greater the amount of transpiration the larger will be the number and size of the conducting elements in the stem and root. Jost found that by cutting off the leaves and buds of seedlings of *Phaseolus multiflorus* and other plants, the vascular bundles, and especially the water-conducting elements, remained in a rudimentary condition. He interpreted this as a kind of self-regulation, keeping the nature of the conducting tract in relation to the particular need of the plant.[1]

In the case of succulent plants we have little information as to the conducting tissue. Volkens, in his observations on plants of the Egyptian-Arabian desert, found that the conducting tissue of the shrubs and trees there was but poorly developed, and many of these would be succulent or semi-succulent plants either in stem or leaf.

The experimental observations of Cannon,[2] however, at the desert laboratory at Tucson, do not bear out Volkens's statement. A number of plants, including *Fouquiera splendens*, *Covillea*, and *Zizyphus,* were grown in the desert region, but were kept constantly irrigated with water for from two to four years. At the end of that time a careful comparison was made between the structure of the wood in irrigated and non-irrigated plants. It was found that in every case the irrigated plants grew much more luxuriantly and organized more woody tissue than the non-irrigated; but they formed a relatively large number of non-conducting parenchymatous elements; whereas in branches of the same diameter of the unwatered plants larger and more numerous tracheal elements were found. Cannon concluded that the desert flora in these cases is a reaction to a minimum water supply, the relative transpiration being greater in the non-irrigated plants, although the total water loss would be less.

In some epiphytes and many halophytes the aqueous tissue is provided with storage tracheides. These may be developed in connexion with the bundle system, and these usually occur as a group of isodiametric elements at the ends of the finest veins of the leaf. Often, however, storage tracheides are developed from the parenchymatous cells of the ground tissue, either singly as idioblasts, or as small groups of cells; but in each case independent of the bundle system. These storage tracheides seem to play an important part in the distribution of the water in fleshy stems and leaves. They occur always near a source of water, and are usually also in connexion with the assimilating cells, which they probably keep supplied with water. There appears to be no direct evidence that these tracheides can exercise a distributive function, but in the case of many species of *Salicornia*, Dr. E. de Fraine[3] tells me that their disposition in the stem would lend support to this view. They appear to be most numerous near the periphery of the fleshiest region of each internode, and they are more numerous in the flowering than in the vegetative shoot. In *Salicornia* the storage tracheides are unlignified, but they are spirally thickened with cellulose, and, according to this observer, retain their shape even when empty of water;

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[1] Haberlandt, Dr. G.: Physiologische Pflanzenanatomie, 1896, p. 296.

[2] Cannon: Water-conducting Systems of Desert Plants. Bot. Gaz., 1905.

[3] Moss, de Fraine, and Salisbury: On the genus *Salicornia* (anatomical section). Linn. Soc. Trans. (in the press).

moreover, in some species of *Salicornia* there appears to be every transition between lignified stereides and spirally thickened unlignified storage tracheides.

II. TRANSPIRATION IN RELATION TO HABITAT.

Probably the factors in the environment which most affect the transpiration of a plant are the water supply and the atmospheric conditions, although undoubtedly other climatic and edaphic influences are also concerned. We have now to consider the effect of the chief physical factors on the principal types of succulent plants.

In the truly xerophilous succulents of desert regions we have a reaction to extreme scarcity of water, to strong illumination, and to the desiccating effects of warm, dry air; adaptations are, therefore, found connected with both absorption and retention of water. The former process is facilitated by the development of deeply penetrating and often much branched root systems, and in many cases there is little doubt that the plants are for this reason almost independent of rainfall. Volkens, indeed, describes shrubs in the deserts of Northern Africa which seem to thrive where there is absolutely no rainfall for eight months of the year. In some cases the root absorption seems to be supplemented in an important way by absorption of dew by means of glands on the leaves, as in the Tamaricineae, also described by this author for the same region.

It is, perhaps, on account of the efficiency of the extensive root systems, that in spite of the very limited water supply the desert plants examined by Cannon developed more conducting tissue when grown in a dry than in an irrigated place. The same explanation may apply to the statements of Prof. F. E. Lloyd that *Fouquiera splendens* and *Verbena ciliata* are characteristic of truly desert regions and yet form leaves which in structure resemble those of a slightly succulent mesophyte; here, however, there is the additional explanation that the leaves are annual and are shed just before the hottest season.

The retention of water within the plant is accomplished partly by the formation of protective structures in the epidermis, and partly by the accumulation of mucilaginous substances and salts of organic acids within the aqueous tissue.

The most frequent protective adaptations are the development of a thick cuticle, which is often covered with a coating of wax, or with finely divided wax particles, the formation of relatively few stomata, which may also be sunken below the level of the neighbouring epidermal cells, protected with depositions of wax particles or surrounded by a raised wax collar as in *Euphorbia Tirukalli*.[1] Less often the stomata are protected by hairs. In all these cases there is a certain amount of reduction of

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[1] Figured in Haberlandt's Physiologische Pflanzenanatomie, 1896, p. 397.

transpiring surface, consequent on the adoption of the succulent habit, and in the epiphytic Bromeliaceae Linsbauer has recently found that the protective means and surface reduction seem to ensure the minimum of transpiration which could be consistent with sufficient aeration.

The experimental work of Aubert shows clearly how important is the presence of organic acids and of mucilaginous substances in the aqueous tissue of all succulent plants. There is little doubt that, owing to the high osmotic activity of these substances, the retention of water in the aqueous tissue is greatly facilitated.

The storage of water which is thus rendered possible may be of the greatest importance to desert plants, enabling them to endure long periods of drought without any evil consequences to the assimilating cells. In extreme cases, like that of *Sauromatum*, the so-called 'Monarch of the East', so much water is stored in a fleshy corm until the beginning of the dry season that the whole inflorescence and, later, the single leaf, are produced entirely at its expense. In the majority of succulent plants there is a definite displacement of water, in time of need, from the older to younger parts, and Pringsheim has shown that this is of value not only in prolonging the life of the more important tissues, but in providing a means of vegetative reproduction. In this way new shoots and buds are for long supplied with sufficient water for growth even when detached from the parent plant; and, in habitats in which germination is attended by great danger from drought, the reproduction is commonly effected by the rooting of such buds and shoots.

Much discussion has been raised over the question of the transpiration of plants of the warm, damp forest regions of the tropics, many of which are either epiphytic, as in the case of the Orchidaceae, or halophytic, as in the case of the genera collectively known as 'Mangroves'.[1]

The experiments of Haberlandt[2] led to the conclusion that transpiration of plants in these regions is less active than in most mesophytes of middle Europe, both in rate and in total amount. The observations of Stahl, Wiesner, Burgerstein, and Giltay, however, pointed to an exactly opposite state of affairs. The explanation of these conflicting results was finally given by Holtermann, who proved that although for hours together the transpiration of these plants may be wholly suspended owing to heavy dews at night and nearly saturated misty air by day, yet for a few hours near midday, under the influence of a tropical sun, the rate of transpiration might become enormous. The plants which are exposed to these alternating extremes are provided with aqueous tissue, although to a less extent than

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[1] Schimper noted the similarity in habit between epiphytes and Mangroves; in the case of *Ficus diversifolia* the same species is found growing equally well as either an epiphyte or halophyte. Holtermann: Der Einfluss des Klimas, &c., p. 125.

[2] Quoted by Burgerstein in Die Transpiration der Pflanzen, 1904.

in many succulent desert types, and during the periods of rapid transpiration the aqueous tissue may become so depleted of its water that the leaf becomes perceptibly flaccid. This, indeed, frequently happens after a hot day, according to Holtermann, in the plants of the Mangrove swamps. The following morning, however, the leaves may be found erect and turgid again after a night of heavy dew, so that Holtermann suggests that an appreciable surface absorption of the dew must have taken place. On the other hand, it is possible, in the light of the experimental researches of Wille[1] and others, that root absorption persisting all through the hours of suspended transpiration would alone account for the change. A similar phenomenon may easily be seen in our British halophytes, and probably the same explanation may be found true.

The experiments of Haberlandt and Holtermann on Mangrove plants indicate that here, as in true xerophytes, there is some power of water displacement, but to a somewhat less extent than in typical desert and rock plants. I have noticed a distinct power of water displacement from old to young leaves during withering, in shoots of *Salsola Kali* and in *Mesembryanthemum edule*, and to a less extent in *Suaeda maritima*; the same power may exist in *Salicornia*, but if so it is not sufficient to prevent the young parts from withering in drought, before the older internodes appear to suffer. The displacement of water from old to younger parts in time of need is by no means limited to succulent plants; it is often to be seen in mesophytes, although here it is less obvious, and probably of little biological importance.

In the majority of halophilous and in many epiphytic plants, the transpiring surface is little protected from water loss, being usually glabrous, with little cuticle or none, and with often numerous unsunken active stomata; relative to the surface area the transpiration may often be far greater than obtains in the majority of mesophytes under the same conditions.

The question of the relation of the halophytic plant to its environment is one of much difficulty. There is no doubt, as Schimper, Lesage, Holtermann, and others have shown, that it is intimately related to the presence of chlorides in the habitat. Probably the conclusion of Warming comes nearest the truth: that, whereas some maritime plants are true halophytes and cannot thrive in the absence of chlorides, others are only halophilous and, whilst showing greater succulence in the presence of chlorides, can thrive almost equally well in their absence; such are *Aster Tripolium* and *Plantago maritima*, and numerous other examples might be given. Moreover, owing to concentration by atmospheric evaporation and to dilution by rainfall, the salt content of the soil water must always be a very variable factor, and the halophyte must, therefore, possess in an unusual degree the power of accommodation to such changes. Further evidence on this

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[1] Quoted by Burgerstein, loc. cit.

point is given by some experiments of Mr. T. G. Hill.[1] Observations of the concentration of the soil water of the salt-marsh at Erquy, before and after heavy rain, showed a concentration of mixed chlorides varying from 3·3 per cent. to 0·7 per cent.; there is thus a definite dilution or 'washing out' of the salts present in the soil. Measurements of the osmotic equivalent of the root-hairs of young plants of *Salicornia* gave an average value equivalent to a 5 per cent. or 6 per cent. solution of sodium chloride when these plants had been growing in soil of which the soil water contained about 3 per cent. of chlorides. After bathing such a sod containing *Salicornia* seedlings with weaker solutions of chloride or with water, the root-hairs were found to have a correspondingly lower osmotic pressure; and, conversely, when seedlings which had acquired a lower osmotic pressure were transferred to successively stronger solutions and re-examined, the root-hairs were found to have regained a higher osmotic equivalent. There is, therefore, a definite accommodation in the cell-sap of these root-hairs to solutions of gradually increasing or gradually decreasing concentration. It would be of great interest to know how far the same property is possessed by 'halophilous' or facultative halophytes like *Aster Tripolium* or *Plantago maritima*, and by mesophytes.

It has long been known that there are some striking parallelisms between the adaptations of some halophytic and alpine plants, especially in the more succulent types. Occasionally exactly the same species are found on mountain-tops and in maritime districts far from each other, as in the case of *Dodonea viscosa*, which, according to Schimper, occurs in Java only in these two positions, and in *Plantago maritima*, which occurs in Europe, either as an alpine or littoral form.

Bonnier[2] has made a careful physiological analysis of the conditions affecting alpine plants. He found that the strong unshaded light in high altitudes was largely responsible for the modifications in structure found; whilst the intense cold and dry or rocky soil produced the well-known dwarf habit with long, deeply penetrating root system. The typical alpine plant, as well as the typical halophyte, shows much greater fleshiness in the assimilating organs, largely due to an increase in the depth of a palisade parenchyma; but in alpine plants this is accompanied by an actual intensification of pigmentation, so that there is a more rapid assimilation in alpine than in lowland forms, whereas in halophytes the thickening of the leaf is connected with feebler development of chlorophyll and less active assimilation, many of the internal cells of the mesophyll being probably almost entirely given up to the storage of water. In the case of alpine plants Bonnier regards the more intense pigmentation as a direct adaptation

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[1] Hill, T. G.: Observations on the Osmotic Properties of the Root-hairs of Certain Salt-marsh Plants. New Phytologist, 1908.

[2] Bonnier, Gaston: Adaptation des plantes au climat alpin. Ann. des Sc. Nat., Bot., 1894.

which enables the whole plant to achieve its period of growth in the shortest time, the rapid assimilation compensating to some extent for the short summer. A somewhat greater transpiration value was found for alpine than for corresponding lowland forms, both relative to surface and to fresh weight; and this in spite of the fact that on the whole there was more cuticular and hairy protection afforded to the alpine types. The greater transpiration, however, is probably due to the relatively greater number of stomata which was found to be a characteristic feature of alpine plants, and to the direct effects of increased assimilation and respiration.

Observations of Holtermann[1] on the vegetation of mountain peaks in Ceylon indicate that here also something more than mere altitude must be responsible for the characteristic modifications which were found. According to Holtermann the peculiarities associated with alpine plants only appear in Ceylon on mountain peaks over 5,000 ft. in height, but not at all in the vegetation of plateaus of an even greater altitude. The most obvious source of explanation was stated to be the absence of humus in the rocky districts of the mountain summits, although possibly other climatic factors might be concerned. If this explanation be true for the alpine vegetation of Ceylon, it is, however, scarcely applicable to that of Switzerland, and it is not borne out by the experience of horticulturalists who now succeed in growing even exclusively alpine plants in suitably prepared rockeries, the interstices of which contain usually a rich humic soil.

Aubert[2] attempted to compare by direct measurement the rate of transpiration relative to both the transpiring area, and to the fresh weight in succulent and mesophytic plants. The method employed was, admittedly, rough, and is open to criticism, but the general conclusion is probably trustworthy, that the Cactaceae transpire the least of any succulent plants as yet tested, and that next in order of magnitude come succulent plants of the type of the Crassulaceae and the Mesembryantheae, which have, themselves, a greater rate of transpiration than many sclerophytes, such as *Picea* and *Nerium*. In these latter types, however, there may be a very large collective leaf surface, so that the total water loss may be very much greater than in more succulent plants. We have seen that in halophytic, epiphytic, and alpine plants the rate of transpiration may be distinctly greater than in the average mesophyte. Such observations as I have made indicate that in our British Crassulaceae, and in those Mesembryantheae which have become acclimatized, the transpiration is greater relative to the surface area than in ordinary mesophytes, although the latter usually possess more numerous stomata. The transpiration relative to the initial fresh weight is, naturally, less in succulent than in mesophytic plants. The explanation of the

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[1] Holtermann, Dr. C.: Der Einfluss des Klimas auf den Bau der Pflanzengewebe, 1907.

[2] Aubert, E.: Turgescence et transpiration des plantes grasses. Ann. des Sci. Nat., Bot., 1892.

greater transpiration in succulents is not altogether clear, but is probably connected with the need for uninterrupted gaseous exchange, by means of the stomata, to compensate for the reduced surface area.

SUMMARY.

In the course of this paper it has been shown that the chief structural peculiarities of succulent plants are connected with (1) the transpiring surface, and (2) the storage of water.

Summing up for the characteristics of the transpiring surface, we see that succulent plants show a varying amount of reduction in leaf surface, and this may or may not be accompanied by arrangements which tend to diminish transpiration. Succulent plants of dry habitat have often either much cuticle or a waxy covering; less frequently protective hairs are found. In many cases water-storing tissue is found, and often the transpiration is considerable relative to the transpiring surface; the stomata are often specially protected in plants of very dry habitats; but in many Crassulaceae, succulent Chenopodiaceae, and halophytes, as well as many epiphytes, the guard cells are unprotected, and lie on a level with the epidermal cells. Many epiphytes absorb water over their whole surface, some also obtain water by means of aerial roots, which are provided with porous water-storing peripheral tissue, forming the so-called velamen. It seems probable that halophytes and desert plants with salt glands absorb water through the hygroscopic nature of the salts excreted; and that other halophytes with a thin cuticle absorb water to a less extent by means of their leaves.

Most succulent plants are characterized by the possession of definite aqueous tissue; others have a great development of mesophyll in the leaves, and then the innermost layers tend to function as storage cells for water. The water stored may be required for long periods of drought as in all truly xerophytic succulent plants. In extreme cases, the whole plant may develop at the expense of the water stored, as in the corms of *Sauromatum*. On the other hand, the water stored may be of value in avoiding the evils of a fluctuating water supply, as in many rock plants; or it may be a provision against temporary rapid transpiration, as in the Mangrove swamps of Ceylon, and perhaps, also, our ordinary British swamp halophytes. How far the aqueous tissue can be replenished from external sources, e. g. rain or dew or standing water, must be regarded at present as an open question; but it is probable that this may at times take place. The formation of aqueous tissue seems intimately related, on the one hand, to the production of organic acids, owing to the influence of limited gaseous exchange on metabolism, and, on the other hand, to the presence of chlorides or sulphates in excess in the soil water. According to Holtermann, however, these influences alone are not sufficient to account for all the peculiarities observed; for, although many plants, such as *Salicornia*, are modified so far as to be obligate halophytes, and others, such as *Aster Tripolium*, are plastic or facultative, yet there are some plants which can endure a saline or non-saline habitat with little or no change of structure, such as *Suaeda fruticosa* and several species of *Atriplex*. In every case, however, the aqueous tissue appears to be mainly of value as a water-storage tissue, the cells of which are always depleted in time of drought before the assimilating cells lose in turgidity, and filled again when a renewed water supply is available.

Finally, we see that many of the peculiarities of succulent plants must be regarded as adaptations to environment of real importance to the plant. They may be produced during the lifetime of the individual, as in the development of additional mesophyll or storage tissue in a facultative halophyte, or they may appear as permanent characteristics, as in the case of plants which are found exclusively in saline situations. In all cases, however, the presence of aqueous tissue and the power of water storage are probably of the first importance in the economy of the individual, and enable it to support a rate of water loss which is very considerable, relative to the transpiring surface.

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The Podocarpeae.[1]

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With Plates XLVI–XLVIII and eight Figures in the Text.

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i. INTRODUCTION.

THE need for comparative researches on the Coniferae cannot be expressed better than in the words of Dr. Scott, who in his Presidential Address to the Linnean Society of London for the year 1910 remarked with regard to this group of plants, 'I am convinced that a thorough comparative re-investigation of the whole family will be needed before the question of their affinities can be cleared up. . . . A broad synthesis is urgently needed.'[2] The investigation described in the following pages is an attempt to treat of the structures met with in one of the less known orders of the Coniferae in a comparative manner. On the other hand, the investigation can by no means be described as thorough, for the difficulties of obtaining material of critical stages of some species and of obtaining material at all of others, have proved in many cases insurmountable. This is especially so in the case of gametophytic structures, where series of stages carefully fixed are essential. Owing to the kindness of Mr. W. T. Saxton it has been possible for me to examine the earlier stages of the gametophytes

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[1] A Walsingham Medal Essay for 1911.

[2] Scott ('10), p. 77.

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of *Podocarpus latifolius* in material collected by him in two gorges near Cape Town on Table Mountain (Stangoolie and Orange Kloof). This material was mostly collected between October 18 and December 17. On the latter date Mr. Saxton found that the ovules in these localities had all aborted, so that a sequence of the later stages has not been available. Dr. Marie C. Stopes has also most kindly provided material of *Podocarpus macrophyllus* and *P. nagi* collected by her in Tokyo. This material consisted chiefly of older ovules, which provided a sequence of later stages than those given by the material of *P. latifolius*.

I have also been enabled to examine material of *Dacrydium cupressinum* sent by Mr. Phillips Turner from Wellington, New Zealand, and material of *Microcachrys tetragona* collected in Tasmania and sent to Professor Seward by Mr. Maiden from Sydney, New South Wales. I would here record my hearty thanks to the botanists mentioned for their kindness in providing me with material, as well as to Mr. T. G. B. Osborn and Miss E. J. Welsford, from whom were obtained roots of *Dacrydium* and *Podocarpus*. I am also much indebted to the Director of the Royal Botanic Gardens, Kew, and to Mr. Boodle for facilities in collecting and fixing material. Most of the material of leaves and stems was collected at Kew, as well as male cones of *Podocarpus andinus* and female 'cones' of *Dacrydium Franklini*. It is a pleasure to me to acknowledge my indebtedness to Professor V. H. Blackman for help in various ways, especially in matters of technique, and to Professor Seward for the trouble he has taken in helping to obtain material for this investigation, and for his putting at my disposal much literature which would otherwise have been unavailable. To my colleague, Mr. J. M. Hector, I am indebted for many kindnesses.

ii. METHODS.

The methods used in this investigation require no very special comment. The material collected by Mr. Turner in New Zealand was preserved in methylated spirit; that of *Podocarpus nagi* and *P. macrophyllus*, collected by Dr. Stopes at Tokyo, was preserved in dilute spirit. The material collected near Cape Town by Mr. Saxton was fixed in the mixture of mercuric chloride, picric acid, and acetic acid, as used by him in his work on the Callitrineae. The vegetative material collected at Kew was mostly fixed in methylated spirit, although some was killed in cytological fixatives. The young female fructifications of *Dacrydium Franklini* were fixed in the mercuric-picric-acetic mixture mentioned above, while the most suitable fixative for the male cones of *Podocarpus andinus* was found to be Flemming's strong fluid.

In the anatomical part of the work microtome series were used wherever possible, as well as hand sections. In a few cases it was found necessary to use the celloidin method. Safranin and anilin blue was found a most useful combination of stains for anatomical work, but several other combinations were also employed. For gametophytic and embryological work Benda's iron-alum haematoxylin was largely used. For thicker sections of 8 μ and upwards combinations of anilin safranin with either Delafield's haematoxylin or light green were found extremely useful, owing to the transparency of the stain. For gametophytes not cytologically fixed these stains gave results equal to those obtained with iron-alum haematoxylin, and so were largely used.

iii. NOTES ON GENERA AND SPECIES: EXTERNAL FEATURES.

The division of the Coniferae into the two primary groups of Pinaceae and Taxaceae appears to date from the work of Lindley in 1836,[1] and has been followed by almost every writer on the Coniferae. A threefold division into Abietinae, Cupressinae, and Taxinae was earlier suggested by L. C. and A. Richard.[2] The main distinction between the two divisions is in the female fructification, this being a well-defined cone in the Pinaceae, whereas cone formation tends to be imperfect in the Taxaceae. This rule is not without exception, but in the case of female cone-bearing Taxaceae there is no doubt of their affinity with other members of the group.

The later grouping of the Taxaceae into Taxeae (Taxoideae, Taxineae) and Podocarpeae (Podocarpoideae, Podocarpineae) first appears in the work of Endlicher in 1847.[3] The differences between the Taxeae and Podocarpeae extend to the male cones as well as the female fructifications. In the first-named group the microsporophylls are peltate or sub-peltate and bear several microsporangia, whereas in the Podocarpeae they are simpler structures bearing constantly two microsporangia on their under surface. As regards their female fructifications, the Taxean ovule is erect and surrounded by two symmetrical integuments; in the Podocarpeae the ovules are generally more or less inverted, and surrounded by a symmetrical inner integument, and an outer integument which only partially surrounds the inner. Here again the distinction is not absolute, for in the genus *Phyllocladus* the male cone is Podocarpean, while the megasporophyll bears a single *erect* ovule, with two symmetrical integuments, a Taxean character. This genus has therefore caused systematists a good deal of trouble. Thus we find it placed among the Taxeae by Endlicher[4] and others, removed to the Podocarpeae by Strasburger,[5] and placed in a sub-family by itself by Pilger.[6] Later work has tended to show more and more that its proper place is among the Podocarpeae,[7] so it is considered with them in this account. The remaining genera are *Podocarpus* with 62 species, *Dacrydium*

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[1] Lindley ('36), p. 316.

[2] Richard ('26), p. 124.

[3] Endlicher ('47), pp. 203, 231.

[4] Endlicher ('47), p. 234; Eichler ('89), p. 108.

[5] Strasburger ('72), p. 16.

[6] Pilger ('03), p. 38.

[7] See Robertson ('06), Young ('10), Stiles ('11).

with 16, *Saxegothaea* and *Microcachrys* each monotypic, and *Pherosphaera* with 2 species.[1] In all genera and species a single ovule is borne in a median position on the megasporophyll. The megasporophylls may be closely aggregated in well-defined cones as in *Saxegothaea* and *Microcachrys*, the cone may be a reduced one as in *Pherosphaera*, a still more reduced one as in *Phyllocladus*, or the sporophylls may be several in number as in some species of *Dacrydium* and *Podocarpus* ; lastly, they may occur singly or in pairs as in other species of these two genera. Again, the ovules may be pedicillate as in *Podocarpus*, or sessile on the sporophyll as in the other genera. In this case, they may be completely reversed as in *Saxegothaea* and *Microcachrys*, only partially reversed as in some species of *Dacrydium*, or erect as in *Phyllocladus* and *Pherosphaera*. Finally, they may have only one integument (*Pherosphaera*), two integuments with the outer only partially surrounding the inner, or the outer integument may be symmetrical (*Phyllocladus*). In spite of these differences there can now be no doubt that the forms here included form a natural family. Other evidence supporting this conclusion will be given in the course of the paper, and indeed such a view is now almost universally adopted.

The following genera and species have been examined :[2]

1. *Saxegothaea*, Lindl.

The single species *S. conspicua*, Lindl., has been described as *Squamotaxus Albertiana*, Senilis, but it would appear that no one but the author of the name has ever employed it. The plant is a small tree of Yew-like habit, native of the Chilian Andes, and is monoecious. A case has, however, been recorded in which some of the branches on a tree were mostly microsporangiate and others ovulate.[3]

2. *Microcachrys*, Hook. f.

The only species, *M. tetragona*, Hook. f., was for long confused with *Athrotaxis*, *Dacrydium*, and *Pherosphaera*. The male cones were first described by Sir W. J. Hooker in 1843 as *A. tetragona*,[4] and again in 1845 by Sir J. D. Hooker as *Microcachrys tetragona*,[5] the female cones then described under that name being those of *Pherosphaera*. Both male and female cones were described by Archer in 1850 as *Pherosphaera*.[6]

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[1] The New Caledonian genus *Acmopyle* (Pilger, '03, p. 117), with its single species *A. Pancheri*, probably belongs to this order, but so little is known of it that it seemed doubtful whether any useful purpose would be served by introducing it into this paper.

[2] The nomenclature of Pilger's monograph ('03) has been followed throughout as the most recent and most complete systematic account of the Podocarpeae. Further information on external features of the species is to be found in that work. Photographs showing the habit of some species of *Podocarpus* and *Dacrydium* are given by Laing and Blackwell ('06). For photographs of *Dacrydium Franklini*, *Pherosphaera Fitzgeraldi*, *Phyllocladus rhomboidalis*, *Podocarpus elatus*, and *P. spinulosus*, see Baker and Smith ('10).

[3] Norén ('08), p. 102.

[4] Hooker, Sir W. J. ('43), Tab. 560.

[5] Hooker, Sir J. D. ('45), p. 149.

[6] Archer ('50), p. 52.

The confusion in nomenclature between the four genera mentioned above was ultimately cleared away by Sir J. D. Hooker in 1860.[1]

This plant in habit is not much more than a straggling shrub found on some of the mountains of Tasmania. As regards reproductive shoots the plant is dioecious. As well as in this it differs from *Saxegothaea*, as far as external characters are concerned, in the form and arrangement of the leaves and in the nature of the fruit.

3. *Dacrydium*, Soland.

The first description of a plant under the generic name *Dacrydium* in 1786[2] contains practically no mention of its structure, but is chiefly concerned with the use to which the plant is put in the making of a kind of Spruce-beer. Lambert[3] appears to be the first writer to append a botanical description to the name.

This genus differs from *Saxegothaea* and *Microcachrys* in the reduction of the number of megasporophylls in the strobilus, and from *Podocarpus* in the absence of a stalk to the ovule, and in the freedom of the inner integument from the nucellus, and of the outer from the inner.

There is some range of habit among the sixteen species of this genus. Some, such as *D. Kirkii*, are trees reaching a height of 100 feet, the majority are small trees about 20 or 30 feet high, while others are low shrubs. The species are generally dioecious, but Kirk[4] describes *D. laxifolium* as monoecious or dioecious.

Two species of the genus have been examined in the course of this work, *D. Franklini*, Hook. f., and *D. cupressinum*, Soland. Both are forest trees, the former being the Huon Pine of Tasmania, the latter the Red Pine of New Zealand.

4. *Podocarpus*, L'Hérit.

The first plant of this genus to be given a botanical name was *P. nagi*, (Thunb.) Pilger, which was described by Thunberg[5] under the name of *Myrica Nagi*. Gärtner in 1778 realized that the plant belonged to a previously undescribed genus, and gave it the name of *Nageia Japonica*.[6] Two species are confused in this description,[7] so the generic name *Nageia* has been replaced by the later name of *Podocarpus*, which was first used by Labillardière[8] in 1806 for the species now known as *Phyllocladus aspleniifolius*. In the next year L'Héritier's name *Podocarpus elongatus* appeared.[9] L. C. and A. Richard in 1826 first gave the name *Phyllocladus* to what had previously been known as *Podocarpus aspleniifolius*.[10] The Vienna Rules regarding botanical nomenclature reject the earlier name *Nageia* in favour

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[1] Hooker, Sir J. D. ('60), i, p. 358.

[2] Forster (1786), p. 80.

[3] Lambert (1803), p. 93, Tab. 41.

[4] Kirk ('89), p. 169, Fig. 87.

[5] Thunberg (1784), p. 76.

[6] Gärtner (1788), p. 191.

[7] Pilger ('03), p. 55.

[8] Labillardière (1806), p. 71, Tab. 221.

[9] Persoon (1807), p. 580.

[10] Richard, L. C. and A. (1826), pp. 23, 124, 129.

of *Podocarpus*, and similarly the name *Podocarpus* as it was first used in 1806 is replaced by the later name *Phyllocladus.*

The chief character distinguishing *Podocarpus* from other genera is to be found in the greater extent to which fusion of parts has taken place in the female reproductive structures. Whereas in the genera already noticed the outer integument is to a great extent separate from the inner, which is itself separate from the nucellus, in *Podocarpus* the fusion of these parts is always more or less complete. In all the species of this genus also the ovule is not sessile on the megasporophyll, but is borne in an inverted position at the end of a short stalk, so that the micropyle faces towards the base of the stalk. For the upper part of its length the stalk is fused with the integuments, so that it is impossible to define the limits of the stalk and of the outer integument.

Pilger[1] divides the genus into five sections:

1. **Dacrycarpus**, in which the fusion of parts has included the megasporophyll, which is connate with the ovulate structure. This has taken place in none of the following sections.
2. **Microcarpus**, in which the leaves are squamiform.
3. **Nageia**, where the leaves are plurinerved.
4. **Stachycarpus**, with linear or lanceolate leaves, but when in fruit without a fleshy receptacle formed by the fusion and swelling up of the sporophylls. In two species, *P. andinus* and *P. spicatus*, the megasporangiate strobilus consists of a branch bearing about eight megasporophylls separated by appreciable internodes. The other species of the section, however, are reduced in this respect like the species of other sections.
5. **Eupodocarpus**, with linear or lanceolate leaves and with a receptacle.

Species have been examined representative of the last three sections mentioned above; it was not found possible to obtain for examination any but herbarium material of the three species of *Dacrycarpus*, or of the single species of *Microcarpus*. One species, *P. nagi*, (Thunb.) Pilger, was examined of the section *Nageia*; two species, *P. andinus*, Poeppig, and *P. amarus*, Blume, of the section *Stachycarpus*; and eleven species of the section *Eupodocarpus*: *P. elatus*, R. Br., *P. spinulosus*, (Smith) R. Br., *P. polystachyus*, R. Br., *P. macrophyllus*, (Thunb.) Don, *P. neriifolius*, Don, *P. salignus*, D. Don, *P. Totara*, A. Cunn, *P. alpinus*, R. Br., *P. elongatus*, (Ait) L'Hér., *P. latifolius*, (Thunb.) R. Br., and an unknown species. It is unnecessary to indicate the external features of each of these species, which are treated of at length by Pilger.

There is a wide range of habitat from tall trees like *P. elatus* reaching a height of 100 feet, through smaller trees such as *P. andinus*, about 20 feet high, to low shrubs like *P. alpinus* and *P. nivalis*, found on the mountains of Tasmania and New Zealand respectively.

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[1] Pilger ('03), p. 55.

Most species of *Podocarpus* are dioecious, though there are cases of monoecism in the genus. Mr. Saxton has made some interesting records when collecting material for this investigation. He found that *P. latifolius* was normally dioecious, but a single case was found of a female plant which bore a single male cone. In the locality in which this record was made male trees were found to be much more numerous than female ones.

It should be noted that *P. neriifolius*, Don, is perhaps more usually called *P. bracteata*, Blume ; similarly *P. salignus*, D. Don, is possibly better known as *P. chilinus*, L. C. Rich., while *P. Thunbergii*, Hook., is the more familiar name of *P. latifolius*, (Thunb.) R. Br. The plant which figures most in past investigations of the genus is *Podocarpus chinensis*, Wall. (or *P. sinensis*). Following Pilger this is *P. macrophyllus*, subsp. *maki*, Sieb.

Of the 63 species enumerated by Pilger, 3 are placed by that writer in *Dacrycarpus*, 1 in *Microcarpus*, 4 in *Nageia*, 10 in *Stachycarpus*, and 34 in *Eupodocarpus*. The remaining 11 species are 'species incertae sedis', 3 of them possibly belonging to *Nageia* and 8 to *Eupodocarpus*. This fact alone shows the imperfect state of our knowledge of the genus at the present time, and it is possible that future systematic study may considerably alter the number of species.

iv. STEMS.

A. **Primary Structure.**

The primary structure of the stem has been examined in the following species: *Dacrydium Franklini*, *D. cupressinum*, *Podocarpus nagi*, *P. elongatus*, *P. amarus*, and *P. andinus*.

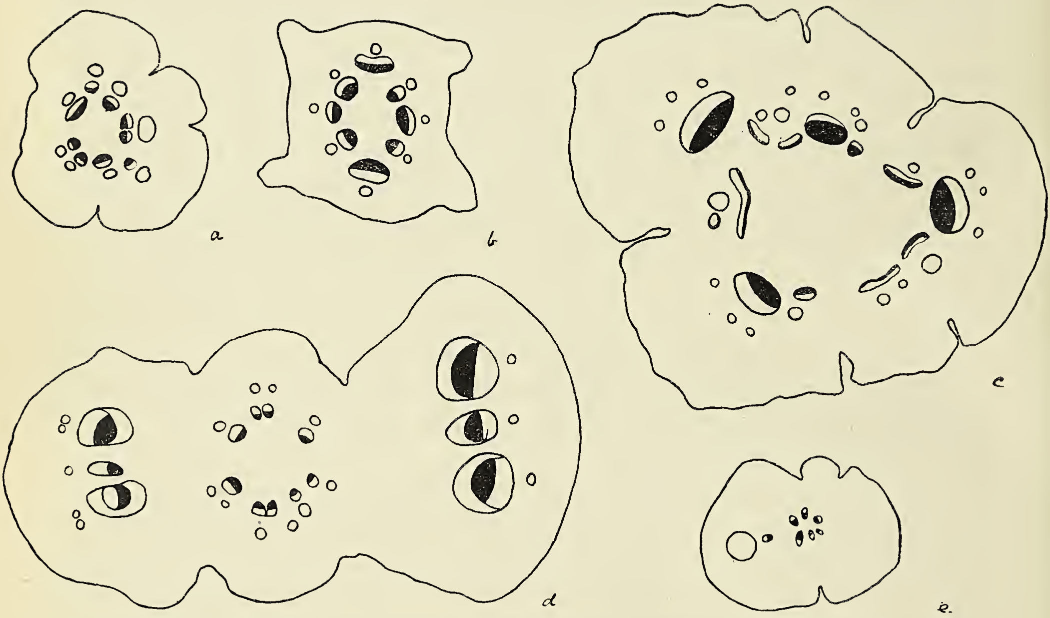
A transverse section through the young stem of *Podocarpus andinus* is roughly circular, and shows generally about four of the decurrent leaf-bases (Pl. XLVI, Fig. 1). There is in the stem a ring of seven or eight endarch collateral bundles. The centre is occupied by a parenchymatous pith ; in the cortex is a ring of resin canals just outside the ring of bundles, one resin canal appearing just outside the phloem of each vascular bundle. In this species the resin canals of the stem are fairly large structures with a well-marked epithelial layer, and the canals are no doubt functional. The protoxylem, as usual, consists of spirally thickened elements, while the cells of the metaxylem have uniseriate bordered pits. The cortex is composed of parenchymatous cells ; stone cells were not observed.

A leaf-trace arises by the division of one of the bundles, so that the two resulting bundles lie side by side in the ring. This is followed by the division of the resin canal in a similar manner. The leaf-trace and its accompanying resin canal then pass upwards and outwards into the leaf base.

Podocarpus elongatus is on the whole similar. A transverse section of the stem of this species is roughly square (Pl. XLVI, Fig. 2, and Text-fig. 1, *b*).

The leaf-insertion in this species is decussate, and the leaf-traces pass rather quickly out of the cortex. Each bundle, as in *P. andinus*, is furnished with a single resin canal.

In *Podocarpus nagi* the stem is usually oblong in transverse section owing to the decussate arrangement of the leaves and to the fusion of the leaf-bases with the stem (Pl. XLVI, Fig. 3, and Text-fig. 1, *d*). As in *P. andinus* there is a ring of endarch collateral vascular bundles surrounding the pith. How small these primary bundles are and how little xylem beyond protoxylem occurs in them is shown in Pl. XLVII, Fig. 18. In the pith, as well as in the cortex, stone cells are of fairly frequent occurrence. In this species they appear to occur singly and frequently near the xylem. The resin canals are distributed as in *P. andinus* and *P. elongatus*, but are relatively much smaller. The great peculiarity in the stem of *Podocarpus nagi* is in the insertion of the leaf-trace. Here one of the stem bundles divides into three, the resulting bundles of the division consisting of two larger lateral ones and one smaller median one (Pl. XLVI, Figs. 3 and 4). Each bundle is accompanied by a single resin canal. These three bundles then pass upwards and outwards, as in the case of *Podocarpus andinus*. As far as the writer is aware, this is the only recorded case among Conifers of a leaf-trace consisting of more than two bundles, and even in *Podocarpus nagi* the three bundles forming the leaf-trace result from a single bundle dividing when about to pass out from



TEXT-FIG. 1. Diagrams of transverse sections through primary stems. × 30. *a*, *Podocarpus andinus*; *b*, *P. elongatus*; *c*, *P. amarus*; *d*, *P. nagi*; *e*, *Dacrydium Franklini.*

the ring of bundles comprising the stele. Probably the condition of affairs is essentially the same as in some of the Araucarieae.[1]

Podocarpus amarus is essentially the same as *P. andinus* as regards the structure of the young stem. This is, as usual, a ring of bundles with a single resin canal outside each bundle. The case is, however, a little complicated as each leaf bundle is accompanied by three resin canals (Text-fig. 1, *c*). These three canals run down into the stem with their corresponding bundle, but the two lateral ones ultimately fuse with neighbouring canals, so that the bundle is left with a solitary canal. In this stem stone cells are extremely abundant in the pith, though before secondary growth had commenced only a few solitary ones were observed in the cortex. As in *P. nagi*, the primary xylem is practically limited to the protoxylem; Pl. XLVII, Fig. 17, shows a primary bundle in the stem just as the interfascicular cambium is forming.

In the stem of *Dacrydium cupressinum* there is a layer of sclerenchyma outside and in contact with the phloem. In the stems examined in the course of this work the sclerenchyma was two or three layers thick. The primary medullary rays in this stem are narrow, in some cases being only two cells wide. A ring of resin canals occurs in the cortex, as in the species of *Podocarpus* examined.

Dacrydium Franklini is the only other species of the genus examined. There is the usual ring of vascular bundles, but unlike *D. cupressinum*, and although resin ducts are present in the leaves, canals are absent from the stem (Text-fig. 1, *e*). The same is apparently the case in *Microcachrys*.

B. **Secondary Xylem.**

The accounts we possess of the structure of the stem in the Podocarpeae are mostly confined to accounts of the secondary wood. They are those of Bertrand[2] on *Podocarpus* and *Dacrydium* (treated as a section of *Podocarpus*), and of Penhallow[3] on *Podocarpus macrophyllus*. Beust[4] examined *Saxegothaea*, and Gothan[5] *Saxegothaea, Dacrydium* spp., and *Podocarpus* spp. Baker and Smith have recently published photographs of the wood of *Dacrydium*, *Phyllocladus*, and *Podocarpus*.[6] The writer has previously examined the stem of *Saxegothaea*,[7] and in conjunction with Mr. Brooks that of *Podocarpus spinulosus*.[8] Miss Gerry[9] has recorded some observations on the occurrence of 'bars of Sanio' in these plants.

Bertrand's observations are largely in terms of *Taxus*. He found that in *Podocarpus* wood parenchyma was often present in greater quantity than

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[1] Seward and Ford ('06), p. 351.

[2] Bertrand ('74).

[3] Penhallow ('04); ('07), p. 216.

[4] Beust ('84), p. 38.

[5] Gothan ('05), p. 57.

[6] Baker and Smith ('10), pp. 405, 428, 439.

[7] Stiles ('08), p. 210.

[8] Brooks and Stiles ('10), p. 306.

[9] Gerry ('10), p. 119.

in *Taxus*, that the phloem fibres early show thick walls, and that resin canals are absent from the wood or phloem.[1] Bertrand's observations were limited to *Dacrydium* and *Podocarpus*, but in *Saxegothaea* and *Microcachrys* his statement with regard to the absence of resin canals in the wood and phloem holds equally well.

On the nature of the pitting, Bertrand only remarks that all the tracheides do not have spiral thickenings as in *Taxus*.[2] Lately considerable attention has been directed to the nature and distribution of bordered pits on the walls of the tracheides. Beust[3] and Gothan[4] find that the pits in *Saxegothaea* are uniseriate, the pits being frequently in close contact and flattened above and below along the line of junction. This observation the writer was able to confirm.[5] Very occasionally the pitting is for a short distance biseriate, when the pits are close together and alternate. As a result of flattening along the line of contact they have a partially hexagonal shape.

Gothan[6] has observed this biseriate arrangement of the pits in the stem of *Dacrydium cupressinum* and two other species of the genus. I have examined the stem of *Dacrydium cupressinum*, but have not been able to confirm this statement. No doubt, as in *Saxegothaea*, the occurrence of the biseriate arrangement, at any rate in stems not many years old, is rather rare.

The writer has examined the wood of the fairly young stems of several species of *Podocarpus*. The pitting on the radial walls is uniseriate, and although the pits are sometimes in contact, this is not always the case, as variations in this respect may occur in the tracheides of the same section.[7] That such a variation should occur is of importance when it is considered that the distribution of pitting is one of the characters used in the determination of fossil woods. On the tangential walls of the tracheides of the summer wood conspicuous and numerous large simple pits ('Eiporen') occur.[8]

The medullary rays throughout the order consist of parenchymatous cells only. They are only one cell wide, and in the young stems, at any rate, are not often more than five cells deep.

Gothan[9] has made observations on the medullary ray pitting, a character on which he lays much stress in the determination of Coniferous woods. He finds four types of medullary ray pitting in the Podocarpeae.

1. *Saxegothaea* has the tangential walls of the medullary ray cells smooth, but the horizontal walls have numerous small simple pits.

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[1] Bertrand, l. c.
[2] l. c.
[3] Peust ('84).
[4] Gothan ('05), p. 57.
[5] Stiles ('08), p. 210.
[6] Gothan, l. c.
[7] Cf. Gerry ('10), Pl. XIII, Fig. 2.
[8] Penhallow ('07), p. 217, for *Podocarpus macrophyllus*; Baker and Smith ('10), p. 405, Fig. 270, for *Dacrydium Franklini*.
[9] Gothan ('05).

2. Typical ‘Podocarpoid’ pitting, consisting of smallish oval pits with a somewhat slit-like opening, occurs in *Podocarpus neriifolius*, *P. salicifolius*, and *Dacrydium laxifolium*.

3. A type characterized by large simple pits (‘Eiporen’) occurs in *Podocarpus andinus*, *P. spicatus*, *Phyllocladus*, *Dacrydium Franklini*, *Microcachrys*, and *Pherosphaera*.

4. A ‘mixed type’, with both the (2) and (3) kinds of pitting, is found in *Podocarpus Sellowi*, *P. falcatus*, *Dacrydium cupressinum*, and *D. elatum*.

v. LEAVES.

The leaves of the Podocarpeae show the usual xerophytic structure characteristic of Conifer leaves,[1] but, nevertheless, the range in structure, at least as far as external characters are concerned, is probably as great as that found in any order of the Coniferae.

A. **External Features.**[2]

The leaves of *Saxegothaea* and of many species of *Podocarpus*—for example, *P. andinus*, *P. spicatus*, and *P. ferrugineus*—are in external aspect very similar to those of *Taxus*. They generally end in a more or less acute tip, and are narrowed at the base. From this type of leaf there can be arranged a series of leaves of various species of *Podocarpus*, similar in shape, but increasing in size, such as *P. Totara*, *P. elongatus*, *P. macrophyllus*, and *P. polystachyus*, culminating in the large leaves of *P. amarus* and *P. elatus*, the leaves of the last-named plant being often 12 to 15 centimetres long and a centimetre wide.

Another kind of leaf is found in the familiar type of small appressed, more or less triangular leaf, which is found in *Pherosphaera*, *Microcachrys*, many species of *Dacrydium* (for example, *D. Franklini*), and in some species of *Podocarpus*. In more than half of the species of *Dacrydium* ‘juvenile’ forms of leaf occur which differ from those of the adult plant in being needle-like in shape. Such ‘juvenile’ forms are found in *D. cupressinum*, *D. biforme*, *D. Kirkii*, *D. Bidwillii*, &c. In *D. cupressinum* the leaves of the adult plant are shorter and flatter than those of the juvenile state, but yet are not triangular nor appressed to the stem, being rather intermediate in shape between the juvenile state and the more general adult state.

The most distinct type of leaf is found in the *Nageia* section of the genus *Podocarpus* (Pl. XLVII, Fig. 24). As is well known, the leaves in this section are comparatively wide and are traversed by a number of parallel veins; in this respect they are sharply marked off from all other species in

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[1] See for example Warming and Vahl ('09), p. 310.

[2] A full account of the external characters of the leaves of the Podocarpeae is given by Pilger ('03), p. 3. See also the specific descriptions in that work.

this order. The leaf of *Podocarpus nagi* is about 6 centimetres long by 2 centimetres wide, and often has as many as 40 veins. The superficial resemblance of the leaves of *Nageia* to those of species of *Agathis* has been frequently commented on, as well as the resemblance of both these types to the leaves of *Cordaites*.

In *Phyllocladus* the true leaves are reduced to scales, being replaced functionally by phylloclades.

In most of the species the leaves are arranged spirally on the axis, but in the *Nageia* section of *Podocarpus*, as well as in *Microcachrys*, *Podocarpus vitiensis*, and *P. elongatus*, a decussate or sub-decussate arrangement prevails.

B. **Internal Structure.**

The internal structure of the leaves of the following species has been examined: *Saxegothaea conspicua*, *Microcachrys tetragona*, *Dacrydium cupressinum*, *D. Franklini*, *Podocarpus nagi*, *P. andinus*, *P. amarus*, *P. macrophyllus*, *P. elatus*, *P. polystachyus*, *P. salignus*, *P. Totara*, *P. elongatus*. In addition accounts of the structure of the following species have already been published: *Podocarpus chilinus*[1] (= *P. salignus*, D. Don), *P. nagi*,[2] *P. neriifolius* and *P. falcatus*,[3] and *P. spinulosus*.[4] The leaf of *Microcachrys* has been described by Thomson,[5] and its structure compared with that of the megasporophyll, a comparison which has brought out some interesting points. Observations on the leaves of *Phyllocladus* have been made by Mrs. Arber.[6] As well, there is the comparative account of the structure of the leaves of *Saxegothaea* and *Podocarpus* (in which the genus *Dacrydium* is included) by Bertrand.[7] A brief general account of the anatomy of the leaves in the Taxaceae is given by Pilger.[8]

From these accounts, and from investigations on the species mentioned above, it is evident that several types of leaf occur in the Podocarpeae.

1. Perhaps the commonest type of leaf is that of *Podocarpus macrophyllus* (Text-Fig. 2, *a*). In this species the leaf is 5 or 6 centimetres long and about 7 millimetres broad. On each side of the xylem of the median vascular strand is found well-developed transfusion tissue (reticulate cells of Bertrand). From either side of this stretches to the margin of the leaf the accessory transfusion tissue of Worsdell (transfusion tissue of Bertrand and hydrostereome transversal of Bernard). Descriptions and figures of both transfusion tissue and accessory transfusion tissue are given by Worsdell[9] and Bernard.[10] On the lower or phloem side of the vascular bundle occur three resin canals, one median with regard to the bundle, the other two, one

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[1] Worsdell ('97), p. 310.
[2] Bernard ('04), pp. 268, 270.
[3] Bernard, l. c., p. 275.
[4] Brooks and Stiles ('10), p. 307.
[5] Thomson ('09^2), p. 349.
[6] Robertson ('06), p. 260.
[7] Bertrand ('74).
[8] Pilger ('03), p. 7.
[9] Worsdell ('97).
[10] Bernard ('04).

on each side of the median one, but yet distinctly underneath the phloem. These canals are small, but the epithelial cells seem to be of the normal type.[1]

A sclerenchymatous hypodermal layer is developed beneath the epidermis on the upper side. On the lower side this layer is only developed below the midrib, and even on the upper surface it is discontinuous. At the base of the leaf, however, hypoderm is developed all round. Stomata seem almost entirely limited to the under side of the leaf. They are sunk below the surface, as is usual in Coniferae, and are of the usual Coniferous type. There is a well-marked palisade layer below the upper epidermis and hypoderm ; the remaining mesophyll of the leaf is well provided with intercellular spaces. It is in this 'spongy' tissue that the accessory transfusion tissue is found.

The structure of the leaves of *Podocarpus elatus* and *P. polystachyus* seems to be essentially similar. In these species three resin canals beneath the phloem appear to be the rule. There are slight differences in the quantity of transfusion tissue, accessory transfusion tissue, and sclerenchyma, but no doubt the quantity of these is to some extent determined by external conditions. It is interesting to note that the leaves in which this type of structure has been observed are amongst the largest of the uninerved ones in the genus.

In leaves referred to *Podocarpus amarus*, obtained from the Royal Gardens, Kew, there are three resin canals, as in *P. macrophyllus*, but they are smaller than usual in this type, and are easily overlooked.

2. *Podocarpus vitiensis*, comprising Bertrand's monotypic subgenus *Polypodiopsis*,[2] according to that author has leaves characterized by a structure different from that found in other species in the genus. The chief difference consists in the distribution of the resin canals, one under the nerve and one in the parenchyma on each side of the leaf and near the margin. The decussate arrangement of the leaves and other characteristics given by Bertrand are also found in other species.

3. In the third type of leaf there is normally a single resin duct under the vascular bundle, as, for example, in *P. salignus* (Text-fig. 2, *b*). There is also a tendency towards reduction of accessory transfusion tissue and sclerenchyma as compared with the *P. macrophyllus* type. In *P. Totara* the accessory transfusion tissue is not very typical, the cells being rather short, and not much lignified, intermediate in character between the mesophyll cells and typical accessory transfusion tissue. Stomata were observed on the lower surface only, while a sclerenchymatous hypoderm was observed on both sides. *Podocarpus elongatus* appears to have leaves with a similar internal structure, but here, as Bertrand [3] correctly observed, stomata are

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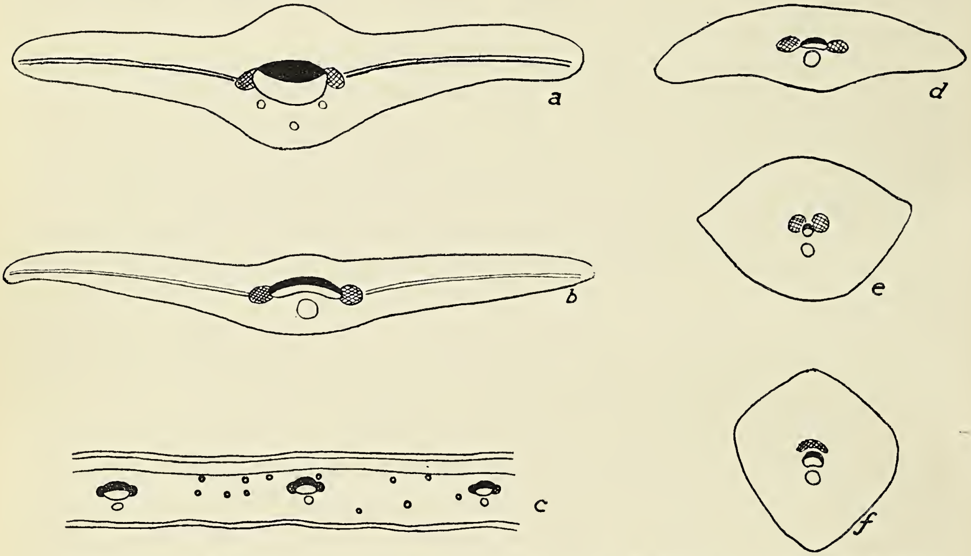
[1] Pilger ('03, p. 8) records the frequent presence of three resin canals under the bundle in *Podocarpus*, but does not give the names of any species.

[2] Bertrand ('74), p. 66.

[3] l. c., p. 60.

found on the upper as well as on the under surface. Pilger[1] would correlate this with the presence of palisade on both sides of the leaf, but in the leaves examined by the writer palisade was very feebly developed. The development of palisade on one side or on both sides of the leaf, as well as the extent of its development, probably, as in Angiosperms, depends mostly on external conditions. Bertrand's statement that, except in *P. elongatus*, *P. vitiensis*, the *Nageia* section of *Podocarpus*, and *Dacrydium*, the upper face of the leaves is without stomata,[2] can be accepted no more than his statement that there is *constantly* under each bundle a single resin canal.[3] In *Podocarpus andinus*, for example, stomata occur on the upper as well as on the under surface.[4] This last species may be regarded as the extreme type of this group as regards leaf structure (Text-fig. 2, *d*), for in its leaves there is no trace of accessory transfusion tissue, nor is a sclerenchymatous hypoderm developed. Together with *P. ferrugineus* this species is classed by Bertrand in his section *Prumnopitys*, the chief characters of the section being want of both hypoderm and accessory transfusion tissue.[5]



TEXT-FIG. 2. Diagrams of transverse sections of leaves of the Podocarpeae. Xylem is shown in black and transfusion-tissue is cross-hatched. In *a* and *b* the distribution of accessory transfusion tissue is indicated, and in *c* the distribution of epidermis, palisade, 'spongy' tissue, and stone-cells. *a*, *Podocarpus macrophyllus*, × 19; *b*, *P. salignus*, × 19; *c*, *P. nagi*, × 36; *d*, *P. andinus*, × 19; *e*, *Dacrydium cupressinum*, × 36; *f*, *D. cupressinum* (juvenile condition), × 36.

4. Bertrand's characteristics of the leaf of *Dacrydium* are absence of accessory transfusion tissue and of differentiation of the fundamental tissue; the constant presence of stomata on the upper surface and of hypoderm below the epidermis.[6] The writer's observations on *D. cupressinum* and

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[1] Pilger ('03), p. 7.
[2] Bertrand, l. c.
[3] l. c., cf. *P. macrophyllus* and *P. amarus*.
[4] The case of *Microcachrys* was unknown to Bertrand.
[5] Bertrand ('74), p. 65.
[6] l. c., p. 67.

D. Franklini quite confirm these statements. The juvenile form of leaf of *D. cupressinum* is tetragonal in transverse section, with a sclerenchymatous hypoderm continuous round the leaf, except in the region of the stomata. These occur in two bands towards those corners of the tetragonal leaf which are morphologically the margins. Palisade tissue is continuous all round the leaf, there being perhaps a slight development of spongy tissue in the corners corresponding to the margins.

There is a single vascular bundle with a well-marked resin canal. A well-marked sheath of parenchymatous cells surrounds the bundle and resin canals, a single layer of similar cells separating the phloem and the canal. Well-marked transfusion tissue is absent, but some lignified parenchymatous cells about the xylem probably represent it. These cells, although in the position of centripetal xylem, have exactly the appearance of parenchymatous cells, except for their lignified walls, and in this case certainly do not appear to be modified xylem-elements (Pl. XLVII, Fig. 19). The leaf of the adult form is more flattened than the juvenile form, and is not markedly tetragonal in section (Text-fig. 2, *e*). The structure, otherwise, is almost identical with that of the juvenile form. The stomata are in a similar position, but more numerous, while the lignified cells probably equivalent to transfusion cells occur in two rather lateral groups (Pl. XLVII, Fig. 20).

5. The leaf of *Microcachrys tetragona* has been described by Thomson,[1] with whose account my own observations agree. There is a distinct palisade with sclerotic hypoderm on the lower surface, while hypoderm on the upper surface is wanting. The stomata, as in *Dacrydium*, occur on the upper surface only. The single resin canal is wholly foliar, not being carried down into the stem.

6. *Phyllocladus*. The writer has not had an opportunity of examining leaves of this genus. Mrs. Arber[2] has described those of *Ph. alpinus*, *Ph. trichomanoides*, and *Ph. rhomboidalis*. They are, as would be expected in such a reduced leaf, of very simple type. On the whole they most nearly recall those of *Microcachrys*, except that no hypoderm or palisade is present. A single resin canal is present under each bundle. There is no differentiation of tissues in the mesophyll of the leaf. Mrs. Arber did not observe stomata in *Ph. alpinus* and *Ph. trichomanoides*, but a few were present in *Ph. rhomboidalis*.

7. *Nageia*. Of the species of the *Nageia* section the only one whose leaves have been examined in the course of this investigation is *Podocarpus nagi*. In this species no stomata were observed on the upper surface of the leaf, a character in which, according to Bertrand, it differs from other species of the section. In the leaves from Tokyo examined there is a scleren-

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[1] Thomson ('09²), p. 349.

[2] Robertson ('06), p. 260.

chymatous hypodermal layer on both sides of the leaf. It is occasionally interrupted, and always so at the stomata.

The palisade layer under the upper hypoderm is well marked; it consists of a single layer of cells occasionally doubled. The rather compact 'spongy' tissue is about eight layers of cells thick; solitary stone cells are scattered in it. A small resin canal is present under each bundle. Accessory transfusion tissue is absent (Text-fig. 2, *c*).

A remarkable character presents itself at the base of the leaf in this species. It has already been stated that the leaf-trace consists of three bundles. Shortly after leaving the central cylinder of the stem a cambium forms, not only between the xylem and phloem, but also on the upper side (as regards the leaf) of the bundle as well. In this way concentric bundles are produced which consist on one side of secondary tissue only (Pl. XLVII, Fig. 21). This state of affairs recalls that found in some of the vascular bundles of the axes of the inflorescences of *Welwitschia*, except that, whereas in *P. nagi* the xylem surrounds a parenchymatous area and is surrounded by phloem, in *Welwitschia* the concentric bundles have an amphivasal structure, with xylem surrounding the phloem.[1]

vi. ROOTS.

An exhaustive account of the structure of the roots of the Coniferales is in process of publication by Noelle; of this account at the time of writing only the first part dealing with the Pinaceae had appeared.[2] In the description of Podocarpean roots to be given here it is not intended to describe these structures in any great detail, but merely briefly to indicate the more important characteristics of the roots of the few species available to the writer.

Perhaps the most conspicuous external character of the roots of *Podocarpus* and *Dacrydium* is the presence of numerous tubercles, which are said to be caused by an ectotropic mycorrhiza. These tubercles have been mentioned by Van Tieghem[3] and described by Shibata.[4]

In all the species examined, *Dacrydium Franklini*, *Podocarpus macrophyllus*, *P. cupressinus*, and *P. alpinus*, the primary xylem forms a rather narrow diarch plate. In this respect it agrees with the roots of the Araucarieae,[5] and most of the Abietineae.[6] In some of the latter, namely,

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[1] See, for example, Sykes ('10), pp. 192, 202. It is perhaps worthy of note in this connexion that Seward and Ford ('06, p. 338) record the presence of such a concentric leaf-trace bundle as I have described, in the cortex of a young stem of *Araucaria imbricata*. Seward and Ford, however, 'prefer to regard this concentric strand as a leaf-trace of unusual structure rather than attribute to what is undoubtedly an exceptional occurrence any morphological significance.'

[2] Noelle ('10), p. 169.

[3] Van Tieghem ('70), p. 195.

[4] Shibata ('02), p. 644.

[5] Seward and Ford ('06), pp. 343, 345; Noelle ('10), p. 170.

[6] Noelle ('10), p. 178.

Pseudotsuga,[1] *Pseudolarix*, and *Abies*,[2] the roots are diarch or triarch, while in *Pinus*, as well as in the Taxodieae and Cupressineae, the roots are polyarch.

The secondary wood is composed mostly of tracheides, but wood-parenchyma cells are fairly numerous. The annual rings are not always well marked, as was the case in my roots of *Podocarpus cupressinus*, whereas in *P. alpinus* the rings were very well marked. These differences are possibly individual rather than specific. The medullary rays are like those of the stem; they are one cell wide and wholly parenchymatous. The bordered pits on the walls of the tracheides are often contiguous; they are usually uniseriate, but a case was observed in *P. alpinus* in which there was a biseriate arrangement. Miss Gerry has recorded the presence of 'bars of Sanio' in the tracheides of the root in *Dacrydium cupressinum* and *Saxegothaea*.[3] She also records the crowding of the pits in many cases.

Considerable emphasis has been laid on the value for phylogenetic and classificatory purposes of the distribution of resin canals in the root.[4] In the roots of the Podocarpeae I have examined these structures were entirely absent.

vii. 'MALE' CONES.

In contrast to the megasporangiate structures, the microsporophylls of the Podocarpeae are always aggregated into cones. These are remarkably uniform structures throughout the whole of the order, but among the different species a fairly wide variation in size is exhibited.

In *Microcachrys* the mature cones are only about 4 mm. long and about 2 mm. broad; those of *Saxegothaea* are about twice this length,[5] while in *Podocarpus andinus* and *P. spinulosus*[6] they may be as long as 1 to 1·5 cm. The cones of *Podocarpus Totara Hallii* vary from 3 to 20 mm. in length.[7] A larger type of cone is found in *Podocarpus latifolius*, while the strobili of *Podocarpus polystachyus* may reach a length of 10 cm.

In *Saxegothaea* and *Podocarpus* the male cones are borne at the apices of short pedicels, which occur either singly or two or three together in the axils of foliage leaves. In *Saxegothaea* one cone is usually found in the axil of a foliage leaf, though there may be two or three.[8] In *Podocarpus andinus* the same appears to be the case. One cone in the axil is the rule in *Podocarpus nivalis*.[9] In *Podocarpus Totara Hallii*, on the contrary, Burlingame reports that the cones are usually in pairs of one large and one small one.[10] In *Podocarpus spinulosus* the number of strobili in the axil

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[1] Jeffrey ('05), p. 3.

[2] Noelle ('10), pp. 194, 205.

[3] Gerry ('10), p. 121.

[4] Jeffrey ('05), p. 24; Noelle ('10), p. 250.

[5] Norén ('08), p. 102; Stiles ('08), p. 211.

[6] Brooks and Stiles ('10), p. 307.

[7] Burlingame ('08), p. 162.

[8] Thibout ('96), p. 179; Stiles ('08), p. 211.

[9] Burlingame, l. c.

[10] l. c.

of one leaf may vary from one to four.[1] The strobili of *Phyllocladus* are borne in clusters of 2 to 8 in the axils of scales at the tips of the lateral branches.[2] In *Microcachrys*, on the other hand, the male cones are terminal on an ordinary leafy shoot.[3]

Coulter and Chamberlain[4] have remarked on the relation of leaves and strobili in the Taxaceae as being such, that if the axis were shortened, a compound strobilus would result. Although this statement does not hold generally for the Podocarpeae (as witness the case of *Microcachrys*), yet it is nevertheless true for *Saxegothaea* and all the species of *Podocarpus* examined by the writer. The resemblance to a raceme of the cone-bearing branch previously noticed in the case of *Saxegothaea*[5] is even still more noticeable in the case of *Podocarpus andinus* (Pl. XLVI, Fig. 5).

In *Microcachrys*, where the foliage leaves are arranged decussately, the sporophylls are arranged in alternating whorls of four. In all other genera, as far as they have been examined, the sporophylls are spirally disposed on the cone axis. At the base of the cone occur a few small spirally arranged bracts.

The writer has only had an opportunity of examining the male cones of *Saxegothaea*, *Microcachrys*, *Podocarpus andinus*, *P. polystachyus*, *P. spinulosus*, and *P. latifolius*, but descriptions are also available of a few other species. These cases are, however, sufficient to show that a good deal of variation in shape and mode of attachment of sporangia exists in the order (Text-fig. 3).

In *P. andinus* each sporophyll is attached to the axis of the cone by a short narrow stalk, which terminates in the relatively large triangular upward-projecting portion ending in a broad point. The two sporangia are inserted on this on the side near the cone axis, one on each side of the stalk and partly attached to it, and are not in contact with one another (Pl. XLVI, Fig. 6, and Text-fig. 3, *a*). In *Podocarpus latifolius* the extent of fusion is greater and the sporangia are radially elongated; a tangential section of the cone shows that they are fused together for the upper half of their vertical width (Text-fig. 3, *b*). In *Saxegothaea*, and presumably also in *Podocarpus Totara Hallii*,[6] the two sporangia are fused together for the greater part of their vertical width (Text-fig. 3, *c*). The same is the case in *Microcachrys*, but here a large cavity is present between the two sporangia and below the phloem of the vascular bundle (Text-fig. 3, *d*). In *Saxegothaea* the line of dehiscence of the sporangium is transverse,[7] and transverse dehiscence of the sporangium of *Dacrydium cupressinum* is figured by Richard and by Lambert,[8] and described for *D. elatum* and

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[1] Brooks and Stiles ('10), p. 307.

[2] Coulter and Chamberlain ('10), p. 319.

[3] Thomson ('09^1), p. 26, Pl. I, Pl. II, Figs. 1 and 2.

[4] Coulter and Chamberlain, l. c.

[5] Lindley ('51).

[6] Burlingame ('08), Pl. VIII, Fig. 6.

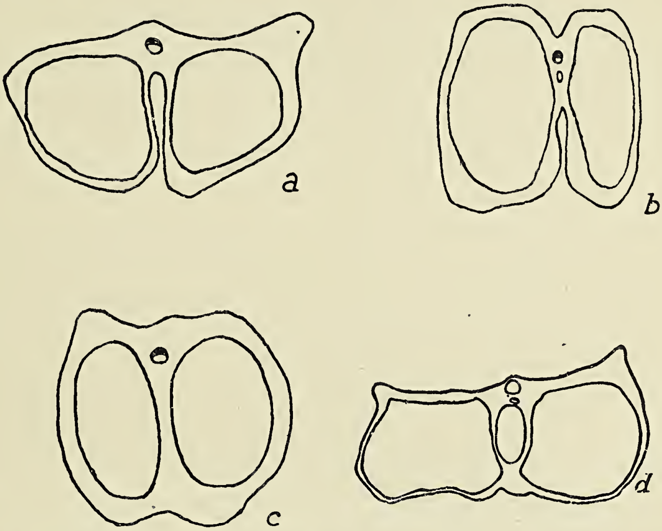
[7] Stiles ('08), p. 212.

[8] Richard ('26), Pl. II, *Dacrydium cupressinum*, Fig. K; Lambert ('32), Tab. 69.

D. Colensoi by Thibout, while the same is said to be the case in *Podocarpus andinus*, *P. dacrydioides*, and *P. Totara*.[1] In *P. spinulosus*[2] and some other species described by Thibout[3] the dehiscence is oblique. Thibout also records oblique dehiscence in *Phyllocladus*.

The vascular structure of the cone axis is very similar to the primary vascular structure of the stem; that is, there is a ring of vascular bundles surrounded by a ring of resin canals, one canal outside the phloem of each bundle. *Saxegothaea* has traces of centripetal xylem in these bundles,[4] but no such thing was found in *Microcachrys* or any of the species of *Podocarpus* examined, nor is any recorded in the case of *Phyllocladus*.[5] No canals are present in the cone axis of *Microcachrys*.

The sporophyll trace originates in the same way as the leaf-trace; that is, by the division of a single bundle into two which lie side by side in the ring of bundles, above which point one of them passes out into the sporophyll. The resin canal divides similarly, and in *Saxegothaea*,[6] *Podocarpus spinulosus*,[7] and *P. latifolius* a canal passes into the sporophyll; in *Podocarpus andinus*, however, the canal does not enter the sporophyll.



TEXT-FIG. 3. Transverse sections through microsporophylls. *a*, *Podocarpus andinus*, × 32; *b*, *P. latifolius*, × 32; *c*, *P. Totara Hallii* (after Burlingame); *d*, *Microcachrys*, × 32.

The single vascular bundle in the sporophyll consists of a few xylem and phloem elements which require no special comment; in the terminal lamina the bundle ends in a development of transfusion tissue.

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[1] Thibout ('96), p. 170.

[2] Brooks and Stiles ('10), p. 308.

[3] Thibout ('96) p. 171.

[4] Stiles ('08), p. 212.

[5] Robertson ('06).

[6] Stiles ('08), p. 212.

[7] Brooks and Stiles ('10), p. 308.

The development of the sporangium has not been traced in detail in any species of the order, but no doubt follows that of other Conifers. In the youngest material examined of *Podocarpus andinus* five layers of cells were visible outside the megaspore-mother-cells; in the mature sporangium only the remains of two layers were visible inside the sporangium wall. The latter in *Saxegothaea* and *Podocarpus latifolius* has conspicuous thickening bands on the vertical walls, but in *P. andinus* the walls are not very conspicuously thickened. In *Saxegothaea*[1] and *Podocarpus* spp. the sporophyll contains cells of presumably a secretory nature with deeply staining contents which are very like those figured by Seward and Ford[2] for *Araucaria Rulei.* In *Podocarpus latifolius* and *P. andinus* the terminal lamina contains a large air-space on the side below the vascular bundle.

viii. 'FEMALE' FRUCTIFICATIONS.

The features distinguishing the various genera of the Podocarpeae have been touched upon in a previous section of this paper, and for this purpose it was necessary briefly to indicate the chief differences between the female fructifications of the various genera. A more detailed account of these organs will now be given.

I. *Pherosphaera.* The sporophylls of this genus, according to Pilger,[3] form small cones with 2 to 5 fertile sporophylls and several sterile ones. Each fertile sporophyll bears a single erect ovule surrounded by a single integument (Text-fig. 8, *a*); in the young condition it is stated by Pilger that the ovule is in the axil of the cone scale.

II. *Phyllocladus.* The female fructifications of this genus consist of small cones, solitary or several together in the axils of scale-leaves, which may be borne either on the branches of unlimited growth or on the phylloclades.[4] The sporophylls may be either spirally or decussately arranged, and this is not even a specific difference, for in *Ph. alpinus*, although the usual arrangement of the sporophylls is a decussate one, yet a spiral arrangement sometimes obtains.[5] In this species there may be one pair or two pairs of fertile sporophylls at the base of the cone, and several upper pairs of sterile cone scales.[6]

Each fertile sporophyll bears a single erect ovule, which appears from one of Mrs. Arber's figures to be borne in the axil of the sporophyll.[7] When young the ovule is surrounded by a single integument, and the resemblance to *Pherosphaera* is thus striking (Text-fig. 8, *b*). Later a symmetrical outer integument (the aril) arises round the inner, and in the older seed forms a papery cup. The inner integument appears to differentiate into

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[1] Stiles ('08), p. 212, Fig. 29.

[2] Seward and Ford ('06), p. 356, Fig. 22, H.

[3] Pilger ('03), p. 39.

[4] l. c., p. 94.

[5] Robertson ('06), p. 261.

[6] l. c.

[7] l. c., Pl. XVII, Fig. 8.

three layers:[1] an outer 'fleshy' layer which does not thicken up,[2] a middle hard sclerenchymatous layer, and an inner soft-celled tissue.

It would appear from Van Tieghem[3] that the sporophyll and ovular vascular supplies are connected independently to the cone axis. The ovular supply terminates below the base of the nucellus in a 'tracheal platform', no strands entering the integuments.[4]

III. *Saxegothaea.* The structure of the cone of this species has already been described by several writers.[5] The cones, which are borne terminally on somewhat elongated branches, are somewhat spherical in shape, and consist of about twenty cone scales. Above the foliage leaves on the cone-bearing shoot, and below the cone, are a number of bracts intermediate in character between the foliage leaves and the lowest of the cone scales. These latter are sterile, and are themselves intermediate between the bracts and fertile cone scales.[6] Foliage leaves and megasporophylls are thus bridged over by numerous intermediate gradations. All these are spirally arranged on the shoot.

The cone scales are ovate-lanceolate or lanceolate structures, imbricate, and becoming fused together later, first at the base. Each fertile megasporophyll bears a single ovule medianly placed on the upper surface of the scale.[7] At the time of pollination the ovule is sunk in a cavity at the base of the scale, and is completely reversed,[8] that is, the micropyle faces towards the axis of the cone. The nucellus of the ovule is surrounded by two integuments; the outer is now generally known as the epimatium. The inner integument completely surrounds the nucellus, the epimatium only partly surrounds the ovule, being present indeed on its upper surface only (Pl. XLVI, Fig. 8).

The youngest condition of the cone has been described by Norén.[9] He finds the young ovule borne near the base of the scale and at right angles to it (Text-fig. 8, *c*), so that the micropyle faces towards the under side of the scale above. He finds the epimatium in this young state to be in close connexion with the integument; only through the subsequent growth do the outer parts of the epimatium and integument come to be free from one another. This does not support Pilger's statement[10] that the

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[1] Robertson ('06), Pl. XVII, Fig. 11.

[2] Young ('10), p. 85.

[3] Van Tieghem ('69), p. 279.

[4] Robertson ('06), p. 261.

[5] Pilger ('03), p. 42; Norén ('08), p. 108; Stiles ('08), p. 213; Thomson ('09²), p. 345; Tison ('09), p. 142.

[6] The application of the term 'bract' has been limited to the reduced leaves below the cone; the 'pièces du cône' are always referred to as 'megasporophylls' or 'cone scales'. It seems best not to use the term 'bract' in regard to the latter.

[7] In several cones Thomson ('09²) has observed ovules borne on the lower surface of the sporophylls at the base of the cone.

[8] See Norén ('08), Taf. 8, Figs. 19 and 20; the former of these figures is reproduced in Coulter and Chamberlain ('10), p. 327.

[9] Norén ('08), p. 109.

[10] Pilger ('03), p. 16.

epimatium is an outgrowth of the cone scale and has no nearer connexion with the integument of the ovule, but Norén refrains from disputing it, simply stating that he does not regard the question as settled.[1] In material a little older, Tison[2] confirms Norén's observations on this point. Tison's observations were made on cones about three weeks before pollination. By this time the ovule is lodged in a cavity at the base of the bract. Tison states that the ovule and epimatium are borne on the upper extremity, and appear to be the prolongation and termination of a mass of tissue differentiated from that of the cone scale. This may be so, but Tison's figures, cited to support this statement, do not in my opinion confirm it. This tissue is that undergoing rapid intercalary growth, as a result of which the ovule is carried further away from the axis, and becomes completely reversed, with the micropyle facing the axis (Text-fig. 8, *d*). As Tison remarks, the ovule in the very young cone is probably borne erect at the base of the scale, and it is by the continued intercalary growth at the base of the scale that the ovule comes to occupy a position some distance from the axis. Thus in the stage described and figured by Norén, the ovule is about one-eighth the length of the scale from the axis; in that described by Tison it is not less than one-fifth the length of the scale away, while at the time of pollination it may be as much as, or more than, one-third of the length of the cone scale from the axis. The later appearance of the cone has been described by Lindley[3] and Pilger.[4] The cone scales appear to fuse into an irregularly globose fleshy mass about a centimetre long; the scales are fused together at their base, but the acute tips remain free. In this fleshy mass are included the seeds, to about the number of six, so that a large number of ovules evidently fail to develop.

The structure of the ovule itself has been most minutely described by Norén. Tison's later description is almost identical with Norén's. The nucellus for the great part of its length is free from the integument. It is not circular in transverse section, but elliptical, the longer diameter being the one transverse to the cone scale.

Before the time of pollination the nucellus grows at a greater rate than the integument and epimatium, so that by the time pollination takes place it not only completely fills the micropyle, but projects beyond it so as to form a large stigmatic surface.[5] Norén has described the peculiar mode of pollination in this species. Pollen, besides falling on the nucellus itself, may get lodged in the cavity round the ovule, in which case the pollen-tube may grow over the tissues of the integument and epimatium into the micropyle. Besides the interest attaching to such a peculiar mode of pollination,

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[1] Norén ('08), p. 109.

[2] Tison ('09), p. 143.

[3] Lindley ('51).

[4] Pilger ('03), p. 42.

[5] Tison ('08), p. 137; Norén ('08), p. 110, Figs. 19 and 20; Stiles ('08), p. 214, Fig. 31; Tison ('09), p. 143, Figs. 8 and 9.

is the greater one of its being essentially similar to the condition found by Thomson[1] in *Agathis*. As pointed out by the writer[2] and by Tison,[3] this nucellar expansion is similar to that occurring in *Araucaria*, but is much more prominent. Tison[4] reports that the nucellus is covered with a sticky secretion which no doubt keeps the pollen-grains attached to the nucellus during their germination. He finds that when the pollen is shed the sporophylls of the female cones on the same plant have already closed up.

A rather remarkable phenomenon is the occurrence of cells towards the apex of the nucellus which have thickening bands on their walls and a somewhat tracheidal appearance.[5] Their function is obscure, but they may serve as water-storage reservoirs.

After pollination the stigmatic expansion continues to grow for about two months.[6] Norén figures an ovule a year after pollination. The extruded part of the nucellus is still conspicuous at this late stage ; fertilization had not taken place in the ovule.

The megaspore-mother-cell arises deep in the nucellus, at the level at which the nucellus and integument become free from one another. It is surrounded by ‘spongy’ tissue.[7]

Accounts of the vascular anatomy of the female fructification have been published by Thomson, Tison, and the writer.[8] The accounts agree in the main, but those of Tison and the writer differ in details.

The axis of the cone contains a ring of endarch collateral vascular bundles with a resin canal outside each in the cortex (Pl. XLVI, Fig. 7). Thomson found near the base of the cone ‘a few centripetal elements associated with the bundles at the sides of the gap left by the exit of the megasporophyll trace’. Similar elements were found, but rarely, in the upper part of the peduncle.[9]

A single bundle leaves the axis of the cone to supply the sporophyll. This bundle arises by the division of one of the bundles of the cone axis, so that the two bundles lie side by side in the ring. A little higher up the resin canal divides similarly. The sporophyll trace and its accompanying resin canal then bend upwards and outwards into the sporophyll. In the young condition observed by Thomson, the ovular supply is given off from the upper surface of the sporophyll bundle at the level of insertion of the integument. After the separation of the ovular supply from the sporophyll bundle, centripetal xylem-elements appear in connexion with the latter.

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[1] Thomson ('07), p. 271.

[2] Stiles ('08), p. 214.

[3] Tison ('09), p. 145.

[4] Tison ('08), p. 137 ; ('09), p. 144.

[5] Norén ('08), p. 112, Fig. 16 ; Stiles ('08), p. 214.

[6] Tison, l. c.

[7] Norén ('08), p. 114.

[8] Stiles ('08), p. 215 ; Thomson ('09²), p. 346 ; Tison ('09), p. 147.

[9] Thomson ('09²), p. 348.

Later, a more complicated, and, in the lower part of the cone scale, a very variable, vascular structure occurs. Generally, the ovular supply given off from the upper surface of the sporophyll consists of about two bundles, although the number is not constant,[1] with inverse orientation of xylem and phloem as compared with the sporophyll. At the same time the sporophyll bundle divides into a row consisting of a variable number of bundles; sometimes there are three, in other cases as many as five. The central bundle often divides partially, inasmuch as although the xylem separates into several groups, the phloems of the corresponding xylem groups remain joined laterally. This state of affairs was described formerly as the sporophyll bundle dividing *more or less* into a row of bundles,[2] a description which M. Tison has evidently misunderstood.[3] The bundles on each side of the central one now bend round through two right angles so as to lie in the same line with the ovular supply given off from the upper surface of the sporophyll bundle; the end bundles of the row, however, gradually die out, often moving further away from the central bundles; these latter, generally to the number of three or four, bend round to the ovule, and fusing into two bundles just below it, enter the base of the nucellus and there terminate.[4] The xylem of these bundles is almost all centripetal in its development. No resin canals accompany the ovular supply. Higher up the scale the sporophyll bundle appears more compact, the xylem masses having reunited. Centripetal xylem makes its appearance in the upper part of the scale in great quantity. The elements are short and wide and of irregular shapes, and have the appearance of transfusion tracheides.[5] Near the tip of one cone scale the single resin canal accompanying the sporophyll bundle had divided into three bundles lying side by side. This is interesting when it is remembered that one type of leaf in the genus *Podocarpus* contains three resin canals underneath the vascular bundle.

IV. *Microcachrys.* The ovoid-globose cones of this genus are terminal on the branches and consist of about twenty sporophylls which are borne in alternating whorls of four; the uppermost are always sterile. These sporophylls are tetragonal in shape, and each fertile scale bears a single ovule in a median position on the upper surface. As in *Saxegothaea*, the ovule is completely surrounded by an integument, partially surrounding which is an epimatium.

The youngest material examined was in the megaspore-mother-cell

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[1] Tison ('09) gives cases in which this supply bundle is absent altogether, but such have not been observed by the writer.

[2] Stiles ('08), p. 216.

[3] Tison ('09), p. 150.

[4] Bertrand ('78), in a paper on ovular integuments, gives a figure of the ovule in which a vascular bundle, presumably the ovular supply, extends through the greater length of the epimatium. This representation is, however, without foundation in fact.

[5] Stiles ('08), p. 216, Figs. 33, 34.

stage. At this time the ovule is inserted nearer the tip of the scale than the cone axis, being about three-fifths of the length of the scale from the cone axis. It is erect at this time, the micropyle facing directly upwards (Pl. XLVI, Fig. 9; Text-fig. 8, *e*). As in *Saxegothaea*, the integument and epimatium are in close connexion, and at this stage are only free from one another for about their upper half. The integument and epimatium both project a good way beyond the top of the nucellus. The integument is much longer on the side towards the cone axis and remote from the epimatium than on the other, and in every ovule examined the upper part of it had bent round through more than a right angle, so that it was actually pointing towards the scale on this side (Pl. XLVI, Fig. 9).

The single megaspore-mother-cell is seated in about the middle of the nucellus, there being about seven layers of cells above it in median section. The cells surrounding it are not much differentiated from the outer cells of the nucellus at this stage.

At a later stage examined by Thomson,[1] the ovule has become quite reversed so that the micropyle faces the cone axis.

The writer has examined a still later stage collected on mountain tops in Tasmania. The cone scales become swollen and fleshy and of a fine red colour; they retain their individuality, not fusing together partially as in *Saxegothaea*. The seed is very small, being from 2 to 3 millimetres long and a little less in width; the lower side is flat, while the upper is convex; the seed thus has the shape of a somewhat flattened tetrahedron. The seed-coat differentiates into an outer thin membranous layer and an inner, thicker, hard sclerenchymatous layer.

The vascular structure of the cone axis consists of a ring of bundles from which the sporophyll bundles arise by division as in *Saxegothaea*. As in the vegetative stems of this plant there are no resin canals, so there are none in the cone axis. Worsdell[2] has described the vascular system of this cone scale as consisting of two distinct bundles very close together: a lower one serving the sporophyll, and an upper one serving the ovule. According to Thomson[3] a single bundle leaves the cone axis and gives off a single supply bundle from its upper surface; the orientation of the xylem and phloem of the latter is inverse as compared with that of the sporophyll bundle. In the old scales I have examined, a single bundle leaves the axis of the cone, and the ovular supply appears on its upper surface very shortly after the entrance of the sporophyll supply into the sporophyll. It lies so close to the latter, however, even up to the insertion of the ovule, that ovular and sporophyll supply might be considered one bundle. Ultimately, the ovular supply bends obliquely upwards and bifurcates near the base of the ovule, the divisions entering the base of the integument. A large resin duct, or

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[1] Thomson ('09^2), p. 349.

[2] Worsdell ('99), p. 538.

[3] Thomson ('09^2), p. 349.

rather resin gland, for it ends blindly in both directions, is present on the under side of the sporophyll bundle.

V. *Dacrydium.*

i. *D. Franklini.* The megasporangiate strobili of this plant are terminal on the branches bearing them, and consist each of a lax spike of eight or nine megasporophylls separated by conspicuous internodes.[1] Each sporophyll is a small structure, in the stage examined about 1·5 mm. long and about 1 mm. wide, and is somewhat triangular in shape, and bears a single ovule medianly placed on the upper surface. Strasburger,[2] who has recorded some observations on this species, states that the ovule arises in the axil of the sporophyll. In the material examined by the writer the ovule was towards the tip of the sporophyll, much as in *Microcachrys.* In another matter the sporophyll is very reminiscent of that of the latter genus, for it contains a resin cavity which ends blindly in both directions, resin canals being absent in the cone axis as in the vegetative stem.

The ovule in the stage examined was about half-reversed; that is, its axis made an angle of about half a right angle with the cone scale, the micropyle pointing inwards. As in *Saxegothaea* and *Microcachrys* the nucellus is free from the integument for most of its length, and the epimatium only partially surrounds the integument just as in those genera. It certainly does not completely surround the integument, as Tison asserts to be the case in *Dacrydium.*[3] The tip of the sporophyll curves up into a point just behind the epimatium, and gives much the appearance of a third integument, and in this respect again it is not unlike *Microcachrys.*

The axis of the fructification contains a ring of five or six small bundles, from which those of the sporophylls are given off. At about the level of the extreme base of the slightly decurrent sporophyll a resin duct appears on the phloem side of the bundle nearest the sporophyll. A very little higher up this bundle divides into three, the two lateral ones being smaller. The central bundle first passes out into the sporophyll accompanied on its under side by a resin canal, and a very little higher up the two lateral ones pass out also. These form the ovular supply and have their xylem and phloem with inverse orientation, as in *Saxegothaea* and *Microcachrys.* As in these genera also they are not accompanied by resin ducts (Pl. XLVII, Fig. 22). The two ovular supply bundles terminate below the nucellus. At the stages examined the xylem of the sporophylls is very little lignified. It is noteworthy that the ovular supply bundles attach themselves laterally to the sporophyll bundle at the extreme base of the sporophyll, much as is the case with the lateral bundles of the ovular

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[1] For figures see Parlatore ('64), Tav. II, Figs. 40–54.

[2] Strasburger ('79), p. 76.

[3] Tison ('09), p. 147.

supply of *Saxegothaea*, where, however, the insertion is higher up the scale. Occasionally, in *D. Franklini*, one of the ovular supply bundles attaches itself to a bundle of the cone axis other than its own sporophyll bundle.

ii. *D. cupressinum.* In this species the fertile branch terminates in a few spirally arranged scales, the uppermost of which bears a single ovule. A case was observed in which there were two fertile sporophylls on the branch. The scales differ but little from the foliage leaves ; they are somewhat shorter and are lanceolate rather than linear, the transition from the linear foliage leaf to the lanceolate fertile sporophyll being gradual.

On the upper surface of the sporophyll and near its base is borne an outgrowth which arches over the ovule in the form of a hood (Text-fig. 8, *f*). This is the epimatium, and the solitary ovule is borne on this and not on the scale itself as in *Dacrydium Franklini.* Owing to lack of material the position of the ovule in the very young state cannot be recorded.

The ovule itself is rather remarkable. The nucellus is a conical structure, and is completely surrounded by a very remarkable inner integument. This consists of four distinct layers: a single outer layer of sclerenchymatous cells forming a kind of epidermis; inside this a layer of anticlinally elongated cells filled with a brown tannin-like substance; a mass of very thick-walled cells inside this, which diminishes regularly in thickness from the micropyle towards the chalaza; and, finally, on the nucellus side there is a region of parenchymatous cells, which increases in thickness as the sclerenchymatous layer diminishes. The micropyle in the stage examined consisted of a very narrow tube, but this was after pollination.

The epimatium is a much larger structure than in the species already described, and, as has been already indicated, bears the ovule on its upper surface, the latter being inserted a short distance above the junction of megasporophyll and epimatium. The latter, like the inner integument, contains a good deal of sclerenchyma. As in *Saxegothaea*, *Microcachrys*, and *Dacrydium Franklini*, it only partially encloses the ovule. It is, however, not pressed closely against the inner integument as in those species, and in a longitudinal section through the ‘strobilus’ reminds one of the ovuliferous scale of *Pinus* rather than of a Podocarpean epimatium. There can, however, be no doubt of its homology with the latter.

The vascular system of the cone scale and axis in *D. cupressinum* is similar to that of *D. Franklini.* The axis below the insertion of the fertile sporophyll contains a small ring of about three vascular bundles, each with a resin canal on its outer side. One bundle of the ring divides into three bundles, the lateral ones bending round so as to lie side by side on the inside of the median bundle. Each of these bundles is provided with a resin canal. The group of three passes into the sporophyll, and the two upper ones then pass into the epimatium and terminate at the base of the ovule.

VI. *Podocarpus.*

i. *Eupodocarpus.*[1] The early development of the female fructification has been traced by Strasburger[2] in the case of *Podocarpus chinensis*, Wall. (*P. macrophyllus*, subsp. *maki*, Sieb.). The ovulate structure is described as arising in the axil of the sporophyll, the integument as growing up round the nucellus, while the horseshoe-shaped aril arises later.

Some young fructifications of *P. latifolius* collected by Mr. Saxton yielded the youngest stages of my material. The ovular stalk is short and the ovule at its apex is almost perpendicular to the megasporophyll, and is not reversed. The integument and epimatium are only free at the tip (Pl. XLVI, Fig. 11, and Text-fig. 8, *g*). In this species the ovular stalk never elongates very much ; by the time the reduction division takes place the ovule is, however, completely inverted (Text-fig. 8, *h*, *k*, and cf. with *l*). Later the bases of the four 'cone' scales become fused together into one fleshy mass.

The ovulate structure of *Podocarpus macrophyllus* is like that of *P. latifolius* in general appearance, but two fertile megasporophylls are perhaps of more frequent occurrence than in that species. The fleshy receptacle is formed in this species as in the last (Pl. XLVI, Fig. 10). The seed-coat is differentiated into two layers ; a harder and thicker outer layer which, however, cannot be described as woody, and a softer inner layer which cannot be aptly described as either fleshy or membranous.

The vascular structure of the female fructification has already been described by Mr. Brooks and the writer[3] in the case of *Podocarpus spinulosus*, (Sm.) R. Br., *P. alpinus*, R. Br., and a third species. These observations have now been supplemented by further ones on *P. macrophyllus* and *P. latifolius.*

In *P. macrophyllus*, as in species previously examined, a single bundle supplies the megasporophyll, while the ovular supply below the point at which the stalk becomes free from the sporophyll consists of two vascular bundles, the xylem and phloem of which are inversely orientated as compared with the xylem and phloem of the sporophyll bundle. These two bundles, by division of one or both, give rise to a row of three or four small bundles (Text-fig. 4, *a–d*). They are accompanied by a row of small canals, about one canal to each bundle, though this relation is not strictly maintained. Towards the chalazal end of the ovule, if the supply bundles number four, one of the bundles at the end of the row may die out, or bend round the ovule in a tangential direction and descend towards the micropyle.

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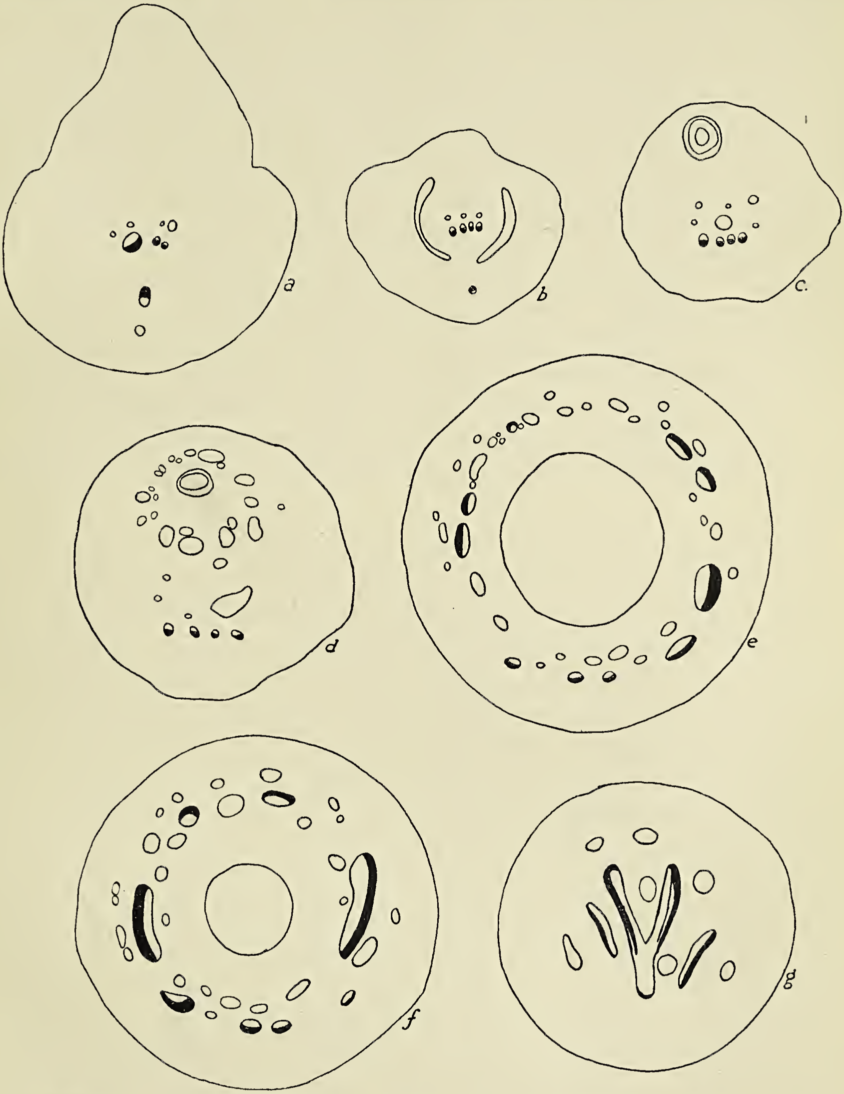
[1] For figures of the female fructifications of *Podocarpus chilinus*, *P. elongatus*, and *P. coriaceus*, see Richard ('26), Pl. I ; and for photographs of these structures in *P. elatus* and *P. spinulosus*, see Baker and Smith ('10), pp. 432, 444.

[2] Strasburger ('79), p. 77.

[3] Brooks and Stiles ('10), p. 311.

Thus at the chalazal end there are only three of the supply bundles present. Of these the median bundle bends over the top of the ovule, and in so doing divides into two (Text-fig. 4, *g*), while the lateral bundles bend round through a right angle (Text-fig. 4, *f*). All these bundles descend towards the micropyle,



TEXT-FIG. 4. *a–g*. Diagrams of a series of transverse sections through the ovule of *Podocarpus macrophyllus*. × 18.

and in so doing any single bundle may divide (Text-fig. 4, *e*, *f*). They die out about half-way between the chalaza and the micropyle.

A conspicuous feature in the ovule of *P. macrophyllus* is the presence of a large number of canals in the ovule apparently unrelated to the vascular supply. These canals form roughly two rings concentric with the micropyle and nucellus (Text-figs. 4, *d–f*).

The vascular supply of the ovule in *P. macrophyllus* is very similar to that of *P. spinulosus*,[1] the chief difference being in the much greater distance the bundles descend towards the micropyle in *P. macrophyllus*.

The vascular supply of the ovulate structure of *P. latifolius* is simpler than that of *P. macrophyllus*. The sporophyll bundle is accompanied by a great deal of transfusion tissue, and in the upper part of its course consists entirely of transfusion elements (Pl. XLVII, Fig. 23). The ovular supply at the base of the stalk consists of two bundles as in other species, but these bundles do not divide further until the chalaza is reached (Text-fig. 5, *a–c*). Here each bundle divides into two, and the outer ones bend round through about a right angle and descend a very short way towards the micropyle (Text-figs. 5, *c* and *d*). The inner bundles proceed further towards the top of the ovule, where they ultimately meet and end in a mass of transfusion tracheides. A small bundle descends from this mass a short way (Text-fig. 5, *e*), but does not reach the level at which the ascending bundles fork. As in *P. macrophyllus*, there is here a large development of canals concentric with the micropyle and nucellus, but two distinct rings cannot always be made out.

It is clear that there is a good deal of variation in the vascular structure of the ovule throughout the section *Eupodocarpus*, and probably an examination of a large number of cases would reveal the presence of a certain amount of variation among the individuals of one species. The essential facts seem clear; the ovular supply consists of two bundles with inverse orientation as regards the sporophyll bundle. These two bundles sometimes divide between the base of the ovular stalk and chalaza, and nearly always in the region of the latter, into a more or less complicated system. This division has probably arisen owing to the needs of a well-developed conducting system for the development of the large ovules and seeds. It has already been suggested that the same cause is responsible for the complete independence of the vascular supply of the ovule from that of the sporophyll.[2]

Having regard to the state of affairs in *Dacrydium cupressinum*, where the ovule is actually borne on the epimatium, I feel disposed to consider the ovular stalk as a further development of that structure. In *Podocarpus*, therefore, the ovule has been carried up a long way on to the epimatium, but the latter is almost completely fused with the integument, a point in which it differs markedly from *Dacrydium cupressinum*.

The vascular system of the ovule of *Podocarpus* is therefore to be regarded as a further development from that of the more primitive Podocarpeae. In a previous paper Mr. Brooks and the writer[3] stated that there was

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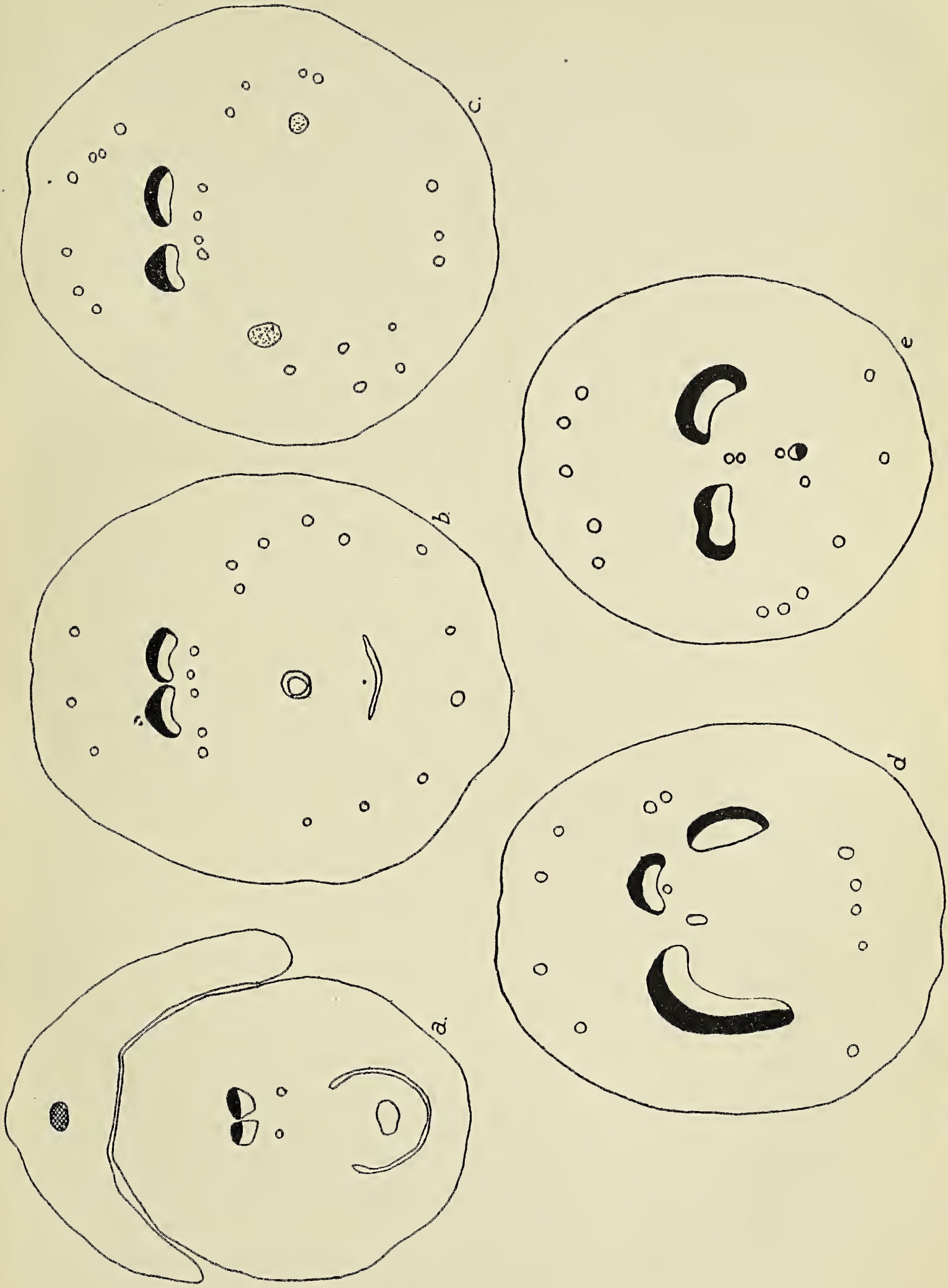
[1] Brooks and Stiles ('10), Pl. XXI, Figs. 15–18.

[2] l. c., p. 314.

[3] l. c., p. 315.



TEXT-FIG. 5. *a–e.* Diagrams of a series of transverse sections through the ovule of *Podocarpus latifolius.*

not sufficient data to decide to what extent the ovular supply in *Podocarpus* was to be homologized with that of other Gymnosperms.[1] If the origin of the ovular vascular system of *Podocarpus* suggested above is correct, it is obvious that the presence of a well-developed vascular system in the ovules of that genus is to be regarded as a fresh development, and is not homologous with that in any older Gymnosperms.

ii. *Nageia.* In *Podocarpus nagi* the fertile branches arise in the axils of ordinary foliage leaves (Pl. XLVII, Fig. 24). Each branch is about 1·5 cm. long and bears about six pairs of decussately arranged scales; one of the uppermost pair bears the solitary ovule. The stalk is short as compared with this structure in some species of *Eupodocarpus*, and is fused at its base with the megasporophyll. The general appearance is that of an ovule terminating the shoot (Pl. XLVII, Fig. 24). The ovule is large compared with that of *P. macrophyllus*; thus ovules collected on July 13, when wall formation had just commenced in the prothallus, were a centimetre long. At this time they are somewhat pear-shaped, the greatest transverse diameter being towards the upper or chalazal end of the ovule. Later, as the ovule becomes the seed, the latter becomes more spherical in shape (Fig. 24), and in material collected on October 5, which contained embryos with well differentiated cotyledons and radicle, the seed is a sphere about 1·5 cm. in diameter.

As in other species, there are two integuments which are only free from one another for an extremely short distance at the micropylar end. Owing to the extent to which fusion of parts has taken place, it is not possible to define the limits of stalk and epimatium. In all the ovules I have seen there is on the side of the ovule remote from the stalk, and about three times as far from the micropylar end as from the chalazal end, a small projecting point which suggests the termination of a leaf or scale. Occasionally, there is more than one such point, as in the case figured in Pl. XLVII, Fig. 24. There is, however, nothing in the internal structure to suggest a meaning for this, nor is there any suggestion of a difference between the tissues of the stalk and epimatium; in the youngest stage examined the tissues of epimatium and stalk are quite similar: this is at about the time of wall formation in the prothallus. The integument and epimatium are at this time quite differentiated from one another, the cells of the epimatium being thicker walled with smaller nuclei, while the integument consists of smaller thinner walled cells with relatively larger nuclei. In the epimatium are numerous canals, as in the species of *Eupodocarpus* examined; these occur in all parts of the epimatium, but are most numerous around its inner limit (Text-fig. 6).

As the ovule develops into the seed both integuments become differentiated into two layers, so that microscopically four layers can be recognized in the seed-coat; a naked-eye examination, however, only

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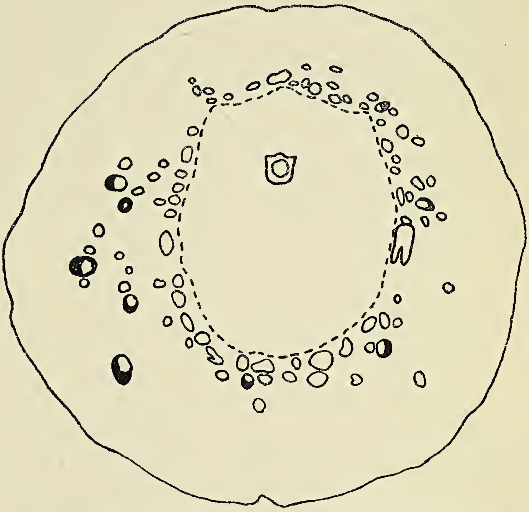
[1] Oliver ('03); Stopes ('04); Arber, A. ('10); Oliver and Salisbury ('11).

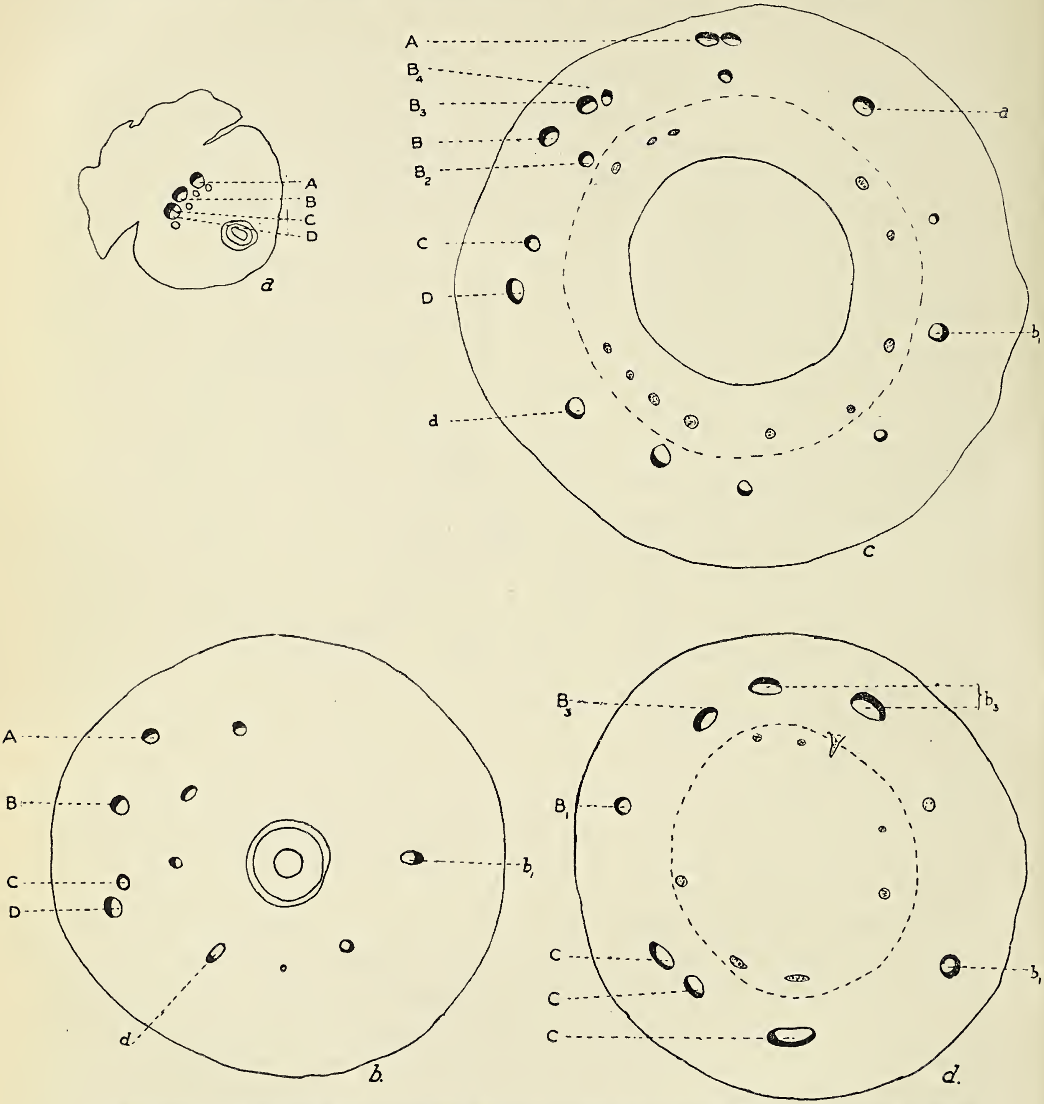
reveals two layers, the outer soft semi-fleshy layer (formed from the epimatium) and the inner woody layer (formed from the integument). By the time the seed contains a young embryo the outer layers of the integument are already becoming thickened and lignified, and are clearly differentiated from the epimatium. The inner layers remain thin and parenchymatous. The thickening and lignification appear to commence at the micropylar end, the chalazal region being the last to become lignified.

The vascular system of the ovulate structure of *Podocarpus nagi* appears to be more complicated than that previously described for the ovule of any Conifer. The sporophyll contains a row of four or five bundles, and is thus parallel veined like the foliage leaves. The ovular supply at the base of the ovular stalk consists of a row of three or four bundles (Text-fig. 7, *a*) having their xylem and phloem orientated inversely as compared with the sporophyll bundles. These bundles ascend towards the chalaza on the sporophyll side of the ovule, although they may sometimes be laterally displaced. As in *P. macrophyllus*, one of the two middle bundles if there are four present, or the median one if there are three, ascends to the chalaza, and bending over the top of the ovule descends on the other side towards the micropyle. As in *P. macrophyllus* and *P. spinulosus*, this bundle divides into two after passing over the top of the ovule, although one of the two may soon die out. However, whereas in the species of *Eupodocarpus* cited the median ascending bundle does not divide between the stalk and the chalaza, in *P. nagi* it may do so. Thus in Text-fig. 7, *c*, the bundles marked B_2, B_3, B_4, have all arisen by division of B. The bundles lateral to the main bundle which passes over the chalazal end of the ovule bend round the side of the ovule as in *P. macrophyllus*, and descend towards the micropyle. Thus in the series shown the bundles A, B_3, C, and D, bend round at various levels and descend towards the micropyle; in this part of their course they are marked *a*, b_3, *c*, and *d* (Text-fig. 7, *c*, *d*). As in *P. macrophyllus*, these descending bundles may divide. They descend almost to the micropylar region (Text-fig. 7, *b*), and in this respect differ



TEXT-FIG. 6. Diagrammatic representation of a transverse section through an ovule of *Podocarpus nagi*, showing the distribution of canals. The dotted line marks the outer limit of the integument. × 8.

from species of *Eupodocarpus* so far examined. In the outer layers of the integument in the young ovules there is a ring of what appear to be undifferentiated vascular bundles connected with the vascular bundles of the epimatium by means of undifferentiated or only partly differentiated strands.[1] Later these strands become more noticeable, until in the old seed



TEXT-FIG. 7. Diagrams of transverse sections through an ovule of *Podocarpus nagi* to show the distribution of vascular tissue. *a*, Near the micropylar end. *b*, A little higher up. *c*, Through the middle of the ovule. *d*, Towards the chalazal end. A–D, ascending vascular bundles. *a–d*, descending vascular bundles.

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[1] Compare the state of affairs in *Torreya*, where the supply of the inner integument is similarly undifferentiated (Oliver ('03), p. 469). On general grounds, however, it does not seem possible to connect the structure of the Torreyan with that of the Nageian ovule.

they stand out distinctly on the outside of the woody integument after removal of the softer epimatium.

The ovule of *Podocarpus nagi* has thus an exceedingly complicated structure, both as regards the differentiation of the seed-coat into four layers, and as regards the vascular system. Everything goes to show that this is a derived and not a primitive condition. The vascular system is extremely unlike that in the ovule of any other genus of Gymnosperms, but can be compared with that of *Podocarpus macrophyllus*. It is more complicated than in that species, but the ground-plan is the same, and the further development is no doubt connected with the large size of the seed. Further evidence in support of the condition being a derived one is found in the greater extent of fusion between the parts; here the integument and epimatium are scarcely free from one another, while the megasporophyll is fused for part of its length with the ovular stalk.

It is possible that this is the species vaguely described by Favre[1] under the name of *P. chinensis*. It has been suggested that this species might throw light on the homology of the vascular system of the ovule of *Podocarpus* with that of other Gymnospermous ovules.[2] Whether this be that species or not it furnishes additional evidence to that already advanced in the course of this section of the present paper, that the vascular system of the ovule of *Podocarpus* is not homologous with that of the ovules of the older Gymnosperms described by various writers. It would seem that the ovular structure in the Podocarpeae has been evolved within the group, and it is in the genus which on general grounds one would consider the least primitive that one finds the most complex development.

ix. FEMALE GAMETOPHYTE.

It is with regard to the female gametophyte and embryology that the greatest gap in our knowledge of the Podocarpeae occurs. Of the female gametophyte of *Pherosphaera* we know nothing; in the case of *Saxegothaea*, *Microcachrys*, and *Dacrydium* our knowledge is limited to a few isolated observations on the megaspore membrane.[3] In *Phyllocladus* and *Podocarpus* a general outline of the gametophytic history is known in one species of each genus (*Ph. sp.* and *P. coriaceus*). Owing to the kindness of Dr. M. C. Stopes and Mr. Saxton I have been enabled to examine gametophytic material of two species of the *Eupodocarpus* section of *Podocarpus* (*P. macrophyllus* and *P. latifolius*), and one species of the section *Nageia* (*P. nagi*). The earliest stages were found in ovules of *P. latifolius*. The megaspore increases in size, and its nucleus divides, so that the free nuclei

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[1] Favre ('65), p. 379.

[2] Brooks and Stiles ('10), p. 315.

[3] Since this paper was written the writer has published a few facts relating to the Gametophytes of *Dacrydium*. See Stiles ('11).

formed by successive divisions come to lie in the parietal layer of cytoplasm surrounding the central vacuole.

During the development of the young prothallus the layers of tapetal cells come to be more sharply marked off from the outer cells of the nucellus. Wall formation was not observed, but from the disposition of the cells before the appearance of the archegonia, there can be no doubt that the ingrowing primary prothallial cells are formed as in other Conifers. Up to the time of formation of the archegonia the cells of the prothallus are all uninucleate; no sign of any binucleate condition was observed such as Lawson[1] found in *Cryptomeria.*

The archegonia arise as superficial cells at the apical end of the prothallus. Coker found that these numbered from five to ten in *P. coriaceus*; there is a similar number in the case of *P. macrophyllus*, but in *P. latifolius,* in the only case examined, there were as many as fourteen archegonia, while in each of the two ovules of *P. nagi* which contained archegonia the number was nineteen. Each archegonium is surrounded by a well-defined layer of jacket cells; these are conspicuous on account of their denser cytoplasm and larger nuclei. Occasionally, in *P. macrophyllus*, two archegonia occur within a common jacket, a fact of interest in comparison with *Phyllocladus,* where the same phenomenon has been observed. In *P. nagi*, to judge from the two cases examined, the phenomenon is more marked, as many as three archegonia being surrounded by a common jacket. The distribution of the archegonia in *Podocarpus* is thus like that in the Abietineae and Taxeae, but shows an approach to the state found in *Cunninghamia*,[3] where the archegonia form a ring all enclosed in a common jacket, and surrounding a central mass of sterile tissue.

Coker has observed a ventral-canal-nucleus in *P. coriaceus.*[4] In *P. macrophyllus* a well-defined ventral-canal-nucleus was not seen, but a safranin-staining mass present in the upper part of some archegonia may have been the disorganizing ventral-canal-nucleus. There is no evidence of this nucleus being cut off by a wall.

In *P. coriaceus*[5] the neck consists of from two to twenty-five cells. Although such an extreme variation was not noticed in *P. macrophyllus*, yet the variation in number is certainly considerable in the mature archegonia, and as many as eighteen cells have been counted in the neck of one archegonium.[6]

Before fertilization the egg nucleus undoubtedly undergoes rapid increase in size. Unfortunately, measurements are not available for the young and old egg nucleus in the same species. In *P. latifolius* the diameter of the nucleus of the young archegonium is about $25\,\mu$, while in

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[1] Lawson ('04^2), p. 427.
[2] Young ('07), p. 87.
[3] Miyake ('11), p. 11.
[4] Coker ('02), p. 98.
[5] l. c.
[6] The structure of the archegonium neck would thus appear to differ markedly from that of *Phyllocladus* and *Dacrydium*. See Young ('10) and Stiles ('11).

P. macrophyllus the egg just before fertilization (Pl. XLVIII, Fig. 31) is about 45 μ or 50 μ in diameter.

Before fertilization takes place the nuclei of the prothallus cells begin to divide, but the products of division are not cut off by cell-walls, and as the process may be and generally is repeated more than once, the prothallus cells in the old seeds may contain as many as eight nuclei. This is evidently a phenomenon of general occurrence in the genus, as it takes place in all species that have so far been examined, namely, *P. coriaceus* by Coker, and *P. nagi*, *P. macrophyllus*, *P. elatus*, and *P. latifolius*, by the writer.

The development of more than one megaspore in an ovule is a phenomenon that has been observed in several Coniferous genera;[1] it has been recorded for *Podocarpus* by Coker in *P. coriaceus*.[2] In both *P. latifolius* and *P. macrophyllus* an example of this has been found among the writer's material; in the former case the prothalli were in a young stage before wall formation had commenced, but in the case observed in *P. macrophyllus* both prothalli had produced archegonia, and one contained a pro-embryo.

In *Phyllocladus*,[3] *Saxegothaea*,[4] *Microcachrys*,[5] and *Dacrydium laxifolium*[6] a thick megaspore membrane of the usual type is developed, but in the two species of *Podocarpus* examined by Thomson, *P. coriaceus* and *P. macrophyllus*, subsp. *maki* (*P. Makoyi*, *P. chinensis*, or *P. sinensis*), no trace of a megaspore coat could be found. From the species examined in the course of this work it is evident there is great variation in the genus as regards this structure. In *P. macrophyllus* and *P. nagi* the membrane is very poorly developed, and never appears to be more than 1 μ thick. Only an isolated late stage has been observed in the case of *P. elatus*, and here also the membrane was very poorly developed. *P. latifolius*, on the other hand, is in marked contrast to these species. At about the 32-nucleate stage of the prothallus the megaspore membrane is well under 1 μ thick, while by the time the neck-cells are cut off from the central cells of the archegonia the membrane forms a thick coat about 4·2 μ thick. It thins out towards the apical part of the prothallus, and is not more than 2 μ thick in the region of the archegonia. By the time the pro-embryos are developing the membrane becomes thinner, and is then about 2·5 μ thick in the middle region of the prothallus.

x. MALE GAMETOPHYTE.

A. **Early History (Structure of Pollen).**

The early history of the male gametophyte, from the microspore stage up to the time of shedding of the pollen, has been investigated in species of all genera of the Podocarpeae except *Pherosphaera*. The later history from

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[1] e. g. *Sequoia* (Lawson, '04^1, p. 10); *Callitris* (Saxton, '10, p. 559).

[2] Coker ('02), p. 97.

[3] Young ('10), pp. 86, 88.

[4] Norén ('08), p. 115.

[5] Thomson ('09^2), p. 349.

[6] Thomson ('05^1).

the beginning of pollen-tube development up to fertilization has only been observed in two cases: in *Podocarpus coriaceus*[1] and *Phyllocladus* sp.[2]

A most excellent general account of the early stages of development has already been published by Coulter and Chamberlain,[3] and as the additional species examined by the writer behave similarly in this respect to those already observed, only a brief account of this part of the subject is necessary.

As regards gross structure, the pollen-grains of *Saxegothaea*[4] are wingless; those of *Microcachrys* possess wings which are, however, small and variable in size and number, three being the most frequent number met with. In *Phyllocladus*, *Dacrydium*, and *Podocarpus* the wings are on the whole larger and are two in number, although three wings are found exceptionally in *Podocarpus ferruginea*,[5] and normally in *Podocarpus dacrydioides*.[6]

The feature of the gametophyte which has attracted most attention is the comparatively large development of prothallial tissue, a feature first noticed by Thibout[7] in *Podocarpus polystachyus*. In all the species so far investigated two prothallial cells are cut off successively, as in the Abietineae. In *Saxegothaea*[8] the second prothallial cell always divides into two daughter-cells, and the first often divides as well. This prothallial complex of three or four cells persists up to the time of pollination, which is as far as the development of the male gametophyte has been observed. An exactly similar condition probably obtains in *Microcachrys*.[9] An essentially similar condition has been found in *Dacrydium Bidwillii*, where, as a rule, only the second prothallial cell divides, and in *D. cupressinum* and *D. laxifolium*, where generally both prothallial cells divide.[10] A less active condition prevails in *Phyllocladus*,[11] where usually neither cell divides, although the second occasionally does so.

In *Podocarpus* there is apparently a more variable condition. In *P. coriaceus*, Coker[12] found that the primary prothallial cells might divide amitotically. Jeffrey and Chrysler[13] found in *P. polystachyus*, *P. ferrugineus*, and *P. dacrydioides* that both the prothallial cells might undergo two successive divisions, making a total of eight prothallial cells. Burlingame[14] recorded a similar condition in *P. Totara*, var. *Hallii*, but reported a great deal of variation in the number of prothallial cells produced, namely, from one to eight, six being the most usual number. Mr. Brooks and the writer have found that as many as eight prothallial cells may be produced in *P. spinulosus*.[15] As many as eight prothallial cells are also produced in *P. andinus*.

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[1] Coker ('02), p. 94.
[2] Young ('10), p. 82.
[3] Coulter and Chamberlain ('10), p. 339.
[4] Norén ('08), p. 105; Stiles ('08), p. 213.
[5] Thomson ('09¹), p. 29.
[6] Thibout ('96), Pl. XIV, Figs. 10 and 11.
[7] l. c., Figs. 8 and 9.
[8] Norén ('08), p. 106.
[9] Thomson ('09¹), p. 28.
[10] Young ('07), p. 192.
[11] Young ('10), p. 82.
[12] Coker ('02), p. 92.
[13] Jeffrey and Chrysler ('07).
[14] Burlingame ('08), p. 169.
[15] Brooks and Stiles ('10), p. 309.

After the cutting off of the two primary prothallial cells, the antheridial cell divides into the tube-cell and the generative cell. The latter is cut off from the former in much the same way as the primary prothallial cells. This division is followed by the division of the generative cell into what may be called the stalk-cell and the body-cell, but the division in all the Podocarpeae is an anticlinal one, so that the products of division lie side by side. Jeffrey and Chrysler have described in *Podocarpus ferrugineus* and *P. polystachyus*[1] an appearance as of a second derivative of the generative cell besides the so-called stalk-cell, and a similar appearance was noted by Mr. Brooks and the writer in *P. spinulosus*.[2] Miss Young has suggested[3] that this appearance is due to the partial encircling of the body-cell by the stalk-cell, and this may well be so in *P. spinulosus*.

In all the Podocarpeae the limiting membranes of the prothallial cells and stalk-cell break down, and the nuclei come to lie free in the general cytoplasm. Thus, at the time the grain is shed the body-cell is the only one surrounded by a definite membrane. In *Podocarpus spinulosus* it was observed that at this stage the tube-nucleus is the largest in the grain, and the same is true of *P. andinus* and *P. latifolius*. In no instance in *P. andinus* were more than eleven nuclei observed in one pollen-grain, so there is presumably no second derivative of the generative nucleus in this species.

B. **Later History (Development of Pollen-tube).**

The development of the gametophyte beyond the stage just described has been observed in very few cases. Miss Young[4] has observed stages in *Dacrydium laxifolium* and *D. intermedium* in which the pollen-tubes have commenced to grow through the nucellus. Only in two cases, however, has the development been followed further, in *Podocarpus coriaceus*[5] and in *Phyllocladus* sp.[6] These are the species in which the least development of prothallial tissue has so far been recorded, so that special interest attaches to the investigation of the later stages of the gametophyte in forms more typical of the order in respect of prothallial development. Observations have been made on the later history of the microgametophyte in *Dacrydium cupressinum*, *Podocarpus macrophyllus*, *P. latifolius*, and *P. nagi*. As in the case of the female gametophyte, it is to be regretted that the difficulties of collection of three of these species did not permit of the use of fixatives suitable for cytological work.

I. *Dacrydium cupressinum*. In this species it would appear that fertilization takes place a year after pollination. Ovules collected near Wellington, New Zealand, in July—that is, in the middle of the resting

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[1] Jeffrey and Chrysler ('07).

[2] Brooks and Stiles ('10), p. 309.

[3] Young ('10), p. 83.

[4] Young ('07).

[5] Coker ('02), p. 94.

[6] Young ('10), p. 84.

period—all showed pollen-tubes that had penetrated a considerable way through the nucellus.

On germination of the pollen-grain the inner layer of the wall bursts through the outer and forms the pollen-tube. Miss Young[1] has described the entry into the pollen-tube of the tube, stalk, and prothallial nuclei in *D. intermedium*, the body-cell remaining behind in the grain. The same thing takes place in the case of *D. cupressinum*. Although the tubes had penetrated a long way into the nucellus, in every case examined the body-cell remained behind in the grain (Pl. XLVIII, Fig. 25). In most cases the tube itself had grown straight down the nucellus and showed no sign of branching. Five or six nuclei were found in most of the tubes besides the nucleus of the body-cell. In the longer tubes, one nucleus, which occupied a position near the growing end of the tube, was much larger than the rest, and was probably the tube-nucleus. The remaining nuclei were indistinguishable from one another, and would be the stalk-nucleus and prothallial nuclei; there would thus be three or four of the latter. Four is the number found by Miss Young in the pollen-grain when ready for shedding, so, unlike *Agathis*, no further divisions occur among the prothallial nuclei after the shedding of the pollen. In one case a peculiar phenomenon was noticed. The growing nucellar tissue had apparently pressed against the pollen-tube so as to pinch off as it were the upper part attached to the pollen-grain, and thus containing the body-cell, from the lower part containing the tube-nucleus. In this case the tube-nucleus had presumably divided, for two large nuclei were found together near the tip of the tube in the place generally occupied by the solitary tube-nucleus (Pl. XLVIII, Fig. 26). Either nucleus of the pair was as large as an ordinary tube nucleus. This is, as far as I am aware, the only case so far recorded of the division of the tube-nucleus. In the pollen-grain and in the upper part of the tube starch grains are very abundant. Unfortunately, later stages of gametophytic development have not been available, so no further information can be given in regard to this genus in this connexion.[2]

II. *Eupodocarpus*. Material of two species of this subgenus has been used in this investigation; these species are *Podocarpus latifolius* and *P. macrophyllus*. Only early stages of the former species were available, and the following description applies almost entirely to *P. macrophyllus*.

The pollen-grains alight in a slightly concave receptacle formed by the apex of the nucellus. It was observed that in one case in *P. macrophyllus* the tube from a pollen-grain, that had come to rest at one extremity of the nucellus, grew along the top of the latter until it reached the middle of the concave portion. In all cases observed the pollen-tubes, after entering the nucellus, grow straight down until they reach the surface of the female

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[1] Young ('07), p. 193.

[2] In *Dacrydium Colensoi* the body-cell nucleus gives rise to two unequal male nuclei (Stiles, '11).

prothallus. Here they expand in a remarkable manner so as to cover almost completely the upper region of the prothallus. From this expanded portion branches grow down in all directions (Pl. XLVIII, Fig. 27). The pollen-tube wall is rather thick, about twice as thick in fact as the megaspore membrane. At this stage wall formation was taking place in the female prothallus.

The body-cell is a conspicuous object, consisting of a dense mass of cytoplasm containing a large nucleus. As described for *P. coriaceus*,[1] and as is also the case in *P. nagi*, the nucleolus is the only part which shows any marked tendency to take the safranin stain. The nucleus at this stage was always excentrically placed in the cytoplasm. The cell itself is not necessarily spherical; in one case it was conical, with the nucleus at the base of the cone, and giving the impression of a moving cell with the nucleus in front.

Small nuclei are found in the tube—in one case they numbered five—while a mass in the pollen-grain staining densely with safranin suggests that some nuclei never leave the grain and disorganize there. No distinction could be made out between the prothallial, stalk-, and tube-nuclei; all the small nuclei in the tube appeared similar. Sometimes two or three of these small nuclei were clustered near the body-cell, as was found in the observed cases of *P. nagi*; but this was not always the case, for sometimes the body-cell was quite by itself. At this stage the body-cell is of considerable size.

No further change is noticeable in the gametophyte until the division of the body-cell. This evidently took place about July 20, for in one ovule collected on this date, of four pollen-tubes which had penetrated the nucellus, one contained an undivided body-cell, while in each of the other three the body-cell had undergone division. Here, as described for *P. coriaceus*,[2] the division of the body-cell results in the formation of two unequal male nuclei (Pl. XLVIII, Figs. 28, 29, 31). The functional one is found embedded in the middle of the body-cell cytoplasm; the other moves, apparently in a very short space of time, to the side of the cytoplasm, and often that side remote from the female prothallus. The functional male nucleus takes deeply the safranin or haematoxylin stain, being in this respect in marked contrast to the body-cell nucleus. The non-functional male nucleus gradually undergoes degeneration. Several ovules showed very prettily the male nuclei just outside an archegonium (Fig. 31). The functional male is still embedded in the middle of the body-cell cytoplasm, while the degenerate male nucleus is still obvious. In all cases observed at this stage it was remarkable that small nuclei, sometimes as many as six in number, were always observed clustered against the body-cell (Pl. XLVIII, Figs. 29 and 30). Whether they are the original prothallial, stalk-, and tube-nuclei, or whether some of these degenerate, while others in the neighbourhood survive and

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[1] Coker ('02), p. 95.

[2] l. c.

divide, is a question that could not be definitely decided, but the facts suggest the former alternative.

III. *Podocarpus nagi.* Owing to the exceedingly small quantity of material available of this species the observations are of a very meagre description. The youngest material examined was collected by Dr. Stopes at Tokyo on July 13. The ovules were abundantly supplied with pollen-tubes, as many as eight having penetrated through one nucellus. Between this and the female prothallus they formed so complicated a tangle that it was found impossible to distinguish between the various tubes in a series of sections. Indeed, the number of pollen-tubes could only be found by counting the number of body-cells contained in them, so in the unlikely event of there being more than one body-cell in one tube the number of tubes would be correspondingly lower. Under these circumstances it was found difficult to estimate the number of prothallial nuclei in one tube; in one case, however, eight small nuclei were found near a body-cell in one tube, while in several other cases at least six nuclei were present. Allowing for the presence of the stalk- and tube-nuclei, which by this time are indistinguishable from the prothallial nuclei, there may be at least as many as six prothallial nuclei in the pollen-tube at this stage.

The further course of events seems to follow closely that in *P. macrophyllus*, two male nuclei being produced, one of which comes to lie centrally in the body-cell cytoplasm, while the other is non-functional, and degenerates on the outside of the body-cell cytoplasm.

xi. EMBRYOLOGY.

The embryology of *Podocarpus coriaceus* has been described by Coker,[1] and that of *P. macrophyllus* is similar. The fusion-nucleus sinks to the basal part of the archegonium and there undergoes successive divisions, so that at least sixteen free nuclei are formed before wall formation takes place. While these divisions are proceeding the basal part of the archegonium wall swells up and becomes conspicuous owing to its power of staining with safranin. This is shown in Pl. XLVIII, Fig. 32, which is a drawing of a section through two archegonia, in one of which the pro-embryo is undergoing the third division, while the other is in the eight-nucleate stage. (All the nuclei are of course not shown in a single section.) The stage directly after wall formation was not observed, but no doubt the same tiers of cells are formed as in *P. coriaceus.*

The embryo consists of a single cell for some time after the suspensors have commenced to elongate. Coker has observed the formation of several embryos from a single egg in *P. coriaceus*; there was no case of this seen in *P. macrophyllus*. The embryo later produces a large cylindrical mass

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[1] Coker ('02), p. 100.

of tissue which ultimately differentiates into the radicle and the two cotyledons (Pl. XLVI, Figs. 12–14). As the latter in the course of their growth destroy the prothallus cells a plug of prothallus tissue is left between them and presents a striking appearance (Pl. XLVI, Fig. 14).

In the mature seed the procambial strands are already obvious, and it is evident that the cotyledons will each contain two vascular bundles at some distance from one another (Pl. XLVI, Fig. 13).

xii. SEEDLING STRUCTURE.

As far as I am aware the only recorded observations on seedling structure of the Podocarpeae are the fragmentary ones of Geyler[1] and Van Tieghem[2] on *Phyllocladus*, and those of Hill and de Fraine[3] on a single species of *Podocarpus*, *P. chinensis* (*P. macrophyllus*, subsp. *maki*). Further observations on this part of the subject are therefore needed; in this work, however, no material of seedlings was available.

From the observations referred to it appears that in *P. macrophyllus*, subsp. *maki* there are two cotyledons, each traversed by a pair of vascular bundles endarch and collateral in nature. Transfusion tissue is developed laterally. The primary root is diarch, each pole of the primary xylem being continuous with the fused pair of xylem-strands of one cotyledon. The same arrangement probably obtains in *Phyllocladus*, but of other genera we know nothing.

xiii. GEOGRAPHICAL DISTRIBUTION.

The Podocarpeae have a very interesting distribution. *Pherosphaera* has two species, one of which is found only in the mountains of New South Wales, while the other occurs exclusively on the mountains of Tasmania. The genus *Microcachrys* has the same very limited range as the latter species, while *Saxegothaea* occurs only on the Chilian Andes. *Dacrydium* is essentially an island type, being almost limited to the islands of the Malay and Australasian region; one species, however (*D. Fonkii*), occurs in the Andes of Chili. The distribution of *Phyllocladus* is similar to that of *Dacrydium*, but is more restricted in its range. In marked contrast to the other genera is *Podocarpus*. This must be regarded as the dominant Conifer in the Southern Hemisphere, as it occurs in South America, the West Indian Islands, South and Central Africa, Australia, Tasmania, New Zealand, and the Australasian and Malay Archipelagoes. It stretches into the Northern Hemisphere in China, Japan, the Malay Peninsula, Burma, and India. On the whole it may be said that *Podocarpus* occupies the place in the Southern Hemisphere that is occupied in the Northern Hemisphere by *Pinus*. These two genera must be regarded, both from the point of view of numbers of species and of wide geographical distribution, as the successful Conifers of the present day.

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[1] Geyler, T. H. ('81).

[2] Van Tieghem ('70).

[3] Hill, T. G., and de Fraine, E. ('08), p. 694.

Perhaps the most noticeable feature of the distribution of the living Podocarpeae is its similarity to that of the Araucarieae.[1] This has been regarded[2] as of significance in regard to the view of the near relationship of these two orders. We know, however, so little of the causes of present-day geographical distribution that it is doubtful what the significance of this similarity of geographical distribution is. The Araucarieae were probably abundant in the Northern Hemisphere in past times,[3] but on the other hand little is known of the past distribution of the Podocarpeae. It has been suggested that the Podocarpeae may have been a southern offshoot of the Araucarieae,[4] but there does not seem to be evidence either in support of, or against this view, from the facts of distribution.

xiv. FOSSIL PODOCARPEAE.

Very little is known as to the existence of Podocarpeae in past times, although many plant remains have been referred to this order. These fossils fall into three groups: impressions of leaves and branches, petrified wood, and impressions of cones, cone scales, and seeds.

I. Impressions of Leaves and Branches.

A good many impressions of leaves and branches have been referred to *Podocarpus* or allied genera ; the more noteworthy of these records are as follows :

Mesozoic. Under the generic name of *Protophyllocladus* Berry has separated the mid and late Cretaceous species formerly included in the genus *Thinnfeldia*.[5] A relationship to the recent *Phyllocladus* cannot, however, be regarded as by any means proved, for the characters on which Berry relies for identification, namely size and shape, petiolation, and venation, do not even show that the impressions are those of phylloclades. Indeed, Berry himself speaks of them as leaves, and mentions that Knowlton formerly pointed out the resemblance of *Protophyllocladus lanceolatus*, (Knowlton) Berry, to various species of *Podocarpus*.

From the Potomac or Younger Mesozoic Flora of the United States Fontaine has described fourteen species of *Nageiopsis*.[6] The leaves of this genus are parallel-veined, and show a general resemblance to the leaves of *Podocarpus* § *Nageia*. In a revision of the genus *Nageiopsis*, Berry[7] has reduced Fontaine's fourteen species to three. Other species have been referred to the same genus by Seward, one from the Wealden[8] and another from the Inferior Oolite of Yorkshire.[9] However, the parallel

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[1] See Seward and Ford ('06), p. 308, Map.

[2] Young ('10), p. 93.

[3] Seward and Ford, l. c.

[4] Coulter and Chamberlain, ('10), p. 351.

[5] Berry ('03), p. 439.

[6] Fontaine ('89), p. 195, et seq.

[7] Berry ('10), p. 189.

[8] Seward ('95), p. 211.

[9] Seward ('00), p. 288.

venation of the leaves, the persistence of the latter, and the non-convergence to a union of the veins at the tip of the leaf are scarcely sufficient evidence of a Nageian affinity. Indeed, *Nageiopsis anglica*, the Yorkshire specimen, is regarded by both Seward and Berry as possibly of Araucarian affinity.

A second genus of Fontaine's[1] from the Potomac is *Phyllocladopsis*. From Fontaine's figure, however, there is little evidence of Phyllocladean affinity.

From the Cretaceous of New Zealand Ettingshausen has described and figured various leaves which he refers to the genera *Podocarpium* and *Dacrydinium*.[2] The evidence of a Podocarpean affinity is however slight, as the leaves do not differ in any essential feature from those of any Conifer with a *Taxus*-like leaf. The impression named by the same writer *Ginkgocladus Novae-Zeelandiae*[3] is interesting, as it is apparently part of a phylloclade which might quite well have belonged to a *Phyllocladus*. As in other cases, however, the evidence is not definite.

Tertiary. Leaves from Tertiary beds referred to *Podocarpus* and *Dacrydium* have been described by Gardner from England,[4] and by Ettingshausen from Australia[5] and New Zealand.[6] These cases are specially mentioned, as fruits in each of these three instances were found occurring with the leaves. As regards the latter it can only be said that although they might be Podocarpean leaves, the evidence is not sufficient to prove they might not have belonged to some other Coniferous genus. Similarly, the impression named *Phyllocladus asplenioides* by Ettingshausen[7] appears to resemble a *Phyllocladus*, but this does not seem to be sufficient indication of relationship.

If all these references of leaf impressions are correct it means that forms near to the modern *Phyllocladus*, *Dacrydium*, and *Podocarpus* were in existence in Mesozoic times in Britain, in North America, and in New Zealand. But it is well known how unsatisfactory are references based on leaf impressions, and this is especially so in the case of the Coniferae, where throughout the group there is such uniformity of type as regards leaf structure. Even references of recent plants based on leaf characters have been very incorrect; thus the plant named by Sir W. J. Hooker *Podocarpus? Diffenbachii*[8] ultimately turned out to be a species of *Veronica*. As Professor Seward has said, 'Tempted by a resemblance in the form of the leaves, one is often inclined to assume a relationship with existing Conifers which rests on wholly insufficient evidence.'[9]

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[1] Fontaine ('89).

[2] Ettingshausen ('87), p. 177.

[3] l. c., p. 179; Taf. VII, Fig. 19.

[4] Gardner ('83–6), p. 48.

[5] Ettingshausen ('86), p. 92.

[6] Ettingshausen ('87), p. 156.

[7] Ettingshausen ('86), p. 94.

[8] Hooker, Sir W. J. ('43), No. 547.

[9] Seward ('11), p. 86.

II. Petrified Wood.

Gothan[1] has recently described several examples of petrified wood which he ascribes to the genera *Podocarpoxylon* and *Phyllocladoxylon* on account of the structure of the wood, special stress being laid on the medullary ray pitting. Another species has also been described by Kubart under the name of *Podocarpoxylon Schwendae*.[2] These woods certainly appear to resemble existing species of Podocarpeae as far as the structure of the wood is concerned; the difficulty lies in determining how far the structure of the Podocarpean woods examined by Gothan can be regarded as characteristic of the Podocarpeae, recent and fossil. A similar wood structure is known to exist in forms not very nearly related, and it is of course possible that this may be the case here. At present, therefore, it seems best not to regard as proved the references of petrified wood to a Podocarpean affinity.

III. Impressions of Cones, Cone Scales, and Seeds.

Ettingshausen has described an impression from the Cretaceous of New Zealand as a fruit of *Podocarpium Ungeri*,[3] while from the Tertiary of Australia[4] and New Zealand[5] fruits referred to species of *Podocarpus* have been described by the same writer. None of the figures of these species can, however, be regarded as at all convincing of Podocarpean affinity.

Two fruits referred to *Podocarpus* have been figured by Gardner as occurring in the British Eocene Flora.[6] These figures certainly show a resemblance to *Podocarpus* seeds, but unfortunately not much detail is evident to make certain their reference.

Of great interest are certain fossils that have been described by Professor Nathorst. The genus *Carpolithes*,[7] as Nathorst says, shows much resemblance to the recent form *Podocarpus spicatus* in that a number of seeds are arranged on a spike.

Two fructifications of Rhaetic age recently described by Professor Nathorst are suggested by him as having possibly a Podocarpean affinity.[8] They are *Stachyotaxus* and *Palissya*. There seems to be good evidence of an epimatium in the former genus at any rate, although it does not seem to be so asymmetrical as the typical Podocarpean epimatium. In any case these genera differ exceedingly from any recent Podocarpean fructification, for *Stachyotaxus* had two ovules to the cone scale, while *Palissya* had as many as 10 or 12 to the scale, and it has been shown

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[1] Gothan ('05), ('07), ('08).

[2] Kubart ('10).

[3] Ettingshausen ('87), p. 177.

[4] Ettingshausen ('86), p. 92.

[5] Ettingshausen ('87), pp. 156, 157.

[6] Gardner ('83-6).

[7] Nathorst ('78), p. 27, Taf. XV, Figs. 14, 15.

[8] Nathorst ('08).

that the most characteristic feature of the Podocarpeae is the single ovule borne medianly in relation to the cone scale. It may be of course that we have in these fossils connecting links between the Podocarpeae and other orders, but at the present time such considerations are at best only conjectures.

A very interesting petrified cone has been described by Hollick and Jeffrey from the Cretaceous of Kreischerville under the name of *Strobilites microsporophorus.*[1] The cones were small and slender, while each sporophyll bore two sporangia containing microspores, each with two wings. The resemblance to a Podocarpean male cone is obvious, while Hollick and Jeffrey's figures are very reminiscent of the structure of the microsporophyll of *Podocarpus andinus.* The authors regard it as belonging to a 'generalized araucarineous type, nearer in the structure of its male sporophylls to the Abietineae, than are any of the existing Araucarineae '.[2] Similarly, it is also nearer to the Podocarpeae than are any of the existing Araucarineae, and to the writer it seems quite possible that it combines features of all three orders, and is in this sense a synthetic type.

Prominence has been given in the past to the similarity of the fossil cone *Walchia filiciformis*[3] to the Araucarieae.[4] This may serve to emphasize the connexion between the Araucarieae and Podocarpeae, for the arguments that are used in favour of the relationship of *Walchia* with the Araucarieae may be used equally well in support of a relationship between *Walchia* and *Saxegothaea.*

Thus the consideration of the fossils referred to the Podocarpeae afford little or no evidence either as to the course of phylogeny in the order, or as regards past geographical distribution, and this is due to the unsatisfactory nature of the plant remains referred to an affinity with the order.

This absence of evidence, however, must not be regarded as equivalent to evidence of absence in past times. The present distribution of the Podocarpeae is almost entirely a Southern one, while it is chiefly the rocks in the Northern Hemisphere that have been explored for fossil plant remains. As Burlingame remarks of the Podocarpeae, 'We know but little of the plant remains of those parts of the world in which these remains would be most likely to be found.'[5]

XV. INTER-RELATIONSHIPS OF THE GENERA AND SPECIES.

That the six genera included in the Podocarpeae in this paper form a natural group cannot, I think, be doubted when the sum of their characters is taken into consideration. The structure of the wood of the stem, the

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[1] Hollick and Jeffrey ('09), p. 66.

[2] l. c., p. 68.

[3] Zeiller ('92), p. 99, Pl. XV.

[4] Seward and Ford ('06), p. 373.

[5] Burlingame ('08), p. 175.

anatomy of the leaves, the remarkable uniformity of structure exhibited by the male cones, the constant relationship of a single medianly placed ovule to a megasporophyll, the simple character of the latter, and the development of prothallial tissue in the male gametophyte,[1] all give evidence of the natural relationship of the genera here included in the Podocarpeae. That we must look for the nearest approach to the common ancestor of them all among the types with the megasporophylls aggregated into cones seems obvious. Apart from the fact that the fructifications of *Dacrydium* and *Podocarpus* give evidence of their reduction from more perfect cones,[2] it is the genera *Pherosphaera*, *Microcachrys*, and *Saxegothaea* which have a very limited distribution, and in every group of vascular plants it is as a rule among such genera of restricted range that we expect to find the more primitive members of the group. Many species and wide geographical range are the indications of a successful genus, and success in the struggle for existence means the evolution of characters capable of struggling with modern conditions. For this reason, although it is possible, yet it is extremely improbable that a genus with a wide range and many species will approach so nearly the primitive type of its order as the genera with few species and restricted range, which are probably old types making their last stand against newer conditions.

Again, it is in the structure of these species with female *cones* that we find most indications of primitiveness. The presence of a certain amount of centripetal wood in the axes of both the male and female cones of *Saxegothaea*, together with the gradual transition from foliage leaves to sporophylls in the case of the female cones, support this view.

The evidence derived from the development of ovulate structures affords fairly conclusive proof that the ovule was originally borne *erect* in the axil of the sporophyll, for it probably arises in this position in all the genera, although its position may be altered by later growth (Text-fig. 8). It was probably surrounded by a single integument, as it remains presumably throughout the whole course of its development in *Pherosphaera*.

The male cones are so uniform throughout this order that they afford little evidence as to the inter-relationships of the genera. The transverse dehiscence of the sporangia of *Saxegothaea* is probably more primitive than the oblique dehiscence in most of the species of *Podocarpus*. The wood of the stem is remarkably uniform throughout the order, but suggests that an arrangement of bordered pits in contact is primitive in the order.

From the account already given of the structure of the leaves of the Podocarpeae,[3] it is evident that, with the exception of *Nageia*, all the leaves examined are modifications of one type. The central type is the leaf of *Saxegothaea* or *Podocarpus andinus*. There is a single vascular bundle with well-marked transfusion tissue developed right and left of the xylem, and

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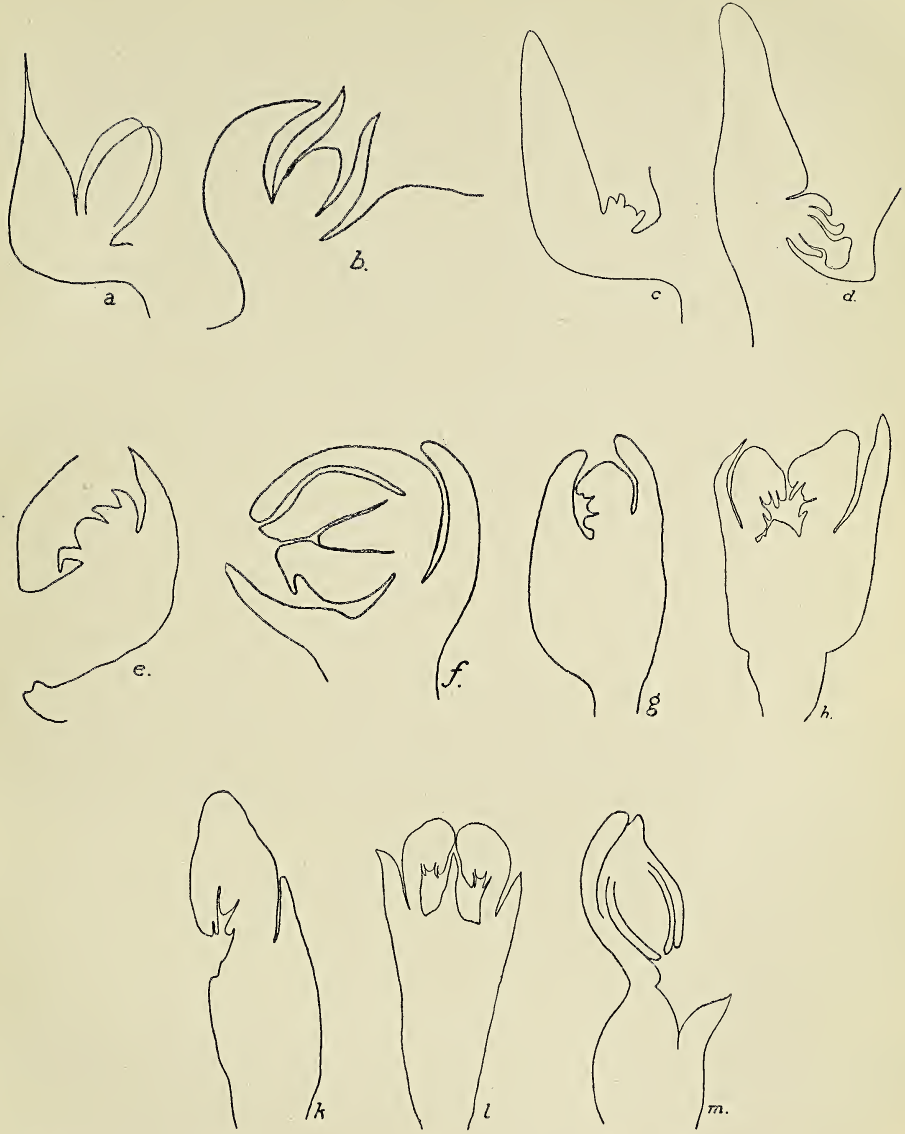
[1] *Pherosphaera* is still unknown in respect of the male gametophyte.

[2] Brooks and Stiles ('10), p. 314.

[3] p. 453.



Text-fig. 8. Diagrammatic representation of megasporophylls to show the relation of ovule and sporophyll throughout the Podocarpeae. *a*, *Pherosphaera* (after Pilger, '03); *b*, *Phyllocladus* (young stage, after Robertson, '06); *c*, *Saxegothaea* (young stage, after Norén, '08); *d*, *Saxegothaea* (older stage, after Norén, '08); *e*, *Microcachrys* (young stage); *f*, *Dacrydium cupressinum* (young stage); *g*, *Podocarpus latifolius* (young stage); *h*, *k*, *Podocarpus latifolius* (older stages); *l*, *Podocarpus spinulosus*; *m*, *Dacrycarpus* (after Pilger, '03).

with a single functional resin canal on the under side. In the leaves of *Podocarpus macrophyllus* and similar ones, the effect of increase in size has probably rendered necessary the development of the accessory transfusion tissue for strengthening purposes. In the larger single-nerved leaves also the single resin canal is replaced by three smaller ones. If Bertrand's description is correct, *Podocarpus vitiensis* would appear to be derived from the *P. macrophyllus* type of leaf, by the removal of the two lateral resin canals away from the median one.

In other species of the order a reverse change has occurred: reduction of leaf surface. Stages in this reduction we see in *Microcachrys*, *Dacrydium cupressinum*, *D. Franklini*, and *Phyllocladus*, the last being the most reduced type of leaf in the order. The juvenile leaves of *Dacrydium* thus approach more nearly the primitive form than those of the adult state, as would be expected.[1] It is also interesting that the first leaves of the seedling of *Phyllocladus* are flat green needles, the transition between these and the very reduced leaves of the adult state being gradual.[2]

In spite of their characteristic venation, the leaves of *Nageia* have a very similar internal structure to the others. A single resin canal underneath the phloem of each vascular bundle, and the development of transfusion tissue on either side of the xylem, occur exactly as in the other species. There can be no doubt that the *Nageia* type and the remaining types of leaf in the Podocarpeae have had an immediate common origin.

There are two possible alternatives; either the parallel-veined type is primitive in the order, and the uninerved type has been derived from it by reduction, or the *Nageia* type has originated from the *Saxegothaea* type in response to demands for extra leaf surface, as the type of *Podocarpus macrophyllus* has, only with a different result.

The evidence in favour of the first hypothesis is practically limited to the fact that parallel-veined leaves are common in the Araucarieae, while this piece of evidence is strengthened by the growing opinion that the Araucarieae and Podocarpeae are nearly related groups of Conifers. Moreover, some Araucarian leaves with only one vein show evidence of having been reduced from a wider parallel-veined leaf.[3] The leaves of Cordaites also are parallel-veined, and at present the more general opinion of botanists is that the Cordaitales and Coniferales are descended from a common ancestor.

But this resemblance of the leaves of *Agathis* to those of *Nageia* is only a superficial one. The internal structure of the leaves of *Agathis* is quite different from that of *Nageia*, especially as regards the distribution of the

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[1] Cf. Goebel ('00, p. 154), who says with regard to the leaves of the Cupressineae, which in external characters closely resemble those of *Dacrydium*, 'the juvenile forms of these plants must undoubtedly be regarded as more primitive.'

[2] Geyler ('81), p. 209; Coulter and Chamberlain ('10), p. 227, Fig. 256, *e*, *f*.

[3] Seward and Ford ('06), p. 350.

canals. In *Agathis* these alternate with the vascular bundles, whereas in *Nageia*, as has been already observed, a single resin canal is present under each vascular bundle, and from the constancy of this character throughout the order it seems reasonable to attach importance to it. There is thus no convincing evidence that within the Podocarpeae the single-veined leaf has been derived from the many-veined one.

On the other hand, the evidence in favour of the greater primitiveness of the uninerved leaf seems more convincing. In the first place, with regard to leaves within the order, we find single-nerved leaves occurring in all the genera, while plurinerved leaves are found in one section of one genus only, and that genus the one which on general grounds must be admitted to be the least primitive in the order. In the second place, leaves of a closely similar structure are found in all other orders of Coniferae. In *Araucaria Rulei*[1] there is a single resin canal beneath the single vascular bundle as in the Podocarpeae, and, moreover, of all the Araucarieae it is this species which approaches most nearly *Saxegothaea*, not only in this respect, but also in the structure of the microsporangium,[2] in the gradual transition between leaves and megasporophylls,[3] and in the vascular anatomy of the megasporophyll.[4] It is also of significance that Seward and Ford regard the leaf of *A. Rulei* as constituting in some measure a link between the two sections of the genus *Araucaria*.[5] In the Taxeae also a leaf with a very similar structure prevails. The leaf of *Taxus* scarcely differs from that of *Saxegothaea* except in the absence of the resin canal from the former. Among the Abietineae the leaves of *Tsuga Mertensiana* and *T. Pattoniana* closely resemble those of *Saxegothaea*, while the leaves of other species may well be derivable from this type. Similarly, among the remaining orders, Taxodieae and Cupressineae, leaves with a not very different structure are found. These facts seem to me to point to this type of leaf as not only primitive in the Podocarpeae, but in the Coniferae as a whole.[6] It is of course possible here, as elsewhere, to invoke reduction, but it seems an extraordinary thing if in all the Coniferous phyla the reduction from the primitive type has been so great, and yet has produced so nearly the same result in each case.

From these considerations it seems to the writer probable that the *Saxegothaea* type of leaf is the primitive one in this order, and that the *Nageia* type has probably been derived from the single-veined leaf, not merely during the course of phylogeny of the Podocarpeae, but during that of the genus *Podocarpus* itself.

The primitive Podocarpean plant can then be pictured as a tree, bearing probably spirally arranged leaves of a Yew- or *Saxegothaea*-like

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[1] Seward and Ford ('06), p. 350.

[2] Stiles ('08), p. 212.

[3] Seward and Ford ('06), p. 361; Stiles ('08), p. 213.

[4] Stiles ('08), p. 217.

[5] Seward and Ford ('06), p. 351.

[6] Compare the remarks of Coulter and Chamberlain ('10), p. 225.

appearance, and with its reproductive shoots bearing male and female cones, with the sporophylls spirally arranged. On the upper parts of the reproductive shoots there was a gradual transition between foliage leaves and sporophylls. Each microsporophyll bore two sporangia, while each megasporophyll bore in its axil a single erect ovule surrounded by a single integument.

From this primitive type we have had two main lines of development. In *Pherosphaera* there has been reduction in the size of the leaves and in the number of sporophylls in the cone; the megasporangium has, however, retained its primitive character. In *Phyllocladus* the reduction has proceeded still further, and there has occurred the special development of stems into phylloclades, physiologically to replace the leaves, here reduced to small insignificant scales. The ovule has also been clothed by a second integument completely surrounding the inner. The prothallial tissue in the male gametophyte consists of two or occasionally three cells; while in the small number of archegonia in the female gametophyte we perhaps have another reduction phenomenon. Owing to our absolute ignorance of the gametophytes of *Pherosphaera*, it is impossible to say whether in this respect *Phyllocladus* is further reduced than its otherwise more primitive relation.

Along the other line of descent we have intercalary growth taking place at the base of the megasporophyll, resulting in the removal of the ovule from the cone axis, and in many cases causing also its greater or less inversion. Probably correlated with this is the development of the incomplete outer integument or epimatium, for this is not present in any other phylum of Conifers, nor in the other Podocarpean line of descent. Whether this epimatium is an outgrowth of ovular or sporophyll tissue, it is at present impossible to say. The evidence of development in *Saxegothaea* and *Microcachrys* suggests the former, while a somewhat older state of *Dacrydium cupressinum* suggests the latter. The most primitive genus of this phylum is *Saxegothaea*, where the cone is still well developed. This is a somewhat smaller structure in *Microcachrys*, but is still a compact cone. The latter genus has also suffered reduction in its leaves and in the suppression of resin canals in the stem, though these are still present in the leaves and megasporophylls.

In *Dacrydium Franklini* the megasporangiate strobilus consists of about eight megasporophylls separated by appreciable internodes. The ovule is partially inverted, and the incomplete outer integument or epimatium is present, as in *Saxegothaea* and *Microcachrys*. As in the latter genus, resin canals are absent from the stem, but are present in the leaves and megasporophylls; in fact the internal structure of this plant, especially as regards the megasporophyll, is remarkably like *Microcachrys*. Curiously enough, both are limited in their distribution to Tasmania, though one is a small straggling mountain shrub, while the other is one of the tallest of forest trees.

In other species of *Dacrydium*, such as *D. cupressinum*, the female fructification contains but a single sporophyll, and so is reduced to the extreme limit. Occasionally, two fertile sporophylls may be borne on one 'strobilus'. The ovule has rather the appearance of being borne on the epimatium, but whether this is due to an outgrowth of the scale below the epimatium, or to intercalary growth of the basal part of the epimatium, cannot at present be said. In any case it is a new development not found in the more primitive members of the order or genus. The complicated structure of the inner integument is probably also a new development. The stems here contain resin canals as well as the leaves.

So far all the genera to which reference has been made in this section have the nucellus, integument, and epimatium free from one another, at least for a good part of their length. In the remaining genus, *Podocarpus*, the integument and epimatium are only free from one another for a very short distance in the neighbourhood of the micropyle. In the section *Eupodocarpus* the nucellus is free from the integument, at least in the species I have had an opportunity of examining, for about half its length. The characteristics of this line of evolution are fusion of parts and reduction of the female strobilus.

The two species, *P. andinus* and *P. spicatus*, in which the megasporangiate strobilus consists of about eight sporophylls on an elongated axis, are presumably to be considered the most primitive, reduction of the strobilus having proceeded to a less degree than in any other species. It is worthy of note also that the leaves are of the *Saxegothaea* type, at any rate in *P. andinus*, while their external appearance suggests a similar structure in the case of *P. spicatus*.

In the section *Eupodocarpus* the fructification is reduced to three pairs of decussately arranged scales, of which the two upper pairs fuse to form the receptacle. Usually only one or two sporophylls are fertile. In the section *Nageia* the fructification is similarly reduced, while the peculiar venation indicates this section as a distinct offshoot of the pre-*Eupodocarpus* stock. *Microcarpus* with its single species would appear to be a reduced type on the *Nageia* line of descent. Finally, *Dacrycarpus* would appear to be the most specialized of them all as far as fusion of parts goes, for the megasporophyll is involved in the fusion. On the other hand, whereas in *Eupodocarpus*, and still more in *Nageia*, there is a tendency towards increased leaf surface, in *Dacrycarpus*, and also in *Microcarpus*, there is a reduction in this respect.

The suggested scheme indicating the inter-relationships of the genera and some of the species of the Podocarpeae is therefore as follows:[1]

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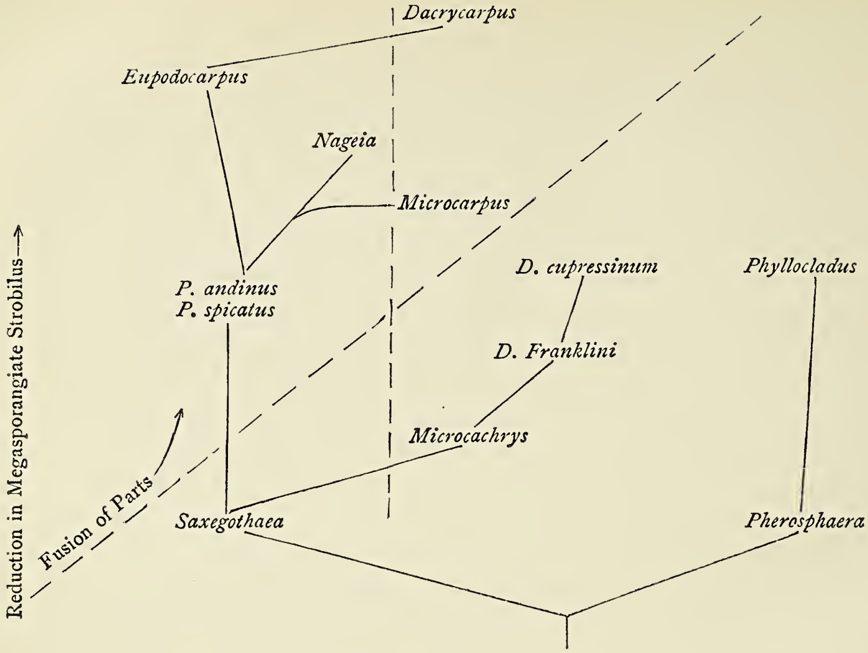
[1] In this scheme genera and species shown above the oblique broken line exhibit fusion of parts in the ovulate structures; while those to the right of the vertical broken line have undergone reduction in their leaves.



XVI. RELATIONS OF THE PODOCARPEAE TO OTHER CONIFERAE; PHYLOGENY.

The question of the inter-relationships of the various phyla within the Coniferae is one on which very diverse opinions are held. The following quotation from the New Edition of Professors Coulter and Chamberlain's 'Morphology of Gymnosperms' shows how various are the views held with regard to the natural position of the Taxaceae (Taxeae and Podocarpeae). 'They have been called the oldest living conifers and the most recent; they have been connected with *Ginkgo* through *Cephalotaxus* and with *Cordaites* on account of the structure of the ovuliferous shoot of *Taxus*; and the podocarps have been related to the araucarians through *Dacrydium* and *Saxegothaea*.'[1]

During the last few years the opinion has been rapidly gaining ground that the Podocarpeae and the Araucarieae are directly descended from a common ancestor, the connecting link between the two orders being found in *Saxegothaea*. The first suggestion of this connexion really dates from as far back as Lindley, who actually classed *Saxegothaea* as one of the Pinaceae on account of its similarity to *Agathis*.[2] Of late years the first suggestion of a revival of this view appears as a result of Miss Young's work on the male gametophyte of *Dacrydium*; this writer being struck with the development of prothallial tissue in the Podocarpeae, Araucarieae,

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[1] Coulter and Chamberlain ('10), p. 350.

[2] Lindley ('53), p. 229 *a*.

and Abietineae, suggested in cautious terms the possibility of a common ancestry for the three orders.[1] The investigation of *Saxegothaea* enabled a more definite statement to be made, so that Dr. Norén and the present writer in 1908 put forward independently the view that the two orders Podocarpeae and Araucarieae are related through *Saxegothaea.* Mr. Thomson and M. Tison, also working independently on *Saxegothaea,* have reached a similar conclusion. As the resemblance between *Saxegothaea* and the Araucarieae is not confined to a single character, but extends to the external features of the female cones, the internal structure of the cone scales, the male gametophyte, and, though not to so marked an extent, to the structure of the microsporangium and the wood of the stem, and in the case of *Araucaria Rulei* to the leaf, there can, I think, be little doubt that the resemblance is an indication of real affinities. One point of resemblance between the two orders, namely, the inversion of the ovule, appears to be due to parallel evolution. The primitive position of the ovule in the Podocarpeae, as Miss Young justly observes,[2] appears to be erect and axillary, as pointed out in the preceding paragraph.

The only recent paper in which an opposed view is taken is that of Miss Gerry,[3] who thinks that her work on the distribution of 'bars of Sanio' in the Coniferae indicates a relationship of the Podocarpeae with the Abietineae rather than with the Araucarieae. The evidence consists in the fact that 'bars of Sanio' are recorded as occurring in the tracheides of the xylem, either of stem or root, but not necessarily of both, in all recent Conifers except *Araucaria* and *Agathis.* Whether this is actually the case cannot be decided for certain, as, judging from the statements in Miss Gerry's paper, only one species of each genus appears to have been examined (at least no more are described), out of the fourteen species of *Araucaria* and ten of *Agathis.* Nor is the additional evidence derived from fossils any more convincing, for although it is stated that 'bars of Sanio' are absent from fossil Araucarian woods and present in woods of an Abietineous affinity, yet the statement means little, for in no case have the so-called Araucarian woods been found in connexion with reproductive organs that are undoubtedly Araucarian, which would alone render certain their reference. As an example of this, mention may be made of Professor Jeffrey's remarks on *Geinitzia gracillima.*[4] The 'purely systematic botanists' referred the plant to *Sequoia.* The author of the paper also says that the cone scales alone, although furnishing him with ample evidence of Araucarian relationships, 'might by those less experienced in the details of Coniferous anatomy still be considered as belonging to the strobili of the Taxodineae or Cupressineae.' Next we learn that 'the pits in no case are contiguous or in more than a single row', which means, of course, that the pitting is

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[1] Young ('07), p. 194.

[2] Young ('10), p. 93.

[3] Gerry ('10), p. 122.

[4] Jeffrey ('11), p. 21.

unlike that occurring in undoubted Araucarieae. Nevertheless Professor Jeffrey finds 'bars of Sanio' absent, and so concludes that the plant is one of the Araucarieae, and further that it furnishes 'evidence for the derivation of the Araucarian stock from an ancestry essentially resembling the existing Abietineae'. Sinnott's *Paracedroxylon*[1] is regarded as 'a new type of Araucarian wood' for the same reason. Indeed, this reference to an Araucarian affinity seems even more unsatisfactory than that of *Geinitzia*.

But even if it be allowed that Araucarian wood is characterized by an absence of 'bars of Sanio', it would seem to be an utterly insufficient reason to assert, therefore, the nearer connexion of the Podocarpeae with the Abietineae than with the Araucarieae. In the first place, Miss Gerry's observations go to show that while 'bars of Sanio' are absent in Araucarieae they are not so abundant in the Podocarpeae as in the Abietineae.[2] Again, it would be quite likely that 'bars of Sanio' should cease to be developed along the Araucarian line of descent and yet remain in the Podocarpean. In fact, when the striking resemblance of the reproductive organs of *Saxegothaea* and the Araucarieae in more than one respect is considered, together with the less-marked resemblances in the vegetative organs, it must be admitted by every impartial critic acquainted with the facts of the case as they are at present known, that this resemblance far outweighs in phylogenetic value the dissimilarity in the case of the 'bars of Sanio'.

A more difficult question is that regarding the relationship of the Podocarpeae and the Taxeae. A relationship between the two orders has for long been assumed by the retention of both in the one family Taxaceae. Mr. Brooks and the writer in a previous paper[3] expressed the opinion that evidence of relationship between the two orders was at present not very clear, though *Phyllocladus* might be a connecting link. M. Tison[4] has stated that he has evidence that the Taxeae are derived from the Cupressineae, but as far as the writer is aware this evidence has not yet been published. Miss Young's recent work on *Phyllocladus* shows conclusively, as Mr. Brooks and the writer suggested might be the case, that this genus is one of the Podocarpeae rather than intermediate between that order and the Taxeae.[5] In the latter the structure of the wood of the stem, the character of the microsporophyll, the position of the ovules in *Taxus* and *Torreya* and the number appertaining to one megasporophyll in *Cephalotaxus*, the absence of prothallial tissue in the male gametophyte, and the absence or poor development of the megaspore membrane[6] are all characters in marked contrast to the equivalent ones in the Podocarpeae. Our knowledge here is at present inadequate to enable a definite statement to be made; it

[1] Sinnott ('09), p. 170.

[2] Gerry ('10), p. 121.

[3] Brooks and Stiles ('10), p. 316.

[4] Tison ('09), p. 156.

[5] Young ('10); see also Stiles ('11).

[6] Thomson ('05^1), p. 43.

is at least safe to say that there is as yet little evidence of any near connexion of the Taxeae and Podocarpeae.

Workers on gametophytic structure have found several points of similarity between the Abietineae and Podocarpeae.[1] In the first place, prothallial cells are developed in the male gametophytes of each order, but whereas these are evanescent structures in the Abietineae, in the Podocarpeae they are generally more numerous and may persist up to the time of fertilization. The distribution and arrangement of the archegonia is also similar, and a third point of resemblance is found in the reduction of the functional male nuclei to one. As regards sporophytic characters, male cones and microsporangia are similarly constructed in the two groups, while in each order the pollen-grains are winged. This last point appears to be another case of parallel evolution, for in the Podocarpeae the wings seem to have arisen within the group itself, for the pollen of *Saxegothaea* is wingless, while in *Microcachrys* the wings are imperfectly developed.[2] Among the Abietineae also the genus *Pseudotsuga* has wingless pollen.[3] As regards the distribution of the archegonia we find a similar arrangement in the Taxeae and in *Sciadopitys*,[4] while the reduction of the functional males to one is probably to be correlated with the absence of archegonial complexes.[5] On the other hand, there are great dissimilarities in the structure of the wood of the stem, and in the female fructification. The relative phylogenetic value of different characters is always a difficult thing to estimate, and personal opinions on such a question will probably always vary to some extent. The opinion of most workers is that it is to the reproductive structures, rather than vegetative characters, such as wood structure, that we should look for indications of relationship. In the case under consideration the differences between the simple cone scale of the more primitive Podocarpeae bearing a single ovule, and the more complicated 'pièces du cône' of the Abietineae each bearing two ovules, indicate that the relationship between the two orders cannot be a very near one. On the other hand, there are several characters which seem to indicate a common ancestry of the two orders, chief among which must be reckoned the similarity of the microsporophylls, the distribution of archegonia in the female gametophyte, the development of prothallial tissue in the male gametophyte, and the inversion of the ovules. In the last two instances the facts indicate that the Abietineae have advanced much further from the common ancestor than the Podocarpeae, for the prothallial tissue is reduced not only in amount but also in the length of time it persists, while the inversion of the ovules is much more complete. The position of the ovule on an outgrowth of the scale in *Podocarpus*, and especially the position

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[1] Coker ('02), p. 102; Young ('07), p. 194; Burlingame ('08), p. 175.

[2] Thomson ('09^1), p. 29.

[3] Lawson ('09), p. 163.

[4] Lawson ('10), p. 412.

[5] Lawson ('07^2), p. 292.

of the ovule in *Dacrydium cupressinum* on the epimatium, which is here developed to a greater extent than the megasporophyll, are very suggestive of a possible origin of the ovuliferous scale in the Abietineae. If the epimatium of *Dacrydium cupressinum* is called an ovuliferous scale we practically have the state of affairs in the Abietineae. It is, of course, not by any means suggested that *Pinus* is descended from *Dacrydium*, nor for that matter that any of the Abietineae have descended from any of the Podocarpeae, or indeed any living Coniferous group from any other as we know them, but *Dacrydium* and *Pinus* may have inherited the same potentialities from the same ancestor. These considerations thus suggest that the Abietineae have sprung from the same primitive stock as the Podocarpean-Araucarian phylum, but in the respects mentioned above have developed much further from their common ancestors than the Podocarpeae, while in the formation of perfect cones they have retained a primitive character lost in the more recent Podocarpean genera, but still present in the more primitive members of the order.

The point of view taken in the preceding paragraph involves the derivation of the complicated ovulate structure of the Abietineae from a simple sporophyll. This is, however, not at all a generally accepted hypothesis, for many botanists have thought that the simple cone scale has been derived by reduction from the more complicated double structure.

The view that the single cone scale groups have been derived from ancestors essentially resembling the existing Abietineae depends for its support largely on abnormalities, and on the vascular structure of the cone scales. The abnormalities consist mostly of structures sometimes found in members of the Abietineae in which the normal cone scale is replaced by a bract bearing in its axil a structure more or less like the short shoot of *Pinus*.[1] From this it has been argued that the double cone scale of the Abietineae is to be regarded as derived from the fertile equivalent of such a brachyblast shoot, and various well-known modifications of this view have been put forward.[2] But abnormalities, especially when they are supposed to be more or less of the nature of reversions, afford by themselves unsatisfactory evidence of phylogeny. As to the evidence of the vascular supply of the cone scales, this depends on the fact that in all groups of Conifers there is inverse orientation of the ovular supply as compared with the lower series of bundles serving the sporophyll (or the lower 'bract' scale in the case of the Abietineae). In most cases the ovular supply and sporophyll supply are independent of one another from the cone axis. These facts are supposed to point to the double nature of the apparently single cone scales of the Araucarieae and Podocarpeae, the two scales as we have them in the Abietineae being very closely fused together. The opinion that the facts do not point to this conclusion in the Araucarieae and Podocarpeae

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[1] See Coulter and Chamberlain ('10), p. 245 et seq.

[2] See Worsdell ('00), p. 39.

has already been expressed by all recent workers on their cone scales,[1] and the evidence is against the double nature of the cone scale in these groups. Indeed the more primitive Podocarpeae are less like the Abietineae in respect of the cone scale than the more modern ones. There is indeed a suggestion of doubleness in *Dacrydium cupressinum* and *Podocarpus*, and here it has most clearly arisen within the group.

So we are left with the alternatives of supposing the forms with double cone scales as derived from a single-scaled ancestor, or of supposing the two lines of descent to be quite distinct. That different groups of Conifers have descended from different Pteridophytic or Pteridospermic ancestors seems to the writer to be quite impossible.

There are at present two views as to the phylogeny of the Conifers: the more popular one that regards the Conifers and Cordaitales as on the same line of descent, and the view that it is in the Lycopodialean alliance that we find the nearest approach to the ancestors of the Coniferae of to-day. Dr. Scott has made out the best case for the origin of the Araucarian alliance from Cordaitales-like ancestors, and the chief points of his argument are as follows:[2]

1. The stem structure of the Araucarieae is Cordaitalean.
2. The roots are like those of *Cordaites*.
3. The leaves are parallel-veined.
4. The staminate strobili and sporangia are like those of *Cordaites*.

Now if the Araucarieae and Podocarpeae are such nearly related groups as the writer supposes them, in considering the Cordaitalean origin of the Coniferae the Podocarpeae must be regarded as of as great importance as the Araucarieae.

Now in the first place the stem structure of the Podocarpeae, although of a simple coniferous type, is not at all Cordaitalean, except as regards the distribution of the primary vascular bundles, a point of resemblance which can be extended to many other forms. As regards the wood there is no reason why the Cordaitalean and Podocarpean types of wood should not have been derived from the same type; on the other hand, there is no evidence from this character of a connexion between the two orders.

In the second place the roots of the Podocarpeae, in so far as they have been examined, are not particularly reminiscent of the roots of the Cordaitales. It is true, indeed, that they are no more reminiscent of Lycopod roots; at least they furnish no definite evidence of a Cordaitalean origin. One interesting fact about the roots of the Podocarpeae is the possession of tubercles and mycorrhiza which have been compared by Osborn with the mycorrhiza in *Amyelon*, the root of *Cordaites*. He says

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[1] Seward and Ford ('06); Stiles ('08), p. 218; Thomson ('09^2), p. 352; Tison ('09), p. 155.

[2] Scott ('09), p. 653.

'... the Coniferae generally have an ectotropic mycorrhiza. It need not, therefore, be a matter of surprise that their Paleozoic ancestors should have had a similar relationship.'[1] This of course assumes the Cordaitalean origin of the Conifers, but when it is considered of what general occurrence among vascular plants is the presence of mycorrhiza, there seems no reason to regard its presence in two groups as any indication of relationship.

The third point, the parallel venation of the leaves, has been dealt with in a previous section of this paper.[2] It was there shown that the evidence was in favour of the primitiveness of the single-veined leaf in the Podocarpeae in particular, and the Coniferae in general, so that in this case the evidence is directly against the Cordaitales theory.

Finally, the last piece of evidence supposed to favour the Cordaitalean origin of the Conifers, that the staminate strobili and sporangia of the Araucarieae are like those of the Cordaitales, no more favours this view when the Podocarpeae are taken into consideration, than the three preceding pieces of evidence, for the strobili and sporangia of the Podocarpeae are quite unlike those of the Cordaitales. It is interesting also that Hollick and Jeffrey's fossil cone, *Strobilites microsporophorus*, which those authors think may be in some ways intermediate between Araucarieae and Abietineae, closely resembles the male cone of the Podocarpeae and is also quite unlike the staminate strobili of *Cordaites*.

Thus, if it is correct, as it seems to the author, to regard the modern coniferous groups as terminating a bunch of radiating lines of evolution from a common ancestor, it seems quite impossible to regard that ancestor as approaching at all nearly the Cordaitales, for in one group, the Podocarpeae, the more primitive members, which exhibit undoubted resemblances to other groups, Araucarieae and Abietineae, as well as exhibiting other features which must be regarded as primitive, are unlike, in almost every character, the Cordaitales as at present known.

One is therefore led to a consideration of the opposed and rather unpopular view of the Lycopodialean origin of the Conifers. This view has received strong support from Campbell[3] from a general consideration of the Conifers, and from Seward and Ford[4] as regards the Araucarieae alone.

The most important piece of evidence in support of this view is derived from a comparison of the cones of the Lycopodiales and the Coniferae. In the Lycopodiales the sporophylls are aggregated into cones, and each sporophyll bears a single sporangium approximately in its axil; in some cases it is true the sporangium arises from stem tissue, but generalizing it may be said that the characteristic of the Lycopodialean phylum is the

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[1] Osborn ('09), p. 608.

[2] p. 492.

[3] Campbell ('05), pp. 534, 604.

[4] Seward and Ford ('06), p. 385.

possession of sporophylls with the single sporangium borne medianly in relation to the sporophyll.[1]

Now in the Podocarpeae and in the Araucarieae, the relation of ovule to megasporophyll is the same. Each sporophyll bears a single sporangium medianly on its upper surface. In most cases in the Podocarpeae the sporangium in its later stages appears on the surface of the sporophyll and not in its axil, and moreover is usually more or less reversed. In every case so far investigated, however, the evidence points to the fact that the sporangium in the very young strobilus does actually arise in the axil of the sporophyll, and in an erect position; it is only later in its ontogeny that it becomes reversed and carried away from the axis. In fact, in the youngest stages, which are almost certainly more primitive than the older, the condition is identical with that in the Lycopodiales. Among the latter the genus *Spencerites* affords an interesting comparison with the Podocarpeae, for here the sporangium is borne on the upper side of the sporophyll away from the axis, and, moreover, may be regarded as reversed.

The chief evolutionary tendency in the Podocarpeae, as has already been pointed out, is reduction in the number of sporophylls in the female strobilus. What are regarded as the more primitive members of the order still, however, retain their sporophylls in cones, and so approach more nearly the majority of Lycopods than do the more specialized Podocarpeae.

The microsporophylls also are easily comparable with those of the Lycopods. The sporophylls are still aggregated into cones, but whereas in the Lycopods there is constantly a single microsporangium in the axil of each microsporophyll, in the Podocarpeae there are constantly two borne on the under side. This difference is not a very serious one. That septation of a single sporangium into more than one has taken place in other cases is more than probable. Indeed, the two sporangia forming the synangium of *Tmesipteris*, and the three sporangia in the sporangiophore of *Psilotum*, are considered by some botanists as derived in this way[2] from the single sporangium as it exists in the Lycopod, and in *Lepidostrobus Brownii* and in *Isoetes* we have cases in which this septation by means of sterilization of formerly sporogenous tissue has proceeded only partially towards completion.[3] Indeed, such a case of incipient septation may be present in the genus *Lycopodium* itself, as in *Lycopodium clavatum*.[4]

The shifting of the sporangia to the under side presents little difficulty to the view under consideration. Shifting of the position of the sporangia in regard to the sporophyll has certainly taken place in other cases. It is possible, though indeed not by any means certain, that in the Ferns the position of the sori has shifted from a marginal to a superficial one.[5]

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[1] Bower ('08), p. 326.

[2] l. c., p. 427.

[3] l. c., pp. 326, 697.

[4] Sykes ('08), p. 50, Text-fig. 7, ix.

[5] Bower ('08), p. 663.

Among the Equisetales in *Palaeostachya* the sporangiophores are found on the upper side of the sporophyll, while in *Cingularia* the sporangia are below the sporophylls. In this case the value of the analogy is perhaps lessened to some extent through the doubt which exists as to the correct reference to the Equisetales of *Cingularia*; the argument, however, still holds good if that genus really belongs to the Sphenophyllales. Moreover, if the microsporophylls and megasporophylls are actually homologous with one another, and all workers on the Podocarpeae are agreed that the female scale is actually a simple sporophyll, a shifting of the sporangium to the other side of the sporophyll must have taken place in the evolution of one or other kind of sporophyll; unless, indeed, the homosporous ancestor bore sporangia on both sides of the sporophyll, a state of affairs which, having regard to the relation of sporangia to sporophylls in all orders of the Pteridophyta, one must regard as extremely unlikely.

Nor is the possession of the seed-habit in the Conifers to be regarded as evidence against the Lycopodialean ancestry of these forms, as Professor Seward and Miss Ford have already pointed out.[1] The seed-habit had already been evolved in the Lycopodiales in Palaeozoic times, as in the cases of *Lepidocarpon* and *Miadesmia*; in the recent genus *Selaginella*, some approach to it appears in *Selaginella apus* and *S. rupestris*,[2] while one of its accompanying phenomena, the reduction of the number of spores in the megasporangium, occurs in several species.[3] There is thus abundant evidence that the potentiality of seed-production existed in this phylum as well as in the Fern phylum.

The vascular anatomy of the sporophylls affords an interesting comparison between the two orders. In the Lycopods the sporangium supply consists of a single medianly placed vascular bundle. In the living genera there is no special sporangial supply apart from this, but in some species of the genus *Lycopodium* there is evidence of the remains of a median sporangial supply given off from the upper surface of the sporophyll bundle.[4] This is the state of affairs which exists in the young state of the megasporophyll in *Saxegothaea* and *Microcachrys*;[5] later it is much modified in the former case owing to the development of ovular bundles which attach themselves laterally to the sporophyll bundle. In the more modified fructification of *Dacrydium Franklini* the central median bundle of the ovular supply is lost, and the lateral bundles alone form the ovular supply; in the species of *Podocarpus* examined these two lateral supply bundles are carried down into the axis of the strobilus independently of the sporophyll bundle. That this condition is to be accounted for by the greater relative

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[1] Seward and Ford ('06), p. 394.

[2] Lyon, F. M. ('01).

[3] Lyon, l. c., p. 138; Mitchell ('10), p. 24; Sykes and Stiles ('10), p. 529.

[4] Sykes ('08), p. 43.

[5] Thomson ('09^2), p. 349.

importance of the ovule as compared with the sporophyll has already been suggested by Mr. R. B. Thomson,[1] and by Mr. Brooks and the writer.[2]

The vascular supply of the microsporophyll of the Podocarpeae is just the same as that of the Lycopodiales as far as its distribution is concerned.

As regards vegetative organs further evidence in support of the view under discussion is derived from a consideration of the leaves. The characteristic of the leaves of living Lycopods and many of the fossil ones is their relative smallness and their possession of a single median vascular bundle. Arguments have been advanced already in the course of this paper to show that the primitive type of coniferous leaf was the common uninerved type characteristic of all Conifers except a few Araucarieae and the *Nageia* section of *Podocarpus*. Some single-veined Araucarian leaves, it is true, have probably been reduced from broader leaves,[3] but there is no evidence of this in the leaves of any of the Podocarpeae or of any other order. The slight tendency to form wider leaves with parallel veins, as exhibited in *Agathis* and *Podocarpus* § *Nageia*, may be compared with the still slighter tendency to the same thing in *Sigillaria*, where in some species (*Sigillariopsis*) the leaves were traversed by two parallel veins.[4]

The presence of leaf-gaps in the stele of the Coniferales has been regarded by some botanists as affording important evidence in the consideration of the origin of the Conifers. This line of argument was first used by Professor Jeffrey in 1902, when he inferred that the Coniferales were descended from the Fern-plexus on the ground that foliar gaps occur in the stele of both these groups, but are absent in the Lycopodiales.[5] Coulter and Chamberlain regard this argument as a very strong one, for in discussing the objections to Seward and Ford's view of the Lycopodialean origin of the Araucarieae they say: 'Perhaps the most fundamental objection is the presence in araucarians of foliar gaps and of wood indistinguishable from that of Cordaitales.'[6]

It is clear that this objection is based on the assumption that the presence or absence of leaf-gaps in the stele is a character fixed and unalterable in the phylum, an assumption for which, although it is so generally accepted as a fact, there is no supporting evidence. There certainly is evidence that in the evolution of the Lycopodialean vascular system, just as in the Cycadofilices,[7] one of the most important tendencies was the elimination of primary centripetal wood. Thus Dr. Scott remarks, 'A gradual transition may be traced from the protostelic type, through the medullated forms of *Lepidodendron* and the ribbed Sigillarias, to the smooth-barked Sigillarias, in which the ring of wood separates, more or less com-

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[1] Thomson ('09^2) p. 351.
[2] Brooks and Stiles ('10), p. 314.
[3] Seward and Ford ('06), p. 350.
[4] Arber and Thomas ('08), p. 148; Scott ('09), p. 230.
[5] Jeffrey ('02), p. 119.
[6] Coulter and Chamberlain ('10), p. 311.
[7] Scott ('02), p. 25.

pletely, into distinct bundles.'[1] In such a series we have the ancient *Lepidodendron pettycurense*,[2] with its solid xylem core, *L. selaginoides* (=*L. vasculare*),[3] where parenchyma was intermingled with the tracheides in the middle of the stele, and *L. Wunschianum*,[4] where a large pith occupied the middle of the stem, and the primary xylem was reduced to a comparatively small ring surrounding the pith. A further reduction of primary xylem is exhibited in *Sigillaria spinulosa*, by the partial division of the narrow ring of primary xylem into bundles, and ultimately in *S. Menardi* the xylem was completely split up into separate bundles, the primary parts of which were very small compared with the size of the stem, for in Brongniart's specimen the pith was about 15 mm. across, while the ring of bundles, the greater part of which was secondary wood, was only about 1 mm. in thickness.[5]

Thus in the evolution of the *Sigillaria Menardi* type of stele from the exarch protostele of the earlier Lepidodendrons, such as *L. rhodamnense* and *L. pettycurense*, the chief tendency has been towards elimination of the primary centripetal wood, which was replaced by secondary centrifugal wood. If we suppose this tendency to have full play we shall at length reach a condition in which the centripetal xylem of the stem has disappeared altogether. The stele in its primary state will then consist of a ring of bundles, each consisting of little more than the proxylem elements, the great mass of wood in the stem being secondary. Under these circumstances it is not only possible but likely that the mode of insertion of the leaf-traces will differ from their mode of insertion on a stele containing much primary centripetal wood.

Thus it appears to the writer that the presence of leaf-gaps in the stele of the Coniferales is a character that cannot be legitimately used as an argument against the derivation of this group of plants from an ancestor without leaf-gaps in the stele.

As regards the structure of the elements themselves the nature of the pitting of the tracheides furnishes an argument against the Lycopodialean ancestry of the Conifers, but here it seems that in Renault's *Sigillariopsis Descaisnei* there were pitted tracheides as well as scalariform ones,[6] though the approach to a coniferous type does not seem very near.

The presence of a double leaf-trace in the Abietineae has been looked upon as furnishing an important piece of evidence in favour of the Cordaitalean relationship of the Coniferae. But in a similar way the presence of a single leaf-trace in the Podocarpeae (except *Nageia*) may be regarded as furnishing equally important evidence in the opposite direction.

Gametophytic and embryonic structures have not been considered in

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[1] Scott ('09), p. 634.

[2] Kidston ('07), p. 207.

[3] Scott ('08), p. 146.

[4] Seward and Hill ('00), p. 907, and Seward ('10), p. 164.

[5] Scott ('08), p. 215.

[6] Renault ('79), p. 270; Scott ('08), p. 230.

this discussion, as exceedingly little is known about these structures in either Cordaitales or fossil Lycopodiales. It is worthy of note that Campbell, who is most competent to speak on this matter, says, 'In the character of the sporangium and the early development of the prothallium, *Selaginella* undoubtedly shows the closest affinity to the Spermatophytes, especially the Gymnosperms, of any Pteridophyte.'[1]

The phylogenetic importance of the presence of a suspensor in the embryo of both Lycopods and Conifers is now considerably lessened, owing to the discovery of a suspensor in some of the Ophioglossales[2] and in *Danaea*.[3]

During the last few years there has been a growing tendency to take for granted the Cordaitalean ancestry of the Coniferales. The favourable reception accorded to this view is no doubt largely due to the acceptance of the Čelakovskian or some similar idea of the brachyblast nature of the ovulate structure of the Abietineae. The Abietineae were at first the most prominently studied Conifers, and it was natural to explain the simple cone-scaled forms studied later in terms of the more complex Abietineae. Satisfactory evidence has, however, yet to be produced in favour of the more primitive nature of the double cone scale, and to the writer the evidence seems to point in the reverse direction, namely, to the primitiveness in the Coniferales of a type bearing female cones composed of aggregations of simple sporophylls, each sporophyll bearing a single erect axillary ovule. Such a type is not found to-day, the various coniferous orders having all departed more or less from it in different directions. This supposed primitive Conifer is very suggestive of the Lycopodiales, but is not reminiscent of the Cordaitales. Further discoveries may necessitate a modification or the withdrawal of this view, but the evidence at present available is, in the opinion of the writer, much in favour of the Lycopodialean ancestry of the Conifers.

xvii. SUMMARY.

1. *Vegetative Organs.* In the Podocarpeae the stem in its primary condition contains a ring of endarch collateral vascular bundles, these in some, and perhaps in all, being very small. Generally there is a resin canal in the cortex outside each vascular bundle.

Except in *Podocarpus* § *Nageia* the leaf-trace consists of a single strand. In *Podocarpus nagi* there is a triple leaf-trace.

Except in *Podocarpus* § *Nageia* there is a single median vascular bundle throughout the length of the leaf. This strand is accompanied by two lateral masses of transfusion tracheides, and usually by a single resin canal on the under or phloem side. Sometimes this single canal is replaced by three

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[1] Campbell ('05), p. 534.

[2] Lyon, H. L. ('05), p. 455; Bower ('08), p. 471; Lang ('10), p. 611.

[3] Campbell ('09), p. 691.

canals. In *Nageia* the leaf is parallel-veined, but each bundle is accompanied by transfusion tissue and a single resin canal as in the single-veined species. In the larger leaved species of the latter accessory transfusion tissue is developed.

2. *Spore-producing Members.* The male cones are very uniform structures throughout the Podocarpeae, each cone consisting of a number of usually spirally arranged sporophylls, each of which bears two microsporangia. In *Saxegothaea* the microspores are without wings; in *Microcachrys* the wings are variable in number and ill-developed; in *Phyllocladus*, *Dacrydium*, and *Podocarpus* there are usually two wings on the microspore, though cases are known in which there are three wings.

The megasporophylls are characterized by the constant relation of one median ovule to the sporophyll. There can be little doubt that the original position of this was an erect axillary one, a position retained to-day by *Pherosphaera* and probably *Phyllocladus*. In most of the genera there has been a considerable alteration from this position due to intercalary growth at the base of the sporophyll. This has resulted in *Saxegothaea* and *Microcachrys* in the inversion of the ovule, and its removal to a situation on the upper surface of the sporophyll remote from the axis. Probably correlated with the inversion of the ovule is the development of the incomplete outer integument or epimatium. In some species of *Dacrydium* the ovule has shifted its position so as to be inserted on the epimatium. In *Podocarpus* fusion of parts has taken place, the integument, epimatium, and nucellus being partly fused in *Eupodocarpus*; the fusion has proceeded further in *Nageia*, and to the greatest extent in *Dacrycarpus*. Another novelty in *Podocarpus* is the development of the ovular stalk. This may be regarded as a further development of the epimatium. In *Phyllocladus*, where the ovule has retained its erect position, there has resulted the development of a second integument which symmetrically surrounds the inner one.

Another evolutionary tendency has been towards the reduction of the female cone to a single fertile sporophyll.

3. *Gametophytes.* The female prothallus has been examined in *Podocarpus*, *Dacrydium*, and *Phyllocladus*. The archegonia occur at the apical end of the prothallus, and as far as at present known to the number of from one to four in *Phyllocladus* and *Dacrydium*, from five to ten in *Podocarpus coriaceus* and *P. macrophyllus* to as many as nineteen in *P. nagi*. Each archegonium is surrounded by a separate layer of jacket-cells, but in the species examined cases have been observed of two or more archegonia surrounded by a common jacket, and this may be the rule in *P. nagi*; a sufficient number of cases has, however, not been examined.

The male gametophyte is characterized by the development of several prothallial cells. These vary in number from two, the usual number in *Phyllocladus*, to eight, a common number in *Podocarpus*. The pollen-tubes in *Podocarpus* are remarkable for their large size and the extent of their branching between the nucellus and the prothallus. The body-cell nucleus divides into two male nuclei, only one of which is functional.

4. A short account is given of the present state of our knowledge of the Embryology, Seedling Structure, and Geographical Distribution of the Podocarpeae, and of the records of fossil plant remains referred to the order.

5. *Theoretical Considerations.* An attempt is made to show the probable lines of evolution in the Podocarpeae, and that the Podocarpeae are probably related to the Araucarieae, and, though to a much less extent, to the Abietineae. Evidence has yet to be produced before a close connexion between the Podocarpeae and Taxeae can be recognized. A consideration of the available evidence shows that there is much to be said for the view that regards the Coniferales as descendants of Palaeozoic Lycopodialean ancestors.

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EXPLANATION OF FIGURES IN PLATES XLVI–XLVIII.

Illustrating Mr. Stiles's paper on the Podocarpeae.

[Figs. 5 and 10 are from photographs by Miss E. Ramsden, and Fig. 24 is from a drawing by Miss E. J. Welsford, University of Leeds. Figs. 1–4, 6–9, and 11–14 are from photomicrographs, and Figs. 15–23 and 25–32 are from drawings made by means of a Zeiss-Abbé camera lucida.]

PLATE XLVI.

Fig. 1. *Podocarpus andinus*. Transverse section through stem in primary condition.

Fig. 2. *P. elongatus*. Transverse section through stem shortly after the commencement of secondary thickening.

Fig. 3. *P. nagi*. Transverse section through stem in primary condition.

Fig. 4. *P. nagi*. The same a little higher up, showing the three vascular bundles of the leaf-base.

Fig. 5. *P. andinus*. Branch bearing male cones.

Fig. 6. *P. andinus*. Transverse section through microsporophyll.

Fig. 7. *Saxegothaea conspicua*. Transverse section through axis of female cone.

Fig. 8. *S. conspicua*. Transverse section through ovule, showing epimatium, integument, and nucellus.

Fig. 9. *Microcachrys tetragona*. Longitudinal section through megasporophyll.

Fig. 10. *Podocarpus macrophyllus*. Branch bearing leaves and female fructification. *ov.*, ovule; *rec.*, receptacle.

Fig. 11. *P. latifolius*. Longitudinal section through young female fructification.

Fig. 12. *P. macrophyllus*. Transverse section through radicle of embryo.

Fig. 13. *P. macrophyllus*. Transverse section through cotyledons of embryo.

Fig. 14. *P. macrophyllus*. Longitudinal section through embryo.

PLATE XLVII.

Figs. 15–16. *Podocarpus andinus*. Leaf-traces in transverse section. × 200.

Fig. 17. *P. amarus*. Transverse section of primary vascular bundle in the stem. × 200.

Fig. 18. *P. nagi*. Transverse section of primary vascular bundle in the stem. × 200.

Fig. 19. *Dacrydium cupressinum*. Juvenile leaf, transverse section of vascular bundle. × 200.

Fig. 20. *D. cupressinum*. Adult leaf, transverse section of vascular bundle. *c.*, canal; *ph.*, phloem; *t.t.*, transfusion-tissue. × 200.

Fig. 21. *Podocarpus nagi*. Transverse section of concentric bundle at the base of the petiole. × 200.

Fig. 22. *Dacrydium Franklini.* Transverse section (in outline) through megasporophyll and young ovule. *s.s.*, sporophyll supply bundle; *r.*, resin-canal; *o.s.*, ovular supply bundle; *e.*, epimatium; *i.*, integument; *n.*, nucellus; *g.*, gametophyte.

Fig. 23. *Podocarpus latifolius.* Transverse section through vascular bundle of megasporophyll.

Fig. 24. *P. nagi.* Part of a stem with leaves and female fructifications. Natural size.

PLATE XLVIII.

Fig. 25. *Dacrydium cupressinum.* Longitudinal section through the apical part of the nucellus of an ovule. The upper parts of two pollen-tubes are shown containing the body-cell and several small nuclei. × 440.

Fig. 26. *D. cupressinum.* Longitudinal section through middle region of the nucellus of an ovule, showing the lower part of a pollen-tube in which the tube-nucleus has divided. × 375.

Fig. 27. *Podocarpus macrophyllus.* General view of the apical part of the nucellus, showing the depression at the top, and a single pollen-tube with body-cell and two smaller nuclei. × 96.

Fig. 28. *P. macrophyllus.* The same with several pollen-tubes. In one tube the body-cell is still undivided; in another the male nuclei are present. × 80.

Fig. 29. *P. macrophyllus.* The functional and non-functional male nuclei with three small nuclei.

Fig. 30. *P. macrophyllus.* A section through the cytoplasm surrounding the same body-cell as that shown in Fig. 29, and showing three more small nuclei.

Fig. 31. *P. macrophyllus.* Apical part of an archegonium just before fertilization. × 235.

Fig. 32. *P. macrophyllus.* Two pro-embryos, one undergoing the division from 4 to 8 nuclei, the other in the 16-nucleate stage. Only the nuclei seen in one section are shown. × 150.

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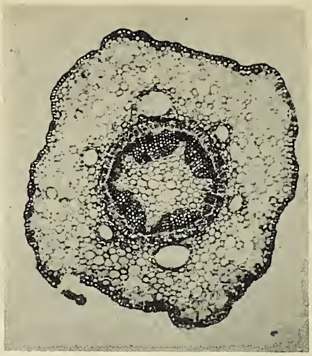

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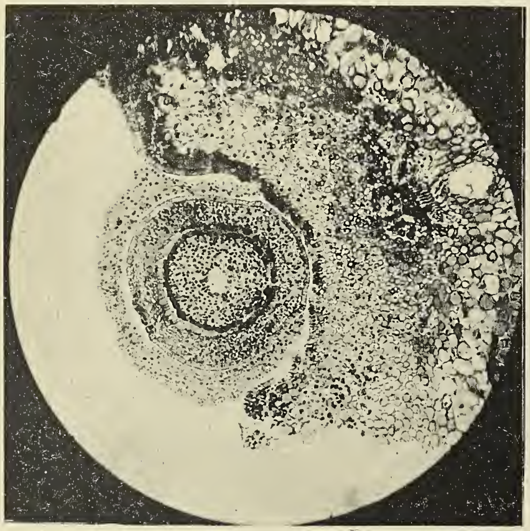

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STILES —— PODOCARPEAE.

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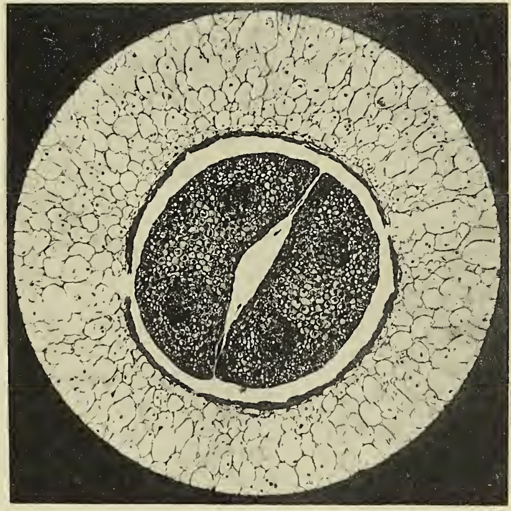

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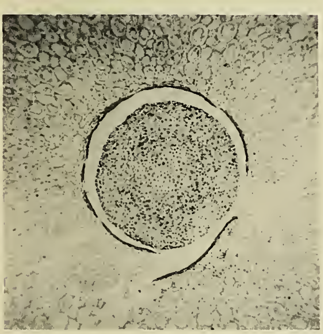

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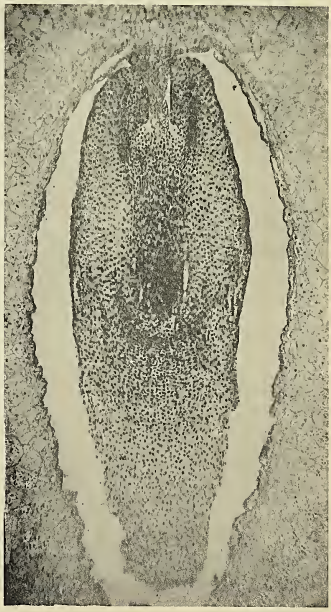

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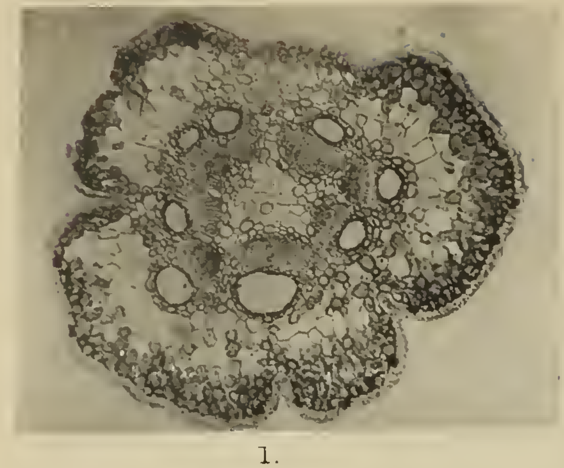
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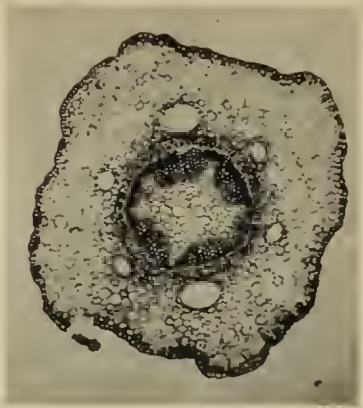
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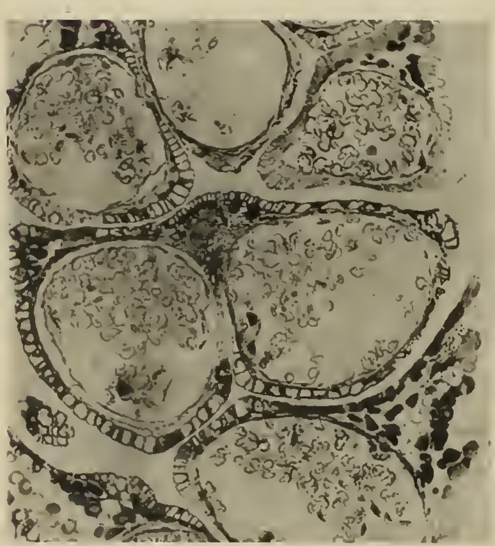
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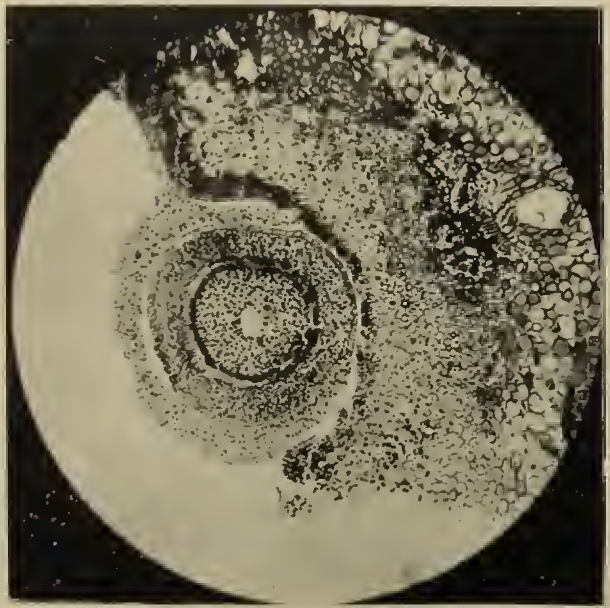
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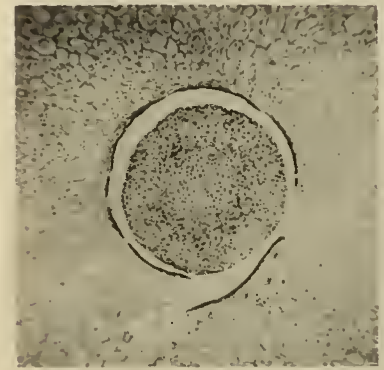
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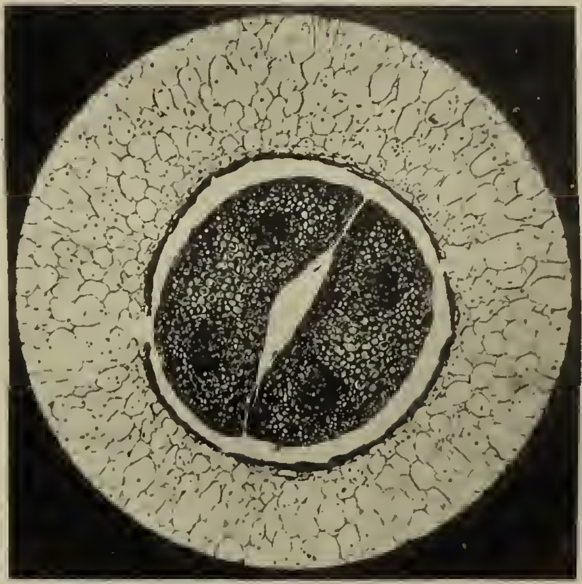
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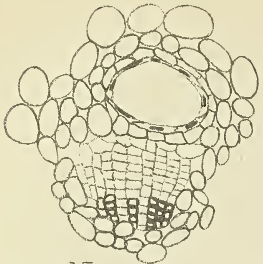

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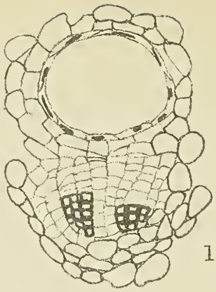

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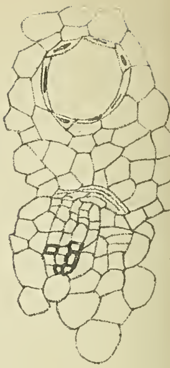

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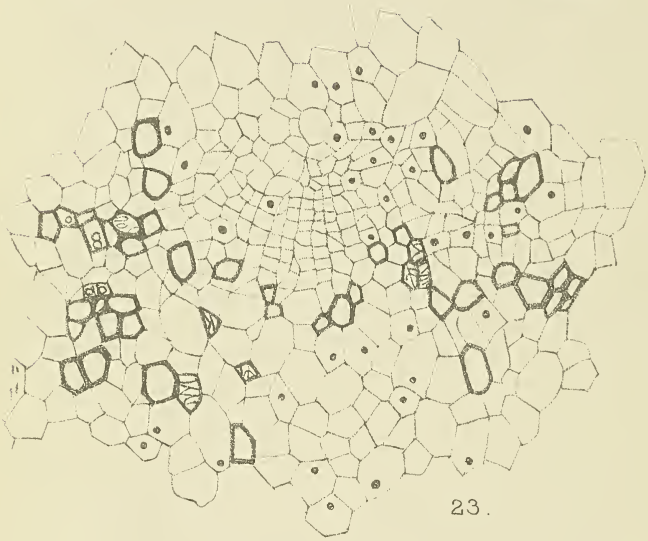

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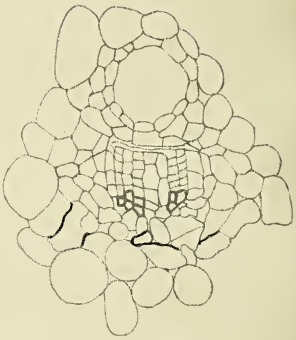

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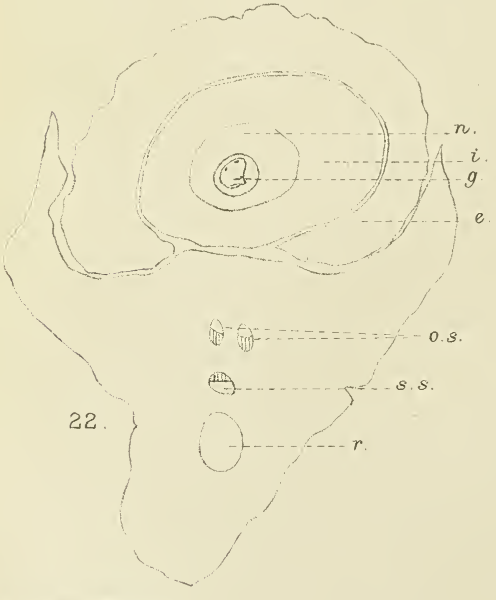

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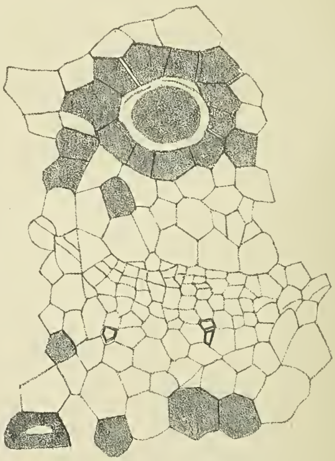

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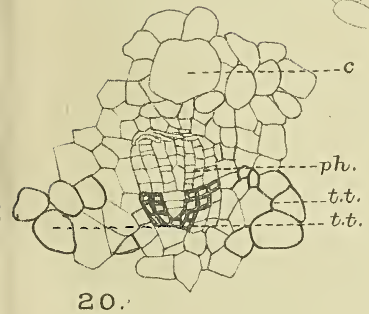

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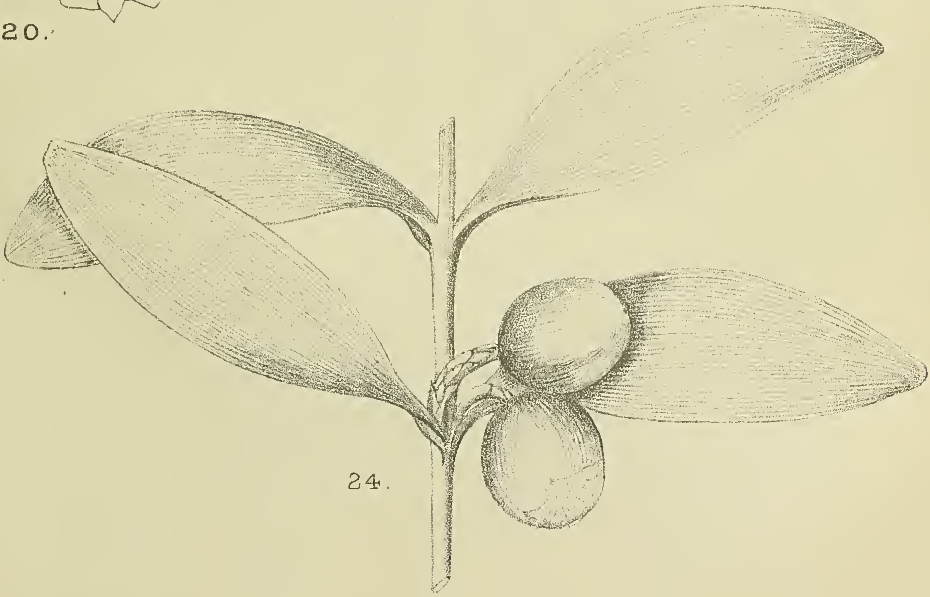

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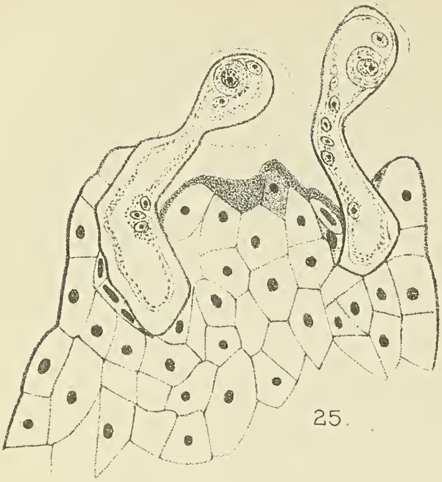
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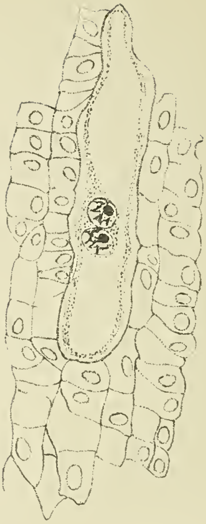
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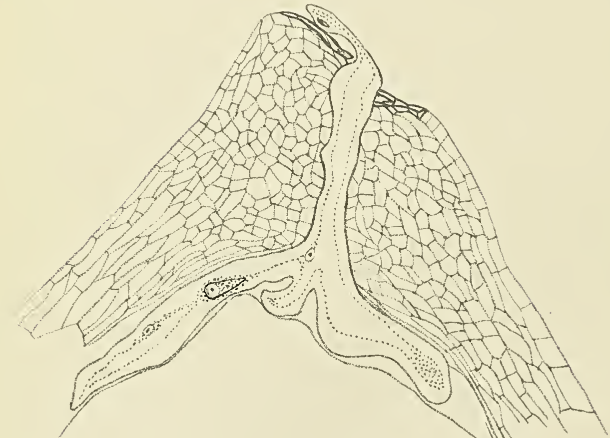
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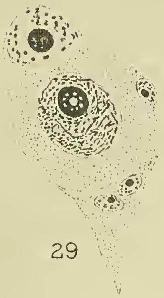

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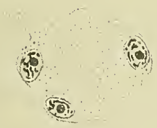

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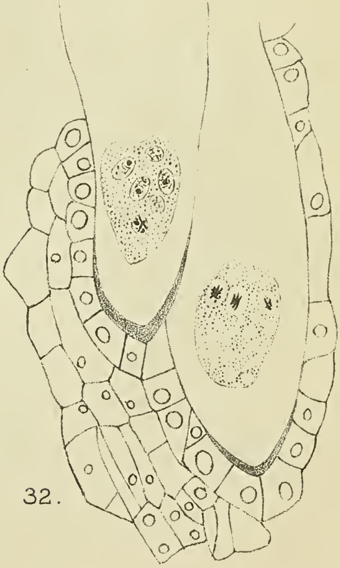

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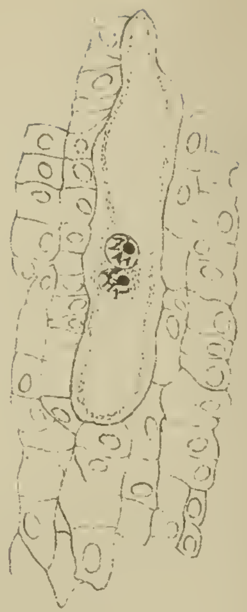

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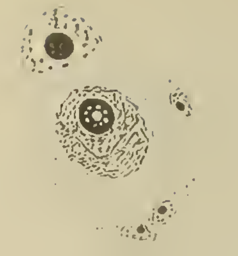

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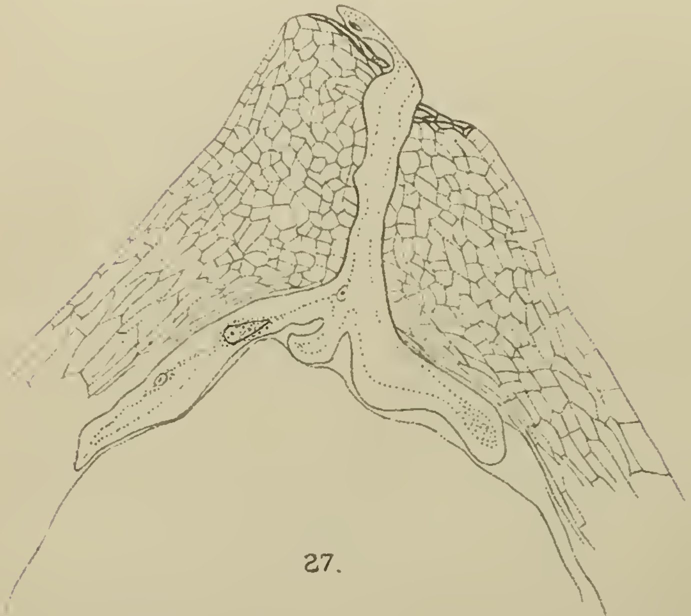

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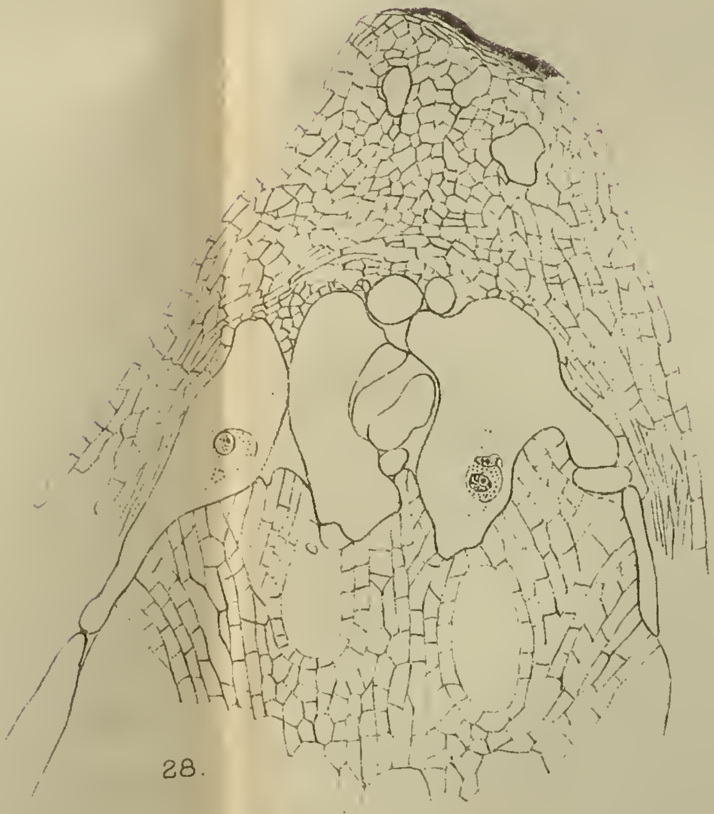

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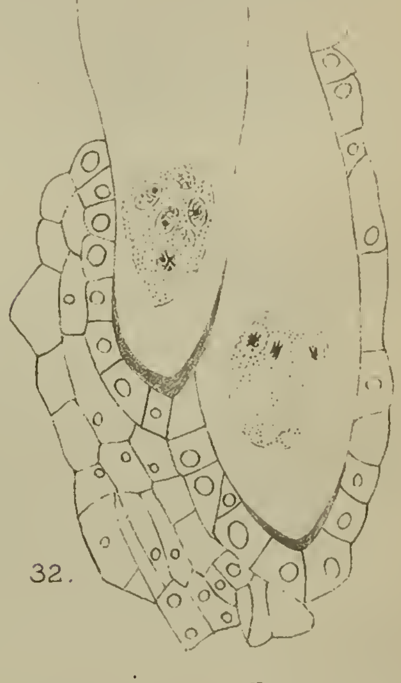

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STILES — PODOCARPEAE.

Huth lith et imp.

On the Development of the Female Strobilus in Podocarpus.

BY

L. S. GIBBS, F.L.S.

With Plates XLIX–LIII.

Introduction.

BEFORE starting for New Zealand and Fiji in 1907, Professor Farmer suggested that it would be a good opportunity for collecting material of the Podocarpoideae, in order to work out the morphology of the so-called 'epimatium', 'aril', or integuments, characteristic of this group. Very little field work soon showed that the study of dried material on which the accepted morphological conception of this family of the Conifers had for the most part been based, had led to conclusions which an investigation of fresh material might considerably modify. Opportunity was therefore sought to collect all available stages in *Phyllocladus*, *Dacrydium*, and *Podocarpus* species. On return a certain amount of work was done which gave valuable data for collecting fresh material on a subsequent expedition to North Borneo in 1909.

The results of both collections are embodied in this paper.

In the following table the list is given of the species collected, with localities and dates, and the stages of the ovules on each occasion. It is hoped this will afford some indication to future workers, as to sequence of development.

Material collected when travelling must necessarily be incomplete, and the methods can only be rough and ready. In this case it was mostly fixed in formalin, and subsequently run up in the laboratory to 75 per cent. alcohol, in which, with the addition of some glycerine, it was preserved.

For the youngest stages this process answered very well, but with the growth of the female gametophyte before fertilization the formalin does not penetrate the ovular envelope quickly enough, and a contraction of the extremely delicate tissue of the prothallus results. Dissecting out the nucellus would therefore be the only satisfactory method, but an impossible one to carry out in the field.

[Annals of Botany, Vol. XXVI. No. CII. April, 1912.]

In the species in which the seed-coat remains unmodified prior to fertilization, in the little material available, archegonia were well shown. On the other hand, in those species which develop a stony layer and show a lignification of the integument before fertilization the material, though excellent for the morphology of the strobilus, was useless for embryological detail.

There is, consequently, in this paper a gap in the sequence of the development of the female gametophyte of the different species described, viz. from the enlargement of the macrospore to the archegonium stage, just prior to fertilization. As far as one can judge from imperfect material these stages seem to agree essentially with what has been observed in other families of the Coniferae.

As soon as embryo formation begins, so much starch is laid down in the prothallus that contraction is eliminated and the stages can be followed.

It will be understood, therefore, that where a point could be substantiated in the embryological development it has been given, in the hope that it may prove of comparative value to future workers, but this investigation is primarily on the morphological side.

The systematic arrangement of the species enumerated in this paper is based on Pilger's Monograph of the Taxaceae in Engler's 'Pflanzenfamilien' series. How far the results of the present investigation modify that arrangement, in the opinion of the author, will be shown at the end of the paper.

A table of the species collected is appended, giving locality, date, and ovular development.

MATERIAL COLLECTED IN PODOCARPUS, L'Hérit.

'*Ovulum, cum carpidio connatum, carpidium ovulum apiculo obtuso superans.*'

Sect. I. DACRYCARPUS, Endl.

P. imbricata, Bl. Nadarivatu, Fiji. Sept. 1907.
Archegonial cavity (sterile).
P. imbricata. Singapore, Bot. Gardens. Dec. 1909.
Pollination (sterile).
P. imbricata. Buitenzorg Bot. Gardens. Dec. 1909.
Pollination (pollen-tube). Archegonia, resting oosphere.
P. imbricata. Kinabalu, Brit. N. Borneo. Feb. 1909.
Pollination. Archegonial cavity (sterile).
P. dacrydioides, A. Rich. Nihotupu, Auckland, New Zealand. Oct. 1907.
Pollination (pollen-grains). Female gametophyte (free nuclei).

P. dacrydioides. Te Aroha, near Auckland. Nov. 1907.
Female gametophyte (free cell formation, contracted).

'*Epimatium ovuli a carpidio liberum, ovulum carpidium parvum longe superans.*'

Sect. II. NAGEIA, Endl.

P. vitiensis, Seem. Nadarivatu, Fiji. Sept. 1907.
Nucellus. Pre-fertilization (archegonia initials, contracted).

Sect. III. STACHYCARPUS, Endl.

P. spicata, R.Br. Ohakune, N. Island, N.Z. Nov. 1907.
Pollination (pollen-tube).
Nelson, New Zealand. Dec. 1907.
Female gametophyte (free cell formation, contracted).
P. ferruginea, Don. Nihotupu, Auckland, New Zealand. Oct. 1907.
Nucellus. Female gametophyte (free cell formation, contracted).

Sect. IV. EUPODOCARPUS, Endl.

P. elata, R.Br. Nadarivatu, Fiji. Sept. 1907.
Pollination (pollen-tube). Megaspore. Young embryo.
P. polystachya, R.Br. Buitenzorg Bot. Gardens. Dec. 1909.
Pro-embryos to young embryo with cotyledons.
P. neriifolia, Don. Nadarivatu, Fiji. Sept. 1907.
Pollination (pollen tube). Pre-fertilization (female gametophyte, contracted).
P. bracteata, Bl. Buitenzorg Bot. Gardens. Dec. 1909.
Embryo (root-cap projecting through seed-coat).
P. saligna, D. Don. = *chilina*, L. C. Rich. Trewidden, Cornwall. April, 1910.
P. Totara, A. Cunn. Te Aroha, New Zealand. Nov. 1907.
Pollination (pollen-tube). Female gametophyte (free nuclei).
P. Totara. Auckland. Oct. 1907.
Pre-fertilization, bracts already swollen and coloured.
P. Hallii, Kirk. Glade House, Lake Te Ano, New Zealand. March, 1908.
Embryo, cotyledons undifferentiated.
P. nivalis, Hook. Mt. Cook, S. Island, New Zealand. Jan. 1908.
Megaspore. Embryo with cotyledons.

In the above table 'nucellus' indicates the homogeneous nucellus of pre-pollination stage, as shown in Figs. 77 and 80, Pl. LIII.

The pollination stage is indicated as 'pollen-grains' when the latter are only seen in the micropylar cavity, or resting on the nucellus, and as 'pollen-tube' when the tubes are penetrating the apex of the nucellus.

In the female gametophyte these stages would correspond to the differentiation of the megaspore and the first free cell-divisions in the enlarging prothallus.

Pre-fertilization shows the female gametophyte filled with endosperm and archegonial initials, or fully developed archegonia at the apex of the prothallus. In pre-fertilization the pollen-tubes have penetrated the stigmatic apex of the nucellus, and with the male prothallus rest over the apex of the female gametophyte (Fig. 18, Pl. L).

In the following pages, the ♀ inflorescence of *Podocarpus* is described as a fully organized strobilus, thus following Bennett and Brown (2), Sperk (50), Bertrand (3), and Tison (57). As this point of view has not previously been accepted by the generality of botanists, it is perhaps as well to give a key to homologize familiar terminology in this genus, as given by Pilger in his Monograph, with that used in this paper.

The different parts of the female flower and axis have, up to the present, always been looked upon as so distinct that even portions of the same organ exact different names.

Inflorescence. Female flower.	Strobilus.
Branch in *Dacrycarpus*, *Nageia*, and *Stachycarpus*.	Stalk in *Eupodocarpus*. Peduncle.
Scales	Scale leaves of the peduncle in *Dacrycarpus*, *Nageia*, and *Stachycarpus*.
Scale leaves . . .	Bracts.
Receptaculum . . .	Swollen bract bases in *Dacrycarpus* and *Eupodocarpus*.
Carpel	Lamina of fertile bract.
Epimatium . . .	Lamina of the ovuliferous scale.
'Stumpfer Fortsatz'	Conical apex of the ovuliferous scale (=semicircular ridge of *P. vitiensis*).

By the fertile bract is meant the bract of the strobilus in the axil of which the ovuliferous scale arises.

It must be explained that in Pilger's terminology 'receptaculum' stands for the bases of all the bracts of the cone when subsequent swelling sets in, as is the case in *Eupodocarpus* and to a lesser degree in *Dacrycarpus*.

When this secondary swelling does not take place as in *Stachycarpus* (Pl. L, Figs. 28 and 29), though the bract bases are equally well developed in the young stages (Pl. L, Figs. 25 and 27), in the maturer stages the laminae are caducous, instead of persisting as points as in the two sections above quoted; the bract bases are therefore spoken of as 'scars'.

The 'carpel' is described by Pilger as developing out of one of the scale leaves. That is to say, one portion only, i. e. the lamina, is specialized as a carpel. There is, however, no vascular connexion between that lamina and the ovular supplies, and it is difficult to see how the carpellary function obtains either when it is free as in *Eupodocarpus*, or when fused with the ovuliferous scale as in *Dacrycarpus*.

This 'carpel' or bract lamina is described as a much reduced and extraordinary small carpel; it is, however, unmodified in the youngest stage in all the sections, even when fused with the lamina of the ovuliferous scale, and does not develop with the ovule, except where there is fusion, and may even be shed entirely, as in § STACHYCARPUS, before the fertilization stage.

The 'epimatium' or ovuliferous scale is considered to be a ligula-like 'excrescence' of the 'carpel'; but here again the vascular system of the 'excrescence' bears no relation to that of the so-called carpel, but maintains complete independence, even where there is fusion of both laminae (Pl. XLIX, Figs. 8 and 9).

The 'carpel' is also served by one vascular strand only (Pl. LIII, Fig. 77) with normal orientation, whereas the 'excrescence' generally shows two (Pl. LIII, Fig. 77), and exceptionally four (Pl. LIII, Fig. 73), all with inverted orientation.

As it is usual to distinguish the sporangium-bearing member in the Coniferae as the ovuliferous scale, that name has been adhered to in this paper.

Pilger's diagnosis for each section is inserted before the description of the species which come under the same, to emphasize better the systematic position, and also for purposes of comparison.

HISTORICAL SUMMARY OF THE LITERATURE.

The literature on the structure of the strobilus or 'female flower' of *Podocarpus* is so voluminous, and the views held on the subject so divergent, that it has been thought advisable to give a summary of the principle work on the subject. It is the sum total of this work which is embodied in our knowledge and opinions of the group at the present day.

With one or two exceptions the bulk of this work has been carried out on herbarium specimens, mostly collected in localities not easily accessible and chiefly in mature condition. It is not surprising, therefore, that interpretations based on laborious work on incomplete material should have resulted in many conflicting theories.

As it is desired to restrict this investigation to *Podocarpus*, all work on the other genera in Taxaceae, where not strictly comparative, has been omitted. This position also applies to all references to the morphological value of the megasporophyll or ovuliferous scale in the Coniferae in general. The voluminous literature on that subject has been admirably summarized by Worsdell (60), and recent investigators disclaim all intention of entering into that vexed question.

The first species of *Podocarpus* known to science was *P. elongata*, L'Hér., from the Cape of Good Hope. It was described by Solander in 1788 (49, p. 415) as *Taxus elongata*, Sol.

L'Héritier, in 1807 (31, p. 580), refers this species to *Podocarpus*, giving the diagnosis as 'Nux ovata, 1-locularis, receptaculum firmo semi-immersa'.

R. Brown, in 1814 (11, p. 47), describes *Podocarpus* as a double cupula, the external one (carpel) forming the drupa, with an aperture near its base or point of insertion. The inner cupula (ovary) is entirely enclosed in the outer one and similarly inverted. He speaks of stigma, style, and fruit.

Richard, in 1826 (41), describes the female flower in this genus as anatropous, the funicle being only slightly indicated. This reversion (p. 125) is due to its being fused for its whole length with a unilateral, fleshy disc, produced at the apex, which corresponds to the base of the flower. This disc may be one of the scales of the involucre, adhering to and forming part of the flower. The pistil is enclosed in a single envelope, corresponding to the calyx, which, however, consists of two portions, an inner one, which thickens and becomes hard, and an outer fleshy one.

Blume, in 1827 (5, p. 88), gives the diagnosis for *P. bracteata*, Bl., as 'Ovarium basi squamula semicinctum. Stigma laterale, sessile'.

Bennett and Brown, in 1838 (2, p. 36), in referring to the position of the Podocarpoideae, write: 'The analogy of the scale with that which supports the seeds in the genus *Pinus* appears originally to have escaped the observation of the elder Richard. It is singular that so unessential a character as the general solitary position of the ovuliferous scale should have induced all the authors who have attempted an arrangement of the Coniferae to separate *Podocarpus* and *Dacrydium* from the true Pines and to associate them with the Yew.

'Their true position is in the Abietineae. The inverted ovula bears the same relation to the supporting scale as in the genera of that group.'

Endlicher, in 1840 (22, p. 262), considered *Podocarpus* as bearing a single anatropous ovule, inserted on a fleshy disc, but later, in 1847 (23, p. 204), he accepts R. Brown's gymnospermous theory, and describes the inflorescence as composed of fertile scales in a lax spike, one or two flowered, the subtending bracts coalescing with the fleshy rachis, their apex alone remaining free. The solitary ovule with two integuments is reversed and sessile near the apex of the fertile scale, with which it is fused in its whole length.

Payer, in 1860 (39, p. 60), describes the female flower as composed of a superior ovary surmounted by a style. This ovary is unilocular, with a naked ovule attached to the base of the cavity.

Eichler, in 1863 (19, p. 430), considered the outer investment of the female flower as an outer integument fused with the inner, and the whole flower therefore equivalent in organization to an anatropous ovule. Later, in 1875 (20, p. 62), he accepted Strasburger's 'cupula' theory.

In 1889 (21, p. 46), in the 'Pflanzenfamilien', he describes, without hesitation, (definitely) the seeds in Podocarpeae as anatropous, a funicle being indicated in *Podocarpus* only; and in this genus two integuments are also present, which are fused together.

Parlatore, in 1864 (38, p. 19), considers the ♀ 'amenta' of the Podocarpeae as presenting a similar type of organization to the Conifers. The female flower he describes as reversed and enclosed in two involucres; the inner urceolate, with a circular orifice at the apex, is formed from the upper bracts, the outer from the two subtending bracteoles, which are fused with each other and the inner involucre.

He apparently considers the nucellus as an ovary.

Favre, in 1865 (24, p. 382), pronounces the flower to be an erect anatropous ovule, with an inner and outer integument, the latter penetrated by a raphe, which terminates in an 'expansion chalazienne'. The ovule is surrounded by the two almost completely fused integuments.

Brongniart, in 1866 (9, p. 239), describes the female branch as terminated by three scales fused together for most of their length, of which one bears an erect anatropous ovule.

Carrière, in 1867 (12, p. 644), describes the ovuliferous scales of *Podocarpus* as naked, or accompanied at the base by a cymbiform bract.

The solitary fruit, inserted on the middle of the scale, or a little below the apex, is free or adnate, broader at the base, with a double integument, the exterior sometimes completely adhering (adnate) to the interior one.

The latter is prolonged in a tube which projects on the outside (' faisant saillie en dehors').

Sperk, in 1869 (50, p. 69), looks upon the structure of the 'stalk' of the flower and the scale into which it merges as partly analogous to the structure of the ovuliferous scale of the Abietineae, with which, as in *Podocarpus*, there is a fusion of the ovary with the scale, also a reversal of the ovary on all sides. On this scale the ovary is sessile and surrounded by an involucrum, which he designates as a carpel. A difference only lies in the degree of the reversion, that is to say, in the thickness of the carpel ('Fruchthülle').

This difference is explained in the structure of the inflorescence. In *Podocarpus* there is more play for unrestricted development than in the Abietineae, where the cones exercise a restricting pressure.

Braun, in 1869 (7, p. 740), was the first to point out, from a study of an abnormal development of the leaves on a branch of *P. chinensis*, Wall., grown in the Botanic Garden in Berlin, that the so-called receptaculum (Endlicher) or discus (Hooker) of the Podocarps, described as the fusion of fleshy bracts with the axis, is in reality due to the swollen bases of those bracts, which become fleshy on maturity, when the free end of each bract can still be distinguished.

Van Tieghem, in 1869 (58, p. 279), is satisfied that the 'stalk' is of foliar structure, the first leaf of an axillary bud which does not develop further.

This 'leaf' is turned towards the dorsal side to form there an anatropous ovule.

Strasburger, in 1872 (52, p. 24), sums up the Taxaceae as being characterized (with the exception possibly of *Cephalotaxus*) by the absence of true strobilus formation.

The Podocarps are distinct from the Taxaceae in that their flowers occur mostly singly, and, with the exception of *Phyllocladus*, are more or less fused with the bracts and more or less reversed.

He does not accept the outer envelope of *Podocarpus*, or the analogous structure in *Dacrydium* and *Phyllocladus*, as an integument, but looks upon it as a 'cupula', a sort of discoid proliferation of the axis of the flower.

In 1879 (53, p. 78) he describes the outer envelope in *Podocarpus* as an arillus, in preference to integument, as it certainly corresponds to the arillus of the Taxaceae. The ovule (p. 79), though resembling an anatropous ovule, cannot be described as such, because the stalk of the latter, the funicle, is always an integral part of the ovule.

In *Podocarpus*, on the contrary, the stalk, bent over in its apical portion, which bears the ovule, is really the axis of the secondary shoot, consisting of a terminal sessile ovule.

Baillon, in 1873 (1, p. 506) in a concise paper, goes thoroughly into the development of *Podocarpus sinensis*, Wall., and from the study of the organogeny entirely refutes the anatropous ovule theory.

As he succinctly states, in the ordinary course of development of an ovule with two integuments (to which, so far, no exception is known), the nucellus appears first to be followed by the inner (secondine) and then the outer (primine) integument.

In the case of *Podocarpus*, however, as he conclusively proves in a series of excellent figures, of these three portions it is the 'primine' which preexists the two other portions; the nucellus appearing next, and finally the 'envelope' which is thus interposed between the two other parts.

Sachs, in 1874 (45), describes the little flowering shoots of *Podocarpus* as consisting of a stalk-like axis, swollen above, which bears three pairs of decussate scales.

The axis terminates between the upper pair, the anatropous ovules, with the two integuments (p. 507), being borne in the axils of the second pair, which may be looked upon as carpels, if such are in any way considered necessary.

Bertrand, in 1878 (3, p. 57), describes the ovules of the Gymnosperms as always orthotropous, erect in the Gnetaceae, Taxineae, Cupressineae and *Cycas*, horizontal in the other Cycads and *Saxegothaea*, reversed in *Podocarpus*, Pineae, Araucariae, and Sequoiae.

In *Podocarpus*, he describes the fertile bract as carrying a large ovuliferous scale, which covers, like a hood, a reversed orthotropous ovule with one integument, and which is fused its whole length with the scale.

From the Abietineae to the Taxineae, through *Saxegothaea* and the Podocarps, he traces a general tendency of the ovuliferous scale to surround, in hood-like fashion, the ovule or ovules which it carries, fusing more and more till finally it merges in the ovular integument in *Torreya* and *Cephalotaxus*.

Schumann, in 1902 (46, p. 60), looks upon *Podocarpus* as not only distinguished by anatropous ovules, taking the bract at the base as a carpel, but these ovules also possess two integuments, of which it can be determined with 'positive certainty' that the second has arisen from the aril. The sequence of these conclusions is rather involved, and he himself refers to the difficulty in arriving at the same, dried material being alone available.

Worsdell, W. C., in 1900 (60, p. 42), cites *Podocarpus* as an anatropous ovule which is fused with the bract, and extends far beyond the latter, owing to its being carried up on a long stalk. It has two integuments, of which the outer is fleshy or coloured. In some species the bracts and axis are fused into a succulent whole.

Pilger, in 1903 (40, p. 2), describes the ovule of the Podocarpoideae as reversed, with the micropyle lying towards the base of the carpel, furnished with an 'epimatium', i. e. an excrescence in various ways connected with the carpel ('excrescentia carpidii vario modo connata'). The testa of the seed is formed from the integument and 'epimatium'.

He thus gets over the difficulty of the morphological nature of the protective envelope of the Podocarp seeds, by designating it an organ *sui generis*, peculiar to the genus Podocarpoideae, consequently of so much importance that the systematic sequence in this group is arranged according to its degree of development. This, through *Dacrydium*, *Microcachrys*, and *Saxegothaea*, attains its maximum in *Podocarpus*, in which family it is curved and completely fused with the integument.

He considers it incorrect to call the 'epimatium' an outer integument, and so create a contrast ('Gegensatz') to the Pinaceae, which is also the case with the Taxaceae. To describe the ovule of *Podocarpus* as anatropous is also questionable ('angängig'), as that constitutes an inadmissible ('unzulässig') comparison with the Angiosperms, necessitating a laterally fused funicle, whereas the ovule hangs direct from the 'epimatium', which surrounds it on both sides.

Pilger concludes (p. 38) that the Podocarps show greater affinity to the

Abietineae than to the Taxoideae, but defers the discussion of the relationship of the 'epimatium' to the ovuliferous scale of the Abietineae ('Ich muss aber die Discussion dieser Angelegenheiten, besonders des Verhältnisses des Epimatiums zu der Fruchtschuppe der Abietineen, einer allgemeinen Einleitung zu den Coniferen überlassen').

Thomson, 1905 (55, p. 43), looks upon the female flower of *Podocarpus* as an anatropous ovule with two well-differentiated integuments, an inner woody and an outer fleshy one.

The fertile bracts are also united in some species to form a 'receptaculum' which becomes berry-like at maturity.

Tison, in 1909 (57, p. 155), working on *Saxegothaea conspicua*, Lindl., finds that the so-called aril of earlier botanists or the 'epimatium' of Pilger is really the ovuliferous scale of the other Conifers, the summit of which is developed to protect the ovule, the coalescence of the different parts of the female cone being less than the other genera.

This reduction of the cone is carried further in *Microcachrys*, where there is less development of the fertile bract above the ovule, whose base is partially protected by the ovuliferous scale.

In the other Podocarps the reduction is much greater, there being only one or two fertile bracts which develop below the ovule, whereas the ovuliferous scale takes on a much greater development, completely encircling the ovule, thus simulating a second integument. Tison recognizes the justice of Bertrand's (3) conclusions.

This view is entirely borne out by the results of the present investigation, which had been already arrived at in 1908, in working out the Fiji and New Zealand *Podocarpus* material collected in 1907.

Coulter and Chamberlain, 1910 (18, p. 323), describe the ovule of *Podocarpus* as inverted and stipitate, arising conspicuously above the bracts.

Young, in 1910 (61), considers the cone (with the exception of *Saxegothaea* and *Microcachrys*) replaced in *Podocarpus* by the single apparently terminal ovule and a few rudimentary scales. The ovule is provided with an arillus or 'epimatium'.

Finally Brooks and Stiles, in 1910 (10), describe *Podocarpus spinulosa* as anatropous, the nucellus surrounded by two integuments, the inner one being fused for half its length with the nucellus, the outer or 'epimatium' being free from the inner at the micropylar end and on the off-side. On the other it is either not present or fused with the 'stalk'.

In a postscript is added, 'Moreover, however, only on Tison's view can it be considered as proved that the "epimatium" completely surrounds the ovule in *Podocarpus*.'

They disclaim Tison's results with emphasis.

DESCRIPTION OF SPECIES.

§ DACRYCARPUS.

Flores terminei terminales: receptaculum parvulum, verruculosum, carpidium fertile unicum, cum ovulo tota longitudine connatum, ovulum breviter apice obtuso superans.

In § DACRYCARPUS we get a very reduced strobilus, but the most highly organized in *Podocarpus* itself. The cone consists of a peduncle or 'fertile branch', clothed with scale leaves, terminated by the strobilus of two to four bracts, of which one to three of the apical ones may be fertile.

The lamina of the fertile bract or 'carpel' is fused with that of the ovuliferous scale or 'epimatium', and bears a solitary ovule, medianly placed, on its ventral surface near the apex.

In the sterile bracts the lamineae are long in comparison with those in the other sections, terete, and through not showing secondary development, remain distinct and green.

The bract bases, on the other hand, though unmodified in the early stages, subsequently swell up, fuse, and change colour, the entire surface showing verruculose swellings. Subsequent growth in the ovuliferous scale carries it well above the bract bases, and it forms with the lamina of the fertile bract the whole protective covering of the ovule, which it surrounds and enfolds completely.

P. imbricata.

Morphology. This species was first described by Blume (5, p. 89) in 1827. It is figured by Bennett and Brown (2, p. 35, t. 10) and by Blume (5, 218, t. 172, f. 2 et 172 B. f. 2). These plates give a very good idea of the foliage of the tree and its dimorphic habit, and both show the same condition as material collected at Buitenzorg in December (Pl. XLIX, Fig. 1), viz. last year's strobilus with this year's above it.

P. imbricata is a graceful forest tree, about 70 ft. high, with straight trunk and compact crown, occurring plentifully in the mixed montane forests of Fiji, and recorded for New Caledonia, New Hebrides, the Malay Archipelago, and N. Burma. Seen in the virgin forest of Java and on the sheltered slopes of Kinabalu in N. Borneo, the tree assumes a larger size and a more branching and spreading habit, with the crown not so markedly compact as observed in Fiji. It is a true mesophyllous mixed forest type, and occurs always singly. As it runs up the exposed slopes of Kinabalu, however, it is finally, in the sclerophyllous dwarf forest subsummit zone (11,500 ft.), reduced to a compact shrub 5–6 ft. high, where, associated with *Phyllocladus hypophyllus*, Hook., *Dacrydium Gibbseae*, Stapf., and *Podocarpus* *brevifolia*, Stapf., it forms one of the chief constituents of that association. There the ultimate branches are erect, the terminal portion showing the cupressoid form of leaves, but arranged radially and five-seriate.

In the mixed forest of lower altitudes the graceful young shoots are always pendulous at the ends of the branches, bearing dorsiventral cupressoid leaves, delicate and light green in colour, and biseriate in arrangement (Fig. 1, *cup. l.*). It is this drooping habit which gives such a pleasing and characteristic 'Taxodium' appearance to the tree. The youth form is characterized by the biseriate leaves, and the ultimate branches are not drooping. This youth form was seen in Fiji up to 10 ft. high, the axis of the stem and of the branches being alone clothed with the imbricate leaves. These two forms of leaf are distinct in anatomical structure, the cupressoid being characterized by groups of idioblasts in the mesophyll.

The strobili terminate small lateral peduncles, which arise in the axils of the imbricating foliage leaves of the stem. These peduncles, bearing only imbricating leaves, are curved just below the strobilus in the younger stage, but straighten later (Fig. 1, *strob.* 1 and 2). The leaves subtending the strobilus pass gradually into the bracts of the latter and spread out round it in both the Fijian (Fig. 4, *s. l.*) and Buitenzorg material (Figs. 1 and 3, *s. l.*), though not in that from the subsummit zone of Kinabalu (Fig. 7, *s. l.*).

The strobilus, as far as seen, may consist of two to four bracts (Figs. 4, 5, and 7) of no specific arrangement; the laminae of these bracts are long and terete, the bases swollen and verruculose.

Pilger (40, p. 15) describes the 'receptaculum' in this species as formed of two bract bases, of which the lamina of one is fertile. Where there are two bracts to the strobilus, they are apparently opposite (Figs. 3, 7, and 8); and usually one is fertile, as Pilger describes, but twice in the limited material collected both have been found fertile (Fig. 2), and Blume (6) describes two, and rare cases of three being fertile. In the present case (Fig. 2) the two fertile bracts oppose a dorsal to a ventral surface, instead of a ventral to a ventral surface.

This suggests that the opposite position is one of convenience, due to the exigencies of packing, and that the bracts really continue the spiral arrangement (Fig. 4) of the scale leaves of the peduncle, as these show a gradual transition from the small leaves clothing the peduncle to the larger ones immediately subtending the strobilus (Figs. 3 and 7). This transition is not so marked in later stages (Fig. 4) as the relative difference in size is masked by the growth of the strobilus; but it shows a resemblance to *Saxegothaea* as figured by Norén (36) and Tison (57, Pl. IX, Fig. 7).

The lamina of the fertile bract is fused with the ovuliferous scale (Figs. 6 and 8) to the apex of the latter. The apex of the lamina in the very young stage is quite free and distinct (Figs, 2, 7, and 8). In one case it projected far above the scale (Fig. 3, *lam. f. br.*). In later stages it may persist as a point (Fig. 4, *lam. f. br.*) or become quite merged with the tissue of the ovuliferous scale (Fig. 6, *lam. f. br.*).

Histology. In a longitudinal section of a strobilus in the gametophyte stage (Fig. 8) the lamina of the bract is seen to have the apex quite distinct with two lines of stomata. A single normally orientated vascular bundle, starting from a plate of tracheides under the epidermal tissue, runs down the whole length of the fertile bract to the swollen base. It is accompanied by a resin canal with well-developed epithelium (Fig. 8 *c*, *epi.*) on the dorsal side. A well-marked cuticularized epidermis with a hypodermal sclerotic layer (Fig. 8 *a*, *h. f.*) covers the lamina of the bract, and is continued uninterruptedly round that of the ovuliferous scale.

This sclerotic layer is also present in the foliage leaves of both cupressoid and imbricate forms, being only interrupted by the stomata, which occur in four rows, two on the ventral and two on the dorsal faces, and seems limited in *Podocarpus* to § DACRYCARPUS. Thomson (56, p. 349) records a hypodermal sclerotic layer for the megasporophyll and the vegetative leaf of *Microcachrys*.

The swollen base of the bract is limited by the verruculose outgrowths (Fig. 8 *b*) which densely cover this portion. They may also characterize the base of the ovuliferous scale (Fig. 5), which is modified like the bract bases, fusing with them completely. This figure is taken from a strobilus on a tree in the Singapore Botanic Gardens, which was covered with young cones in December. Mr. Ridley informed me that this tree never set seed. The ovules proved all sterile, showing no proper nucellus formation. The epidermal layer of these bract bases is cuticularized, and the cells show dense tannin contents like those of the lamina, but there is no hypodermal sclerenchyma. The other cells are thin-walled, large, and practically devoid of contents. They suggest water-storage tissue, and this may possibly be the function of the so-called swollen 'receptacle' throughout the genus. This tissue is well developed, as we have seen, in post-pollination (Figs. 2, 3, 4, and 7), and increases in size with the growth of the ovule, the increase being due to proliferation of the mesophyll cells.

The structure of the sterile bract resembles that of the fertile. The lamina shows a cuticularized epidermis and hypodermal fibrous layer, and the vascular bundle terminates in a group of tracheides and is accompanied by a resin canal with epithelium. The swollen base is equally verruculose. These swellings are characteristic of § DACRYCARPUS and possibly increase the surface of the water-storage tissue, which is much less developed in this section than in § EUPODOCARPUS, and is entirely absent in § NAGEIA and § STACHYCARPUS.

The ovuliferous scale completely encloses the ovule, and is fused with the integument throughout its whole length, except in the region of the micropyle. It shows the same structure in its epidermal, hypodermal, and mesophyll tissues as the lamina of the bract. Most of the cells of the mesophyll show tannin and associated starch contents, and many also are packed with starch.

The vascular system consists of two bundles with inverse orientation, each accompanied by a resin canal with epithelium. They unite to form a ring at the base of the ovule, but separate again and remain distinct to the base of the bracts, where the bundles run with normal orientation on to the ring of leaf-traces of the strobilus.

This converse orientation is effected by the rotation of the two bundles as they descend the scale, where they form an arc, the phloem gradually diverging outwards and the xylem converging towards the centre (Pl. LIII, Fig. 77).

This rotation increases in the passage down the bract bases, until the two bundles run on the ring of collateral bundles of the strobilus, one on each side of the strand of the fertile bract, with normal orientation. The ovular supply bundles in *Saxegothaea*, as figured by Tison (57, Pl. X, D), insert themselves with a similar semi-rotation on to the single vascular strand of the fertile bract, but in *Podocarpus* the bundles remain distinct.

The *integument* stands out well from the tissue of the ovuliferous scale, owing to its smaller and more regularly arranged cells. At its free portion, which forms the micropyle (Pl. XLIX, Fig. 8, *o. s. int.*), the epidermal layer of cells is densely filled with starch and tannin. In the free apex at the micropyle the sub-epidermal cells are strongly lignified, staining with safranin, are pitted, showing spiral thickening, and elongated in a vertical direction (Pl. LI, Fig. 58, *a.*). This feature is characteristic of all the species examined. The base of the integument is well defined by a zone of nutritive cells. Of these cells some are packed with starch, while others contain both starch and tannin, the starch grains showing up conspicuously with iodine, by their blue coloration, through the tannin (Pl. XLIX, Fig. 8, *tan.* and *st. c.*). A similar zone occurs at the base of the nucellus, a fact Tison has also recorded for *Saxegothaea* (57, p. 144), designating them 'cellules tannifères'; but he does not specify the presence of starch. These cells appear to be initiated at the same stage in both genera, viz. at pollination, with the enlarging macrospore; but in *Podocarpus*, in conjunction with the zone at the base of the integument, their number increases with the growth of the female gametophyte until starch formation is initiated in the prothallus. Saxton (44, p. 559) in *Callitris* mentions, in relation to the absence of any kind of tapetum in that genus, that 'physiologically its place is taken possibly by the basal part of the nucellus, the cells of which are densely packed with contents'. He further adds that growth of the nucellus takes place mainly in this basal part. This also holds for both integument and nucellus in *Podocarpus*.

The *nucellus* is much contracted in all material at this stage (Fig. 8, *nuc.*), but pollen-tubes can be traced in the apex, whose development, judging from other material, would coincide with free nuclear division in the female gametophyte.

In Fig. 6 a longitudinal section of the ovule in the archegonium stage shows the lamina of the fertile bract no longer so distinct, it having elongated and stretched with the growth of the ovuliferous scale ; it is served by one bundle with normal orientation arising from a hypodermal group of tracheides. The mesophyll cells show lignified thickening of the cell-walls, reacting to phloroglucin (Fig. 6 *c*). This lignification is also seen in the mesophyll of the ovuliferous scale, in which the two vascular bundles, on expanding in a ring round the base of the ovule, send secondary branches into the lamina of the scale. This development, which marks a later stage than shown in Fig. 8, extends half-way down the lamina which surrounds the ovular tissues (Fig. 6, *in. v. b. o.s.*).

The nutritive cells of the *integument* and *nucellus* show considerable increase in number (Fig. 6, *tan.* and *st. c.*) and the micropyle is closed, a result effected by proliferation of tissue in the basal portion of the ovuliferous scale and the elongation of the lamina of the same. The integument shows slight peripheral lignification of the cell-walls.

The cells of the stigmatic apex of the *nucellus* show dense starch contents. Stopes and Fujii (51, p. 11) note in *Pinus* the deposition of starch in considerable quantities in the tip of the nucellus, in very young ovules in which archegonia were not present. This is also the case in *Podocarpus* (Fig. 6, *nuc. st.*). Starch is initiated with the formation of the macrospore, and its presence is no doubt related to the growth of the pollen-tubes, which, however, do not absorb it entirely, as some is generally present even when the nucellus itself is reduced to this apical portion, which persists as a cap to the prothallus (Pl. L, Fig. 38, *nuc. cap.*). The periphery of the nucellus is marked by large cells with very little contents, whereas the two or three parietal layers of smaller tabular cells which surround the female gametophyte, and are constantly being absorbed by the growth of the latter, show large and active nuclei and denser staining contents, forming a well-marked tapetum.

Coker (15, p. 103) in *P. coriacea* records the complete absence of any tapetum and ‘ spongy layer ’, but in the present investigation it was found in every species from the earliest stages till the laying down of starch in the prothallus and in embryo development.

The female gametophyte has increased at the expense of the nucellus, and in this stage contains five archegonia. These are situated at the apex of the prothallus, and are very long and attenuated, each being surrounded by a well-marked jacket layer of denser staining cells with large and active nuclei. Archegonia are separated from one another by sterile prothallial tissue (Pl. XLIX, Fig. 6, *arch.*), and are long and tapering, similar in shape to those described by Lawson in *Sciadopitys verticillata* (30, p. 413), and in *Cephalotaxus Fortunei* by Coker (17, p. 5). All the five archegonia are in the maturation stage, when according to Blackman (4, p. 404) the uniformly reticulated nucleus increases enormously in size and moves into the centre of the egg (Fig. 6 *a*, *e.n.*) Lawson (30, p. 44) describes the same result in *Sciadopitys verticillata*, where the egg nucleus enlarges enormously and moves down to the centre of the cell. The protoplasm of the archegonia is much vacuolated, and contains densely staining proteid bodies.

As far as could be ascertained in the scanty material available the neck cells (Fig. 6 *a*, *n.c.*) consist of two to six cells. Coker (15, p. 98) gives them as from two to twenty-five in *P. coriacea*. Below the archegonia there is a central cylinder of small-celled tissue (Fig. 6, *cen. cyl.*) which Coker has also described for *P. coriacea* (l. c., p. 96). The cells of the prothallus are mostly binucleate. Saxton (36, p. 175) records in *Widdringtonia* that this binucleate condition in the prothallus is initiated on the maturation of the oosphere. The cells increase in size towards the periphery, which is limited by an epidermal layer of small uninucleate cells with denser staining contents. This modified epidermal layer, which remains almost free from starch, is characteristic of all species examined (Pl. LI, Fig. 39 *b*). Coker (15, p. 97) has described a similar layer for *P. coriacea*, and suggests that it is modified for secretion and correlated with the absence of a spongy layer in that species; but in all the *Podocarpus* material examined in the present instance, well-marked 'spongy tissue' or tapetum was invariably present (Pl. LIII, Figs. 74 and 75) in conjunction with the differentiated epidermal layer. Pl. XLIX, Fig. 6 shows the prothallus contracted on one side, due to imperfect fixation, the tissue being so very delicate before starch formation is well under way. Contraction is also seen between the necks of the archegonia, where the tissue often becomes folded by pressure and projects, forming a point at the apex of the gametophyte (Pl. L, Fig. 37). This contraction, in microtome sections, often gives the appearance of the archegonia lying in pits.

The prothallus is surrounded by a delicate two-layered megaspore membrane, of which the fibrous layer turns yellow both with iodine and chloro-zinc iodine. The whole membrane can be dissected off the prothallus in its entirety.

In every species examined a megaspore membrane was found to be present. This membrane is conspicuous in microtome sections, taking a mauve-red colour in gentian violet and Bismarck brown (Pl. LIII, Figs. 74 and 75), and staining red in safranin when dissected off. It resists the action of iodine and sulphuric acid, but in every case responded to the iron sulphate test for tannin.

Coker (15) records no megaspore membrane for *P. coriacea.* Thomson (55, p. 42) in *P. coriacea* remarks on the absence of this membrane in the early embryo stage, and also in *P. Mokoyi* in mature condition, and questions if the coat is present in the earliest stages. He concludes that *Dacrydium*, in which the megaspore membrane is well developed, must be much more primitive than *Podocarpus* from the supposed absence of this feature of the latter (l. c., p. 54). In the present case it has been seen in normal development in every species examined, which suggests that *P. coriacea* is an exception in the order in this respect, as it is also in the absence of a tapetum.

The one prothallus available from Fijian material, dissected out of a normally developed strobilus, proved to be sterile. A cavity immediately above the small-celled central cylinder replaced the archegonia. No tracheides occurred in this tissue, in which the cells were two to four nucleate. The larger peripheral cells were uni- to binucleate and contained very few starch grains. There were, however, many tannin cells, which was not the case in the one described above, from Buitenzorg.

A sterile prothallus from the subsummit zone of Kinabalu (11,500 ft.), dark brown in colour on the surface, showed on sectioning almost every cell with tannin contents, the central cylinder being alone excepted.

In another prothallus, of which the name of the species was lost, seven archegonia were present, two being in one jacket (Fig. 6 *b*). In this case there was no contraction of tissue, and the archegonia lay with their necks plane to the apical surface of the prothallus in two distinct groups.

Podocarpus dacrydioides.

Morphology. This species, familiarly called Kahikatea or White Pine, is a well-known New Zealand tree. In this list it is the only species which grows gregariously, forming dense forests in swampy localities, and predominating along river beds, where, before the usual drastic clearing on so-called 'settlement', it must have been a valuable agent in fixing shingle banks, which now spread unrestricted over miles of country. In the mixed forest it takes its chance with the other members of an association, in which no one species seems able to prevail. It is a slender tree, with the straight trunk and the small conical crown so characteristic of the New Zealand mixed forest type. According to Cheeseman (14, p. 651) the tree may attain the height of 150 feet, and he describes the wood as straight-grained and easily worked, but not durable if exposed to damp. It is general in lowland forest through the three islands.

The young strobili are very abundantly produced in October and November. They are minute, but conspicuous from the waxy bloom which completely covers them, and are borne on peduncles about 5 mm. long, which may either terminate a shoot, as Tison figures for *Saxegothaea* (57, Pl. IX, Fig. 6), or they may form little lateral branches, as in *P. imbricata.* The peduncle is clothed with imbricating scale leaves (Fig. 10, *s. l.*), of which those immediately subtending the strobilus are larger and more spreading (Figs. 10 and 11, *s. l.*).

The bracts may be two (Fig. 11, *brs.*) or three (Figs. 10 and 12), with long terete free laminae, which remain erect or open out. The swollen bases are verruculose as in *P. imbricata.* There is one fertile bract, the lamina of which is fused with the ovuliferous scale to the point of insertion of the ovule (Figs. 9, 10, and 13). The extreme apex of the lamina is more or less free in the younger stages (Figs. 9 and 10), but becomes completely fused as growth continues (Figs. 12 and 13). Fig. 10 is in the pollination stage.

Histology. A longitudinal section of the ovule shows the lamina of the bract with its normally orientated vascular bundle ending in a group of tracheides (Fig. 9, *v. b. br.*). The epidermis and hypodermal sclerotic layer is continuous with that of the lamina of the ovuliferous scale as in *P. imbricata.* The hypodermal layer, as in that species, also characterizes the foliage leaves, where it is only interrupted under the stomata which occur in four lines, two on each surface of the leaf. This layer is recorded by Van Tieghem (59) for the leaves of both these species. The inverted vascular supply of the ovule runs down the ovuliferous scale in two bundles, as previously described, forming a ring round the base of the nucellus (Figs. 9 and 13), but in *P. dacrydioides* they were not seen to send branches into the lamina of the scale. These bundles run separately, as in *P. imbricata*, to the base of the bracts, gradually rotating as they descend, till they insert themselves normally on to the ring of bundles of the strobilus. They are each accompanied by a resin canal with epithelium. These canals, in the region of the ovule, limit the tissue of the integument from that of the ovuliferous scale (Figs. 9 and 13, *r. c.*).

Pilger (40, p. 23) describes the upper portion of the integument as being very thin in this species and the large resin canals as lying in the outer seed-coat ('äussere Samenschicht'), presumably the 'epimatium', i. e. lamina of the ovuliferous scale. In the younger stages (Figs. 9 and 13, *r. c.*) it will be seen that there is nothing abnormal in the texture or size of the integument or the position of the resin canals, which are, as Pilger states, very large in this species.

The *integument* shows the usual differentiation of cells, those in the region of the base being marked by starch and tannin contents which also characterize the epidermis of the free apex at the micropyle (Pl. LIII, Figs. 77 and 78).

The nutritive zone of tannin cells is present at the base of the nucellus, and the stigmatic surface of the apex shows pollen-grains lodged on it, and the cells dense starch contents.

The megaspore cell divides about the time of pollination, as described by Miyake in *Cunninghamia* (34) and by Miyake and Yasui in *Pseudolarix* (35). It is limited by the slightly cuticularized megaspore membrane (Pl. XLIX, Fig. 9, *m. m.*) and shows nuclear division. The cells of the nucellus surrounding the female gametophyte form two or three tapetal layers of smaller rectangular cells with denser contents and more active nuclei, which are constantly being absorbed by the growing megaspore (Fig. 9, *tap.*). In an older stage (Fig. 13) where the female gametophyte is much enlarged, filling the centre of the nucellus, the former is too contracted to make out the exact stage, but no cell-walls can be traced in the endosperm, which is definitely outlined by the megaspore membrane. Starch and tannin are present in the cells of the stigmatic apex of the nucellus. The tapetum, showing disintegration of cells, limits the cavity formed by the prothallus. The usual nutritive region of tannin and starch cells is seen at the base of the nucellus and of the integument (Fig. 13, *tan.* and *st. c.*).

§ NAGEIA.

In § NAGEIA and § STACHYCARPUS, in the species described, the cone consists of a peduncle, or so-called 'fertile branch', clothed with scale leaves, and succeeded by six to eight strap-shaped bracts, of fleshy consistency, with their long bases fused with the axis.

The apparent opposite and decussate arrangement of the younger stages is subsequently lost on elongation of the axis, as the bract laminae are shed before fertilization, leaving only scars which show a spiral sequence.

The strobilus organization is therefore limited to the younger stages; there is no secondary modification of the bract bases, so that the 'receptaculum' is described as not being developed.

P. vitiensis.

P. vitiensis was provisionally placed in § NAGEIA by Pilger (40, p. 59) on the strength of its vegetative leaves being opposite and broad towards the base. He admits that the leaves are narrower than those of the other species in this section, also that the ♂ flowers do not agree. The ♀ flowers were unknown to him and are now described for the first time.

In a former paper (26, p. 182) the position of this species in § NAGEIA is questioned, and its inclusion in § STACHYCARPUS suggested, from the similarity in habit and in the position of the ♂ and ♀ flowers to *P. ferruginea*. On anatomical investigation the leaves in *P. vitiensis* were found to possess one vein, whereas it is one of the distinguishing features of § NAGEIA that in that section alone the leaves are pluri-veined (40, p. 5).

In the strobilus, however, the stone cells, which according to Pilger

(l. c., p. 23) are peculiar to § STACHYCARPUS (Pl. LIII, Fig. 75) in the seed-coat, proved absent in *P. vitiensis.*

The peduncle of *P. nagi*, as figured by Pilger (40, p. 61, Fig. D) and in material examined at the British Museum, is thick and shows scars, suggesting a resemblance to older stages of *P. vitiensis* (Pl. XLIX, Fig. 16), but it is difficult in this group to draw conclusions from dried or mature material.

The branching of the peduncle, so accentuated in the Fijian plant (Fig. 14), is also to a certain degree represented in § NAGEIA.

It is therefore thought advisable to follow Pilger, and leave *P. vitiensis* provisionally in § NAGEIA, though the very characteristic branching of the peduncle (Fig. 14), the four vascular bundles of the ovuliferous scale (Pl. LIII, Fig. 73), and the semicircular ridge which terminates it (Pl. XLIX, Fig. 16, *o. s.*), together with the beak-like prolongation of the nucellus into the micropyle, are features which seem to distinguish it from all the other sections (Pl. L, Fig. 18, *nuc.*).

Morphology. This species, the Dakua Salu Salu of the Fijians, is the most beautiful forest tree of that country, or, in the writer's estimation, of the Conifers of the world. Moreover, the wood is the most valuable of the Fijian timbers, being not over hard and very durable. The magnificent symmetry of this species is striking. The splendid shaft, clothed in smooth white bark, rises sheer through the surrounding forest to expand in a crown of spreading branches, of which the ultimate shoots bear leaves so evenly arranged that they suggest pinnules on the frond of a huge fern. These leaves are light green in colour, coriaceous and shining in texture.

The strobili are borne on branching peduncles which arise on the axils of the lower leaves of the shoot in great profusion. The frond-like habit of the branches exposes the strobili, which are conspicuous from their bright magenta colour and glaucous bloom.

This colour, according to Cheeseman (14, p. 650), is also characteristic of *P. ferruginea.*

These strobili were collected from one tree, and show two stages, as already described for *P. imbricata* (see p. 525). The current year's cone shows the ovule in the unmodified nucellus or pre-pollination stage (Fig. 15), which can be compared with the longitudinal section given for *P. ferruginea*, the structure being identical in both cases (Pl. LIII, Fig. 77). These cones were found at the apex of the axis on the youngest wood, on which the leaves had not yet expanded.

In a few cases ovules in the dividing macrospore stage were found on the same axis as the older cones, as figured for *P. imbricata* (Pl. XLIX, Fig. 1, *strob.* 1 and 2). These older cones (Fig. 16) occur in the axils of the lower leaves of the secondary branches of the immediately older wood (Fig. 14, *strob.*). Whether this wood is last year's or not it would be impossible to decide from a single observation on a tropical tree of which no data for judging the rate of growth are available. In the older cones the ovules show the prothallus contracted in every case. These ovules are in the pre-fertilization stage, as archegonia can be traced in the contracted prothallial tissue, and the pollen-tubes have completely penetrated the apex of the nucellus.

In *Podocarpus*, in all sections except § DACRYCARPUS, the 'epimatium' (lamina of the ovuliferous scale) is described (40, p. 18) as being free from the short fleshy point of the 'carpel' (lamina of the fertile bract), which the ovule greatly exceeds.

The 'carpels' are described by Pilger (40, p. 13) as extremely rudimentary, and of the scale-like leaves which in their entirety represent the 'female flower' of the Podocarpoideae (meist zu einem Teil fertil als Carpiden entwickelt) generally only one is fertile and can function as a 'carpel'. In § NAGEIA in some species a 'receptaculum' (l. c., p. 15) is developed, in others not. When there is no development the stalk of the 'receptaculum' is not naked but bears rudiments of scale leaves, while the scales (Schuppen) of the 'receptaculum' are not decussate, being present in greater numbers in spiral sequence (l. c., p. 16).

In *P. vitiensis* the peduncle is covered with imbricating scale leaves as described in the two former species, which do not appear 'rudimentary' when examined in this early stage. The strobilus consists of six to ten bracts (Fig. 15, *strob.*) which show the same spreading laminae and elongated bases fused with the axis of the peduncle general in the order. The terminal, or sometimes the two terminal bracts (Fig. 16, *f. br.*) are fertile. These bracts are sub-opposite and the laminae show a protective function in the young stage (Fig. 15, *o. s.*), folding over the fertile bract and the ovuliferous scale, and opening out later (Fig. 16, *s. br.*). To describe these bracts as scales is hardly logical, as their organization is entirely similar to that of the foliage leaf; also the spiral sequence of the scars of the caducous bract laminae seen on the mature peduncle may be due to subsequent development during the elongation of the axis. In this species the laminae of the bracts persist till the fertilization stage (Fig. 16, *f. br.*). A bract can hardly be described as a rudimentary scale merely because the lamina is caducous, the scars alone being present at maturity. This fact would rather point to an advance in economical organization, a useless member being shed when no longer required.

In Fig. 15 the lamina of the fertile bract encloses the ovuliferous scale (*f. br.* and *o. s.*) with ovule, reaching to the apex of the same. In longitudinal section of *P. ferruginea* in the same stage, the structure of which, with the exception of the broadened apex, is identical with that obtaining in *P. vitiensis*, the lamina of the fertile bract is seen to be perfectly free from that of the scale (Pl. LIII, Fig. 76). In Fig. 77 in transverse section the base of the bract is shown coalescing with the base of the scale, but, as in § DACRYCARPUS, there is no fusion of bundles; both vascular systems remain distinct till they are separately inserted on the strobilus system. It is therefore obvious that one cannot describe the fertile bract properly as a 'carpel', considering that there is no vascular connexion with the ovular tissues.

In the older or pre-fertilization stage the ovuliferous scale has grown well above the bract (Fig. 16, *o. s.* and *f. br.*).

The scale or 'epimatium' can, however, hardly be described as greatly exceeding the 'carpel', nor can we speak of these 'carpels' as extremely rudimentary. They are, on the contrary, well-developed bracts, showing a transitory strobilus organization instead of a permanent one as in *Saxegothaea*. This organization is for the development and protection of the ovule till the ovuliferous scale can assume independent function.

The peculiar semicircular ridge formed by the apical portion of the ovuliferous scale is very prominent. Sometimes it projects in two or three points (Fig. 14, *o. s.*), but this effect is no doubt due to contraction of tissue. More than half the ovules sectioned were found to be full of tiny insect eggs, which replaced the integument.

Histology. In longitudinal section of the pre-fertilization stage the lamina of the fertile bract is now very inconspicuous in relation to the well-developed ovuliferous scale (Pl. L, Fig. 17, *lam. f. br.*).

The *ovuliferous scale* shows the same foliar structure as in § DACRYCARPUS. There is a cuticularized epidermis and well-developed mesophyll with many tannin cells, but as yet no lignified tissue in the scale which reacts to phloroglucin.

In transverse section of the dividing megaspore stage, taken through the centre of the nucellus, eight bundles are shown in the adherent lamina of the ovuliferous scale (Pl. LIII, Fig. 72). The vascular system of the ovuliferous scale consists of four distinct bundles with inverted orientation and accompanied by four resin canals, which arise in the apex of the scale, and form a ring-like expansion at the base of the ovule, where each bundle divides again, and the eight branches run down the adherent lamina of the ovuliferous scale surrounding the ovule.

The epidermal tannin layer of the micropylar apex of the *integument* is well shown, also the tanniferous nutritive zone at the base. A similar layer limits the nucellus, and phloem strands show on each side penetrating both zones of tannin cells. Surrounding the megaspore is a tapetum of several layers of cells.

In Fig. 73 a transverse section of the same series, taken through the micropyle, shows the four main bundles, which have again approached each other, on the axial side of the scale towards the base of the latter. On the opposite side the apical portion of the integument is seen, still limited by the zone of tannin cells. In the lamina of the ovuliferous scale which surrounds the integument, the resin canals belonging to the ring of vascular bundles shown in Fig. 72 are still apparent, as they are continued beyond the strands. These resin canals must branch independently, as more canals appear in the lamina surrounding the ovule than there are vascular strands (Pl. LIII, Fig. 73).

In longitudinal section of the pre-fertilization stage the branches from the inverted vascular system surround the ovule in the lamina of the scale (Pl. L, Fig. 17, *v. b. o. s.*) and are seen running down to the micropyle. These strands end in the usual group of tracheides (Fig. 17, *tra.*). This branching of the vascular system to serve the lamina of the scale is also present in *P. imbricata* (Pl. XLIX, Fig. 6, *v. b. o. s.*). Several strands of phloem elements, of narrow elongated cells with densely staining contents, pass from the phloem of the bundles across each zone of nutritive tannin cells to the base of the nucellus (Pl. L, Fig. 17, *ph. str.*) as previously described in transverse section (Pl. LIII, Fig. 72), where they show on both sides penetrating the zone of tannin cells of the integument. The strand rising a little above the bract, which runs parallel to the vascular bundle for a short distance (Pl. L, Fig. 17), was present in every ovule sectioned. A phloem strand penetrating to the nucellus is also present in the ovules of *Agathis*, connecting them with the vascular supply, a fact which has been recorded by Worsdell.

The usual zone of starch and tannin cells limits the base of the *integument*, of which the apex shows elongated spirally thickened and pitted cells (Fig. 17, *lig. c. int.*).

The base of the *nucellus* is marked by another zone of tannin cells (Fig. 17, *tan.* and *st. c.*). The nucellus has a markedly long beak formed of serial rows of cells (Fig. 18, *nuc.*) which are not thickened but merely crushed at the extreme apex (Fig. 18, *stig. ap.*). This beak is penetrated by pollen-tubes which run more or less vertically down it. In one case the ♂ prothallus was seen in the archegonial cavity (Fig. 18, ♂ *pro.*), containing two generative nuclei with stalk and tube nucleus. In this stage, according to Lawson in *Sciadopitys verticillata* (30), there is an interval before actual penetration into the archegonia.

The nucellus is limited on the interior by two or three tapetal layers with disorganized cells surrounding the gametophyte (Fig. 17, *tap.*). The ♀ gametophyte has absorbed about two-thirds of the nucellus and (Fig. 17, *pro.*) shows a prolongation into the beak of the latter.

In all material obtained, though much contracted, a megaspore membrane is traceable (Fig. 17, *m. m.*).

§ STACHYCARPUS.

Receptaculum nullum ; flores feminei spiciformes vel ovula 1–2 ad apicem ramulorum abbreviatorum.

P. spicata.

Morphology. *P. spicata*, the Matai or Black Pine of New Zealand, is a very graceful tree, with a round and densely branching crown, and leaves unusually delicate in texture and dark in colour, with silvery undersides. It is general in forests throughout the country, up to 2,000 ft., according to Cheeseman (14, p. 651), who describes the wood as hard and durable.

The flowering shoots, both ♂ and ♀, are exceedingly numerous, but both in the Ohakune and Nelson material the ovules were mostly attacked by some insect grub, aborted and abnormally swollen.

Miyake, in *Abies balsamea* (33, p. 134), describes more than half the ovules examined as being infested by insect larvae; in early stages they were impossible to distinguish from those not infested. In the present instance *P. vitiensis* (l. c., p. 536) was found to be similarly infected. In a purely endemic species limited to a small country, where even in its native habitat it is only of local occurrence, such infection, spread over a series of years, would very soon work out its extinction. This is the case in California with *Pinus radiata*, now practically limited to the Monterey Peninsula, and to more or less isolated groups through the coast country, where it is subject to specialized insect attack. In accounting for the dominance or recession of species this very evident but passive factor, as far as the plant is concerned, is as a rule neglected, and most emphasis is laid on the possible active participation of the plants themselves in the struggle for existence, the survival of the fittest being attributed to superior organization and response to ontogenic requirements or to natural selection.

The flowering shoot consists of an elongated axis, borne in the axils of the foliage leaves of the young wood (Pl. L, Fig. 19, *strob.*). The shoots show the protective bud scales at the base; these are succeeded by apparently opposite and decussate modified leaves, an arrangement subsequently lost with the elongation of the axis. The laminae of these modified leaves are suppressed, the bases being adpressed to the stem axis (Fig. 20, *l.*). That these are modified leaves is shown in Fig. 21, where the laminae have grown out as ordinary foliage leaves. These leaves, as a rule reduced to their bases, are followed by the fertile bracts, exactly similar in structure, with the bases fused with the axis and laminae more or less suppressed. Above the bracts is an apical bud (Fig. 20, *ap. bud*) always pressed to one side by the development of the last fertile bract.

Pilger (40, pp. 13, 14) describes the ♀ flower in this species as a spike, consisting of a short limited branch, with a thin axis, bearing spirally arranged scale leaves at equal distance on it, which run down the axis, and at their base bear each an ovular rudiment. He describes the branch as bearing only 'carpels', but this 'Verhalten' is often modified so that the lowest leaves may be sterile. In the present instance the sterile leaves were invariably found to be present, as described above (Figs. 19, 20, 21, *l.*). The fertile bracts are given by Pilger as about 8, whereas Cheeseman (14, p. 650) gives them as 2–8.

In the present investigation, one axis was found with one fertile bract

(Fig. 21, *f. br.*) and a great many with 3–5 (Figs. 19 and 21). In every case the axis was terminated by a bud pushed on one side by the growth of the last fertile bract (Fig. 20, *ap. bud*). In no case, and a great deal of material was available, were the bud-scales and modified leaves of the axis and the apical bud absent.

In Fig. 21, where only one ovule was present, a cicatrized scar showed where the apical bud had been broken, or more probably eaten off. This fact had possibly caused the modified lower leaves to develop laminae and so grow out as ordinary foliage leaves.

In the ♂ shoots, as stated by Pilger, the same conditions obtain. Ten to thirty flowers are massed on an axis about 5 cm. long, where they may arise in the axils of small bracts only, or the shoot axis may bear foliage leaves at the base, which gradually pass into the bracts, the lowest flowers being borne in the axils of the foliage leaves, and an apical bud may develop (40, p. 10).

The ♀ 'spike' of *P. spicata* may be regarded as an entire strobilus, and fossil evidence may be referred to in support of this interpretation. Nathorst (62) describes in *Stachyotaxus septentrionalis*, C. A. Agardh, and *Stachyotaxus elegans*, Nath., an almost identical structure. He figures the strobilus of the former (62, Figs. 1 and 2, t. 2) and the resemblance is very striking; these figures show scale leaves densely clothing the peduncle, which are succeeded by a number of bracts, all of which are fertile, inserted at regular intervals on the axis. Nathorst refers to the similarity in structure of the ♀ shoot of this plant (which he rightly holds to be a *Dacrydium*) with *P. spicata* (62, p. 15). The ♂ strobili of *Stachyotaxus* show the elongation of the axis with the spacing of the sporophylls which is so striking in the ♀ strobilus in this genus. Nathorst figures these ♂ strobili with reserve, as they have not been found in organic connexion with *Stachyotaxus*, but they are so typically Dacrydioid that there can be little doubt that they belong to the same plant.

In § STACHYCARPUS the lamina of the fertile bract remains free from the ovuliferous scale, and does not keep pace with its growth, as described and figured in *P. vitiensis* (Fig. 27). The region of the ovuliferous scale referred to in the description of *P. vitiensis* as the semicircular ridge is in § STACHYCARPUS more prominently developed, and appears as a conical apex projecting from the free side of the ovuliferous scale above the place of insertion of the ovule. Pilger describes this pointed apex as a narrowing of the 'epimatium' into a blunt continuation ('stumpfen Fortsatz'), but as this continuation is furnished with two well-developed vascular strands with resin canals (Fig. 22, *v. b. o. s.*), and the foliar structure of the scale in the shape of epidermis, stomata, and stone cells is equally and continuously distributed, it seems to indicate that it is an integral portion of the scale itself. The greater development of this region may be related to the water supply of the ovule, in the absence of all swelling and proliferation of tissue in the bract bases or so-called 'receptaculum' of the axis. A double apex is often apparent in the ovuliferous scale (Fig. 20, *o. s.*), this appearance being caused by contraction of the mesophyll tissue between the two vascular bundles, as previously noted for *P. vitiensis* (see p. 536).

Histology. In longitudinal section the ovuliferous scale is marked by a well-developed epidermis with stomata. Pilger (40, p. 23) notes that in § STACHYCARPUS a thick stone-cell area arises evidently from the integument, but it will be seen that this area arises in the ovuliferous scale (Fig. 22, *o. s. stone c.*), the cells occurring in groups of two or three (Fig. 22) at regular intervals in the mesophyll. In this species stone cells were not found in the foliage leaves. The vascular system tissue consists of bundles which arise in the above-described apex of the scale. They lie facing each other, or phloem to phloem, separated at the apex by a little mesophyll. The separation increases as they descend till they expand at the base of the ovule in a ring of branching strands. Each bundle then divides so that four strands, all showing inverted orientation, and accompanied by small resin canals, surround the ovule in the lamina of the ovuliferous scale. On the dorsal side, or midrib region, below the ovular insertion, the two bundles approach each other again, but laterally, that is to say, side to side, until in the micropylar region the xylems almost converge, the phloem gradually rotating outwards.

Phloem strands penetrate to the nucellus (Fig. 22, *ph. str.*) as described in *P. imbricata* and *P. vitiensis.*

The *integument* is outlined by tannin and starch cells round its whole periphery (Pl. LIII, Fig. 75), and at the apex, at the micropyle, shows ingrowth of lignified closing cells (Pl. L, Fig. 22 *a*, *lig. c. int.*), as Saxton has described for *Widdringtonia* (42, p. 32), the cells similarly showing no septation and not entirely closing the opening.

The apex of the *nucellus* is prolonged, the cells containing starch, and the cell-walls show slight collenchymatous thickening (Pl. LIII, Fig. 74). It is this difference in texture and contents which is the cause of the slight contraction apparent at the base of the apex (Pl. LIII, Fig. 75), which is sometimes so marked in species where the apex is long, that the nucellus appears bottle-shaped, and this cause must always be allowed for in the presence of apparently abnormal shape. Pollen-tubes are seen penetrating the stigmatic apex (Fig. 75). These tubes extend above the actual apex, still attached to the spore-case; the loose cells forming the stigmatic surface of the extreme apex on pollination gradually become contracted and pressed together, the upper portion of the tube being in consequence exposed.

The base is outlined by the nutritive zone (Fig. 74). The tapetum is well marked in the region of the growing gametophyte (Figs. 74 and 75), which in Fig. 75 has absorbed nearly the whole of the body of the nucellus.

In Fig. 75 the ♀ gametophyte is almost filled by the centripetal growth of the endosperm, in which cell-wall formation is beginning, but so much contraction has taken place that the tissue is folded in and out, the outlines being traced by the megaspore membrane, which follows the folds (Fig. 75).

Of the two microphotographs of longitudinal sections given, Fig. 75 is older than Fig. 74, which shows the same stage as Pl. L, Fig. 22.

P. ferruginea.

Morphology. *P. ferruginea*, or Miro, is a very handsome New Zealand tree, which with *P. dacrydioides* forms an exception amongst the Podocarps of that country in not suggesting the Yew in foliage and asymmetrical habit.

The branches of this tree are not crowded and recall *P. vitiensis* in their rather horizontal spreading, and light green and shining leaves, with distichous arrangement. The fruit is also the same colour.

Cheeseman (14, p. 650) gives it as abundant throughout the country, the wood hard and compact, but not durable in exposed situations.

The strobili are borne on the apex of small lateral peduncles which arise in the axils of the lower foliage leaves of this year's wood (Fig. 24, *ped. strob.*), and as in *P. imbricata*, last year's ovules, in the pre-fertilization stage, and this year's with a homogeneous nucellus and dividing macrospore stage, were obtained on the same tree.

The lower portion of the peduncle of the strobilus is densely covered with imbricating scale leaves in the youngest stages, as in *P. vitiensis* (Fig. 27, *s. l.*). These scale leaves gradually pass into the bracts of the strobilus. The strobilus terminates the apex of the peduncle and is composed, as far as could be determined, of six to eight bracts of fleshy consistency, arranged in a decussate manner, with the bases fused with the axis, and with expanded laminae. One of the terminal pairs of bracts (Fig. 27, *f. br.*) or both (Fig. 28) are fertile.

The laminae of these bracts do not persist as long as in *P. vitiensis*, but soon fall off, including even the lamina of the fertile bract, leaving only cicatrized scars (Fig. 29, *scar lam. f. br.*), but the scale leaves of the peduncle persist.

Pilger (40, p. 14) emphasizes the spiral sequence of the bracts or 'carpels' in § STACHYCARPUS in comparison to their opposite and decussate position throughout the Eupodocarps, but the tendency is really the same in both cases, viz. an opposite and decussate arrangement due to economy in packing, in the youngest stages, which is gradually lost sight of on the elongation of the axis when the spiral phyllotaxy of the leaves come into play as the permanent arrangement.

These bracts surround and protect the fertile ones in the young state (Fig. 25, *br.*), expanding and reflexing later (Fig. 27, *s. brs.*). The lamina of the fertile bract at first covers the ovuliferous scale almost as if it were fused with it, as in § DACRYCARPUS. However, in longitudinal section it is seen to be free from the latter (Pl. LIII, Fig. 76). Where two bracts are fertile, they appear to be opposite, but, as stated before, this is only a question of packing, as one is invariably younger than the other (Pl. L, Fig. 28, *o. s.*).[1]

Histology. In a microphotograph of a radial longitudinal section of the youngest stage available (Pl. LIII, Fig. 76) the initial distribution of the tannin cells shows well. They are seen in three or four layers outlining the bases both of the fertile bract and scale, also the laminae of both, to continue in the epidermis only of the latter in the region of the micropyle, and in that of the integument and nucellus, where free (Fig. 76). This distribution is further emphasized in transverse section of the same stage, taken through the nucellus (Fig. 77)

There are, as yet, no tannin cells at the periphery and base of the integument and nucellus. This zone is initiated, as in *Saxegothaea* (57, p. 144), with the enlarging megaspore on pollination. In Fig. 76 the cells at the base of the ovule are in active division, the zone of growth being basal, as Saxton (44) describes for *Callitris.* The apical cells of the integument at the micropyle are not yet lignified.

The base of the ovuliferous scale on the ventral side shows swellings, which recall the structure of the scale bases in § DACRYCARPUS (Pl. XLIX, Figs. 8, 9, and 13).

Two bundles, with resin canals, showing the usual reversed orientation, run down the scale (Pl. LIII, Fig. 77). The course of the bundles is essentially as described for *P. spicata* (see p. 540). These bundles arise in the apex of the scale, which is produced (Pl. L, Fig. 29, *o. s.*), though not to the same extent as in *P. spicata.*

The two strands, each with a resin canal, do not exactly face each other in the extreme apex, as in the above species, but converge more or less as they descend the scale (Pl. LIII, Fig. 77), gradually rotating until the phloems are side by side. Then the strand of the fertile bract slips in between and the three bundles are inserted on the ring of distinct bundles in the strobilus.

In the fertilization stage from the same tree (Pl. L, Fig. 29, *o. s. scar brs.*) the ovuliferous scale rises well up above the bracts. The imbricating scale leaves of the peduncle still persist, but the laminae of the bracts of the strobilus have been shed, including that of the fertile bract, leaving only cicatrized scars.

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[1] In *P. ferruginea* we get an interesting reduction in the organization of the strobilus as compared with that of *P. spicata.* The peduncle, clothed with scale leaves, is shortened, as is also the axis of the strobilus, on which the bracts are massed close together, and not only reduced in number but also in fertility, the lower ones being all sterile, with one or two of the apical bracts alone fertile, and there is no apical bud. This reduction is also indicated in the *Stachyotaxus* series, as in *S. elegans* (62, t. 3, Figs. 1 and 2). Nathorst figures the strobili in a similar lateral position, with the peduncle reduced in length and in the number of bracts, all of which are fertile, but, though still showing an appreciable interval between each bract, the spacing is more than is the case in *S. septentrionalis.*

In the *ovuliferous scale* groups of stone cells are arranged regularly in the mesophyll as in *P. spicata*, but, unlike that species, they also occur in the mesophyll of the foliage leaves. The vascular strands start from hypodermal groups of tracheides (Fig. 34) at the apex of the scale and spread ring-like round the base of the ovule, where a phloem strand penetrates to the base of the nucellus, as in *P. imbricata*, *vitiensis*, and *spicata*. The bundles show no secondary branching in the lamina of the scale (Fig. 29, *v. b. o. s.*).

The *integument* shows the greatest differentiation yet met with, as the peripheral zone of tannin cells tends to elongate laterally, broadening out towards the centre and tapering towards the micropyle (Fig. 29, *tan. c.*). This regular arrangement is peculiar to this species amongst all those included in the present investigation.

Of the *nucellus* all but the apex, of which the cells show thickening of the cell-walls and starch contents, is absorbed by the ♀ gametophyte. The apex is penetrated by numerous pollen-tubes, the spore-cases being still attached to the stigmatic surface. The pollen-tubes penetrate more or less vertically, crushing the tissue on either side as the contents become absorbed. In all species this crushing of the thick cell-walls gives a certain stability to the apex, which persists till the embryo stage, forming a little brown cap which can be removed from the apex of the ♀ prothallus when the latter is dissected out.

The ♀ *gametophyte* is very contracted in the material available, but cell-wall formation can be traced.

It is limited by a megaspore membrane of two layers, the outer one striate.

§ EUPODOCARPUS.

Flores feminei singuli axillares, sub-sessiles vel plerumque longius pedunculati; receptaculum evolutum, saepe basi foliolis 2 angustis instructum.

In § EUPODOCARPUS the strobilus occurs, as usual, in the axils of the lower foliage leaves of lateral shoots. In these shoots there is always a well-marked fertile area of about an inch to an inch and a half. Above and below this area no strobili are to be found. It is only noticeable in the young wood up to pollination stage, as after that the unfertilized strobili drop off in great numbers, and it is no longer apparent that every leaf in this limited region bears a cone in its axil.

The strobilus may be almost sessile or pedunculate. If pedunculate the peduncle is naked, the strobilus consisting of two to six bracts, the two lower ones in some species (which Pilger in his monograph places first, and whose presence he considers divides § EUPODOCARPUS into two large groups, geographically separated (40, p. 14)) being thin, strap-like, and generally unmodified. The upper bracts show the small laminae and relatively large bases, which swell up as growth proceeds, and fuse with the axis and with each other, as previously described in § DACRYCARPUS. But in § EUPODOCARPUS the swelling is carried much further, resulting in a berry-like structure, in which the laminae are not traceable, or merely indicated by points.

This berry-like structure of swollen tissue forms the so-called 'receptaculum' which A. Braun (8, p. 740) was the first to reduce to its proper status.

That the two strap-like 'folioles' of Pilger really belong to the strobilus is seen in *P. spinulosa*, R.Br., where Pilger (40, p. 77, Fig. 15, H–L) shows that their bases may become swollen and so resemble the other bracts of the strobilus.

In their strap form these bracts are very caducous and so approach those which compose the strobilus in *P. vitiensis* and *P. ferruginea.*

Flores feminei infra receptaculum foliolis 2 parvis linearibus ornati.

P. elata.

Morphology. *P. elata* was found as a slender tree with sparingly branched crown in the montane mixed forests of Fiji, growing about 30–50 ft. high; on higher ridges, of shrubby habit, about 20 ft. and fruiting at 9–10 ft. The leaves are Willow-shaped, about 3 inches long, crowded towards the ends of the branches. The recorded distribution is for Queensland, New South Wales, and New Caledonia.

In this case also two stages were found on the same tree, as previously recorded for other species. The strobili arise in the axils of the lower leaves of the secondary branches of the young wood, and in September were in the pre-pollination or homogeneous nucellus, to the formation of the functioning megaspore stages (Figs. 30 and 32).

On the next oldest wood, the ovules were much larger, showing young embryos, with the cotyledons just forming, the bracts of the strobilus being already swollen and fused (Fig. 33).

This species shows the unmodified lower bracts (Fig. 33, *l. brs.*), which in the youngest stage are not so differentiated from the upper ones, and form an integral portion of the strobilus (Fig. 30, *l. brs.*), enclosing and protecting the upper bracts. They are evidently the reduced remains of the more highly organized bracts that obtain in *P. vitiensis* and *P. ferruginea*, whose function is also protective (Figs. 15, 25, and 27). No geminate cones were seen in this species.

In Fig. 30 the sterile bract has been removed to show the ovuliferous scale, with the lamina of the fertile bract well above it (*o. s.* and *f. br.*).

On the dorsal side of the same strobilus (Fig. 30 *a*) the fertile bract is seen to be unmodified in structure.

The ovule at this stage would show a homogeneous nucellus (Pl. LIII, Fig. 79). In Pl. L, Fig. 31, in an older stage, the bracts and the ovuliferous scale are about the same level, and the ovule would be showing sporogenous tissue. In Fig. 31 *a* the lower bracts have been dissected off, exposing two bracts, one fertile, of which the laminae begin to show marked contraction and the bases are swelling up. In Fig. 31 *b* the micropyle of the ovule can be seen. In Fig. 32 the ovule is well above the bracts; there is more swelling in both the latter, with greater contraction of the laminae (Fig. 33, *lam.* and *br. bs.*). This would be approximately the dividing macrospore stage.

In Fig. 33 the embryo shows cotyledons differentiating. The unmodified lower bracts have expanded, and in most cases were already shed. The bract laminae are reduced to points, while the bases have swollen enormously. The structure of these bases is essentially the same as described in § DACRYCARPUS. The tissue shows the same uniform cells with colourless contents, freely interspersed with tannin cells, especially (Pl. LIII, Fig. 79) round the periphery. The increase in size is due to the proliferation of the mesophyll tissue cells, which with very thin walls and no cell contents suggest a water-storage function; the fact, too, of the very early development of this tissue points to some provision for the wants of the growing ovule, which in its isolated position is rather remote from the supplies of the sporophyte. The increase in this tissue, which characterizes the Eupodocarps, may be possibly correlated with the absence of scale leaves on the peduncle.

In § DACRYCARPUS, where the scale leaves are present, this tissue is reduced (Pl. XLIX, Figs. 4 and 12). In § STACHYCARPUS, where, though fused for their whole length to the peduncle, the bract bases do not swell up (Pl. L, Fig. 28), the provision in the younger stages is much better, as the bracts then are more numerous and more specialized, each is of fleshy consistency, larger scale leaves support the base of the strobilus, and these merge into the smaller and more densely imbricating scale leaves which clothe the peduncle.

Histology. It is interesting to compare a photomicrograph of the homogeneous nucellus, which would be represented in Fig. 31 and Pl. LIII, Fig. 79 of *P. elata*, with Fig. 76 of a slightly younger stage of *P. ferruginea*, in § STACHYCARPUS, as the different development of the bract bases, also variation in form of the ovuliferous scale in each section, can then be better appreciated.

Fig. 79 is a radial section and shows well the foliar structure of the scale, from which, as far as seen in *Eupodocarpus*, hypodermal fibres are absent, both from the ovuliferous scales and the foliage leaves. The beginning of the nutritive starch and tannin zones is present at the base of the integument and nucellus. This section also shows how distinct the organization of the ovuliferous scale is from that of the fertile bract, the former having no vascular connexion with the lamina or so-called ‘carpel’.

In Fig. 79 the base of the ovuliferous scale is distinct and forms an integral portion of the strobilus, being pressed between the bract bases and fused with them. In the series of sections of the strobilus the three vascular strands can be traced separately to the base of the same. This distinctiveness is shown in the section figured, by the separation of the two resin canals, viz. that of the sterile bract to the right and that of the ovuliferous scale in the centre. Only a trace of the vascular bundle of the fertile bract is visible (Fig. 79) to the left, but that is sufficient to show how widely it is separated from that of the ovuliferous scale with its inverted orientation.

In transverse section of the same stage, a microphotograph (Pl. LIII, Fig. 80) shows a section taken through the base of the nucellus where the two bundles spread round the base of the ovule. It is slightly oblique, but the foliar structure of the scale comes out well. The lamina of the fertile bract appears on the dorsal side to the left (Fig. 80).

A prothallus, showing an embryo, was sectioned. The structure was identical with that described for *P. imbricata* (see p. 530).

P. polystachya.

Morphology. This material was collected from a large tree growing in the Buitenzorg Botanic Gardens. The leaves are long and Willow-like, aggregated towards the end of the branches. The strobili arise in the axils of the foliage leaves and consist of apparently two bracts, of which one is fertile. The unmodified lower bracts are no longer present (Pl. L, Fig. 35). The ovules all show different stages of pro- and embryo formation (Figs. 36 and 37, and Pl. LI, Figs. 38 and 39).

Histology. Some of the ovules were much smaller, suggesting earlier stages (Pl. LI, Fig. 41), but, on sectioning, these showed sterile prothallia, in which pollen-tubes, however, had penetrated the whole length of the persistent apex of the nucellus in each case (Pl. LI, Fig. 42, *p. t.*).

In both fertile and sterile prothallia there is a well-marked epidermis, showing small uninucleate cells which contain little or no starch (Pl. LI, Fig. 39 *b*, *epi.*) with thickening of the outside cell-walls. Coker has also described and figured a similar layer for *P. coriacea* (15, p. 97). This epidermis in the sterile prothallia was folded in and out over the apex and even into the neck of the archegonial cavity, owing to contraction of prothallial tissue.

Below these convolutions is a cavity (Pl. LI, Fig. 42, *dis. arch.*) where the archegonia would normally occur, but of which no trace was to be seen. In the central cylinder of small cells which reached below the cavity to nearly the base of the prothallus were a great many tracheides (Fig. 42, *tra.*, and Fig. 43). Neither the central cylinder nor the rest of the prothallus contained starch in the cells, though most of them were binucleate. This absence of storage contents was strikingly shown in a similar prothallus cleared in cedar-wood oil. The transparency was so great that the cylinder of small cells showed clearly through the centre, giving a very slight opaque effect, otherwise the prothallus would have been invisible. On cutting, it proved quite sterile, showing the apical cavity and just one or two tracheides in the central cylinder.

Coker (15, p. 97) gives a similar instance in *P. coriacea.* In that case there were two prothallia in one ovule; neither had formed archegonia, and in one three to four tracheides occurred in the central cylinder.

In the fertile prothallia the earliest stage is shown in Pl. L, Fig. 36, where many proembryos form a complex which has excavated a cavity in the prothallial tissue below the archegonia, from each of which a proembryo seems to be formed. This is also the case in *P. coriacea* (15, p. 101). These embryos were, with the suspensor tubes, rather contracted, but as far as could be made out resembled essentially those figured by Coker for that species.

One suspensor had penetrated vertically into the prothallus (Fig. 36, *sus.*), showing a tiny embryo of about twenty cells at the basal end (Fig. 36, *emb.*). Immediately below it, in the central cylinder, the cells were densely packed with starch (Fig. 36, *cen. cyl.*). All the cells of the cylinder were multinucleate, mostly containing four nuclei (Pl. LI, Fig. 40). This division must be very simultaneous, as the daughter nuclei are still enclosed in the limiting membrane. Miss L. Digby, in looking over the sections, came to the conclusion that the divisions are mitotic. This agrees with Coker's (15) and Miyake's (34, p. 10) conclusions. The large peripheral prothallial cells showed very little starch, but dividing nuclei.

In Pl. L, Fig. 37 the disorganized remains of the embryos and suspensors still persist (*dis. emb.* and *sus.*), but the cavity they lie in has shrunk, while the embryo has increased in size, the suspensor tubes being still traceable. Contraction of tissue is shown at the apex (Fig. 37) and similar contractions occur round the periphery of the prothallus, whose tissues are filled with starch.

In Pl. LI, Fig. 38 a section of the ovule with the ovuliferous scale is given. Stone cells are thickly scattered through the mesophyll, with no group arrangement. The vascular bundles show laminal branching (*v.b. o. s.*).

The *integument* has very much shrunk, centripetal lignification having begun in the cell-walls, while nothing remains of the nucellus but the cap (Fig. 38, *nuc. cap*), still showing starch and traceable pollen-tubes.

The megaspore membrane, though present, is very difficult to see, and cannot be dissected off, but in sections a thin line of cuticle persists in iodine and sulphuric acid.

The cavity of the proembryo complex has quite closed up and the embryo is seen in the centre of the prothallus, with digested cell layers on each side, and a plug of the crushed and empty cells of the central cylinder at the base (Fig. 38, *cen. cyl.*). This embryo shows no differentiation.

In Fig. 39 the cotyledons are differentiated, also the root apex. The embryo shows resin canals surrounding the phloem, which are present even in the cotyledons. Pressed between the cotyledons and spreading below them is the plug of the crushed axial tissue. The prothallial cells are packed with starch (Fig. 39, *st.*) which thins off towards the periphery, and where embryo digestion is in progress.

As many as eight nuclei may occur in the cells, which show up clearly in iron-alum and haematoxylin, counterstained with Congo-red (Fig. 39 *a*, *nuc.*)

In the Podocarps, in the endosperm tissue, all the nuclei seem to remain active, and their division suggests a correlation with the demands of starch formation, as this division is initiated at the beginning of the latter after fertilization and keeps pace with its demands.

P. bracteata.

P. bracteata, like the last species, was only seen in the Botanic Gardens of Buitenzorg. There it was a large tree, with very long narrow leaves. The leaves in this species are verticillately arranged and very large, about four inches long and three-quarters of an inch broad, markedly dark green in colour. Pilger (40, p. 80) has sunk *P. bracteata*, Bl., in *P. neriifolia*, Don, but as the plant was labelled by the former name, that designation is naturally adhered to. Only mature cones were collected.

The strobili occur singly in the axils of the foliage leaves on very short peduncles, the bract bases being much swollen, full size, and of a dull purple colour, with all trace of individual parts entirely lost (Fig. 44, *br. bs.*). The mature seed-coat is thick and resisting; lignification takes place in the cells of the integument, and involves the adjacent tissues of the ovuliferous scale as well.

Histology. The youngest prothallus in longitudinal section shows the hypocotyl with root-cap of the embryo nearly reaching to the apex (Fig. 45, *hyp.* and *rt. cap*), where it is capped by the crushed remains of the proembryos and suspensors (Fig. 45, *crushed sus.*). Empty tissue surrounds the embryo, with a plug of the crushed cells of the central cylinder at the base (Fig. 45, *cen. cyl.*). In the embryo the outside walls of the epidermis are thickened, and two small roundish cotyledons show at the base, succeeded by the hypocotyl, in which the plerome cylinder is outlined by resin canals (Fig. 45, *r. c.*), and these occur also in the cotyledons, cortex, and root-cap. The root apex is well differentiated (Fig. 45, *rt. cap*).

In the fully developed embryo (Fig. 46, *emb.*) the root-cap projects out beyond the prothallus (Fig. 46, *rt. cap*), breaking through the opening left by the archegonial cavity, and pressing aside the remains of the crushed suspensor tubes (Fig. 46, *crushed sus.*).

The limiting layers of the prothallus show crushing all round the embryo, while the basal plug is still in evidence (Fig. 46, *cen. cyl.*). The rest of the prothallial tissue has undergone little diminution in area and is packed with starch (Fig. 46, *st.*). This fact suggests that the embryo, in the oldest stage available material shows, has not yet reached its full development on the parent plant, though it is unusually well organized, in comparison with some Angiosperms, at this stage. Lloyd (32) has described vivipary in *P. Makoyi*, Bl., a species referred by Pilger (40, p. 79) to *P. macrophylla*, Don, var. *maki*, Sieb. Naaml, from Japan, grown in the United States. He describes the hypocotyls as always extruding from the ovules, while the latter are still attached to the sporophyte. No histological details are given, so that it is impossible to compare the development of the embryo in each case.

In Fig. 46 all the growth in the embryo in comparison with the previous stage (Fig. 45, *emb.*) seems limited to the hypocotyl, the cotyledons remaining almost stationary in their development.

The structure of the hypocotyl is essentially that of a root. There is a limiting epidermis with small nuclei and no starch contents; this is succeeded by the cortical layers packed with starch and penetrated by resin canals, which are chiefly aggregated towards the periphery, and surrounding the plerome cylinder. The plerome consists of elongated elements with small nuclei and no cell contents. Two procambial strands, of two or three spiral vessels, occur, one on each side of the plerome cylinder (Fig. 46, *procam. st.*), alternating with the two areas of primary bast. The central pith consists of long, narrow, rectangular cells with no contents and several resin canals. Two strands pass into each cotyledon, which arise from the respective poles of protoxylem in the hypocotyl. Resin canals are distributed as in the latter.

Fig. 47 shows an embryo dissected out.

P. neriifolia.

This species in Fiji is of Willow-like habit, and regularly outlines the streams in that country, the branches spreading over the face of the water. The strobili are conspicuous, as the bracts and ovuliferous scale are unusually long in structure, and so stand well above the dark leaves, to which their glaucous bloom forms a pleasing contrast.

It was determined as *P. bracteata*, Bl., by Seeman (48, p. 266), who first collected it in Fiji; but it does not appear to be the *P. bracteata*, Bl., as grown at Buitenzorg and put in *P. neriifolia*, Don, by Pilger (40, p. 80), nor is the Fijian plant quoted in his monograph. The structure of the leaves is quite distinct. In the latter there are two lateral resin canals, in close association with the transfusion tissue, which forms a small group on the outer side of each resin canal; whereas in the former the resin canals are medianly placed, and quite distinct from the two lateral groups of transfusion tissue.

The leaves are not quite as long as in the Javan plant, and are much narrower. The Fijian habitat also, always on the banks of streams, has not been recorded for *P. neriifolia* in any other locality.

The distribution of the latter is from East India, through Indo-China, Malaya, to New Guinea and Fiji. But the species in question from the latter country would be endemic.

The strobili occur in the axils of the upper leaves of the shoots, the peduncle varying in length from 3–5 mm. The bracts are from five to six in number, the lowest pair unmodified and caducous, the upper with the bract bases being exceptionally long and attenuated (Figs. 48 and 49, *br.bs.*); the free laminae, as usual in the order, drying up in maturer stages (Figs. 50 and 51, *lam. brs.*). The upper bracts may be either equal (Figs. 48, 50, and 51) or sub-equal in size (Fig. 49); the arrangement also varies from sub-opposite (Figs. 48–50) to sub-spiral (Figs. 49 and 51).

One (Figs. 48, 49, and 51) or two (Fig. 50) bracts may be fertile, but in the latter case, though opposite, one ovule is always younger than the other (Fig. 50), which shows, as before noted, that the opposite and decussate arrangement is merely apparent, and due to economy of space in the arrangement of the aerial portions of the members of the shoot, an arrangement always liable to modification, but that is not the inherent basis of construction.

Histology. In a microphotograph of a radial longitudinal section of this species (Pl. LIII, Fig. 78) in about the same stage as Pl. LI, Fig. 49 (dividing megaspore), the ovuliferous scale forms with the integument an unusually long micropyle. It is also very elongated in the basal region (Pl. LIII, Fig. 78).

The vascular system is of the usual type, consisting of two inverted bundles as previously described. Secondary branches run half-way down the lamina of the scale (Fig. 78), an unusual development at so young a stage. The secondary branches terminate each in a group of tracheides in the region of the micropyle. Each bundle is accompanied by a resin canal with functioning epithelium.

The *integument* shows the zone of starch and tannin cells with epidermal layer of the same. The hypodermal cells of the tip of the integument have the usual lignified and spiral thickening.

The *nucellus* is limited at the base by the zone of nutrient cells (Fig. 78). A tapetum of several layers surrounds the female gametophyte, which is in the dividing megaspore stage (Fig. 78). Older stages show many cells of the mesophyll tissue of the scale developing as stone cells. The micropyle is attenuated, the integument keeping pace in length with the long lamina of the scale, which possibly homologizes with the length of the foliage leaves in this species. The apex of the nucellus is not produced as a beak, but rather forms a depression. The nutritive zone of basal cells is very large round the nucellus, which is limited on the interior by a well-marked tapetum surrounding a contracted ♀ gametophyte in the fertilization stage.

Flores feminei foliolis infra receptaculum destituti.

P. saligna, D. Don.

P. saligna is well grown in several gardens in Cornwall, as *P. chilina*, L. C. Rich. The present material was obtained at Trewidden, Mr. Bolitho's place near Penzance, in March, 1909. The trees were still in their youth form of shrubby growth, fruiting at ten feet. There were very few cones, and they occurred in the axils of the lower leaves of last year's wood, and consisted of very long peduncles and three (Pl. LI, Fig. 52, *br. bs.*) or four (Fig. 53, *br. bs.*) bracts, of which one was fertile. The bract bases were green and somewhat swollen.

In Fig. 53 two bracts are of unequal size and all show a spiral tendency. This spiral tendency is carried still further in *P. spinulosa*, R. Br., judging from Pilger's figures (40, Fig. 15, H–L). In this stage stone-cell formation has invaded the whole integument, which made it impossible to get good microtome series; the female gametophyte, too, was contracted and shrunken. It seems to be the rule that, where there is a difference in the relative size of the bracts of one cone, the fertile one is generally the largest; this enlargement is probably a subsequent development correlated with nutritive requirements.

P. Totara.

Morphology. *P. Totara* is one of the finest denizens of the New Zealand forest, and may be said to be the classical tree of that country. Its dark green foliage, rather pendulous in outline, massed branching habit, and stringy bark strongly recall the Yew, a resemblance accentuated by the similar colour of the berry-like strobili. According to Cheeseman (14, p. 648), the wood is durable and much used for building purposes, and it was the favourite timber of the Maoris in the construction of their houses and war canoes. It is common in forests from the North Cape to the south-west of Otago, up to 2,000 ft.

The youngest material was collected during a two weeks' stay at Te Aroha, in the beginning of November, from a young ♀ tree in the public gardens. The stage of development ranges from the pollination or differentiated megaspore to free cell formation in the prothallus.

A great many of the ovules remained unpollinated, though there were several ♂ trees in their immediate vicinity. These strobili died off by degrees in great quantities, and, though undistinguishable in the living state, they could always be picked out in formalin by their dead appearance.

As far as it is possible to judge by present experience, it seems to be characteristic of the Podocarps, no doubt associated with the reduced strobili, that the ovules are in much the same stage on the whole tree at one time. Even when last and this year's strobili are found there is little gradation in the stage of either.

Though constantly looked for, gradation in sizes was rarely obtainable on the same tree or even on others in the same locality.

Professor Macfarlane, on a recent visit to this country, mentioned the case of an isolated ♀ tree of *Ginkgo*, growing near Philadelphia, on which very few fruits matured each year. One year he tried the experiment of tying a bunch of ♂ flowers to the branches, with the result that the tree set a large crop of seed. The next year the experiment did not come off; he therefore decided to adhere to the exact date of the first successful result, which was again repeated, and that special date to the day has invariably proved reliable. The male trees were found to never vary in the maturation of the pollen, and by observing that date in the transportation of the ♂ flowers the crop of seeds on the isolated ♀ tree could be controlled.

The experience at Te Aroha suggests a similar condition. Several ♂ trees were quite near and covered with strobili; the one ♀ tree also bore an abundance of young cones in a lower fertile zone of the young shoots in such profusion as to be noticeable, notwithstanding their small size, on account of the glaucous bloom covering the entire strobili. Yet it was surprising how many were not fertilized. The cones would grow vegetatively for a little time and then drop off.

Possibly only those ovules in a physiologically receptive state when the pollen is shed can retain it. Stopes and Fuji (51, p. 10), in *Ginkgo*, note great uniformity in the development of the different ovules of the same tree, or even on different trees, and it has been remarked in the course of this work how uniform the stages are for the different species.

The strobili of *P. Totara* consist of three (Fig. 54, *brs.*) to four (Fig. 55, *brs.*) bracts, which in the pre-pollination stage are quite unmodified (Figs. 54 and 55, *brs.*). Two are usually fertile (Figs. 55, 58, and 59), but, as we have seen before, one ovule is always younger than the other, showing that the apparently opposite and decussate arrangement of the bracts is merely an ontogenic convenience, not related to the actual organization of the plant, and probably induced by the shortness of the axis of the strobilus, to be lost on the elongation of the same, or an increase in the number of the bracts.

When three bracts are present (Figs. 54 and 56) the arrangement is evidently spiral. Pilger does not take into consideration this contingency, where the opposite and decussate theory cannot hold good. For *P. Totara* (40, p. 84) he gives the 'receptaculum' as formed of two or four 'scales'; whereas, in the youngest stages, two bracts were not seen on the present occasion, always three (Figs. 54 and 56) or four (Fig. 55), but the third one is completely lost sight of in the subsequent swelling of the bract bases. In section, however, the vascular strand is always traceable (Fig. 58, *v. b.*, 3 *br.*).

As growth increases the bract bases begin to swell a little, but the lamina are still distinct (Figs. 56 and 58); by the pre-fertilization stage, however, their identity may be said to be entirely lost (Fig. 59).

Histology. In longitudinal section of the pollination stage the ovuliferous scale has grown well above the bracts (Fig. 57, *o.s.*), and it shows a production of the apex above the insertion of the ovule. The vascular system of two bundles starts in this apex, each from a hypodermal group of tracheides. It spreads ring-like below the insertion of the ovule, but as far as the available material goes no laminal branching occurs (Figs. 57 and 58, *v. b. o. s.*). Resin canals with epithelium accompany the vascular supply on the ventral side, limiting the tissue of the *integument*, in which the nutritive cells are arranged in the normal zone (Fig. 59, *tan.* and *st.c.*).

The hypodermal cells at the micropyle show spiral thickening and elongation (Fig. 57, *a.*). The *nucellus* is roundly pointed, but not beaked; pollen-grains rest on the apex, but no tubes are traceable. Two or three layers of tapetal tissue limit the nucellus in the vicinity on the dividing macrospore (Fig. 57, *tap.*).

In an older stage (Fig. 58, *pro.*) the ♀ prothallus shows free nuclei. In this case the tracheides of the vascular system of the third bract come into the section, though the presence of the former is not otherwise indicated (Fig. 58, *v. b.*, 3 *brs.*).

In older stages again, collected at Auckland in October, the bract bases were already swollen up to their mature size and identical in colour with that of the Yew aril, points alone indicating the laminae (Fig. 59, *br. bs.*).

The cells of the bract bases show an increase in size and number, but not in contents, being evidently filled with a watery cell-sap. They retain their foliar structure; the proliferation of cells taking place in the mesophyll, the arrangement of the cell layers in this tissue is retained (Pl. LIII, Fig. 79).

The ovuliferous scale shows increase in length, and centripetal lignification of the cells of the integument, involving half its tissue, is in progress. A two-layered megaspore membrane surrounds the ♀ prothallus, which is very much contracted. Archegonia can be traced in the apex of the prothallus. Many of the cells show two nuclei, which would point to the maturation of the oosphere (see p. 530). This would agree with the development of the ovular tissues, and the contraction of the prothallus shows that endosperm formation has not yet been initiated.

In this case we see that the swelling and coloration of the bract bases, which are turgid with a sweet cell-sap, are present long before maturity. This development can therefore be of no use to the plant in facilitating distribution by the agency of birds, as stated by Pilger (40, p. 22).

It may possibly have this secondary result, as the berry-like swelling and sweet cell-sap persist to maturity; but such a result can hardly be described as a function, it is in fact incidental. When a modification sets in at so early a stage, and is so gradual in its accomplishment, it shows the initial cause must be more fundamental in character than a merely secondary result would demand, and it is more probably correlated with the function of the nutrition of the young ovule, as previously suggested.

The ground under the tree from which these strobili were collected was strewn with the Yew-like berries, and many were put up in formalin, both from the ground and from the tree, under the impression that they had been shed at maturity, but all were subsequently found to be in the pre-fertilization stage, as far as could be judged from the contracted prothallia.

Oliver, in a paper on the ovules of the older Gymnosperms (37, p. 455), discusses the fact that all the seeds of these plants preserved are in the stage just preceding fertilization, only occasionally being met with in an earlier state. He says:

'In the course of evolution, probably, the time of banding the integument was postponed till embryonic changes had set in, so that well-marked ovular or seed phases became very recognizable; but in the Palaeozoic seeds known to us, such a distinction can hardly be drawn. In referring to them the term *seed* is usually employed, though in recent Gymnosperms the corresponding stage would be called an unfertilized ovule. This usage in terminology has doubtless arisen from the appearance of maturity which their integumentary tissues present, a maturity which seems to preclude all possibility of subsequent expansion.'

The latter sentence might equally well have been written of the same stage in *Podocarpus*. Merely some enlargement in growth, progressive lignification of the tissue of the mesophyll and integument, and the loss of function in the resin canals, which apparently coincides with the cession of further vegetative growth, are the only vegetative features in the embryonic stages in these strobili, the ovuliferous scale being fully developed for protective purposes by the pre-fertilization stage.

Coulter and Chamberlain (18, p. 47) consider it possible that the Palaeozoic seed matured in developing a testa before fertilization, but becoming detached after pollination, developed a proembryo which subsequently aborted. Scott, in reviewing the above work (47, p. 171) in 'Nature', says: 'The interesting question of the constant absence of an embryo in all Palaeozoic seeds hitherto investigated is discussed. This has been regarded as the normal condition, the development of the embryo not having begun until the seeds were shed, and then having passed over at once into germination. The author inclines to the view that all Palaeozoic seeds investigated were abortive, having been shed prematurely. The fact that nearly all the seeds observed were in the same stage of development, and the usual presence of normal pollen in the pollen chamber, scarcely seem consistent with this view.'

These conditions are also fulfilled in the modern Podocarps, as we have seen, as far as the shedding of the seed before or after fertilization, as quoted above. Such shedding may very well be due to altering conditions of temperature or water supply, or to unknown physiological conditions which demand a reduction in the number of fruits the sporophyte can mature. The demands of the purely vegetative tissues of the strobilus on account of the advanced organization of the ovuliferous scale is as great before fertilization as it is after; therefore, once initiated, the embryonic development is practically a fresh charge on the energies of the sporophyte.

P. Hallii.

P. Hallii is a scraggy, untidy looking tree, distributed over New Zealand, and is considered a variety of *P. Totara* by Pilger (40, p. 84), who makes it var. *Hallii*, Pilg. Cheeseman (14, p. 648) keeps it up as a species, and this point of view, which would be supported by any botanist who had seen both species in the field, is also borne out by histological results.

The strobili occur singly in the axils of the lower foliage leaves of lateral shoots and consist of a short peduncle, and in material investigated apparently two bracts, of which one (Pl. LI, Figs. 60, 61, 62, and Pl. LII, Fig. 64) or both may be fertile (Pl. LI, Fig. 63). The bract bases may remain either normal (Figs. 61, 62, and Pl. LII, Fig. 64), or their bases become swollen (Pl. LI, Fig. 60). The ovule is very elongated, and may be as long (Fig. 60), or even longer than the bracts (Pl. LII, Fig. 64), or shorter (Pl. LI, Fig. 61) than the same.

Histology. The material, which was collected in March, shows embryo formation, but the exact stage could not be determined, as the prothallial tissues having dried to a certain extent, the paraffin in consequence could not penetrate sufficiently well for successful sectioning.

The ovuliferous scale shows a very much produced apex, equalling that seen in *P. spicata* (Fig. 62).

The two inverted bundles, running down from the tip, show no lateral branching in the lamina (Fig. 62, *v. b. o. s.*); each sends a phloem strand from the base of the ovule into the nucellus (Fig. 62, *ph. st.*). The resin canals are very large and no longer functional, all epithelium having disappeared.

This condition was also noted by Brooks and Stiles (10, p. 311) in *P. spinulosa*, but to state that they are always functionless, as they infer, is judging cause from effect.

We have seen that in the earlier stages of the ovuliferous scale the resin canals are initiated with the vascular tissue system (Pl. LIII, Fig. 77, *r.c.*), and invariably show a well-marked and active epithelium of thin-walled cells and dense contents (Pl. XLIX, Fig. 8 *c*). Their constant presence and distribution (on the phloem or ventral side of the bundles) suggests that they play an important part in the very active metabolism of the ovuliferous scale, which controls the developing ovule. The complete organization of the former in the pre-fertilization stage, when even the lignification processes in the tissue of the same and of the integument have begun, also the metamorphosis of the bract bases, show how vital these rapid metabolic processes must be, as their action is so limited in period.

With the growth of the embryo the activity of the zone of tannin cells at the base of the integument and nucellus seems to cease, as in the first case the walls of the cells lignify, and in the second the entire tissue is digested by the rapidly growing ♀ gametophyte. The prothallus is about two-thirds its mature size at fertilization, and independently elaborates its own starch supplies for the nutrition of the embryo, merely drawing on the sporophyte for crude materials. The conveyance of supplies must be limited, once the nucellar tapetum no longer functions, to the phloem strands, which, as we have seen, penetrate the lignifying tissues (Pl. LIII, Fig. 72) in greater or less degree, through specialized areas. This secondary induration is different in form from that which obtains from about the pollination stage in the hypodermal cells at the apex of the integument. These cells are spirally thickened, the walls being very thick (Pl. LI, Fig. 57 *a*), and limited to a very small area.

The cells of the integument, on the contrary, show ordinary lignification of the walls with centripetal development, as seen in *P. Totara*, where in a young state only the peripheral layers were lignified, which had increased to half the tissue in the pre-fertilization stage, and it is evidently initiated in the zone of the nutrient tannin and starch cells as their available supplies are absorbed and their activity ceases.

In Fig. 62 a longitudinal section of the strobilus of this species shows three bracts, one of the upper pair fertile, and a lower sterile one, the presence of which was quite unnoticeable on examination before selection for embedding, and only revealed in the microtome series. Attention may also be called to the fact that, were the third bract placed decussately to the upper pair, the apex would not come into the radial section figured (Fig. 62, *br.* 3), but only the vascular strand seen, as figured in *P. Totara* (Fig. 58, *tra.* of 3 *v.b.*).

The tissue of the *integument* is entirely indurated and lignified, turning yellow with potash and red in phloroglucin. The nucellar tissue is crushed and empty (Fig. 62, *nuc.*).

The megaspore membrane can still be traced, limiting the ♀ prothallus—indeed it peels off on disarrangement of the tissues; the prothallus cells are packed with starch, with an embryo about half the length of the prothallus in the centre, of which the exact stage could not be distinguished, the tissue having to a certain extent dried up.

P. nivalis.

This species is a much branched erect or prostrate shrub, occurring in sub-alpine localities in New Zealand, from 2,000 to 5,000 ft. During a stay of four weeks at Mt. Cook, in the South Island, a serial collection of strobili was made, the species being very abundant about there. The material is in pollination stage, showing the breaking of nucellar tissue round the megaspore and vacuolization of the latter, and is in usually good condition.

The older shoots of this species seem to innovate, this year's strobili occurring singly in the axils of the lower leaves of this year's shoot (Pl. LII, Fig. 65, *strob.*), whereas last year's, containing young embryos, would be on the next lowest portion, separated by the scars of the bud scales.

The fertile area on the young shoots is as well marked as in *P. Totara* (see p. 552).

The strobili consist of a short peduncle bearing two (Figs. 66 and 67) or three (Figs. 65 and 68) bracts, of which one (Figs. 65–7, 69, 70) or two (Fig. 68) may be fertile. Pilger (40, p. 85) for this species, as for *P. Totara,* gives only two 'scale leaves' with one ovule, but the two ovules were as often seen as not present, as were also the three bracts (Figs. 65 and 68); the presence of the third is sometimes indicated at maturity (Fig. 69), but often all evidence is lost in the basal swelling and fusion.

In *P. nivalis* the bracts, as usual in the Eupodocarps, are unmodified in the earliest stages (Figs. 66 and 67). It is on pollination that the bract bases begin to show distinct modification.

In the oldest or young embryo stage, as in *P. Hallii,* about an equal number of examples were seen with unmodified bracts (Figs. 70 and 71), which in this species are suffused with red from the pollination stages and probably before, but there is no glaucous bloom.

Where the bases do swell up they show the same colour and consistency so familiar to us in the Yew arils, and also characteristic of *P. Totara* and *P. Hallii*; and, similarly, all trace of the third bracts, where present, is lost, the blades being reduced to mere points.

Pl. LIII, Fig. 81 is a photomicrograph of a section taken through the apex of the integument, which is enclosed in the base of the ovuliferous scale. Two bundles are shown in the scale, as yet hardly organized, the ovule being in the homogeneous nucellus stage. The lamina of the fertile bract is seen to the left, and that of the sterile one to the right.

In this species the larger ovules were in the early embryo stage, with no cotyledons. The bract bases, however, were fully coloured and developed, as was the case with *P. Totara* (Pl. LI, Fig. 59) in the pre-fertilization stage, and *P. Hallii* (Fig. 63), in which the embryo was a little more advanced. In other instances the bracts remained unmodified (Pl. LII, Figs. 70 and 71), as described for *P. Hallii* (Pl. LI, Fig. 61 and Pl. LII, Fig. 64). Sometimes a small dead ovule would be found still attached to the strobilus (Fig. 71).

The development of the species is so similar to *P. Totara* and *P. Hallii* that no longitudinal sections are given. The chief difference is in the micropyle, which is very long, which is also the case with the integument and the apex of the prothallus. The produced conical apex of the ovuliferous scale is intermediate between the two former species. Certainly in their development as in appearance, these three species seem to be very closely allied.

CONCLUSIONS AND SUMMARY.

In the foregoing pages we have seen that much of the debatable structure of the different parts of the fruiting axis in *Podocarpus* is due to interpretation based on the study of secondary modifications alone, which are easily explained by a serial study of the process of development in early stages.

The structure of the different parts of the cone is then homogeneous; therefore it is only by tracing the serial development of the axis as a whole from the bud to the fruit that the progressive sequence of the evolution of the structure and the specific development of its different parts can be offered.

This study at the same time strikingly reveals the relationship of the axis to the strobilus or cone of the Abietineae and its component parts, a relationship previously suggested by Bennett and Brown (2), Sperk (50), and Bertrand (3), and recently supported by Tison, in *Saxegothaea* (57).

These authors all consider the ovuliferous envelope of the Podocarpoideae as analogous with the ovuliferous scale of the Abietineae, which, to quote Bertrand (3), 'from the Abietineae to the Taxineae through *Saxegothaea* and the Podocarps shows a general tendency to surround in a hood-like fashion the ovule or ovules which it carries, fusing more and more till finally it merges in the ovular integument in *Torreya* and *Cephalotaxus*'. Bertrand traces a further reduction in strobilus organization in the fewer number of bracts and increased independence of the ovuliferous scale for protective purposes, through *Microcachrys* and *Dacrydium* until the final reduction is reached in *Podocarpus*.

Tison has confirmed this interpretation for *Saxegothaea*, in which he considers the so-called aril of earlier botanists and the 'epimatium' of Pilger to be the ovuliferous scale of the other Conifers, the summit of which is developed to protect the ovule, the coalescence of the different parts of the female cone being less than in the other genera, where there is less development of the fertile bract above the ovule, whose base is partially protected by the ovuliferous scale. In other species of *Podocarpus* the bracts only develop below the ovule, which is encircled by the ovuliferous scale.

Correlated with the gradual independence in function of the ovuliferous scale, we get a gradual change in the relation of the ovule to the scale.

In the Abietineae two ovules are placed at the base of the scale, one on each side, which is the most economic arrangement where the cone itself is organized for protective purposes. In Araucarineae one ovule is inserted medianly on the scale. In the Podocarpoideae, as Pilger has noted (40, p. 16), we get a gradual pushing up of the ovular rudiment on the ' epimatium ' or ovuliferous scale.

In *Saxegothaea* the apex of the ovuliferous scale is a homogeneous membrane, without vascular structure, merely protective in the pollination stages, the ovule being inserted towards the base of the bract and remaining free from the same, except at the base. The two inverted strands which supply the ovule are spoken of by Tison as the ovular supply ; they are described as being without resin canals, and insert themselves on the vascular bundle of the bract at the base of the same, with a semi-rotation similar to that obtaining for the bundles of the ovuliferous scale in *Podocarpus* when they insert themselves between the bundles of the strobilus. It is impossible to draw any close comparison between *Saxegothaea* and *Podocarpus* in these respects, as the exact delimitation of the tissues of the ovuliferous scale and bract respectively are not insisted on.

From a cursory examination of *Dacrydium* (on which it is hoped to publish results later) it is possible that in that genus good intermediate series between *Saxegothaea* and *Podocarpus* will be found, showing the retrogression in the development of the fertile bract above the scale, and the increase in the protective function of the latter, which, however, does not fold round the highly inserted ovule, while on the other hand the coalescence of the different parts of the cone is still marked.

In *Podocarpus* we find a similar series, showing the course of reduction in the cone, the actual strobilus organization appearing only in the youngest stages, as subsequently the laminae of the bracts composing the cone do not develop further. The ovuliferous scale takes on an entirely independent organization, analogous to that of the foliage leaves, and completely surrounds and protects the ovule, which, as it grows up, it carries well above the bracts of the strobilus, the insertion of the ovule being towards the apex of the scale. This independent function is correlated with a great reduction in strobilus organization, the fertile bracts decreasing in number until they are limited to one or two in an apical position as the cone decreases in size and protective organization.

In Pilger's excellent monograph of the Taxaceae the Podocarpoideae are classified according to the progressive development of the ' epimatium ', which he regards as an accessory organ, a ligula-like excrescence of the ' carpel ', through *Microcachrys*, *Saxegothaea*, and *Dacrydium* to *Podocarpus*, in which family this organ reaches its maximum development.

It is interesting to note that this arrangement accords completely with the views of the authors quoted above, and also with the results of the present work on *Podocarpus*, as progressive development of the ovuliferous scale is correlated in each stage with reduction in strobilus formation.

The systematic position therefore remains on Pilger's basis, by merely substituting ovuliferous scale for ' epimatium '.

Morphologically the results may be summarized as follows:

In *Podocarpus*, in the youngest phases, a strobilus formation obtains which is masked by subsequent modification.

An actual series of successive stages in the reduction of the strobilus is apparent in the different sections of the family.

In § DACRYCARPUS the peduncle is clothed with scale leaves, in spiral sequence, which show increase in size just below the strobilus.

The strobilus is reduced to two to five bracts, of which one to three may be fertile.

In these bracts, which are more or less unmodified in the younger stages, the bases show subsequent swelling and coalescence, ultimately changing colour, but the laminae remain green and unaltered.

The lamina of the fertile bract is fused with the ovuliferous scale to the apex of the latter, showing the cohesion between the scale and the supporting bract seen in *Microcachrys* and *Saxegothaea*, as also in *Agathis*, *Araucaria*, and the Abietineae.

In § DACRYCARPUS this fusion may be complete, as in *P. dacrydioides*, or sometimes incomplete, as in *P. imbricata*, where the apex of the fertile bract often remains free, and may even exceed that of the scale.

The ovuliferous scale entirely enfolds the ovule, leaving an orifice at the micropylar end where the integument protrudes on pollination; but subsequent growth in the lamina of the scale, and swelling at the base of the same on the ventral surface, cause the micropyle to be pressed against the lower portion of the scale, closing up the orifice.

In § NAGEIA (if *P. vitiensis* can be taken as typical of that section) and § STACHYCARPUS the peduncle (branched in the former) is still clothed with scale leaves, showing increase in size below the strobilus.

The strobilus consists of eight to ten bracts, of which all, or only one or two of the apical ones, are fertile. These bracts are fleshy and strap-like in the youngest stage, and opposite and decussate in arrangement. There is no subsequent swelling of the bases, and the laminae may be shed on or before fertilization, leaving only scars, which show a spiral sequence, due to elongation of the axis.

The lamina of the ovuliferous scale develops quite independently of the lamina of the fertile bract, which enfolds and protects the former in the youngest stages, but either remains undeveloped as a ring at the base, or is subsequently shed.

§ EUPODOCARPUS offers the greatest extreme in the reduction of the strobilus in the genus, from the point of view of number of members, but the cone-like organization is nevertheless obvious in the young stages.

The peduncle is naked, and the bracts of the strobilus may be reduced to two. In some species the two lower retain the strap-like unmodified form we get in § STACHYCARPUS, and are merely protective in the young stages, being subsequently shed as in that section. In *P. spinulosa* (40, p. 77) the bases often swell up like those of the upper bracts. In other species they are finally eliminated altogether.

The upper bracts, of which one or two are fertile, are unmodified and protective in the young stage, but their bases soon swell and become coloured as in § DACRYCARPUS; the laminae, however, do not persist, as in that section, but become entirely merged in the basal swelling, all subsequent development being arrested.

The secondary swelling and coloration of the bract bases in some cases is already present in the pre-fertilization stage; its presence can therefore be of no use to the plant in the dissemination of seeds, as stated by Pilger, but is probably correlated with ovular development by serving as water-storage tissue.

The ovuliferous scale is well protected by the sterile bracts in the young stages, but gradually rises above them until, on pollination, the lamina of the fertile bract alone encircles the base as a ring, remaining stationary in development. The ovuliferous scale takes on not only the whole protective covering of the ovule, but by the early and complete organization of its metabolic activities supplies the material for the development of the ovule as well.

Judging from the stages obtained in the material collected, development occurs in the early spring. In Fiji this season is in August and September, and material collected in those months showed pollination and young embryo stages. In New Zealand at the same season, in September and October in the North Island, *P. ferruginea* and *P. Totara* showed pollination and post-pollination stages and pre-fertilization. In the South Island, at Mount Cook, at an altitude of 2,500 ft., *P. nivalis* showed pollination to young embryo stages in December and January, while further south, at Glade House in March or autumn, *P. Hallii* was found in the embryo stage.

At Buitenzorg, enlarging megaspore and pre-fertilization stages were found associated in December, and on other trees embryos, mature and immature, were also collected.

As is the case with the Abietineae, last year's cones were generally found concurrently on the old wood with those developed on this year's wood above them.

There is a very marked uniformity in the stages of development of the ovules in the strobili of each period. It is probable that this feature is associated with the great reduction in the number of fertile bracts to each cone. The same fact has been recorded for *Ginkgo*.

HISTOLOGICAL RESULTS.

The ovuliferous scale shows the complete organization of a foliage leaf with epidermis, stomata, and mesophyll tissue. It is provided with a well-developed vascular system with inverted orientation, and accompanied by resin canals.

In § DACRYCARPUS a hypodermal sclerotic layer is present in both the ovuliferous scale and the foliage leaves.

In § STACHYCARPUS, on pollination, groups of stone cells form a regular zone in the mesophyll tissue. In *P. ferruginea* these were also present in the foliage leaves.

The cells of the mesophyll show secondary thickening throughout the group, which is initiated before fertilization.

The vascular system of the ovuliferous scale consists of two bundles (*P. vitiensis* four), each accompanied by a resin canal. These arise each at the extreme apex of the scale in a hypodermal group of tracheides, as is also the case in the foliage leaves. They branch in a ring below the insertion of the ovule, half-way down the scale. From this ring secondary branches may run into the lamina of the scale, so encircling the ovule; these branches stop half-way down the lamina or continue right to the micropyle, and are always terminated by groups of tracheides.

Below the ovule the main bundles separate, and by a gradual semi-rotation the phloem becomes peripherally placed when they insert themselves, one on each side of the vascular strand of the fertile bract, on to the ring of bundles in the strobilus.

Resin canals accompany each bundle on the phloem side. These are very active, with well-developed epithelium, and evidently play an important part in the metabolism of the scale. Their function gradually ceases on the organization of starch formation in the female prothallus. In later embryo stages the epithelium is no longer traceable.

The resin canals limit the tissue of the integument in the plane of the mesophyll tissue of the scale.

The integument shows basal growth, and its small regular cells stand out well from the irregular mesophyll of the scale. Long lignified hypodermal cells are differentiated in the apex, at the micropyle, at pollination, at which stage also a nutritive zone of starch and tannin cells (*cellules tannifères* of Tison) is laid down round the base and sides of the nucellus till the prothallus is fully organized, when, as is the case with the resin canals, the function of these cells apparently ceases and the walls gradually lignify, areas being left where phloem strands penetrate to the nucellus.

In *P. ferruginea* these tannin cells are organized in regular superposed layers, laterally elongated, which surround the integument.

The nucellus, composed of larger and less compact cells, is also characterized by a smaller zone of tannin cells. Before pollination the apex shows rows of loose stigmatic cells, which catch the pollen-grains. These cells subsequently become crushed on the elongation of the tubes; the latter are often exposed in consequence.

No pollen-grains or cases of penetration by pollen-tubes were observed from any portion of the nucellus other than the stigmatic apex.

In § NAGEIA and § STACHYCARPUS, also some species of § EUPODOCARPUS, the apex of the nucellus is very much elongated.

From the pollination stages starch is present in the cells of the apex in all species, and the walls often show thickening.

A well-differentiated tapetum of several layers is present in all the species examined, the cells with active nuclei and denser contents showing continuous disintegration in the outer layer, from the development of the megaspore to the absorption of the body of the nucellus by the female gametophyte.

Tetrad formation was not seen.

In megaspore formation the nucleus divides, a parietal layer of free nuclei surrounding the central vacuole, which gradually fills up with prothallial tissue. Further stages were not traceable, owing to contraction of tissue, but are evidently paralleled with Coker's results on *P. coriacea* and similar development in the Abietineae.

A megaspore membrane of two layers was observed in all the species surrounding the developing prothallus, which persists till the embryo stages. In *P. polystachya* it was not traceable with certainty in the embryo stage.

The female prothallus at fertilization consists of a central cylinder of small elongated and rectangular cells, and large, mostly binucleate, polygonal cells extending to the periphery, and it is limited by an epidermal layer of small uninucleate cells, with denser contents and active nuclei.

The number of archegonia seen was five to seven.

The archegonia, arranged in one or two groups, are very long and flask-shaped. The neck consists of two to six cells, and they are each surrounded by a single layer of jacket cells.

In one case two archegonia were enclosed in one jacket.

No ventral canal cell was seen.

Sterile prothallia, with a cavity replacing the archegonia, were observed. In one case no tracheides were present, but on two occasions well-developed tracheides were found in the central cylinder of small-celled tissue.

A complex of proembryos with suspensors forms a cavity at the apex of the prothallus. One suspensor develops an embryo which penetrates the central cylinder. Starch formation is initiated in the central cylinder cells immediately below the embryo, and mitotic divisions occur in the cells. The two divisions must follow one another in rapid succession, as four daughter nuclei are generally seen enclosed in the same limiting membrane. These nuclei further divide up to eight, remaining active and normal even when the cells are packed with starch.

The cavity formed by the complex gradually closes up as the embryo increases in size. Resin canals surround the plerome cylinder before the cotyledons are differentiated, and appear in their tissue as soon as the latter develop.

In the mature embryo the root-cap extrudes through the apex of the prothallus.

The cortical tissues of the embryo are packed with starch, but none is seen in the cells of the plerome cylinder.

The structure of the hypocotyl is that of a diarch root, and has two areas of primary bast alternating with two poles of the protoxylem, showing spiral vessels. Two branches from each pole pass to each cotyledon.

Resin canals are present in the root-cap, hypocotyl, and pith of the mature embryo.

The acquisition of a considerable quantity of material in various young stages has rendered it possible for the first time to bring forward a certain amount of evidence in favour of the view that in *Podocarpus* we are dealing with a much modified cone. The cone character is well seen at an early stage, but subsequent developments, including the swelling of the bract bases at the expense of the lamina, or the shedding of the latter and the great development of the ovuliferous scale, together with the reduction in the number of fertile members (usually only one), completely mask the strobilus organization. This view was formulated by Bennett and Brown (2) as early as 1838, and later by Sperk (50) and Bertrand (3), and quite recently by Tison (57), working on *Saxegothaea*. With the exception of Tison these authors were limited to herbarium material.

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EXPLANATION OF PLATES XLIX–LIII.

Strob., strobilus; *ped.*, peduncle; *s.*, scale; *bud s.*, bud scales; *l.*, leaf; *s. l.*, scale leaves; *br.*, bract; *s. br.*, sterile bract; *f. br.*, fertile bract; *b. br.*, base bract; *lam.*, lamina; *ap. bud*, apical bud; *ap.*, apex; *o. s.*, ovuliferous scale; *mic.*, micropyle; *i.*, integument; *nuc.*, nucellus; *o.*, ovule; *v. b. f. br.*, vascular bundle, fertile bract; *v. b. o. s.*, vascular bundle, ovuliferous scale; *x.*, xylem; *ph.*, phloem; *ph. str.*, phloem strands; *tr.*, tracheides; *r. c.*, resin canal; *mes.*, mesophyll; *c.*, cells; *stone c.*, stone cells; *st.*, starch; *t.*, tannin; *h. f.*, hypodermal fibres; *e.*, epidermis; *stom.*, stomata; *idio.*, idioblasts; *tis.*, tissue; *lig.*, lignified; *m. m.*, megaspore membrane; *pro.*, prothallus; *meg.*, megaspore; *tap.*, tapetum; *arch.*, archegonia; *in.*, initials; *n.*, nucleus; *e. n.*, egg nucleus; *j. c.*, jacket cells; *n. c.*, neck cells; *p. g.*, pollen-grain; *p. t.*, pollen-tube; *nuc. cap*, nucellus cap; *pro. emb.*, proembryos; *sus.*, suspensor; *cen. cyl.*, central cylinder; *dig. c.*, digested cells; *cots.*, cotyledons; *pl.*, plerome; *plu.*, plumule; *hyp.*, hypocotyl; *rt. cap*, root-cap; *pro. cam. str.*, procambium strands; *cor.*, cortex; *stig. ap.*, stigmatic apex; *gen. n.*, generative nucleus; *stalk n.*, stalk nucleus; *tube n.*, tube nucleus; *cup. l.*, cupressoid; *imb.*, imbricate; *epi.*, epithelium.

PLATE XLIX.

Fig. 1. *P. imbricata.* Branch showing strobili in two stages: this year's with unmodified bracts, in early pollination stage; last year's with bract bases swollen at the expense of the lamina, in pre-fertilization stage. Nat. size.

Fig. 2. Strobilus with two bracts, each fertile; bract bases swollen and verruculose; scale leaves subtending strobilus removed. × 16, red. ½.

Fig. 3. Strobilus with two bracts, one fertile; showing the apex of the fertile bract unusually free and prolonged, and larger size of the scale leaves of the peduncle immediately subtending the strobilus. × 16, red. ½.

Fig. 4. Strobilus with four bracts, one fertile, with apex still free, in fertilization stage; the peduncle is covered with imbricating scale leaves, those subtending the strobilus showing less modification as the latter increases in size. × 8, red. ½.

Fig. 5. Sterile strobilus, showing base of ovuliferous scale swollen and verruculose (Singapore). × 8.

Fig. 6. Longitudinal section through ovuliferous scale and ovule in pre-fertilization stage, showing lamina of fertile bract, with normal vascular bundle, fused with the lamina of the scale, with inverted vascular system, both enclosing the ovule; epidermis, hypodermal layer of fibres, and mesophyll with lignified idioblasts are continuous for both; the integumental tissue is limited by the resin canals and shows a zone of nutritive cells, i.e. tannin and starch; lignified cells occur in the apex, at the micropyle; the nucellus shows also a zone of nutritive cells, and starch is deposited in the cells of the apex; a tapetum showing continual breaking down of tissue limits the nucellus on the inside; the female gametophyte is invested by a two-layered megaspore membrane, and shows archegonia; the cells of the prothallus are binucleate. × 16, red. ½.

Fig. 6 *a*. Archegonium with contracted protoplasm and resting egg nucleus, showing neck cells at apex, surrounded by jacket cells. × 625.

Fig. 6 *b*. Two archegonia in one jacket, seen in a prothallus of another species. × 625.

Fig. 6 *c*. Idioblasts in mesophyll tissue.

Fig. 7. Strobilus with two bracts, one fertile, from the subsummit zone of Kinabalu (11,500 ft.), in which the scale leaves do not spread out below the bracts, as in Fijian and Buitenzorg material; pollination stage; the apex of the lamina of the fertile bract projects above the ovuliferous scale. × 16, red. ½.

Fig. 8. Longitudinal section of strobilus in pollination stage or after; the fertile bract shows distinct apex, which becomes obscured as ovule matures; the nucellus is contracted, but pollen-tubes can be traced in the apex. × 16, red. ½.

Fig. 8 *a*. Hypodermal layer of fibres in the lamina of the fertile bract and that of the ovuliferous scale, with epidermis. × 625.

Fig. 8 *b*. Verruculose outgrowths on the swollen bract bases, the epidermis showing tannin contents; no hypodermal layer of fibres. × 625.

Fig. 8 *c*. Resin canal with epithelium of thin-walled cells with active nuclei and darker staining contents, surrounded by cells containing tannin and starch. × 625.

Fig. 9. *P. dacrydioides.* Longitudinal section of pollination stage; tannin and starch cells show at the base of the integument and nucellus, with starch in the apex of the latter, and a well-marked tapetum limiting on the inside; megaspore shows dividing nuclei. × 16, red. $\frac{1}{2}$

Fig. 10. Peduncle with imbricating scale leaves, terminated by strobilus of three bracts, one fertile, the lamina of which is fused with the ovuliferous scale. × 8.

Fig. 11. Strobilus showing two bracts, one fertile, older stage. × 8.

Fig. 12. Strobilus of three bracts, one fertile. × 8.

Fig. 13. Longitudinal section of above stage: the prothallus is contracted, but shows centripetal filling by free nuclear division. × 16, red. $\frac{1}{2}$.

Fig. 14. *P. vitiensis*, showing position and branching of peduncles, each branch bearing a strobilus with one fertile bract; the ovules are in the fertilization stage, the upper ones on simple peduncles being younger. Nat. size.

Fig. 15. Young strobilus, showing peduncle with imbricating scale leaves; the strobilus is composed of five pairs of bracts, opposite and decussately arranged, with one of the upper pair fertile; the lamina of the fertile bract is free from the ovuliferous scale, which bears an ovule with undifferentiated nucellus. × 16, red. $\frac{1}{2}$.

Fig. 16. Peduncle with eight bracts, of which the upper two are fertile; the ovuliferous scales bearing the ovules have grown far above the lamina of the fertile bracts; these laminae, with those of the sterile bracts, subsequently desiccate and fall off, leaving only the elongated bract bases, as in the last species, which remain unmodified; fertilization stage. × 16, red. $\frac{1}{2}$.

PLATE L.

Fig. 17. Longitudinal section of above stage, showing the growth of the lamina of the ovuliferous scale, that of the fertile bract remaining stationary; the prothallus shows contraction, but archegonia are traceable in its tissue: ♂ prothallia show above the archegonia, the stage being just prior to fertilization. × 16, red. $\frac{1}{2}$.

Fig. 18. Male prothallus with pollen-tube, which has penetrated the unusually long apex of the nucellus, showing two generative nuclei, with stalk and tube nucleus. × 245.

Fig. 19. *P. spicata.* Bbranch showing strobili in the axils of the foliage leaves. Nat. size.

Fig. 20. Strobilus bearing three fertile tracts, showing bud scales at base, and modified foliage leaves above, which are succeeded by the fertile bracts and an apical bud. × 16, red. $\frac{1}{2}$.

Fig. 21. Strobilus of which the apical bud has been damaged, leaving only a cicatrized scar; this fact has probably stimulated the development of the modified leaves into foliage leaves; one fertile tract on the shoot. × 16, red. $\frac{1}{2}$.

Fig. 22. Longitudinal section of strobilus, showing produced apex of ovuliferous scale. A regular zone of stone cells occurring in groups of two or three is seen in the mesophyll; phloem strands penetrate to the nucellus, and the vascular bundles send secondary branches into the lamina of the scale; the prothallus shows contraction, but free cell-division can be traced. × 16, red. $\frac{1}{2}$.

Fig. 22 *a*. Section through micropyle, showing non-septate closing cells. × 625.

Fig. 23. Stone cells in the mesophyll of the ovuliferous scale. × 625.

Fig. 24. *P. ferruginea.* Strobilus with peduncle in the axil of a foliage leaf; the fertile bract slightly covers the ovuliferous scale; pollination stage. × 8.

Fig. 25. Strobilus in younger stage with scale leaves dissected off the peduncle; the bracts are still folded and protect the ovule enclosed in the scale; the nucellus is undifferentiated. × 16, red. $\frac{1}{2}$.

Fig. 26. Strobilus and peduncle still enclosed in the scale leaves investing the latter. × 16, red. $\frac{1}{2}$.

Fig. 27. Peduncle bearing strobilus composed of six pairs of bracts, of which one of the uppermost pair is fertile; the ovuliferous scale shows above the bract; scale leaves clothe the peduncle. × 16.

Fig. 27 *a*. Ventral view of same, showing micropyle. × 16, red. $\frac{1}{2}$.

Fig. 28. Strobilus with two fertile bracts; ovuliferous scale has grown up above the bracts, of which only the bases persist, the laminae having shrunk to pin points; scars indicate the sterile bracts, of which the laminae have already been shed; pre-fertilization stage. × 8, red. $\frac{1}{2}$.

Fig. 29. Longitudinal section of strobilus slightly older than above stage; phloem strands penetrate to the nucellus from the vascular bundles; the tannin cells of the integument show a symmetrical, laterally elongated arrangement; pollen-tubes have penetrated half-way down the apex of the nucellus, and the very contracted prothallus shows cell-wall formation; a scar is all that remains of the lamina of the fertile bract; in section the arrangement of the bracts in the strobilus is seen to be more spiral than opposite and decussate, as the morphology would suggest. × 8, red. $\frac{1}{2}$.

Fig. 30. *P. elata.* Earliest stage, showing strobilus of four bracts, the lower pair being narrower than the upper, of which one is fertile; all four enclose the ovuliferous scale. Ventral view, the opposite sterile bract having been removed. × 16.

Fig. 30 *a.* Dorsal view of fertile bract, showing unmodified lamina and base. × 16.

Fig. 31. Older stage, ovuliferous scale showing above the bracts, which are becoming broader and thicker. × 16.

Fig. 31 *a.* Lateral view, showing both upper bracts, with the lower pair dissected off. × 16.

Fig. 31 *b.* Ventral view of same, showing micropyle. × 16.

Fig. 32. Strobilus showing lower bracts which remain undifferentiated; the upper pair show shrinking laminae and swollen bases. Pollination stage. × 16.

Fig. 32 *a.* Ventral view of above, showing sterile bract cut off, to expose micropyle. × 16.

Fig. 33. Strobilus with ovule showing young embryo; the unmodified bracts still persist, the upper bracts show very swollen bases and laminae shrunk to points; the ovuliferous scale extends above the fertile bract. × 16, red. $\frac{1}{2}$.

Fig. 34. Group of tracheides terminating a vascular bundle in the lamina of the ovuliferous scale. × 625.

Fig. 35. *P. polystachya.* Strobilus with ovule, showing embryo; the unmodified lower bracts have been shed and in the upper ones all foliar structure is lost, the lamina being no longer indicated, while the bases are fused in a succulent whole. Nat. size.

Fig. 36. Longitudinal section of prothallus, showing complex of proembryos, one being formed from each archegonium; the suspensor bearing successful embryo is seen penetrating the central cylinder of prothallus, the cells of which are binucleate, and those immediately below the embryo show dense starch contents. × 245.

Fig. 37. Longitudinal section of prothallus, showing embryo increasing in size at the expense of the central cylinder; the remains of the suspensor tubes are still traceable, also the cavity formed by proembryos, with disorganized remains of complex; contraction of tissue is shown at the apex of the prothallus, causing the epidermal layer to fold in and out. × 16.

PLATE LI.

Fig. 38. Longitudinal section of ovule enclosed in the ovuliferous scale, showing stone cells scattered in the mesophyll of the latter, also laminal branching of the vascular bundles; the integument shows lignification of the cell-walls and the nucellus is reduced to the apex, which, still showing starch and pollen-tube, forms a mere cap; the prothallus has grown at the expense of the nucellar tissue, and shows an undifferentiated embryo; the cavity left by the complex of proembryos has almost closed, though still filled with the crushed remains; digested cells surround the embryo, and the crushed and empty tissue of the cells of the central cylinder is being pushed down as a plug by the vertical growth of the embryo. × 16, red. $\frac{1}{2}$.

Fig. 39. Longitudinal section of embryo, showing differentiation of the cotyledons, root apex, and resin canals; the dotted area shows the relative distribution of starch in the prothallus, of which the cells, excluding the peripheral layer, are multinucleate. × 8, red. $\frac{1}{2}$.

Fig. 39 *a.* Multinucleate cells of the prothallus, packed with starch grains. × 625.

Fig. 39 *b.* Peripheral layer of the prothallus, with megaspore membrane. × 625.

Fig. 40. Nucleus in prothallial cells, on initiation of division, showing four daughter nuclei still enclosed in the limiting membrane, as seen in Fig. 36, in the cells of the central cylinder, just below embryo. (L. Digby del.)

Fig. 41. Sterile strobilus borne on same tree as the fertile one drawn in Fig. 35. Nat. size.

Fig. 42. Longitudinal section of above. In this prothallus, which contains no starch, tracheides are developed in the central cylinder; a cavity occurs where the archegonia would normally be found. × 16.

Fig. 43. Tracheides as seen in the central cylinder of small-celled tissue of sterile prothallus. × 625.

Fig. 44. *P. bracteata.* Mature strobilus, with swollen bract bases, deep purple in colour (Buitenzorg). Nat. size.

Fig. 45. Longitudinal section of embryo, showing cotyledons, with plerome cylinder and cortical resin canals. × 8, red. $\frac{1}{2}$.

Fig. 46. Longitudinal section through prothallus, showing embryo with extruding root-cap; in the embryo resin canals occur both in the cortex and the pith; procambium strands of spiral vessels mark the plerome cylinder; the cotyledons show similar development, each being served with two vascular strands. × 8, red. $\frac{1}{2}$.

Fig. 47. Embryo dissected out. × 8, red. $\frac{1}{2}$.

Fig. 48. *P. neriifolia.* Strobilus in earliest stage available, with four bracts, one of which is fertile; the lowest pair of unmodified bracts have been cut off, showing two pairs of upper bracts, apparently opposite and decussate (Fiji). × 8, red. $\frac{1}{2}$.

Fig. 49. Later stage, strobilus with three bracts, one of which is fertile, showing approach to spiral arrangement. × 8, red. $\frac{1}{2}$.

Fig. 50. Strobilus of three bracts, of which two are fertile; the caducous lower bracts have already been shed; the upper ones show shrunken laminae and swollen bases. × 8, red. $\frac{1}{2}$.

Fig. 51. Fertilization stage. Nat. size.

Fig. 52. *P. saligna,* showing a strobilus of three bracts, of which one is fertile; the peduncle, in this species, is very elongated. × 8, red. $\frac{1}{2}$.

Fig. 53. Basal portion of a strobilus with four bracts, showing a modification of the decussate arrangement. × 8, red. $\frac{1}{2}$.

Fig. 54. *P. Totara.* Strobilus of three bracts, still undifferentiated, one of which is fertile; ovule in pollination stage. × 8.

Fig. 55. Strobilus of four bracts, two of which are fertile; the oldest ovule shows nuclear division in macrospore. × 8.

Fig. 56. Strobilus with three bracts, of which two are fertile; the oldest ovule shows free cell formation in the prothallus. × 8.

Fig. 57. Longitudinal section of Fig. 55, showing well-differentiated tapetum and enlarging macrospore. × 16, red. $\frac{1}{2}$.

Fig. 58. Longitudinal section of a strobilus with three bracts; in the largest ovule the female gametophyte shows free nuclear division, while the youngest is in the enlarging megaspore stage. × 16, red. $\frac{1}{2}$.

Fig. 59. Strobilus with four bracts, of which two are fertile; the laminae are barely indicated, but the bases have swollen to a large size, and are of a bright red colour; the ovules are in the pre-fertilization stage, the peripheral half of the integument showing lignification of the cells.

Fig. 60. *P. Hallii.* Strobilus with bases of bracts swollen and red in colour, one bract being fertile; the ovule is in young embryo stage. × 8, red. $\frac{1}{2}$.

Fig. 61. Strobilus in which the bract bases have remained unmodified; one bract is fertile, with ovule in young embryo stage. × 8, red. $\frac{1}{2}$.

Fig. 62. Longitudinal section of a strobilus with three unmodified bracts, the upper one fertile; the ovuliferous scale shows stone-cell thickening in the mesophyll and very large resin canals, also phloem strand penetrating the integument; the integument is entirely lignified, with the exception of a few cell layers in proximity to the nucellus, which is mostly digested by the prothallus; the latter is surrounded by a well-developed megaspore membrane and contains a young embryo. × 16, red. $\frac{1}{2}$.

Fig. 63. Strobilus with two fertile bracts. Nat. size.

PLATE LII.

Fig. 64. Strobilus with bract bases unmodified and shorter than the ovule.

Fig. 65. *P. nivalis,* showing a strobilus with three bracts, still undifferentiated, one of the lower bracts being fertile; the strobilus is in the axil of a foliage leaf of the axis. × 8.

Fig. 66. Strobilus with two undifferentiated bracts, one of which is fertile; the ovule is in the pollination stage. × 8.

Fig. 67. Strobilus with two bracts, one of which is fertile; enlarging macrospore stage. × 8.

Fig. 68. Strobilus with three bracts, two of which are fertile; macrospore stage with dividing nuclei. × 8.

Fig. 69. Strobilus with three bracts, one of which is fertile; the lamina of the bracts are reduced to points, while the bases are much swollen and of a bright red colour; young embryo stage. × 8, red. $\frac{1}{2}$.

Fig. 70. Strobilus with two bracts, both unmodified; ovule in young embryo stage. × 8, red. $\frac{1}{2}$.

Fig. 71. Strobilus with three bracts; the lamina of one bract with an undeveloped ovule is dead. × 8, red. $\frac{1}{2}$.

PLATE LIII.

Sections cut 2–3 μ on a Minot microtome.

Fig. 72. *P. vitiensis.* Transverse section through an ovule in the pollination stage, taken below the base of the nucellus, where the four bundles of the scale break up into branches which run down the lamina of the same (Fig. 17). In this case eight branches can be counted; the integument shows a thick ring of tannin and starch cells, the walls of which are becoming lignified. This ring is seen penetrated in two places by phloem strands from the vascular system (Fig. 17). The tannin cells surrounding the base of the nucellus are seen in the integument, which is limited by an epidermal layer of tannin cells. Similar cells mark the peripheral layer of the nucellus, which is bounded on the inside by the tapetum in which lies the dividing macrospore. × 24.

Fig. 73. Transverse section, same series, taken through the micropyle and the base of the ovuliferous scale, just above the apex of the nucellus where the four bundles have again approximated. × 25.

Fig. 74. *P. spicata.* Longitudinal section of nucellus only, showing the well-marked tapetum, megaspore membrane, and free cell formation in the prothallus. × 120.

Fig. 75. Longitudinal section of ovule, showing zone of stone cells (torn) in the ovuliferous scale, with the zone of tannin and starch cells lining the integument and the nucellus. The cells at the apex of the nucellus contain starch and show some pollen-tubes; on the inside the nucellus is limited by the tapetum. The prothallus is very much contracted, but the megaspore membrane can be traced folding in and out with the prothallial tissue, which shows cell-wall formation. × 45.

Fig. 76. *P. ferruginea*, longitudinal section of young strobilus in the stage of Fig. 25. It shows an undifferentiated nucellus with basal cell growth and a limiting layer of tannin cells to the nucellus and the free portion of the integument; in the ovuliferous scale the tannin cells at this stage are limited to the epidermis and two or three hypodermal layers. × 27.

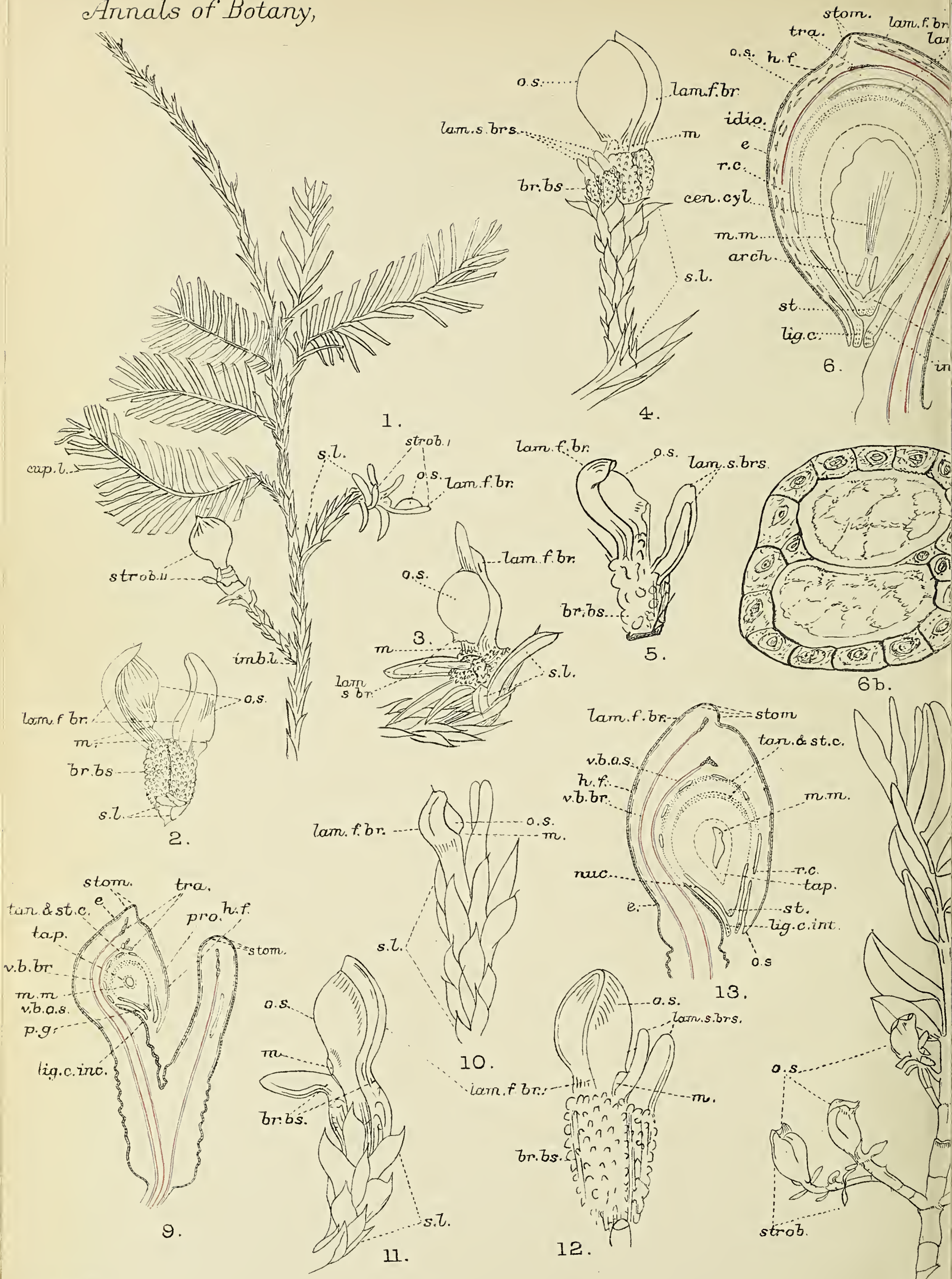
Fig. 77. Transverse section through the nucellus, integument, and bract bases of the same stage. The two vascular bundles are seen with the xylems converging, whereas in the apex of the scale the phloems face each other. This rotation finally leads to a normal orientation as the bundle slips into its place in the ring of leaf-traces in the strobilus. × 42.

Fig. 78. *P. neriifolia.* Longitudinal section of strobilus in pollination stage (Fig. 49), showing a very attenuated ovuliferous scale; a well-marked tapetum is seen in the nucellus, enclosing the enlarging megaspore. × 7.

Fig. 79. *P. elata.* Longitudinal section of strobilus with undifferentiated nucellus (Fig. 31), showing modified bracts with swollen bases, proliferation of mesophyll tissue; also the independence of the vascular system of the fertile scale and the sterile bract at the base of the strobilus, indicated by the two resin canals far apart. × 26.

Fig. 80. Transverse section of strobilus in above stage, taken at the apex of the nucellus, showing lamina of the fertile bract. Slightly oblique. × 40.

Fig. 81. *P. nivalis.* Transverse section of an ovuliferous scale taken across the micropyle, showing the foliar structure of the scale. × 45.



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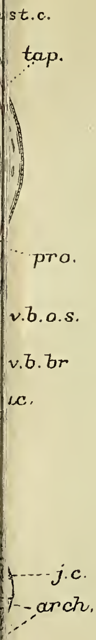

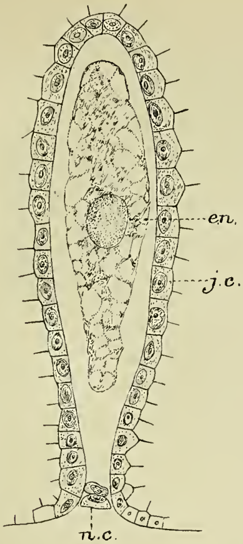

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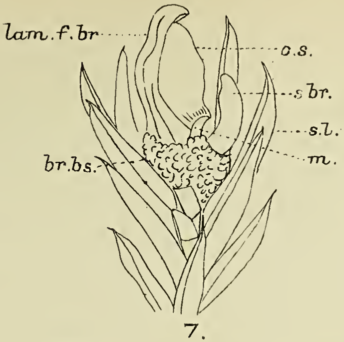

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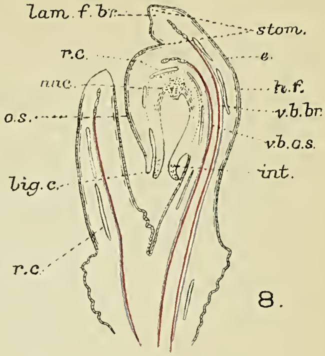

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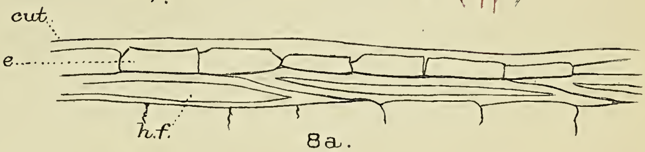

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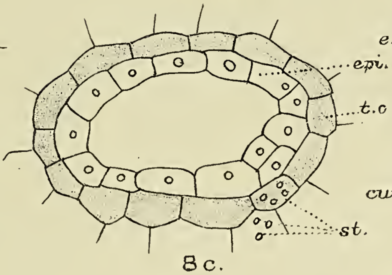

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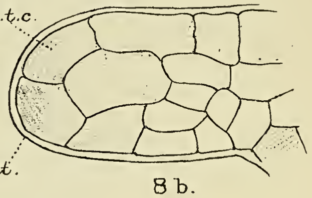

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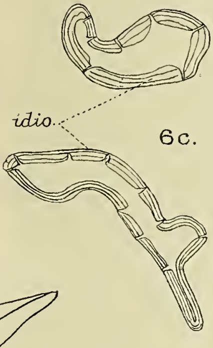

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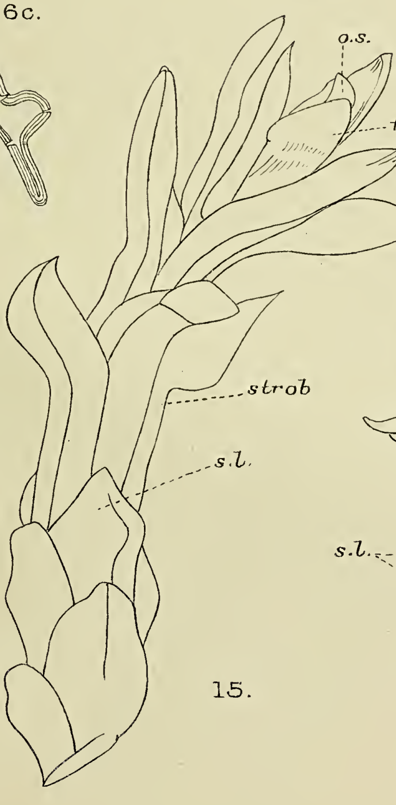

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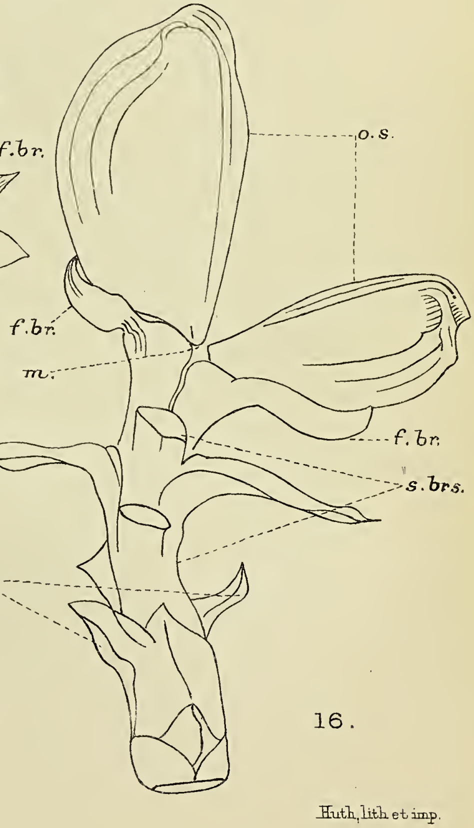

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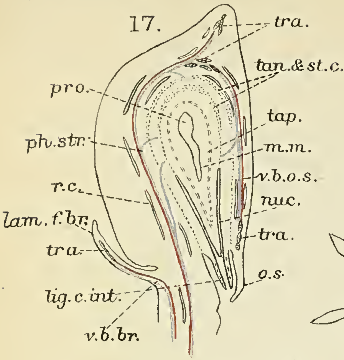

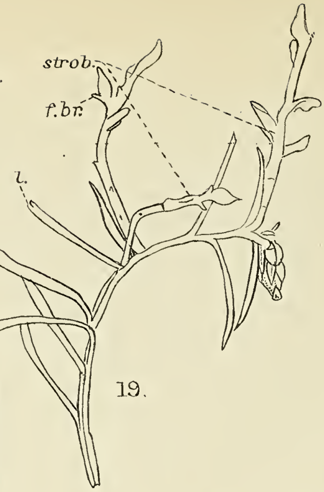

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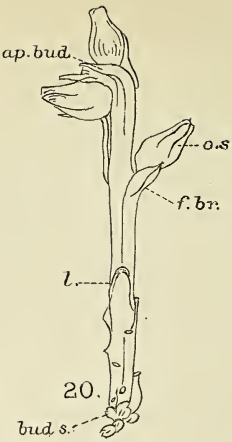

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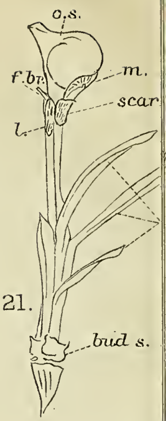

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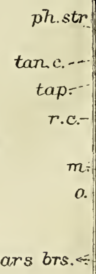

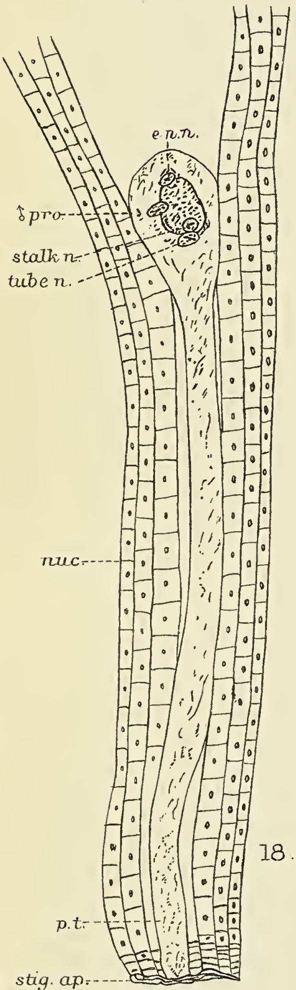

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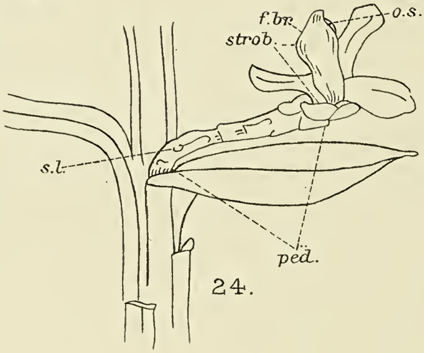

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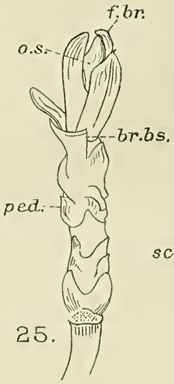

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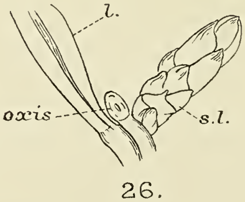

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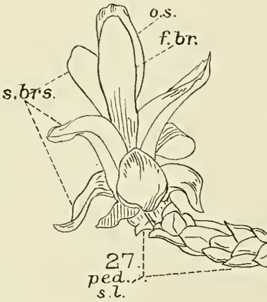

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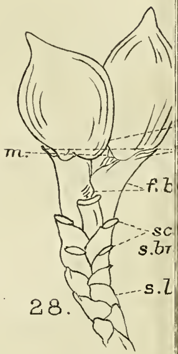

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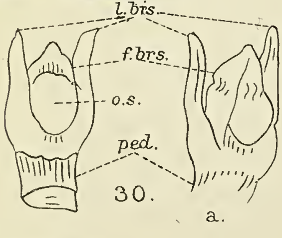

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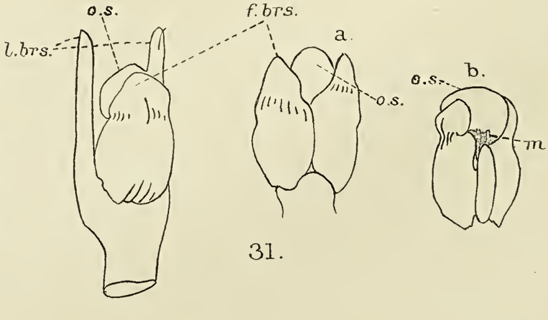

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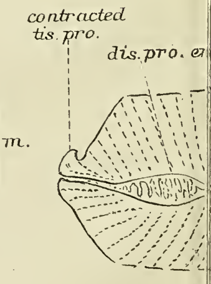

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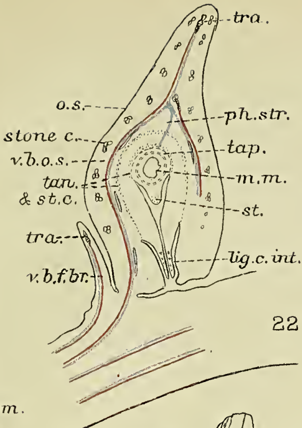

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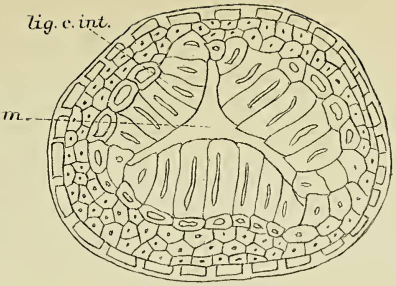

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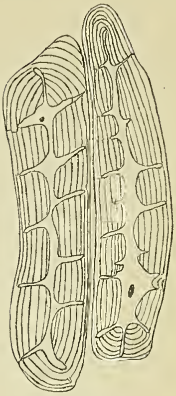

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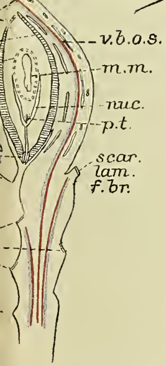

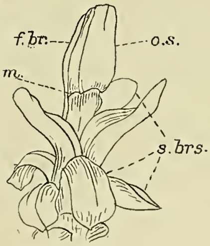

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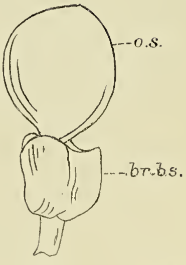

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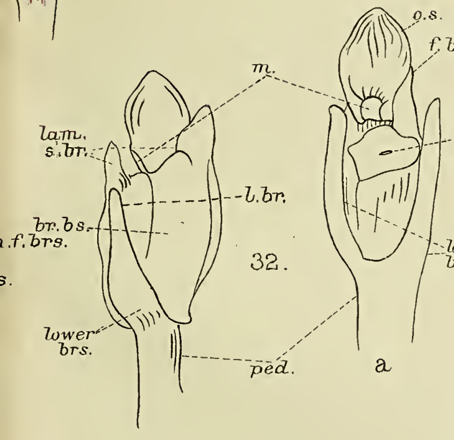

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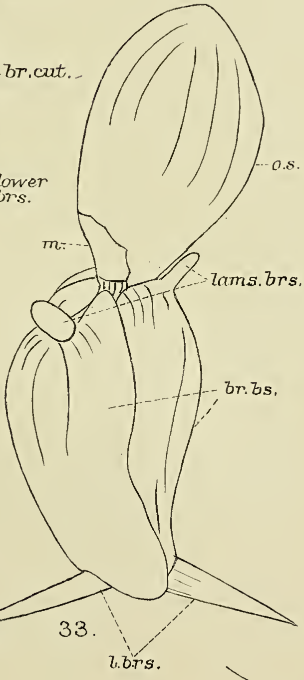

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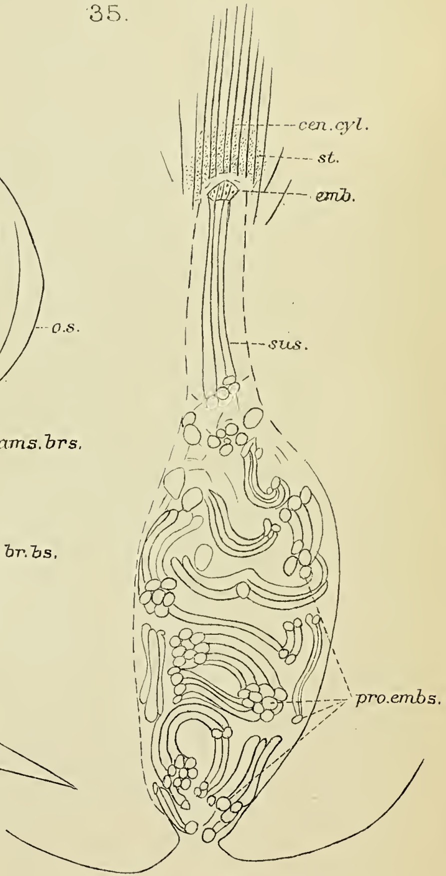

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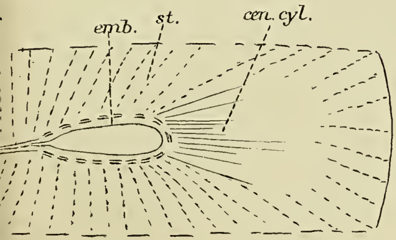

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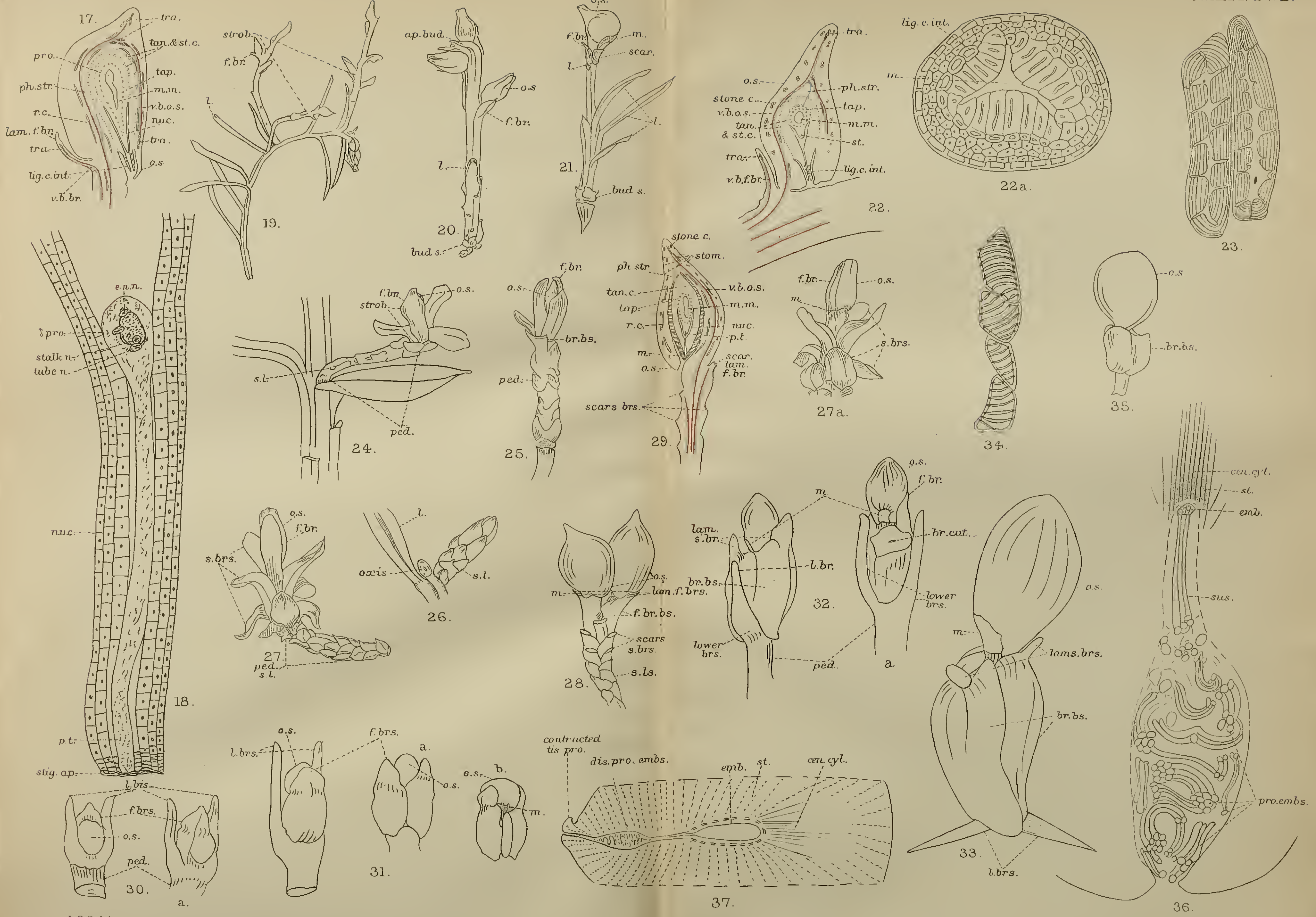

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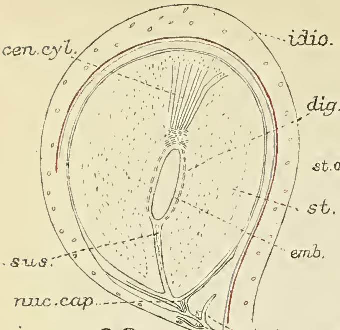
GIBBS —— PODOCARPUS.

Huth, lith. et imp.

Annals of Botany,



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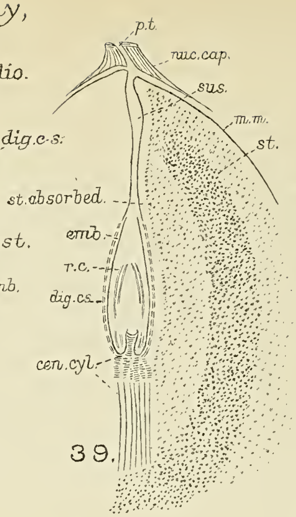

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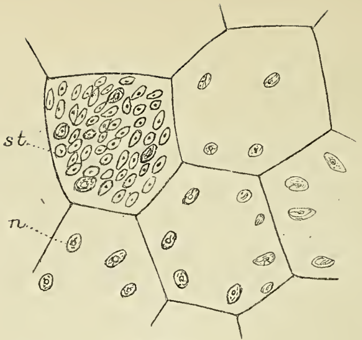

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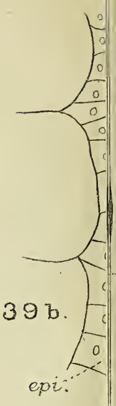

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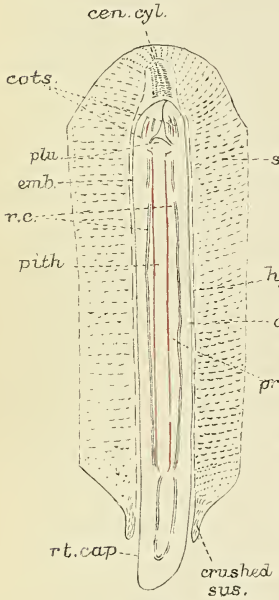

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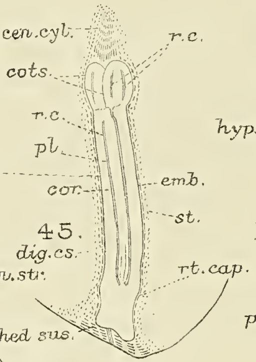

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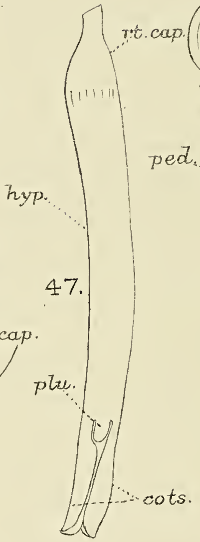

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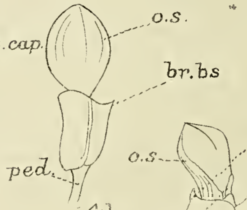

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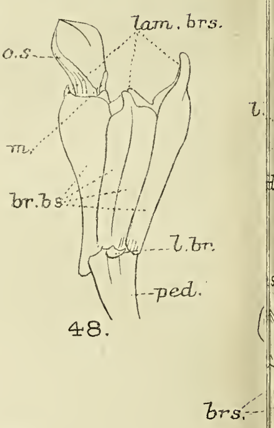

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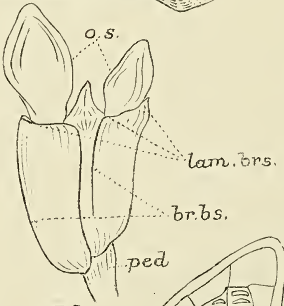

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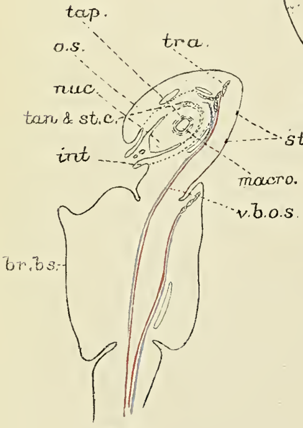

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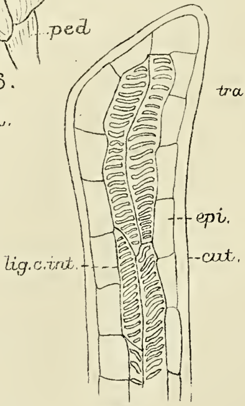

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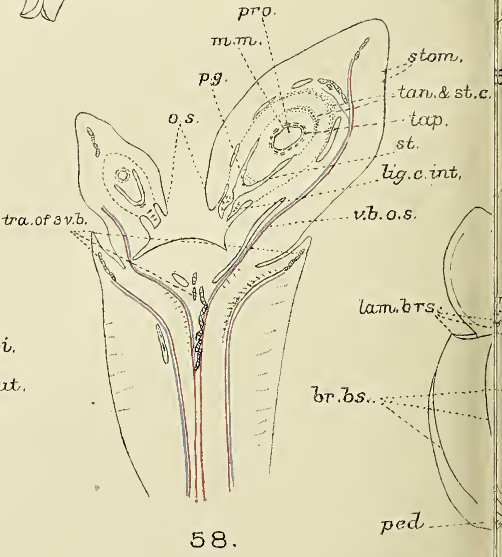

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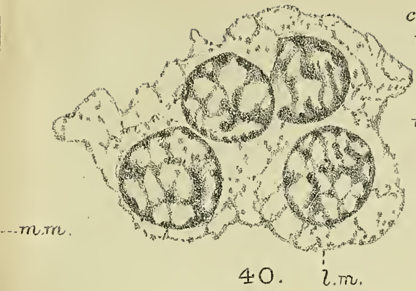
L. S. G. del.

GIBBS — PODOCARPUS.



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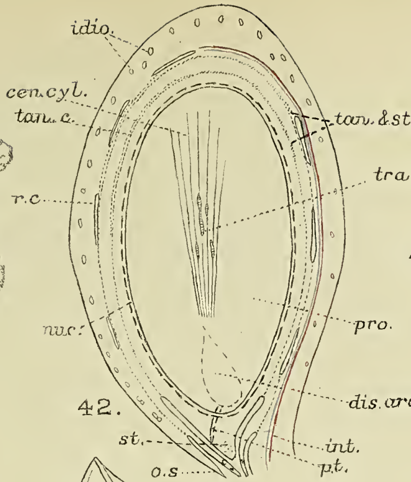

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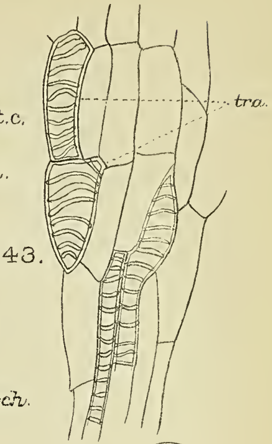

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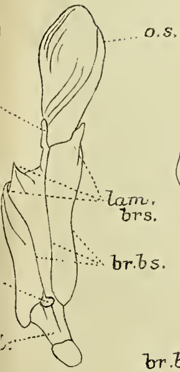

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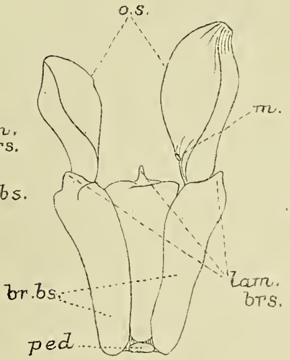

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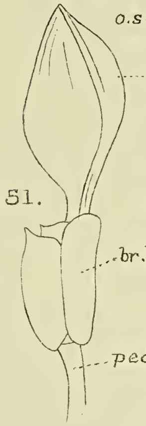

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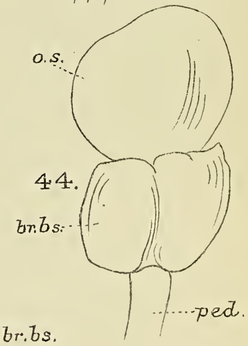

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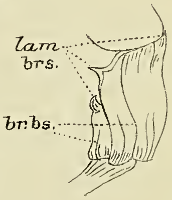

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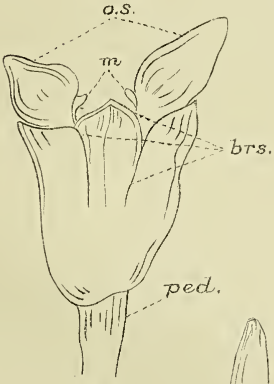

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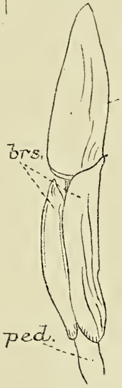

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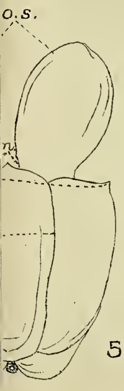

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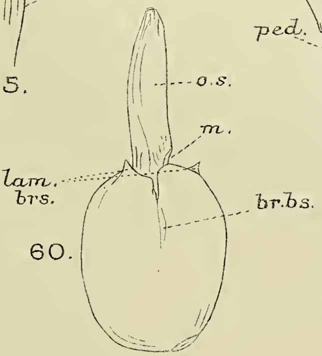

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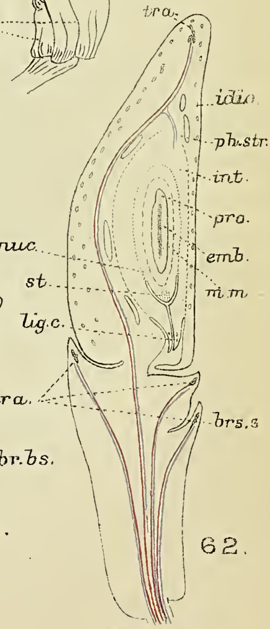

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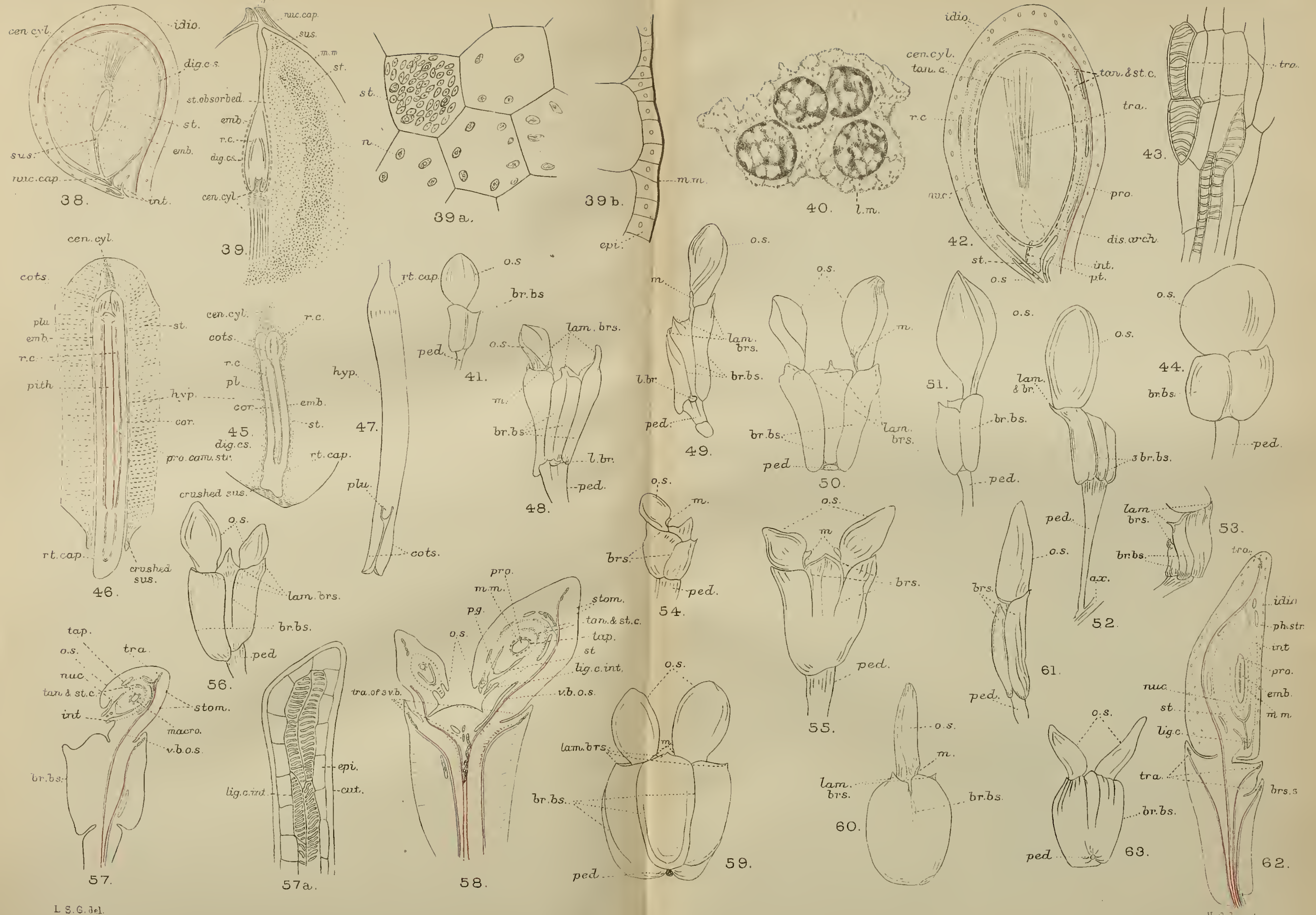

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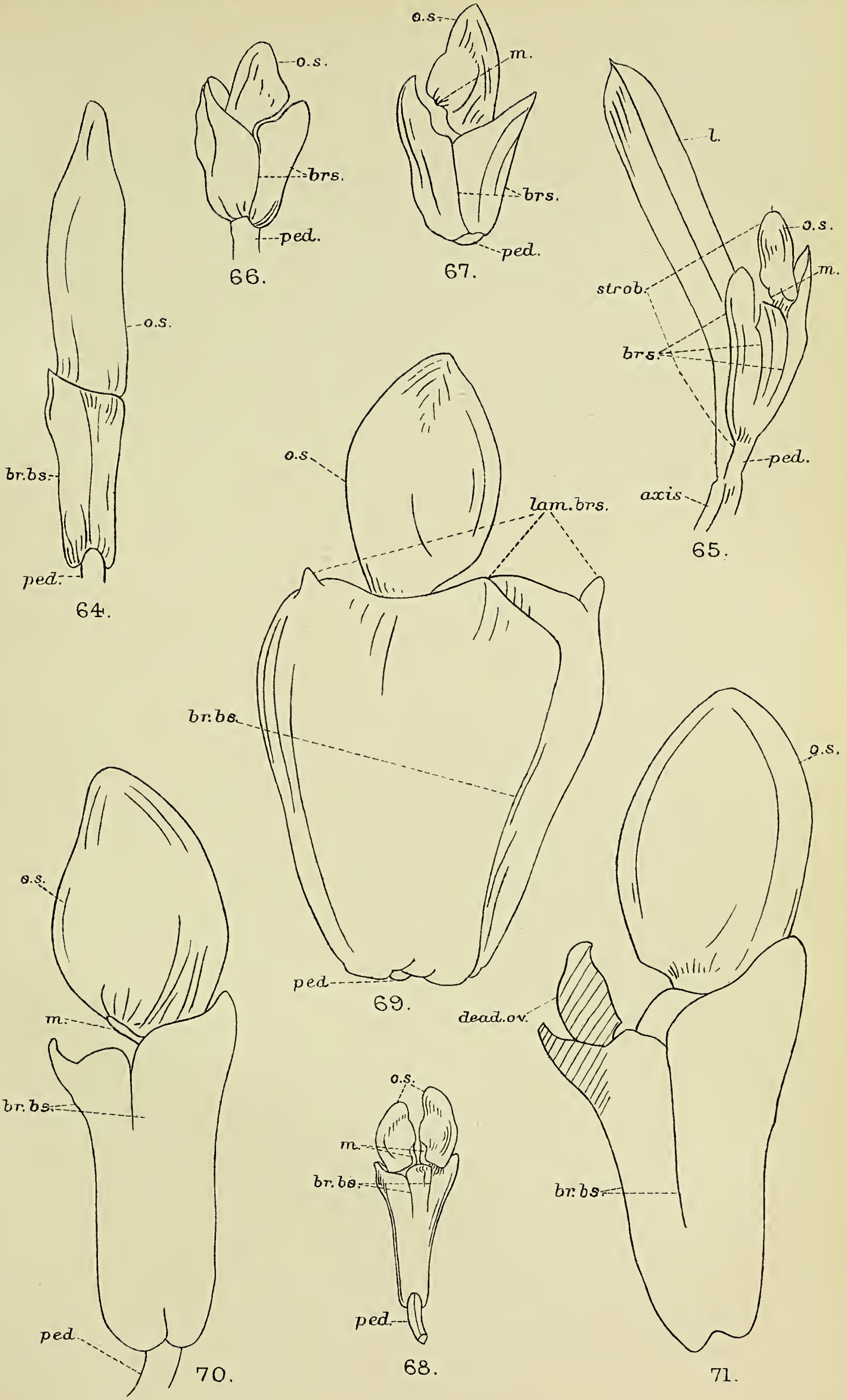

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GIBBS —— PODOCARPUS

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W. Tams, phot.

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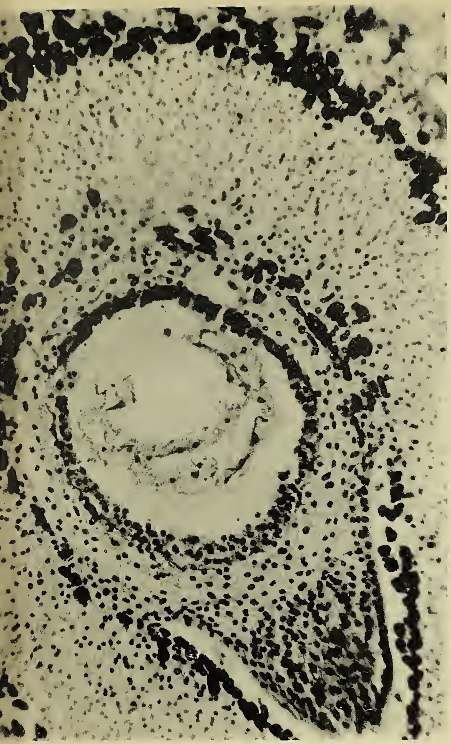

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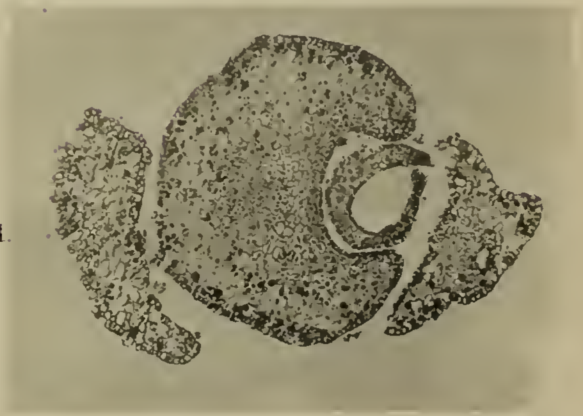

81.

W. Tams, phot.

GIBBS.—— PODOCARPUS.

Huth, coll.

The Histology of the Sieve Tubes of Pteridium aquilinum, with some Notes on Marsilia quadrifolia and Lygodiumdi chotomum.

BY

E. M. MARGARET HUME,

Bathurst Student of Newnham College, Cambridge.

With Plates LIV and LV.

NO re-examination of the sieve tubes of any Vascular Cryptogam has been made since more accurate methods of histological investigation have been introduced. It seemed therefore desirable to make an examination which would render possible a comparison between the sieve tubes of some Vascular Cryptogam and those of certain Angiosperms[1] and of *Pinus*,[2] as worked out by A. W. Hill, and of *Macrocystis* and *Laminaria*,[3] as worked out by M. G. Thoday (Sykes).

HISTORICAL.

De Bary,[4] in his 'Comparative Anatomy of Phanerogams and Ferns', seems to have been the first to recognize the parietal layer of protoplasm which forms the characteristic lining of the sieve tubes of Vascular Cryptogams. He also noticed that the refringent granules contained in the protoplasm were aggregated against the sieve plates, and figures[5] a connexion between granules of adjacent sieve tubes, across the pit-closing membrane. De Janckzewski[6] also mentions the presence of the refringent granules, as do most persistently all writers upon the subject. He mentions that starch is very rarely present in the sieve tubes of either Vascular Cryptogams or Monocotyledons; the latter, he says, also possess the refringent granules. He recognized that portions of the pit-closing membrane in *Pteridium aquilinum* give the same reactions as the callus of Angiosperms, but he regarded it as quite exceptional among Vascular Cryptogams, and held that their sieve plates are really unperforated.

Russow[7] confirmed de Janckzewski's observation of the presence of refringent granules in Monocotyledons. His examination of Vascular Crypto-

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[1] Hill ('08).

[2] Hill ('01).

[3] Sykes ('08).

[4] De Bary ('84), pp. 180–2.

[5] De Bary, loc. cit., Fig. 79 B.

[6] De Janckzewski ('82), pp. 59–65.

[7] Russow ('82), p. 207.

[Annals of Botany, Vol. XXVI. No. CII. April, 1912.]

gams was not very extensive, but he observed the presence of callus in most Ferns, with the exception of the Marattiaceae and Ophioglossaceae, and noticed that the refringent granules adhere to the free ends of the callus rods. He came to the conclusion that the sieve plates are perforated, but does not seem to have been able to demonstrate both callus rods and connexions at the same time.

Poirault,[1] in the course of his extensive researches into the structure of Vascular Cryptogams, also came to the conclusion that the sieve plates must be perforated by connecting threads, but he too was unable to prove it; neither could he determine whether the paired callus plugs, on either side of the sieve plate, were or were not continuous with one another across the middle lamella.

The question has remained in this uncertain condition ever since, as far as the Vascular Cryptogams are concerned. The only additional observations worthy of note are due to Boodle,[2] who, using Grübler's azoblue as a callus reagent, showed the widespread presence of callus among the Ferns, with the exception of the Schizaeaceae, Hymenophyllaceae, and, as he at first thought, of the Gleicheniaceae. Examination of material of the rhizome of *Gleichenia pectinata*, which had not been allowed to come into contact with spirit, revealed the presence of callus,[3] and suggested the probability of its presence in the other two orders as well, if suitable material and methods could be found to demonstrate it. Boodle [4] mentions that the refringent granules are 'often distinctly grouped on the sieve plate'. The question of the perforation of the sieve plates was not attacked by him, but the callus is described as a rod running through the wall, and having expanded heads. He also notes the presence of callus in the pits between sieve tubes and phloem parenchyma cells.

In *Pinus* [5] and the Angiosperms,[6] A. W. Hill has traced the development of the connexions between adjoining sieve plates. He has shown that, in their earliest condition, these appear as delicate protoplasmic threads, with a darkly staining node. Later, probably by the action of ferments working along them, the threads become converted into thicker slime strings, while, at the same time, the cellulose of the cell-wall immediately surrounding the slime string is altered into callus. Several slime strings may be contained in the same callus rod, as in *Pinus*, or each string may have its own callus rod (*Vitis*). It is only the cellulose portion of the cell-wall which becomes converted into callus; hence the callus does not form a continuous rod right across the pit-closing membrane, but is interrupted at the middle lamella. Here, however, the ferments also bring about some change, and immediately round each median protoplasmic node the pectic

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[1] Poirault ('93), pp. 191–5.

[2] Boodle ('01), pp. 395–9.

[3] Boodle, loc. cit., p. 708.

[4] Boodle, loc. cit., p. 397.

[5] Hill ('01).

[6] Hill ('08).

substances of the middle lamella become altered, so that they stain darkly with protoplasmic dyes. The portion of the middle lamella which is thus altered separates the callus rod into two halves, and is called the median nodule. The slime strings may be further bored out until they become very thick, and meanwhile the median nodule is apparently dissolved away and callus deposited as a tubular lining right across the pit-closing membrane. In *Wistaria* the wall between adjoining slime strings may be completely dissolved away, and several small strings may coalesce to form one large one. In *Vitis* the original slime strings remain independent. Callus is present on the sieve tube side of the pits connecting sieve tubes and bast parenchyma cells. The sieve tubes of *Vitis* function from year to year, and become plugged during the winter with callus, which is dissolved away in the following spring when the phloem resumes its activity.

In the light of the foregoing facts, an examination of the sieve tubes of *Pteridium aquilinum* was undertaken in order to determine whether the protoplasm of adjacent sieve tubes is, or is not, continuous across the sieve plate, and if it is so, what relation such a connexion bears to the distribution of the callus. It was also hoped, if possible, to throw some light upon the nature of the refringent granules. *Pteridium aquilinum* was chosen for the purpose, because it possesses exceptionally large sieve tubes; for the same reason *Marsilia quadrifolia* was also selected. In the latter case everything seems to indicate that the development of the sieve tubes closely resembles that of *Pteridium*, but the actual sequence of development was not worked out. The absence of secondary thickening in Ferns makes the accurate seriation of the various developmental stages of the sieve tubes a rather tedious business, as a number of blocks have to be cut, and after preparation the stages contained in them are often found to be only duplicates of those already obtained. The development was worked out in the rhizome, as it contains all the older stages as well as the young ones.

METHODS OF RESEARCH.

The methods used were, in the main, those introduced by W. Gardiner[1] and adapted by A. W. Hill[2] and M. G. Thoday.[3]

A dilute solution of iodine in potassium iodide was used for fixation, and swelling was effected by treatment, for two or three weeks, with a much stronger solution of iodine. Kolossow's mixture was used for mordanting, and for staining, safranin and London blue. Excess of safranin was washed out in dilute glycerine, and the washing-out process is continued in the London blue.[4] In cases where staining with safranin was insufficient the sections

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[1] Gardiner ('97), p. 100; ('98), p. 504.

[2] Hill ('01, '08).

[3] Thoday (Sykes) ('08, '11).

[4] For the use of this stain as a callus reagent and for washing out safranin from cell-walls, see Sykes ('08), p. 299.

were remordanted in dilute iron alum and stained in water blue made up with aniline.[1]

The material of *Marsilia quadrifolia* and *Lygodium dichotomum* was obtained from the University Botanic Garden, by the kindness of the Curator; that of *Pteridium aquilinum* was collected in Gamlingay Wood.

DISTRIBUTION AND GENERAL CHARACTERS OF THE SIEVE TUBES.

Both in *Marsilia* and in *Pteridium* the sieve tubes are characterized, as has already been stated, by their remarkable size (Pl. LV, Fig. 32).

In *Pteridium* they occur as a complete ring surrounding the xylem in each of the vascular strands of the rhizome (Pl. LV, Figs. 31 and 32). The vascular strands are flattened dorsiventrally, corresponding to the horizontal position of the rhizome, and it is on the dorsal and ventral sides of these strands that the phloem attains its greatest development. Laterally it is much thinner, the phloem elements being very small and scarcely distinguishable from the protophloem. The ring of large sieve tubes is very regular, and is not usually more than one cell thick, so that each sieve tube abuts on another on either side of it, but centrifugally and centripetally it abuts upon phloem parenchyma, protophloem, or pericycle (Pl. LV, Figs. 31 and 32). The sieve plates of the lateral walls, therefore, occur almost invariably upon the radial walls, i. e. between two sieve tubes, and being in *Pteridium* of frequent occurrence, they form a very perfect, continuous conducting channel round each of the vascular strands. The importance of this will be realized when it is recalled that the roots occur all along the ventral side of the rhizome, while a single frond is given off laterally each year; there is no aggregation of roots and leaves at a definite node, as will be described in *Marsilia.*

The protophloem (Pl. LV, Fig. 29) occurs as an almost continuous ring of very small and, in the mature state, thick-walled elements, situated just outside the ring of large sieve tubes. Irregularly distributed, and abutting on the tangential walls of the sieve tubes, are the phloem parenchyma cells (Pl. LV, Fig. 31), which are elongated, with dense contents and a large elongated nucleus. Pits can be seen in the walls between sieve tubes and phloem parenchyma cells (Pl. LV, Figs. 30 and 32). The pericycle is generally one cell thick, and the cells are very obviously sister cells of the endodermis. Pits are particularly abundant on the tangential walls between pericyclic and endodermal cells. As regards the general characters of the mature sieve tubes, they are large, very thick-walled elements, with a thin parietal layer of protoplasm containing refringent granules. The lumen of the cell appears empty in cross section, but no doubt, in life, the contents

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[1] Hill ('08).

are a watery fluid. The terminal walls of the sieve tubes are generally very much inclined (Pl. LIV, Fig. 26), so much so that in longitudinal section they are often indistinguishable from the lateral walls. Both in the terminal (Pl. LIV, Fig. 13) and in the lateral (Pl. LIV, Fig. 14) walls large thin areas occur ; these are the sieve plates. They are generally more numerous on the terminal walls, but no definite rule can be laid down, and it is almost impossible to distinguish with absolute certainty between a lateral wall with numerous large plates and a terminal wall with few small ones ; the two classes merge into one another, and a separation of the two types is not founded on any fundamental difference in their structure. In a transverse section terminal plates can often be distinguished by the fact that they may run right across the diameter of what would otherwise be a single circular sieve tube, dividing it into two elements, each having a half-moon-shaped cross section (Pl. LIV, Fig. 26). The distinction of sieve areas into sieve fields and sieve plates, employed by Hill in describing the Phanerogams, will not be of value here, since there is no sharp differentiation of the two types. The development of sieve plates on the terminal walls is sometimes very extensive indeed, the major part of the wall being thin, and the thickened portion consisting only of bars of cellulose running transversely from side to side of the wall ; such a wall is very reminiscent of that of a tracheide.

In *Marsilia* the general relations do not differ very materially ; the stele is a solenostele in which the sieve tubes form two fairly regular concentric rings, or rather two pairs of arcs, for laterally phloem development is feeble as in *Pteridium*. The sieve tubes have somewhat thicker walls than those of *Pteridium*, but in general appearance they resemble them very markedly. The development of sieve plates on the lateral walls of the internodes is not so extensive as in *Pteridium*, and gives more the impression of scattered pits. At the nodes, however, the lateral sieve plates seem to be developed fully. This is interesting in view of the fact that roots are developed at the nodes only in *Marsilia quadrifolia*, roots, petiole, and sporocarp all growing out at the same level round the procumbent rhizome. There is, therefore, a special demand for conduction around the stem at the nodes, while the internodes are comparatively passive in this respect. Russow[1] asserts that terminal walls in the sieve tubes occur, as a rule, only at the nodes, the tube running unbroken from node to node, but this statement has not been found to be correct. Terminal walls are not very frequent in the internodes, but they do occur. They are often considerably inclined, which may have caused Russow to overlook them ; and they bear numerous sieve plates ; in other details the phloem of *Marsilia quadrifolia* does not differ materially from that of *Pteridium*.

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[1] Russow ('72), p. 5.

HISTOLOGY.

In the earliest stages of development of the sieve tubes of *Pteridium aquilinum*, the same difficulties of observation were experienced as seem to have been encountered by A. W. Hill. The walls of the sieve tubes, at an early age, are thin (Pl. LV, Fig. 29), and it is not until they have undergone quite a marked increase in thickness that it becomes possible to demonstrate the presence of threads traversing the walls. The contents of the sieve tubes are at first quite considerable, consisting of rather darkly staining protoplasm, which tends to contract very easily with any reagents and, in transverse section, often falls out altogether. Such behaviour would probably rupture the connecting threads, but even in cases where the contents are still present and are little or not at all contracted, no certain indications can be seen of any connexions. As the diameter of the sieve tube increases the protoplasm becomes more and more drawn out into a thin layer, lining the walls, to which it is very closely applied. The walls are by this time considerably thickened, the areas of the sieve plates are mapped out, and in particularly favourable cases, delicate protoplasmic threads may be seen crossing the membrane (Pl. LIV, Fig. 1). At the middle lamella they show a rather more darkly staining protoplasmic node. The recognition of the protoplasmic threads before the appearance of callus is extremely difficult in the large sieve tubes, but it is easier in the elements of the protophloem. These elements become very much thickened, and show deep pits with long drawn-out pit fillings. Callus often cannot be detected at all; in fact it would appear as if the protophloem elements were not much used by the plant, and retain their juvenile characters right through life (Pl. LIV, Figs. 2 and 3). The connecting threads in the sieve plate are not as a rule aggregated into groups, but are dotted about singly all over the sieve plate. Up to, and even after, the first appearance of callus the refringent granules so often described appear to be small and distributed through the sieve tube, though mainly collected about the sieve plates (Pl. LIV, Fig. 1). It cannot be said with absolute certainty that, at this stage, they bear a definite relation to the threads, since they often appear to have no connexion with them; how far this can be attributed to disturbance brought about by cutting, however, it is not very easy to say.

The callus appears first as a shallow basin about the head of each thread (Pl. LIV, Fig. 4); callus formation seems generally to take place simultaneously on both sides of the pit-closing membrane, though some instances[1] were observed in which it was advanced further on one side than on the other (Pl. LIV, Fig. 4). Callus formation advances from either side towards the middle lamella, but stops short at it, while a corresponding

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[1] Cf. Sykes ('08), Pl. XIX, Fig. 15; and Hill ('08), Pl. XVII, Fig. 24.

alteration of the substance of the middle lamella between the callus plugs takes place. This change in the middle lamella causes it to have the property of staining with protoplasmic dyes, and after treatment with safranin the altered places are very conspicuous as red dots (Pl. LIV, Figs. 5 and 6). The threads stain more darkly at this stage, but are still very slender, so that they are not very conspicuous; before treatment with London blue they can generally be traced fairly easily, but after the callus has been stained, the deep blue colour is often sufficient to obliterate the outline of the slime strings altogether (Pl. LIV, Fig. 4). Each slime string possesses its own distinct pair of callus basins (Pl. LIV, Fig. 9), though when two slime strings are sufficiently near together their callus basins may touch one another, and give the appearance of more than one string contained in a single mass of callus (Pl. LIV, Fig. 6). Soon after this stage has been reached, it is quite apparent that the arrangement of the refringent granules bears a very definite relation to that of the slime strings and callus basins (Pl. LIV, Fig. 12). The granules are larger than in the earlier stages, and their appearance suggests that they are more of the nature of mucilaginous droplets than of granules. A refringent granule is attached to each end of the slime string (Pl. LIV, Figs. 12 and 17), and in a section of a sieve plate each slime string has the appearance of a hatpin with a knob at each end. Whether, however, the callus is of a sticky nature and causes the granules to adhere, or whether they are actually connected with the slime strings, could not be determined. In a surface view of a sieve plate the refringent granules, which stain darkly with safranin, make the callus basins invisible (Pl. LIV, Figs. 13 and 14), though on focusing carefully the granules take a mauve tinge, due to the blue of the callus below them; in sections of this stage, stained in London blue only, there can be seen successively, on focusing downwards, the clear outline of the upper refringent granule, then a blue spot (the callus rod), and lastly the outline of the lower refringent granule. In sections stained with both London blue and safranin the callus can only be seen here and there in surface view as a blue spot, where the refringent granule above it has been dislodged (Pl. LIV, Figs. 15 and 16); in such cases the median nodule can be focused as a red spot in the midst of the blue (Pl. LIV, Fig. 15). The median nodule is also visible as a dark spot on focusing down through the granules (Pl. LIV, Fig. 15). The granules seem to grow larger after their attachment to the ends of the slime strings becomes apparent (Pl. LIV, Fig. 16). When this stage becomes visible the callus basins begin to be dissolved away (Pl. LIV, Figs. 18 and 19), possibly through the action of the granules, which sink down into the cavity left by the disappearance of the callus. The median nodule, also, next becomes entirely dissolved away, so that there is now a thick slime string (Pl. LIV, Figs. 18–26) stretching uninterruptedly across the pit-closing membrane, and derived apparently, in part at least, from the refringent granules or mucilaginous droplets of each end of the slime string. The solution of the callus does not always go on quite at the same rate all over the same sieve plate, so that various stages of the sinking in of the granules may be observed. Sometimes the sinking in has not proceeded quite at the same rate (Pl. LIV, Fig. 19) on both sides of the plate; and sometimes the granule becomes dislodged by cutting after it has sunk in some distance, and the deep pit into which it had sunk then becomes visible (Pl. LIV, Fig. 24); the pit thus left empty has noticeably the same shape and size as the callus basin from which it was derived. Whether the callus always becomes completely dissolved away, or whether a thin shell or tubule is sometimes left, is not quite certain. Certainly stages were often seen in which no remaining trace of callus could be detected, but others were also observed in which a very faint blue tubule could be seen before the slime string was stained in safranin. Probably the solution is ultimately completed, and as far as could be seen no redeposition of callus ever takes place. Portions of the rhizome, which must have been many years old, were examined and the sieve tubes were found to be still functional; thick slime strings, such as those described above, generally perforated the sieve plates; in a few places traces of callus were observed still, but there is little doubt that these represent paths, which, for some reason or another, have not functioned very extensively. The whole aspect of the sieve plates suggests most strongly that it is as the result of use that much of the foregoing process of evolution is gone through. Thus the most rapidly developed slime strings represent the most frequented paths, and in support of this view it may be mentioned that in the terminal plates, which necessarily see the most service, the development is most rapid.

In the pits connecting sieve tubes with phloem parenchyma cells (Pl. LIV, Figs. 27 and 28), callus was never observed,[1] even in elements in which it was fully developed in the sieve plate. The pits are simple depressions of considerable depth, usually containing a single connecting thread. The wall of the sieve tube being a great deal thicker than that of the adjoining parenchyma cell, the middle lamella is asymmetrically placed, lying very much nearer to the lumen of the parenchyma cell than to that of the sieve tube. The connecting thread can be seen as a granular protoplasmic filament running to the middle lamella, where there is a dark dot—the median node. The wall between the lumen of the parenchyma cell and the middle lamella is so extremely thin that the detection of any pit or thread on that side is very difficult, but in some cases they can be seen to exist. It would seem, therefore, as if there is no boring out of the thread even on the sieve tube side, such as Hill[2] has described for *Pinus* and the Angiosperms, and his view that the ferment action is initiated

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[1] Contrast Hill ('01), Pl. XXXIII, Fig. 23, and pp. 600–2, and Hill ('08), Pl. XVIII, Figs. 50, 51, 52, and pp. 278–80.

[2] Hill ('08), p. 281.

within each sieve tube, and necessarily works outward in all directions towards the middle lamella, would hardly be supported by the facts in *Pteridium*. Of course it may be that callus does occur very transiently in these pits at some stage, examples of which have not been hit upon. This seems hardly probable, as, did it occur, the most likely place would be in sieve tubes where the sieve plates show the presence of callus; but in these no callus was found in the pits in question. If the slime strings become bored out by use, by the travelling of ferments and other substances along them, it seems strange that the same process should not take place in the case of threads leading into the phloem parenchyma cells, which have every appearance of being in a state of activity.

Accumulations of callus (Pl. LIV, Figs. 10 and 11) were occasionally observed, but their presence could not be connected with any particular stage in development, nor was their distribution at all regular; possibly their formation is attributable to some pathological condition in the particular elements in which they were found. Callus formation is generally entirely limited to the paired basins connected with each slime string. Material was gathered in December and examined for accumulations of callus, blocking the sieve tubes during the winter rest, but no evidence of anything of the kind could be seen. Sections were examined, from the apex of the rhizome backwards to regions as much as five years old (the giving off of a frond being taken as an annual event), and in no part was a development of callus beyond the ordinary detected. It does not seem therefore as if any blocking of the sieve tubes during winter takes place.

MARSILIA QUADRIFOLIA.

No systematic examination of *Marsilia* was made, as has been stated already, but the stage at which callus is developed was detected, and everything indicated that the mucilaginous droplets behave in the same way as in *Pteridium*. The lateral plates in the internodes are few and small, often taking the form of isolated threads in pits; they seem to be but little used, as even in sections of comparatively older material their threads appear to be granular, protoplasmic, and unaffected by ferments.

Some material of *Lygodium dichotomum* was also examined, especially in order to investigate the presence or absence of callus, in view of Boodle's inability to demonstrate it in the Schizaeaceae.[1] None could be detected, but, as the material available was all of nearly the same age, this was not surprising. Preparations stained with safranin were not made, so that no definite statements can be made as to the perforation of the sieve plates, but the aspect of the unstained sieve tubes suggested that they did not differ materially from those of *Marsilia* and *Pteridium*.

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[1] Boodle ('01), p. 396.

CONCLUSIONS.

According to de Janckzewski, as summarized by Russow,[1] the sieve tubes of Vascular Cryptogams differ from those of Phanerogams in their shape, in the absence of callus, and in the fact that the pores of the sieve plate are always closed. Russow was able to show that Cryptogams are not characterized by the absence of callus from their sieve tubes, and the foregoing account shows that the pores are not closed, but are as open as they are in Phanerogams ; and that the process of development is similar in many ways. The outstanding differences therefore are in shape and contents ; the sieve tubes of Vascular Cryptogams are larger and thicker walled and contain refringent granules.

The size of the sieve tubes and the thickness of their walls in Vascular Cryptogams are, it would seem, very likely dependent on the fact that, owing to the absence of secondary thickening, they have to function for a long time, in some cases for as much as twenty years, while in some Dicotyledons and Gymnosperms they are renewed each year. In some Phanerogams (as in *Vitis*), it is true that they do function for more than one year, after being blocked with callus in the winter. *Vitis*, being a climber, perhaps needs more than the average amount of phloem, and therefore makes use of more than the current year's growth of phloem. It was suggested to the writer that the absence of blocking by callus in winter in *Pteridium* might be due to the fact that the rhizome is an underground organ, and is therefore not so severely affected by the frost as an aerial one would be. With a view to determining whether this theory is borne out by the state of affairs in other underground organs, a rapid examination was made of winter material of the roots and stem of *Vitis vinifera*. The sieve tubes of the stem showed the great pads of callus blocking the sieve plates which Hill has described,[2] but in the roots no such pads could be found, and the sieve tubes showed masses of slime pressed against the sieve plate, the pores of which were clearly traversed by the slime strings. Little doubt, therefore, seems to exist that the sieve tubes of *Pteridium* remain unblocked by callus in winter because the rhizome is a completely subterranean organ and is, on that account, not subjected to the severities of weather to which an aerial organ would be exposed during the winter.

The facts ascertained concerning the finer histological details of the sieve tubes of *Pteridium aquilinum* correspond closely with Hill's observations on *Pinus* and the Angiosperms. The presence of the protoplasmic threads in the early stages, and the way in which they are bored out to form slime-strings associated with callus, are all materially the same. The salient differences are the ultimate total disappearance of the callus while the sieve

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[1] Russow ('82), p. 208.

[2] Hill ('08), pp. 275-6, and Pl. XVIII, Figs. 41 and 54.

tube is still functional, and the presence and behaviour of the mucilaginous droplets, which still remain somewhat of an enigma. The fact that the droplets or granules occur in Monocotyledons also, and that in the early stages of the development of the sieve tubes in Vascular Cryptogams they cannot be distinguished in the granular mass of protoplasm forming the contents of the sieve tubes, seems to suggest that their manifestation may be connected in some way with the prolonged functioning of the sieve tubes. Their general behaviour and appearance suggest that they are viscous drops of slime akin to the substance of the slime strings, but whether they really function in the formation of the slime string, or whether they merely sink mechanically into the pits left by the solution of the callus, cannot be affirmed absolutely; their final fate would certainly appear to be incorporation in the substance of the slime string. It is possible that they are attached to it *ab initio*, and are at first very small, and that afterwards they gradually swell until their relationship to it is easily discernible. In that case they would seem to be simply the swollen heads of the slime strings. The separate individuality of the mucilaginous droplets would naturally become more clear as the parietal layer of protoplasm becomes thinner, consequent upon the increase in size of the sieve tube.

The constant connexion of the droplets with the sieve plate was noticed by both Russow[1] and de Bary,[2] and the latter figures the droplets as connected with one another by threads crossing the sieve plate.

With regard to the callus, it cannot be disputed that there is no very great development of callus in Vascular Cryptogams. In the life-history of each sieve tube, however, in *Pteridium*, at any rate, there is a stage at which callus is quite abundant and easily detected. An observer, examining material in the stages before callus formation or after its dissolution, would be disposed to say that callus was absent, and this no doubt led to the contradictory statements that were for a long time made upon the subject. The stage in which callus is present does not seem to last very long; the amount of the rhizome of *Pteridium* in which this stage is to be found would therefore be comparatively small in relation to the total mass, and the chance of the region containing callus being overlooked would be considerable. The same is true of *Marsilia quadrifolia*; many sections were examined, and the callus stages were only present in a few. No stages with callus were found in *Lygodium dichotomum*, but the material examined was all derived from petioles of approximately the same age, so that the absence of callus was not surprising; examination of a sufficient quantity of material of varying ages would very likely reveal it. Boodle records his inability to detect callus in the Schizaeaceae, attributable probably to the same cause, as the general appearance of the sieve tubes in *Lygodium*, though they are

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[1] Russow ('82), p. 209.

[2] De Bary ('84), Fig. 79 B.

smaller, does not suggest that their development is likely to differ fundamentally from that in *Pteridium.*

The short duration of the stage in which callus can be detected, in the sieve tubes of those Vascular Cryptogams examined, confirms the view advanced by other authors,[1] that callus can be formed as an early stage in the solution of the cellulose of the wall. The existence of callus in various states of aggregation or hydration is mentioned by Mangin[2] and M. G. Thoday;[3] these different states perhaps represent different stages of solution. Deposition of callus directly from the protoplasm does not seem to occur in *Pteridium*, unless the exceptional cases figured in Pl. LIV (Figs. 10 and 11) originated in that way.

SUMMARY.

1. The distribution of the sieve tubes and sieve plates in *Pteridium aquilinum* provides for conduction all round the vascular strand, both in the node and internode. In *Marsilia quadrifolia*, conduction around the stem is especially provided for in the nodes, but in the internodes there is very little provision for it. The variations in the distribution of the sieve plates in the two types are connected with the association together of the leaf, roots, and sporocarp at the node in *Marsilia*, while in *Pteridium* the roots have no regular distribution in relation to node and internode.

2. The sieve plates in *Pteridium aquilinum* are traversed at the earliest stage by delicate protoplasmic threads, each with a median protoplasmic node.

3. Callus is developed as a pair of basins in connexion with each individual thread, and the middle lamella between the callus basins becomes altered to form the median nodule.

4. The protoplasmic thread begins at the same time to be bored out to form a slime string, and at this stage the refringent granules or mucilaginous droplets can be seen to be arranged regularly, one at each free end of each slime string.

5. The callus next becomes dissolved away, and the mucilaginous droplets sink down into the cavities thus left.

6. Finally, the median nodule becomes dissolved away, and a homogeneous thick slime string is produced, derived partially, as far as can be seen, from the paired mucilaginous droplets.

7. The sieve tubes function for years, but no callus seems to be deposited in winter to block them. This is very probably because the rhizome of *Pteridium* is a subterranean organ. Winter material of the roots and stems of *Vitis vinifera* showed callus blocking the sieve tubes in the stem, but not in the root.

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[1] Oliver ('87), p. 112; Hill ('01), pp. 597-600; Hill ('08), p. 278; Thoday ('11), p. 673.

[2] Mangin ('92), p. 263.

[3] Thoday ('11), p. 315.

8. Connexions between sieve tubes and phloem parenchyma cells are frequent, but no callus was ever detected in connexion with them.

9. From a limited examination, the impression was received that the development of the sieve tubes in *Lygodium dichotomum* and *Marsilia quadrifolia* resembles that in *Pteridium aquilinum*.

My thanks are due to Mrs. Thoday, to whom I am deeply indebted for her never-failing kindness and counsel, and for an initiation into histological methods of research.

BOTANY SCHOOL, CAMBRIDGE, 1911.

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EXPLANATION OF PLATES LIV AND LV.

Illustrating Miss Hume's paper on the Sieve Tubes of *Pteridium aquilinum*.

PLATE LIV.

Lenses used were Swift's $\frac{1}{8}$ 'apochromatic' objective, with 6, 8, and 12 Zeiss's compensating oculars. Staining was with safranin and London blue except where otherwise stated. The blue colour in the figures represents callus. The figures throughout are of *Pteridium aquilinum*.

Fig. 1. Longitudinal section of portion of sieve plate, abutting on parenchyma cells on the right of the figure, on another sieve tube on the left. Protoplasmic threads, showing a median node

traverse the lateral sieve plates. A certain amount of granular protoplasm lines the sieve tube and is aggregated at the sieve plate. × 650.

Fig. 2. A group of protophloem elements in longitudinal section. The contents are comparatively dense, especially in the pits. The middle lamella is not visible, and only one of the protoplasmic threads shows a median node. × 650.

Fig. 3. Longitudinal section of two protophloem elements connected by protoplasmic threads. Middle lamella and median nodes are faintly visible. × 730.

Fig. 4. Transverse section of two adjoining sieve tubes. Aggregation of the granules is more marked and callus formation has commenced on both sides of the plate, but has advanced somewhat further on one side than on the other. Alteration of the middle lamella to form the median nodule has begun, and the staining of the threads is darker. × 600.

Fig. 5. Transverse section. A very common aspect of stages containing callus, in which only the median nodule and the paired callus basins can be seen. The layer of protoplasm covering the plate is thicker but less granular than usual. × 1,000.

Fig. 6. Transverse section, showing groups of paired callus basins with their median nodules in the same stage as Fig. 5. The large empty lumen and parietal layer of protoplasm of the sieve tubes and the dense contents of the abutting phloem parenchyma cells are shown. × 550.

Fig. 7. Longitudinal section, showing lateral sieve plates of radial wall in surface view. Stained with London blue only so that the callus basins alone are shown as blue dots. (Semi-diagrammatic.)

Fig. 8. Longitudinal section, showing a much inclined terminal wall in suface view. Cf. Fig. 7; the sieve plates are larger and much more numerous than in a radial wall. Stained with London blue only. (Semi-diagrammatic.)

Fig. 9. Longitudinal section of a sieve tube wall bearing numerous sieve plates. Callus basins and median nodules have been developed and the protoplasmic threads are undergoing conversion into the more darkly staining slime strings. Granules in some cases show attachment to slime strings. × 650.

Fig. 10. Longitudinal section, showing a much inclined terminal wall in surface view. An exceptional case in which there is a considerable development of callus over the surface of the plate. The granules show a regular arrangement in relation to the ends of the slime strings. × 700.

Fig. 11. Transverse section of group of abutting sieve tubes, showing accumulations of callus on two lateral sieve plates. × 480.

Fig. 12. Longitudinal section of a lateral wall with numerous sieve plates; a slightly more advanced stage than Fig. 9. The slime strings are thicker, and the relation of the granules to the slime strings is more definite. Solution of the callus has commenced, and its outline has become less sharp. × 650.

Fig. 13. Surface view of a large inclined terminal plate. The sieve plates are very numerous and show the distribution of the refringent granules all over them, each granule concealing a slime string beneath it. × 750.

Fig. 14. Surface view of radial wall. The sieve plates are smaller and much less numerous. Refringent granules as in Fig. 13. × 600.

Fig. 15. Surface view of two sieve plates in optical section. The refringent granules conceal the underlying callus and slime strings, except in one or two cases where the granule has been dislodged and the callus has become visible. The median nodule is focused as a dark dot in the midst of the callus basins and is also visible through one or two of the granules. × 800.

Fig. 16. Two sieve plates in surface view. The granules have increased in size. One granule dislodged, showing corresponding underlying callus basin. × 800.

Fig. 17. Longitudinal section of a stage corresponding to surface view in Fig. 16. Paired callus basins, median nodules, and refringent granules, some of which have been dislodged. × 800.

Fig. 18. Transverse section of adjoining sieve tubes with lateral plate between them. Three slime strings, considerably thickened, with granules attached, cross the sieve plate. The callus is partially dissolved away, leaving only a thin tubule; the median nodule is still present. × 600.

Fig. 19. Longitudinal section of single sieve plate. Callus, with slime strings not much thickened, but with granules attached on one side of the plate. Callus dissolved away on the other side, one granule sinking in and another detached not far off. × 800.

Fig. 20. Longitudinal section of three slime strings, the top one cut obliquely; the callus is

completely dissolved. The paired granules of the middle slime string have sunk in unequally. The paired granules of the bottom one are connected by a thick slime string and the median nodule is still visible. × 1,000.

Fig. 21. A longitudinal section showing four slime strings. The granules have sunk more deeply in and almost abut upon one another. × 1,000.

Fig 22. Longitudinal section of a single slime string. The granules connected by a short thick slime string. × 1,000.

Fig. 23. Longitudinal section of a single slime string. Two granules in contact across the middle lamella. × 1,000.

Fig. 24. Longitudinal section of a single slime string. The granule on one side has sunk in almost to the middle lamella. The granule of the other side has become dislodged in cutting and is lying near. The secondary pit formed by the solution of the callus and from which the granule has been dislodged is visible. The sieve plate is one containing only the single slime string illustrated, and the thickened portions of the wall limiting the sieve plate are visible behind the granules. × 1,000.

Fig. 25. Transverse section of two sieve tubes with a lateral plate connecting them. Thick slime strings with median nodules and paired granules. × 420.

Fig. 26. Transverse section, showing an inclined terminal wall crossing the lumen of the large sieve tube and dividing it into two separate elements. The granules are in various stages of sinking in and the slime strings are thick. Owing to the fact that the wall is inclined, many of the slime strings being cut obliquely are incomplete, and others have had their granules detached. × 600.

Figs. 27 and 28. Transverse sections of a portion of a sieve tube abutting upon phloem parenchyma cells. The pits each contain one, or more rarely two, protoplasmic threads. The median nodule is visible as a dark spot. The wall of the sieve tube is much thicker than that of the phloem parenchyma cell, so that the portion of the thread traversing the wall of the latter is very short and only just recognizable. × 600.

PLATE LV.

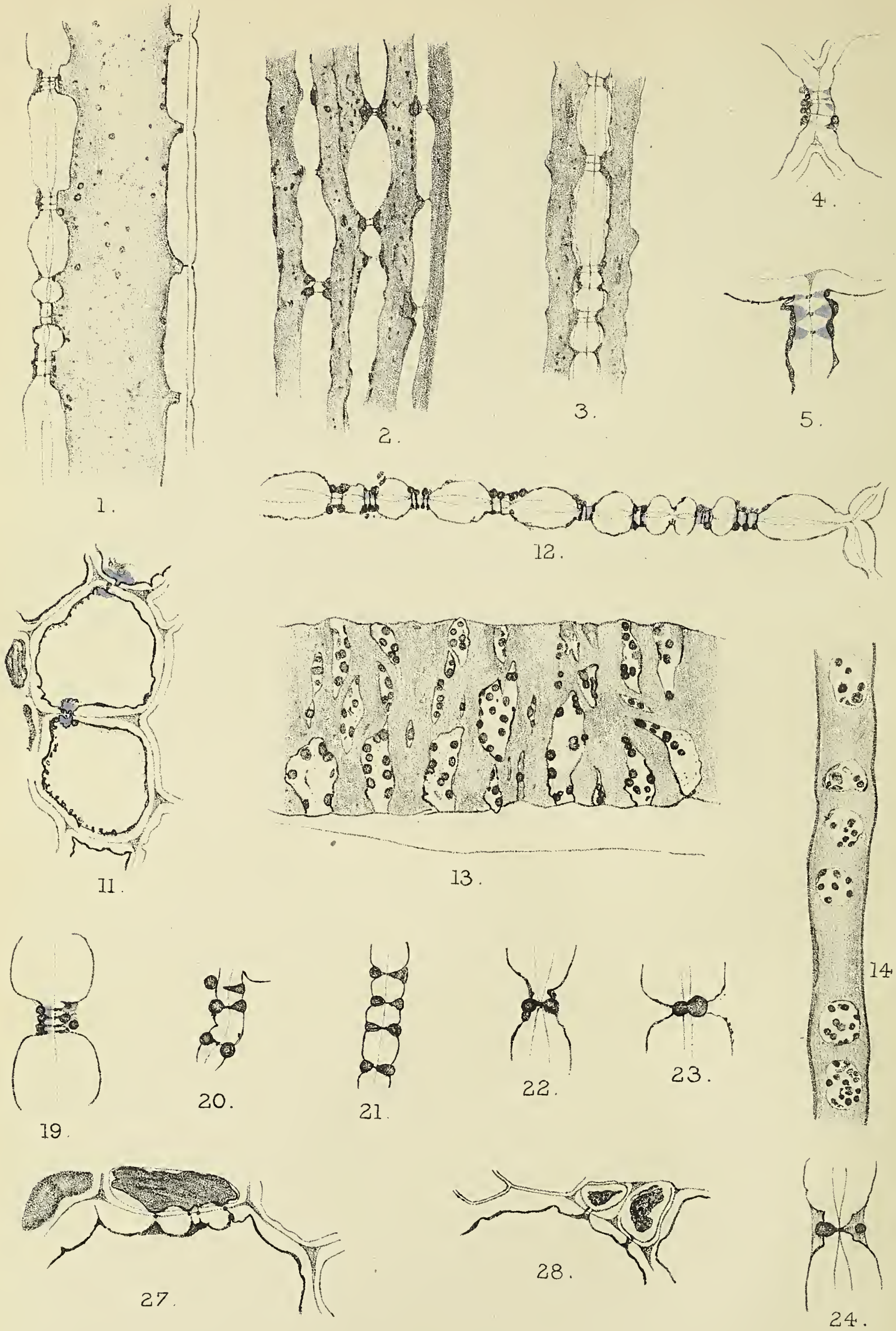
C = cortex; *E* = endodermis; *P* = pericycle; *PPh* = protophloem; *Ph* = phloem;
PPr = phloem parenchyma; *X* = xylem.

Fig. 29. Transverse section of young rhizome. The sieve tubes (*Ph*) are unthickened and the contents have fallen out. The protophloem elements are considerably thickened, and many of them still retain their contents, but no connecting threads are visible. × 470.

Fig. 30. Transverse section of rhizome, old. A group of sieve tubes with lateral sieve plates connecting them. Traversing the sieve plates, the slime strings can be seen indistinctly. The refringent granules are visible as dark dots embedded in the walls in connexion with each slime string, especially on the largest plate of the largest sieve tube *A*; the pits connecting a sieve tube with a phloem parenchyma cell can be distinguished. × *c.* 500.

Fig. 31. Transverse section of portion of mature rhizome. The distribution of the sieve tubes as a peripheral ring of elements, connected with one another by sieve plates on the radial walls, is shown. The position of the sieve plates is indicated, slightly diagrammatically, by the blue colour of callus. Protophloem is almost absent from this section. × 450.

Fig. 32. Transverse section of portion of rhizome, old. The peripheral row of sieve tubes skirting the xylem is shown. The sieve plates on the radial walls are large, and in places the granules and slime strings can be seen. × *c.* 500.



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HUME — PTERIDIUM AQUILINUM.

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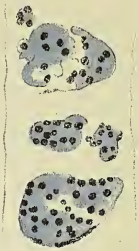

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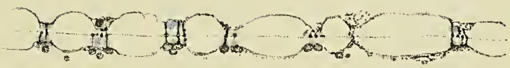

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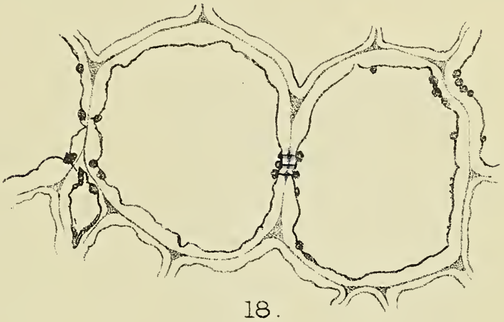

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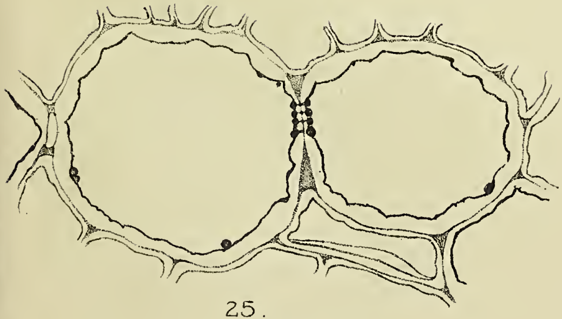

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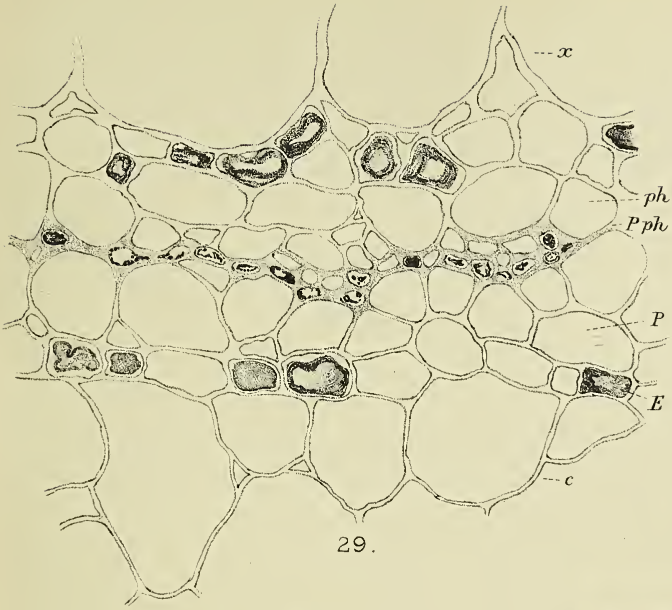

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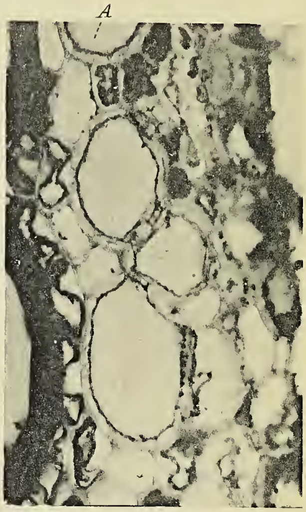

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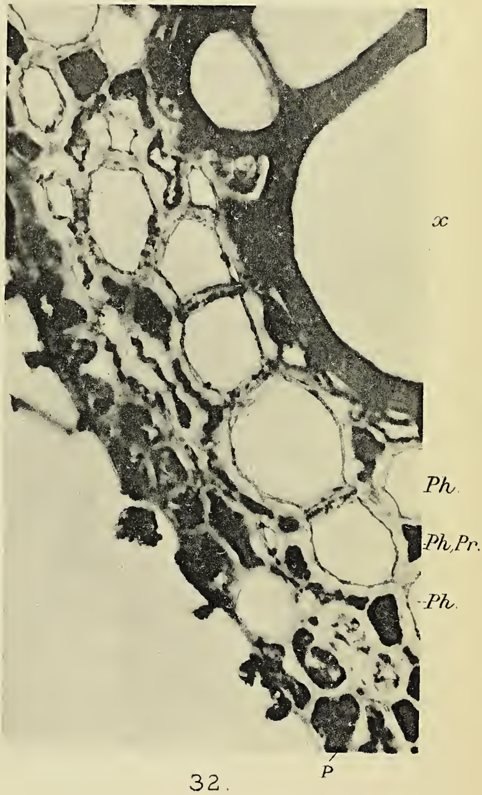

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HUME —— PTERIDIUM AQUILINUM.

The Production of Hairs on the Stems and Petioles of Tropaeolum peregrinum, L.

BY

ARTHUR W. HILL, F.L.S.

With Plate LVI and seven Figures in the Text.

THE common Canary-creeper or 'Canariensis'[1] of gardens is generally assumed to be a typically glabrous plant, and an examination of specimens grown in the British Isles or of material collected in South America preserved in herbaria fully bears out this assumption. Occasionally, however, it may be noticed that plants growing under favourable conditions show a very few hairs on young stems or petioles, but where they are growing normally and luxuriantly no traces of hairs can be seen, and the plants are absolutely glabrous.

In the summer of 1910, I noticed that some small plants of *Tropaeolum peregrinum*, growing in my garden, which had received injury to the leaf laminae, had developed a few hairs, and from this observation it seemed desirable to try and find out the cause of the production of hairs, and to ascertain the conditions under which it may be artificially induced. In the first case in which hairiness was noticed, snails were found to have eaten the laminae of the leaves more or less completely, leaving the young plants with only stems and bare petioles.

When making experiments the laminae were removed from healthy plants, and after an interval of a few days hairs were quite conspicuously developed on the young portions of the stem and on the young petioles. Experiments have been repeated during the past summer with greater care. Seedlings were grown until they had developed about ten leaves, and all the laminae were then removed; as each new leaf began to develop, its lamina was also removed, so that, except for the green stems and petioles, the plants were deprived of their main organs for assimilation and transpiration. Some of these plants so mutilated have been grown in the open, and some under a bell-jar in a moist atmosphere. It was found in August last (Aug. 19) that such plants developed hairs in the open in about four or five days, but

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[1] *Tropaeolum canariense*, Hort. *T. aduncum*, Sm. = *T. peregrinum*, L.

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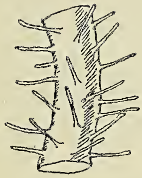
in September, both in the open and under the bell-jar, hairs were noticed on the formerly glabrous plants in three days' time (laminae removed 12 o'clock, Sept. 12, 1911, examined Sept. 14, 1911, and a few developing laminae removed, no hairs seen; Sept. 15, 1911, scattered hairs especially on younger parts. Rain fell on the night of 13th and air cool). Again, in October, the experiment was repeated during cold, dull, foggy weather (Oct. 14–16), and a plant under a bell-jar deprived of all its laminae developed a fairly dense felt of hairs on the younger parts in a space of three days. A similar plant in the open developed hairs, but to a less extent. Control plants with the laminae undisturbed, grown both in the open and under a bell-jar, showed no trace of hairs. It was noticed, however, that under a bell-jar a very few hairs were developed occasionally if the plant had been covered for about two weeks. The plants which had been stripped of their laminae were continued in this condition by removal of all developing leaves for about ten days, with the result that quite a dense felt of hairs was developed, clearly visible to the naked eye. In the case of the plants under a bell-jar the hairs were much longer than those of plants in the open, and hairs measured from 0·5–1 mm. in length. If, after this copious development of hairs has proceeded for some days, the plant is again allowed to grow normally and the laminae are not removed, the production of hairs gradually diminishes, and in a short time ceases, with the result that the stems and petioles are again as glabrous as when the experiment was started. The hairy condition can be again induced in the growing portions by removal of the laminae, and the glabrous by their retention, so that by the alternate removal and retention of the laminae the hairy or glabrous condition may be produced. Should, however, the leaves which are allowed to form after a glabrous period be malformed and remain more or less aborted, the stem and petioles will still retain their hairy character.

On a plant which had been kept defoliated for some weeks under a bell-jar out of doors, it was noticed that hairs had developed on the midribs and under sides of the young leaves which were allowed to form at the end of October.

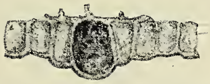
The hairs in *T. peregrinum* are unicellular, the outgrowth of a single epidermal cell. In a transverse section of the stem of a glabrous plant all the epidermal cells appear to be identical in character, and no cells can be distinguished as potential hair cells. When, however, hairs are about to be developed, certain epidermalcells become filled with dense granular contents, and such cells, at a slightly later stage, form small protruding papillae which will grow out to form the long unicellular hairs.

The full-grown hair has thin walls, and contains a copious supply of granular vacuolated protoplasm with a conspicuous nucleus suspended either near the base or towards the middle of the hair. The apex of the hair is rounded and slightly swollen, which makes it appear somewhat glandular, but this appearance is only due to the refractive character of the protoplasm at the tip of the hair.

The hair cells are seen to occupy a position comparable to that of the stomata in relation to the ordinary epidermal cells of the stem when a surface view or longitudinal section of the stem is examined. The epidermal cells are elongated, while the cell from which a hair arises is almost circular in outline. No such small cells could be seen except when a hair was either in process of formation or had already been formed. It would seem, therefore, that the stimulus caused by the removal of the laminae induced division in some of the epidermal cells, and that one of the products of the division gives rise to a hair, while the other elongates to form an ordinary epidermal cell. Such cell-division appears to take place near the apex of the stem when the tissues are still quite young, but it is also clear that hair mother-cells may be cut off from epidermal cells in older portions, as it has been noticed that the longer the plant is deprived of its laminae the denser the hairs become throughout the whole portion of the stem which is still capable of growth.

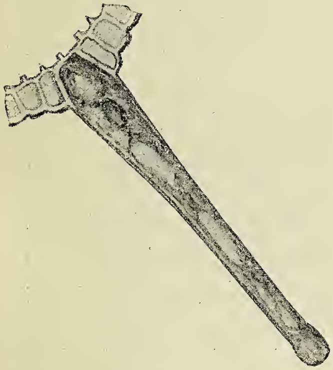


TEXT-FIG. 1. A small piece of stem showing hairs.

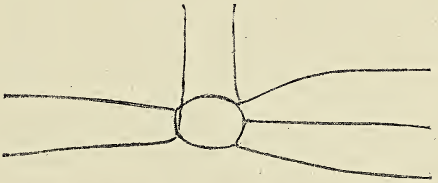


TEXT-FIG. 2. An epidermal cell, destined to become a hair, with dense granular contents.

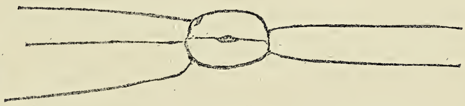
TEXT-FIG. 3. A slightly later stage, the cell forming a papilla.



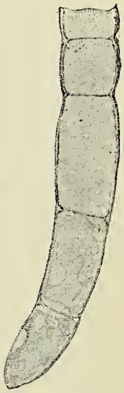
TEXT-FIG. 4. A fully developed hair with nucleus suspended near the base.



TEXT-FIG. 5. Surface view of an old piece of epidermis showing a small hair cell in optical section.



TEXT-FIG. 6. A stoma in optical section to compare with Fig. 5.



TEXT-FIG. 7. A young hair of *Tropaeolum majus*.

As far as I am aware very little has been done in the way of attempts to produce a hairy or glabrous condition, though characters of hairiness or the reverse are known to be variable[1] among plants according to circumstances.

Vesque[2] discusses the development of hairs, and agrees with the theories of Kraus and Mer that the formation of hairs is due to the excess of nutritive matters and to the arrest in growth of the organs which carry them. He adds, 'Des plantes différentes sont plus ou moins propres à développer des poils sous l'influence du milieu. Tandis que certaines espèces deviennent velues au point d'être méconnaissables, je n'ai jamais réussi à faire développer un seul poil sur une plante réellement glabre, sur le pois, par exemple.'

Vesque would possibly not consider *Tropaeolum peregrinum* to be a truly glabrous plant, and it is unfortunate that his paper was not brought to my notice until it was too late to make similar experiments on peas to those made on the Canary-creeper.

It is of interest to notice that the allied species, *Tropaeolum majus*, the common 'nasturtium' of gardens, is always hairy on the under surfaces of the leaves, and when grown under unfavourable conditions the starved plants tend to show an extension of the hairs down the short petioles. But when the plants receive favourable treatment the petioles become elongated and glabrous. The hairs, however, in *T. majus*, which are normal organs of the plant, differ from the straight unicellular hairs of *T. peregrinum* in being multicellular and curved. The hairs which arise from single epidermal cells may consist of from three to several cells, the terminal cell being usually the longest.

The cause of hair production in *T. peregrinum* may perhaps be due to the excess of water supplied to the plant in its abnormal condition, and to the sudden arrest of growth owing to the removal of the large evaporating and growing surface represented by the laminae. It was not obvious that the hairs performed any marked function connected with transpiration, though it may well be that they represent the plant's efforts to provide a substitute for the large transpiring surfaces which it has lost.

EXPLANATION OF PLATE LVI.

A photograph of the upper part of a plant deprived of its laminae and showing a considerable development of epidermal hairs. Slightly enlarged.

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[1] Yapp on *Spiraea Ulmaria*. Brit. Assoc., Portsmouth, 1911.

[2] Sur les causes et sur les limites des variations de structure des végétaux. M. J. Vesque, in Ann. agronomique, vol. ix, pp. 495–6.

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HILL — TROPAEOLUM.

Anatomy of Gnetum africanum.[1]

BY

AUGUSTA V. DUTHIE.

With Plates LVII–LIX.

THE following account of the anatomy of *Gnetum africanum* is based on material collected by Professor H. H. W. Pearson in Montobello, on April 7, 1909. My thanks are due to Professor Pearson for handing over the material to me for investigation, and for much helpful advice with regard to the work.

In view of the systematic position of *Gnetum* and the difficulty of obtaining suitable material for investigation, it seemed worth while to place on record the facts ascertained with regard to the anatomy.

Brief Review of the more important Published Work on Gnetum Anatomy.

Griffith's 'Remarks on *Gnetum*', which appeared in the Linnean Society's Transactions of 1859, contains a few references to the anatomy of the genus. Professor Bower in 1882 published an account of the germination and seedling structure of *G. Gnemon*, while the seedlings of *G. scandens* and *G. moluccense* have been investigated recently by Hill and de Fraine. A few references to the anatomy of *G. Gnemon*, *G. scandens*, and *G. Thoa* occur in de Bary's 'Comparative Anatomy of Phanerogams and Ferns'. Strasburger, in 1872, investigated the course of the bundles in leaf and stem, and the vascular supply of the axillary buds in several species of *Gnetum*. In 1894 Boodle and Worsdell published a paper on the 'Comparative Anatomy of the Casuarineae, with special reference to the Gnetaceae and Cupuliferae'. The species of *Gnetum* examined were—*G. Gnemon*, *G. paniculatum*, *G. scandens*, *G. neglectum*, and *G. Thoa*.

External Characters.

The genus *Gnetum* comprises between 20 and 30 species of tropical trees and climbers, two of which—*G. africanum* and *G. Buchholzianum*—are natives of the African continent. *G. africanum* was first discovered

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[1] Percy Sladen Memorial Expedition in South-West Africa, 1908–9, Report No. 20.

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by Welwitsch in the Cazengo district of Central Angola in 1858, and was described by him in the Linnean Transactions of 1869. The species is a climber with long slender stems which are swollen and jointed at the nodes; the much elongated internodes are sometimes more than 15 cm. long. The plant exhibits marked heterophylly. In the material examined the lateral branches spring from the axils of short, opposite scale leaves; while the expanded foliage leaves produce only abortive buds in their axils. On superficial examination the scale leaves resemble the persistent bases of the petioles of foliage leaves, and were apparently mistaken for such by Welwitsch.[1] No root material was available.

STEM.

The oldest material examined shows a stem 5 mm. in diameter, its surface covered with a thin layer of cork. The younger internodes are marked with numerous, irregularly arranged dots and striations of a dark brown colour, while the youngest branches are smooth and green. The secondary thickening is perfectly normal; there is no trace of the successively renewed thickening rings which are present in *G. scandens*[2] and *G. Thoa*. It is possible that this abnormality may occur in older stems than those investigated. The number of vascular bundles seen in transverse section of the axis varies considerably; from 12 to 20 were observed in the internodes examined.

Xylem. The wedge-shaped xylem-masses are separated by conspicuous medullary rays (Pl. LVII, Fig. 1). The first-formed elements of the protoxylem have loosely coiled, spiral thickenings occasionally combined with annular markings. Those formed later have very close spiral or reticulate thickenings and often communicate by means of a row of large pits on the oblique walls. Reticulately thickened tracheides are specially abundant in the region of the node. Boodle and Worsdell[3] have pointed out that in *Gnetum* the vessels first formed by the cambium have usually several perforations in their end walls, resembling in this respect the well-known vessels of *Ephedra*. In several cases two rows of large, circular, bordered pits were seen in the sloping end of a reticulate nodal tracheide of *G. africanum*, and one of the vessels was found to possess two rows of perforations on the oblique wall, as described by the above authors in the node of *G. paniculatum*. In general, however, only a single row of perforations occurs in these first-formed vessels. Transitional stages between pitted tracheides with a row of large, closed, bordered pits on their terminal wall, and true vessels were observed, the central pits of the oblique walls being perforated, those at the ends closed. Transitional stages between vessels with several circular perforations and those with a single long narrow one

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[1] Welwitsch (9). [2] De Bary (3). [3] Boodle and Worsdell (1).

were also noticed (Pl. LVIII, Fig. 8, *a–d*). The later formed vessels usually have inclined end walls with a single oval or circular perforation (Fig. 8, *e–f*). In one case two large circular openings were observed. In transverse sections the remains of the oblique septa of the vessels with their perforated bordered pits are often seen (Pl. LVII, Fig. 1). Associated with the true vessels of the secondary xylem are numerous gymnospermous tracheides. The thickness of the walls and the size and shape of the bordered pits in both vessels and tracheides vary considerably. In addition to the tracheal elements large numbers of elongated cells with protoplasmic contents and simple pits occur in the secondary xylem. They do not contain starch, but occasionally numerous minute crystals of calcium oxalate are found in them. Near the cambium these parenchymatous cells are usually seen to be arranged in regular rows continuous with the albuminous cells in the phloem (Pl. LVIII, Figs. 9, 12); in the older wood they are scattered, apparently owing to the enlargement of the vessels. Occasionally, the lumina of the vessels were found to be blocked with resinous substances.

Phloem. The boundary between the xylem and phloem masses of the vascular bundles, as seen in transverse section of the younger internodes, has the shape of an arc whose concave side is directed outwards. In older internodes this appearance is generally lost. The phloem elements are extremely regular;[1] but when sections are placed in water, considerable swelling and distortion occurs. No hard bast elements are present. The large sieve tubes are arranged in radial rows (Figs. 9, 10, 12). Compound sieve plates occur in large numbers on the very oblique end walls, but are less numerous on the vertical walls (Figs. 13 and 14). On staining with iodine after heating in glycerine, minute starch grains may be seen in the albuminous contents of many of the sieve tubes. Considerable callus formation covers the older sieve plates (Figs. 15, 17, 18). These callus pads stained readily with water blue, Russow's callus reagent, and corallin soda, but were unaffected even after prolonged staining with aqueous solutions of aniline blue and eosin. Particularly good results were obtained with water blue.

The albuminous cells are also arranged radially, and, as was remarked above, they are generally continuous with the elongated parenchymatous cells of the xylem (Fig. 12). In longitudinal section they are seen to be long and narrow, with dense proteid contents and well-defined, often elongated, nuclei (Fig. 14). Their end walls are at first transverse, but later become oblique. Numerous minute crystals of calcium oxalate are often collected at one end of these cells, and small starch grains are occasionally present. A doubling of the radial rows of albuminous cells and sieve tubes often takes place. In the older phloem, owing to the displacement of the elements, the albuminous cells come to lie at the corners of the sieve tubes

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[1] Boodle and Worsdell (1).

and the likeness to companion cells is then very marked (Fig. 12). At the periphery of the bundle lies the crushed mass of protophloem. In tangential sections through the phloem of older stems, fusiform rays are often seen. These vary much in size, some of them being only one cell wide and from one to three cells high; others are several cells wide and higher in proportion (Figs. 11, 16). These rays are specially numerous near the nodes of old stems. The cells are thin-walled, with protoplasmic contents, and are packed with minute crystals of calcium oxalate. A few starch grains are occasionally present. Intermediate stages were observed between the smaller rays and albuminous cells with crystallogenous contents (Fig. 16).

Pith. The pith consists of elongated cylindrical cells with pitted walls which are conspicuously lignified in the region of the vascular ring. In most of the older internodes the central thinner-walled cells have been disorganized (Pl. LVII, Figs. 1, 4), though this is not always the case. The older stems have the pith cells packed with rounded starch grains; these are entirely absent from the pith of the younger internodes. In the pith region below the growing point, and in the pith of the nodes, large numbers of crystals of calcium oxalate were found. Running longitudinally through the pith are latex tubes similar to those of the cortex which will be described below. In the pith of the node large numbers of branched, lignified, fibrous cells occur (Fig. 5). Similar sparingly branched lignified elements are very occasionally found in the pith of the internode.

Primary Medullary Rays. As is well known, the primary medullary rays are very conspicuous, and extend from pith to cortex, gradually broadening outwards (Fig. 1). Their width varies considerably in different internodes. These rays are composed of parenchymatous cells which are often radially elongated. Many of the cells are lignified and provided with simple pits; others are packed with calcium oxalate crystals and crystal sand. The amount of lignification of the ray varies. In young stems most of the ray cells are lignified; all have conspicuous protoplasmic contents, and some are packed with crystals. Between the phloem masses large thin-walled crystallogenous cells are very numerous. In older internodes a very large number of thin-walled crystallogenous cells occur, and in the wider parts of the ray there are stone cells with lignified, pitted, and conspicuously striated walls (Fig. 1). These sclerosed cells are very numerous in the ray parenchyma between the masses of bast. Starch was found to be absent from the rays of the young internodes, but is present in considerable quantities in those of older stems. The crystals in the ray cells are usually rhombic in surface view and vary greatly in size (Pl. LIX, Fig. 30), many of them being extremely minute. They resemble closely those found in the crystal sacs of phloem and cortex. In tangential section the rays are often seen to be broken up into partial rays by vascular tissue (Pl. LVII, Fig. 2). Single bridging xylem elements were frequently observed (Figs. 2, 3). In one section a large pitted vessel was seen bridging a ray, and in a few cases bridging phloem elements were also noted.

Cortex. The ground tissue lying next to the phloem masses consists, in young stems, of thin-walled parenchymatous cells through which a few latex tubes and fibres are scattered. In older stems an irregular ring of large parenchymatous cells becomes strongly lignified, forming the conspicuous sclerenchymatous zone (Fig. 1 and Pl. LVIII, Fig. 9). The walls of these stone cells are stratified and pitted, and resemble the sclerotic elements of the old medullary rays. The fibres are elongated and unbranched, with cellulose walls and pointed ends. They are very numerous in the outer cortex, but are occasionally found inside the zone of stone cells. The white lustrous thickening layer is often found to have separated from the primary wall (Pl. LIX, Fig. 27). The marked contraction which sometimes occurs may have been caused by the alcohol in which the material was preserved. The cell lumina of many of the fibres are almost entirely obliterated; the walls of some show well-marked stratification (Fig. 27). In the cortex of the node, stellate fibrous cells also occur (Pl. LVII, Fig. 5), but they are extremely rare in the internode. In the younger internodes the parenchyma of the cortex, especially near the epidermis, contains numerous chloroplasts. A considerable quantity of storage starch is present in the cortex of older stems, and is very abundant in the parenchyma between the zone of stone cells and the phloem masses. Crystallogenous cells, similar to those of the rays, also occur in the cortex.

Latex Tubes. Among the most striking elements of the cortex are unbranched secretory tubes similar to those found in the pith and leaf-stalk. These laticiferous vessels of *Gnetum africanum* do not differ from those of *Gnetum Gnemon* described by Bower.[1] Their origin from vertical rows of elongated parenchymatous cells was observed in microtome sections of the young stem below the apical cone. Two or three nuclei were observed in several of these cells. Occasionally short, lateral protrusions of these vertical tubes were found forcing themselves between the neighbouring cells. The coagulated, albuminous contents turn a golden yellow on treatment with iodine. On placing longitudinal sections of the stem in strong sulphuric acid, the cellulose walls are dissolved and the strands of coagulated latex show up very distinctly. After prolonged treatment with alkanet tincture the contents of the tubes turn a reddish brown.

Epidermis of Stem. The epidermal cells of the young internodes are papillate, with much thickened outer wall and well-defined cuticle. The wall thickening in older internodes often projects irregularly into the cell cavity. The stomata are arranged in vertical rows and sunk below the level of the epidermis (Pl. LIX, Fig. 23). Their structure resembles that of the leaf stomata which will be described below.

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[1] Bower (2).

Cork. The formation of cork in the internodes of *G. africanum* was found to be very irregular. The phellogen originates in circumscribed areas of the stem epidermis (the dots and striations mentioned above) and then spreads peripherally (Pl. LVIII, Fig. 21). It is not unusual to find part of an internode covered with 8–10 layers of cork and the rest with no sign of phellogen formation. This is probably connected with the climbing habit of the species. Some of the older internodes are surrounded by a thin and regular mantle of cork whose surface shows many minute cracks. In several of the stems examined, however, periderm formation cuts deeper and deeper into the cortex at certain points, and thus cortical fibres and secretory tubes come to be embedded in the outer cork. Occasionally, groups of fibres are completely isolated by cork cells (Pl. LVII, Fig. 4). In longitudinal sections these cork rings are found to be connected with the peripheral layer of cork. Similar cork rings surrounding a core of fibres were found in *G. scandens* and *G. Buchholzianum.* Layers of thick-walled phelloderma cells containing numerous crystals are sometimes found inside the phellogen. In several internodes the ground tissue cells inside the sclerenchymatous zone were found to have undergone division, often to such an extent that part of the zone of stone cells was ruptured and pushed out to the periphery of the stem, while large parenchymatous cells next to the phloem masses had become sclerosed. If an anomalous zone of xylem and phloem occurs in this species, the cambium must arise in this secondary tissue inside the zone of sclerenchyma (Pl. LVIII, Figs. 19, 20). A curious feature of some of the sections examined was the mucilaginous disorganization of groups of parenchymatous cells in the region of the protophloem masses (Fig. 20).

Stem Apex. According to Stapf,[1] Dingler[2] made the surprising statement that he had traced the growth of the stem apex of *Ephedra* to a 4-sided apical cell. Microtome sections were cut through the apical cone of branches of *G. africanum*, but in every case a small-celled meristem was found.

LEAF.

As was mentioned above, *G. africanum* possesses pairs of scale leaves in addition to the expanded foliage leaves. In transverse sections of the node below the swollen bases of the foliage leaves, eight bundles are seen to leave the stele, four passing into the base of each leaf. The two median leaf-traces are the first to separate from the vascular ring (Pl. LVII, Fig. 5). Sections through the bases of the pair of scale leaves immediately below the female cone showed the same bundle supply. On tracing the bundles through the scale leaf they were seen to fuse laterally, so that near the apex the separate strands could no longer be distinguished. A small twig which possessed six pairs of scale leaves was also examined. The vascular supply resembled that described above. Undeveloped buds were

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[1] Stapf (7).

[2] Dingler (4).

found in the axils of these leaves. Sections were also cut through old nodes with opposite lateral branches in the axils of the scale leaves. Again eight bundles leave the stele as in the cases already described. These bundles branch in passing through the cortex,[1] the median leaf-traces often bifurcating before the lateral are quite clear of the vascular ring. A number of these branches enter the axillary bud, while others pass into the leaves. The material available did not admit of all the details of the leaf and axillary branch supply being made out, but in the sections examined a brush of xylem elements appears to pass up into the free part of each scale leaf.

Petiole. The short petiole of the foliage leaf is slightly furrowed above and convex beneath. Numerous latex tubes and fibres traverse the petiole and pass into the midrib. A few lignified spicular cells are also present. The four leaf-trace bundles remain distinct through the entire length of the petiole. A considerable amount of secondary tissue is formed by the cambium of each of these bundles.

Lamina. The four bundles of the petiole enter the base of the lamina and fuse laterally in pairs about one-fourth of the way up the midrib. Complete fusion occurs at some little distance above this point. The cambium of the midrib forms a considerable amount of secondary xylem and phloem. As is well known, the reticulate venation of the *Gnetum* leaf is of the normal dicotyledonous type (Figs. 6, 7). The leaf lamina, though fairly thin, is extremely tough. Below the cuticularized upper epidermis lies a single row of short palisade cells. The many-armed, spongy mesophyll occupies a considerable part of the substance of the leaf. The sclerenchymatous framework of the lamina consists of branched acicular cells and elongated fibres. The former have lignified, finely striated walls, occasionally perforated by simple pits. They are stellately branched and differ from the spicular cells of *Welwitschia* in the absence of an outer crystal-bearing layer of cellulose from their walls (Pl. LIX, Fig. 29). They occur for the most part in the stellate parenchyma close to the lower epidermis, the more prominent arms usually lying in the plane of the lamina. The fibres, which are far more numerous, are of enormous length. Their cellulose walls are so strongly thickened that in places the cell lumen is almost entirely obliterated. On isolating these fibres after maceration the ends are frequently found to be shortly and unequally branched. Similar branches occasionally occur at other points, but are seldom of great length (Figs. 28, 31). A bundle of these fibres strengthens the leaf margin and prevents tearing. These marginal fibres resemble those of the axis in being much straighter than the fibres described above and entirely unbranched.

The ordinary epidermal cells of both surfaces have strongly undulated walls (Pl. LVIII, Fig. 22), but opposite the larger veins they are narrower and more regular in outline. Nodose thickenings of the walls are fairly common.

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[1] Strasburger (8).

The epidermis is provided with a well-marked cuticle which shows up distinctly on treatment with alkanet root or a fresh solution of chlorophyll.

The stomata are small, numerous, and arranged irregularly over the whole of the under surface with the exception of the veins and veinlets. A few are also present on the upper surface over the midrib.[1] They occur on the scale leaves as well as on the foliage leaves. Each stoma is accompanied by two subsidiary cells lying parallel to the pore (Fig. 22), but the material did not admit of the determination of the origin of the subsidiary cells. Several cases of 'twin stomata' were observed. In the leaf the stomata are about on a level with the subsidiary cells (Pl. LIX, Fig. 24), but in the young stems the stomata, which are here placed parallel to the longitudinal axis, are considerably sunken (Fig. 23). Seen from above the stoma often bears a striking resemblance to the under surface of a cowrie shell (Pl. LVIII, Fig. 22). The outer wall of each guard cell is provided with a lignified flap marked by delicate striations (Fig. 22). The guard cells, as seen in longitudinal section, are enlarged at the ends and somewhat contracted in the middle (Pl. LIX, Fig. 25). They contain large quantities of starch. As in most investigated Gymnosperms, ridge-like protuberances of the membrane are absent.

SUMMARY.

Gnetum africanum is heterophyllous, producing pairs of scale leaves which subtend the lateral branches, and foliage leaves with abortive buds in their axils. The stems examined showed normal secondary thickening.

The number of vascular bundles seen in transverse section of the axis varies considerably.

The first-formed vessels have several perforations on their sloping end walls, and transitional stages were found between these and the later-formed vessels with a single circular perforation. As is usual in climbers, the vessels of the secondary wood are of considerable size.

The phloem tissue is composed of regular rows of sieve tubes alternating with rows of elongated albuminous cells. Numerous compound sieve plates occur on the oblique end walls and also on the lateral walls of the sieve tubes. Considerable callus formation takes place.

In addition to the conspicuous primary medullary rays, smaller fusiform rays packed with calcium oxalate crystals are sometimes found. Transitional forms between the latter and the elongated albuminous cells of the phloem occur. Rhombic crystals of calcium oxalate, which vary greatly in size, are present in large numbers in certain cells of the xylem, phloem, medullary rays, cortex, and phelloderma, also in the pith of the nodes.

Sclerenchymatous elements of various forms occur in the stem. Stone cells with lignified and pitted walls form a conspicuous zone outside the

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[1] Griffiths (5).

vascular bundles, and also occur in the broader parts of old medullary rays. Branched acicular cells with lignified and finely striated walls occur in the pith and cortex of the nodes. Elongated fibres with enormously thickened cellulose walls are present in large numbers in the cortex.

Latex vessels with cellulose walls and dense albuminous contents occur in pith and cortex, also in petiole and lamina of the foliage leaf.

Cork formation is exceedingly irregular. The phellogen originates in circumscribed areas of the epidermis and spreads peripherally. It is not unusual to find periderm formation cutting deep into the cortex at certain points, and occasionally groups of fibres are completely isolated by cork cells. Division of the ground tissue cells within the sclerenchymatous zone was observed.

The stem apex was found to possess a small-celled meristem.

Four bundles pass into the base of each foliage leaf, the median leaf-traces being the first to leave the vascular ring. The bundles which supply the scale leaves subtending the lateral shoots branch on passing through the cortex, a number of the bundles passing into the axillary shoots.

The reticulate venation is of the usual Dicotyledonous type.

A single layer of short, palisade cells occurs below the upper epidermis. The spongy parenchyma consists of many-armed, loosely packed cells.

Fibres and branched acicular cells are present in large numbers in the mesophyll of the leaf.

The stomata are arranged irregularly over the under surface of the lamina with the exception of the veins and veinlets. They also occur on the upper surface over the midrib, and are about on a level with the surrounding epidermal cells. The outer wall of each guard cell is provided with a lignified flap. The stomata of the young stems are sunken below the level of the epidermis.

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[1] In several cases the entire paper was not available and extracts only were seen.

EXPLANATION OF FIGURES IN PLATES LVII–LIX.

Illustrating Miss Duthie's paper on the Anatomy of *Gnetum africanum.*

PLATE LVII.

Fig. 1. Part of a transverse section of the stem. × 165.

Fig. 2. Part of a longitudinal section of the stem, showing vessels of the xylem and medullary rays. × 500.

Fig. 3. Part of a medullary ray with bridging xylem tracheides. × 500.

Fig. 4. Transverse section of stem, showing irregular cork formation and cork rings.

Fig. 5. Transverse section through node, showing leaf-trace bundles.

Fig. 6. Photograph of skeleton of young leaf. Natural size.

Fig. 7. Portion of above. × 3.

PLATE LVIII.

Fig. 8 *a*. Terminal wall of pitted vessel with row of circular perforations. × 375.

Fig. 8 *b, c*. Terminal walls of segments of vessels, showing fusion of circular pits. × 375.

Fig. 8 *d–f*. Terminal walls of vessels of old wood with single perforations. × 220.

Fig. 9. Transverse section through phloem mass, showing radial rows of sieve tubes and albuminous cells. × 375.

Fig. 10. Transverse section through a portion of a bundle, showing medullary ray cells packed with crystals of calcium oxalate. × 375.

Fig. 11. Tangential section through medullary rays in phloem. × 375.

Fig. 12. Transverse section through a portion of a bundle, showing albuminous cells packed with crystals of calcium oxalate. × 375.

Fig. 13. Radial section through phloem, showing compound sieve plates on sloping terminal walls. × 375.

Figs. 14 and 15. Tangential section through phloem, showing sieve tubes and albuminous cells. × 375.

Fig. 16. Tangential section through phloem, showing short parenchymatous cells filled with crystals abutting on elongated albuminous cells. × 375.

Fig. 17. Transverse section through phloem, showing large callus pads on walls of sieve tubes. × 375.

Fig. 18. Tangential section through phloem, showing large callus pads on sloping end walls of sieve tubes. × 375.

Fig. 19. Transverse section through part of stem, showing formation of phelloderm and cork in the ground tissue inside zone of stone cells. × 240.

Fig. 20. Transverse section through part of stem, showing formation of mucilage cavity and secondary tissue inside zone of stone cells. × 240.

Fig. 21. Portion of a transverse section of a stem, showing origin of phellogen in epidermis. × 375.

Fig. 22. Surface view of stoma. × 800.

PLATE LIX.

Fig. 23. Median transverse section through sunken stoma of young stem. × 800.

Fig. 24. Median transverse section through stoma of leaf. × 800.

Fig. 25. Longitudinal section through stoma of leaf. × 800.

Fig. 26. Transverse section through end of stoma of leaf. × 800.

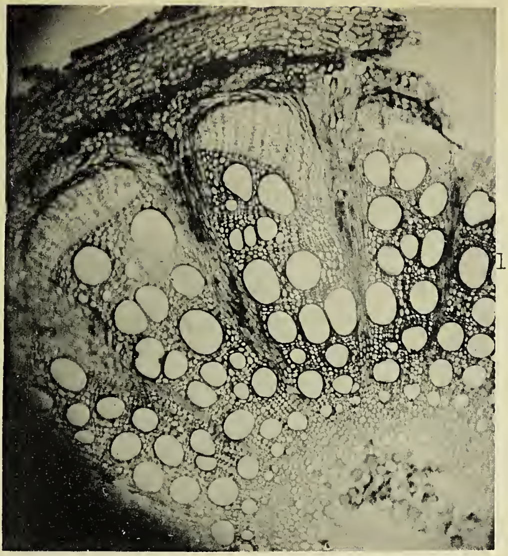
Fig. 27. Transverse section of stem cortex with fibres. The thickened walls are unshaded. × 375.

Fig. 28 *a*. Bone-shaped end of elongated fibre of leaf. × 220.

Figs. 28 *b* and 31. Fibres of leaf with short branches. × 220.

Fig. 29. Branched acicular cell of leaf. × 220.

Fig. 30. Cell of medullary ray with rhombic crystals of calcium oxalate. × 375.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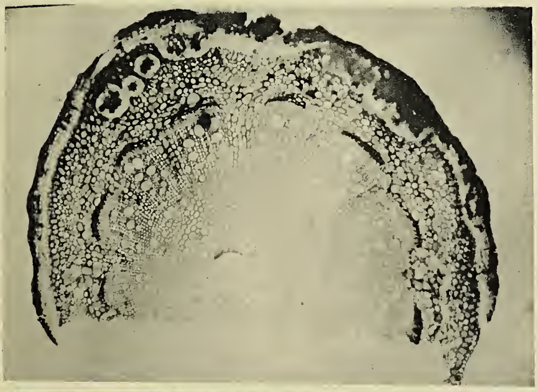


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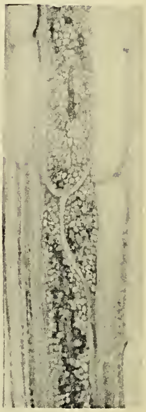
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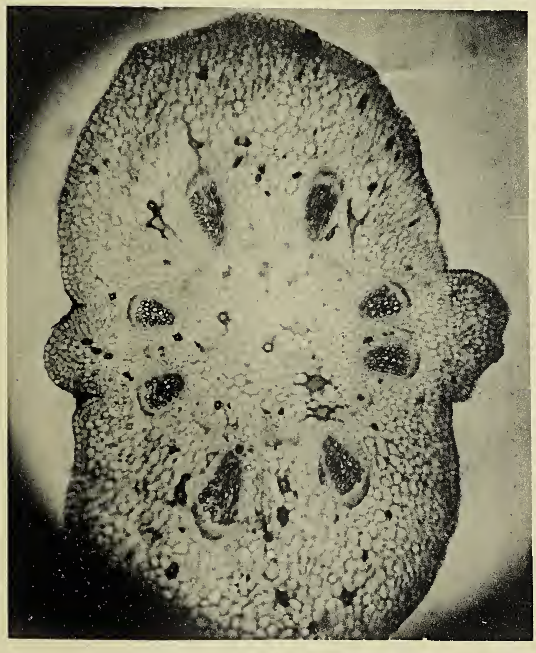
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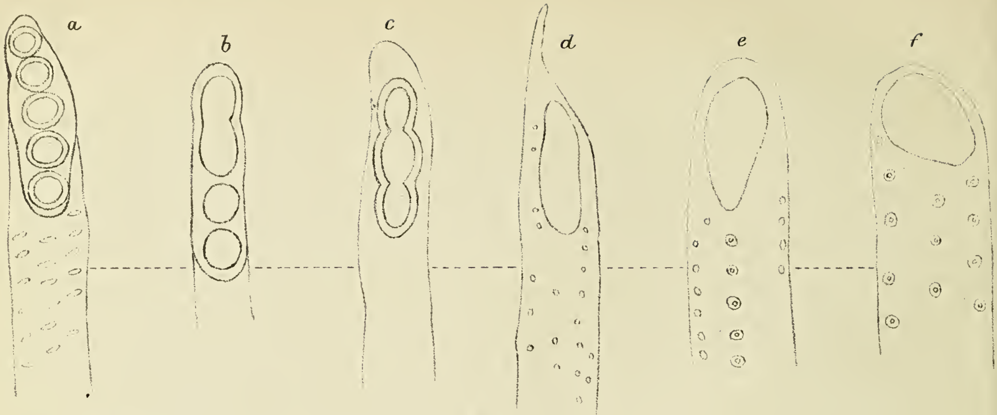
DUTHIE — GNETUM



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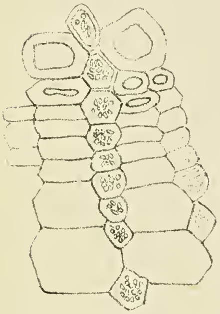

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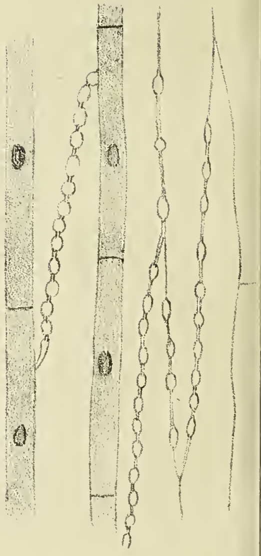

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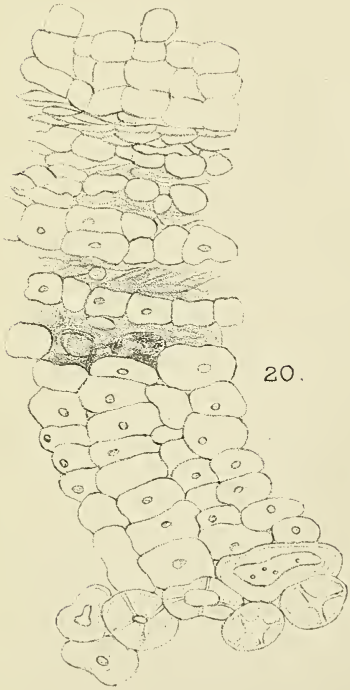

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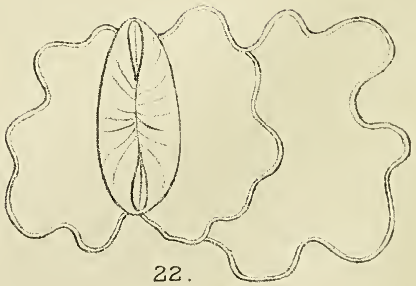

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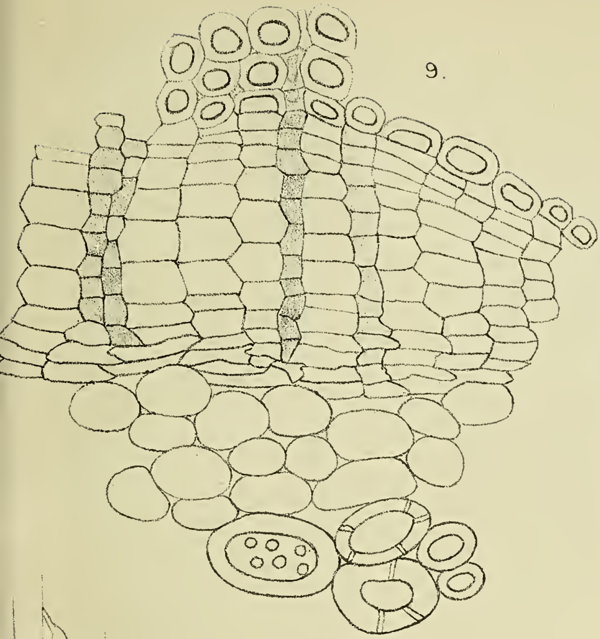

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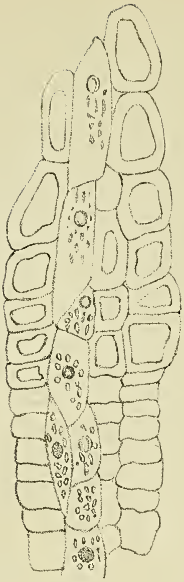

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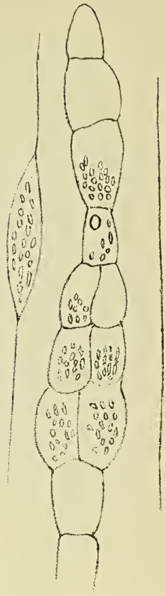

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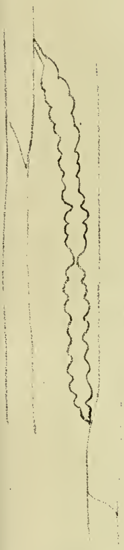

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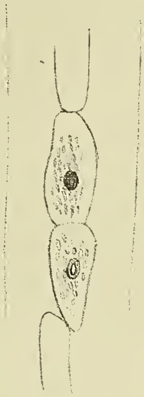

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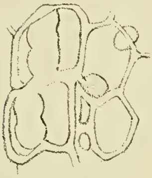

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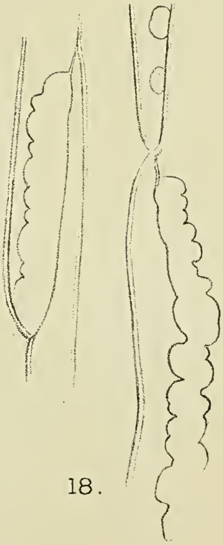

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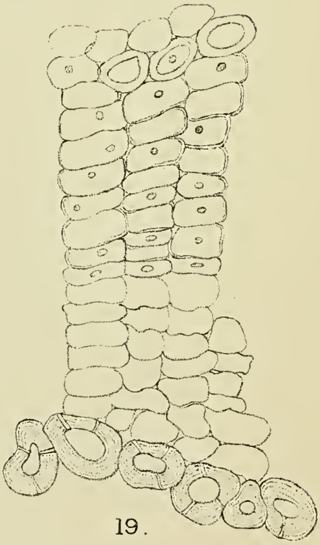

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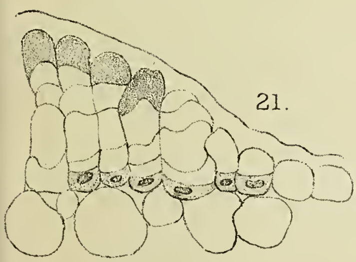

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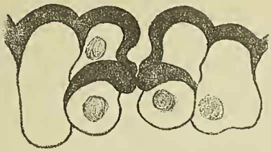
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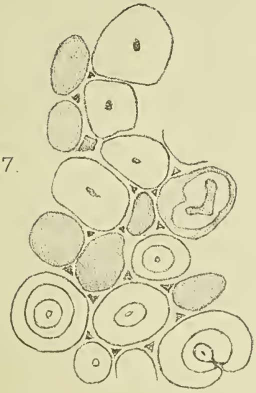
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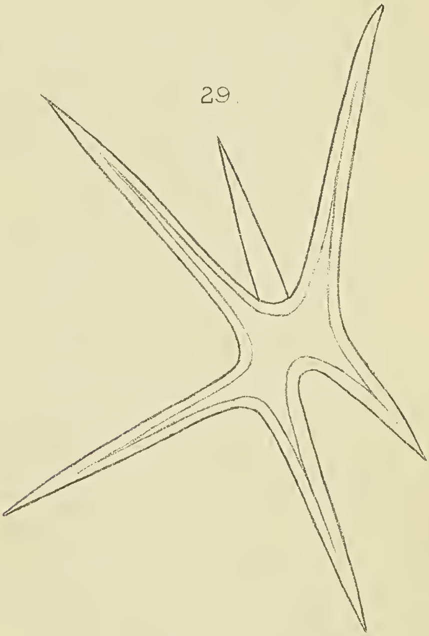
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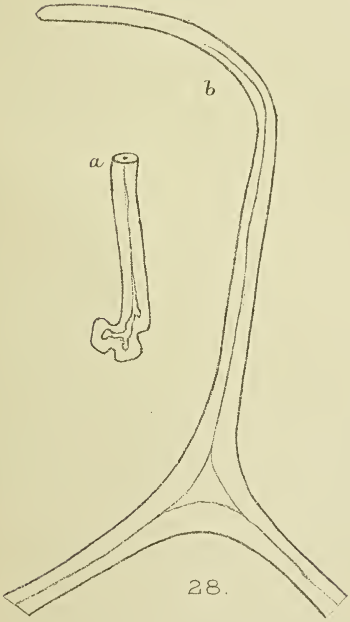

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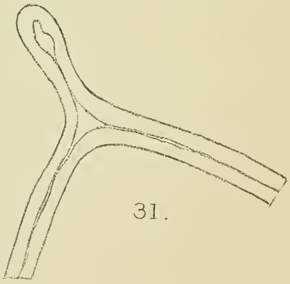
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DUTHIE — GNETUM.

On the Microsporangium and Microspore of Gnetum, with some Notes on the Structure of the Inflorescence.[1]

BY

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Harry Bolus Professor of Botany in the South African College.

With Plates LX and LXI and six Figures in the Text.

THE study of the life-history of *Gnetum* has been attended by considerable difficulty; the results hitherto obtained are incomplete and not entirely harmonious. This is due mainly to the lack of adequate material for investigation. The localities in which most of the known species occur are not easy of access. The characters of the inflorescence are such as to preclude the possibility of obtaining a long series of stages at one gathering. In the female inflorescence in particular, the range of development defined by the youngest and the oldest flowers is a small one, and at any one time the flowers of all the inflorescences of a particular plant represent but a short series of stages. This is in striking contrast to the spike of *Welwitschia*, for example, in which, for a period measured by weeks, a large number of flowers developing in strictly acropetal succession furnish a crowded series ranging perhaps from the stage of the macrospore mother-cell to that of the advanced proembryo. In the female inflorescence of *Gnetum* a further difficulty arises from the fact that in many species (*G. africanum*, *G. Buchholzianum*, *G. scandens*, &c.), a large proportion of the ovules become arrested early in their history; this may be due to an obvious disease, as in *G. scandens*,[2] or to more obscure causes, among which may perhaps be reckoned an insufficiency of available food. There are therefore good grounds for Lotsy's conclusion that it is 'absolutely necessary to be a resident in the tropics if one wants to obtain a pretty complete series, and even then it is slow and difficult work'.[3] Failing this we must resort to the less satisfactory process of piecing together the isolated facts established by various investigators working by different methods, at different times, on different species.

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[1] Report of the Percy Sladen Memorial Expedition in South-West Africa, 1908–9, No. 15. This expedition was assisted by a grant from the Royal Society.

[2] Lotsy ('99), p. 47; ('03), p. 397.

[3] Lotsy ('99), p. 47.

[Annals of Botany, Vol. XXVI. No. CII, April, 1912.]

Among the objects of the Percy Sladen Memorial Expedition of 1908–9 was that of obtaining material for the study of the life-history of *G. africanum.* This species was collected at Montobello in the Cazengo district of Angola, by Mr. J. Gossweiler, F.L.S. and the writer, in April, 1909. The ovules then obtained have yielded information regarding the condition of the embryo-sac and its contents both before and after fertilization, but they have not made it possible to describe completely the origin and development of the endosperm. It is hoped that, by the kindness of Mr. Gossweiler, a further supply of ovular material will be available in the course of a few months. Meanwhile, the following facts established for the male inflorescence and flower are placed on record.

As far as was possible with the information then available, the visit to Central Angola was timed to coincide with the later stages of ovular development, and it was hardly expected that young male inflorescences would be seen. While ovules were obtained in abundance only four male spikes were found; these have yielded all the stages shown in Pls. LX, LXI, Figs. 5–20. A further supply was gathered in the same locality by Mr. Gossweiler in January, 1910; these were preserved in alcohol and have been used in the investigation of the grosser morphological characters now to be described. Herr G. Zenker has most kindly sent me material of the male inflorescences of *G. Buchholzianum* from the Kameruns. A comparison of the internal morphology of these with that of *G. africanum* has led to very interesting conclusions regarding the degree of affinity between the African species and the important differences which separate them from the Indo-Malayan forms which have been studied. I am further greatly indebted to Mr. I. H. Burkill, Professor Gammie, and Mr. W. Talbot, C.M.G., for supplies of material of *G. scandens.*

The structure of the male inflorescence and the development of the parts of the flower of various species of *Gnetum* have been described by Griffith, Strasburger, Karsten, and Lotsy. Their results are scattered and some of them are in danger of being overlooked; it may therefore serve a useful purpose to summarize them briefly.

Griffith[1] describes the inflorescence in a few species. In *G. Brunonianum* he notes the presence of two sterile bracts on the peduncle. The bracts subtending the flowers are connate at the base and form an obsolete involucral ring; in its axil are a single ring of female flowers and many male flowers developing basipetally ('pluriseriatis, superioribus primo evolutis'). The female flower consists of a central fleshy ovate body which 'appears to be a naked ovule'. 'The nature and structure of the two envelopes indicate them to be perianthial.' Articulate hairs occur between the flowers. In a climbing species whose specific name is not given, he observes the presence of two vascular bundles in the axis of the male flower:

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[1] Griffith ('54).

'Diandrum esse conjicio, filamento vasorum fasciculi 2 laterales adsunt, nec centrales ut in filamentis monandris et anthera vere didyma e loculis 2 omnino discretis.'

Strasburger[1] gives a detailed account of the male inflorescence of *G. Brunonianum.* The cupule subtending each nodal ring of flowers represents two fused bracts. The internodes are short (l. c., Taf. xxi, Figs. 1 and 2). In each ring the uppermost flowers are female (incomplete); below these there may be as many as sixty male flowers. The basipetal order of development of the flowers in each node is indicated in Figs. 2 and 5 (l. c.). Each flower is surrounded by a number of filamentous hairs. The two anthers stand laterally on the summit of the axis, the two perianth leaves being placed antero-posteriorly. In the axis of the inflorescence the bundles are arranged in a ring from which the numerous leaf-traces arise; these pass out direct to the leaf-cupule; before entering the latter they give off branches which proceed upwards and outwards to supply the flowers. These latter form a somewhat indefinite ring lying outside the main cylinder and within the circle of leaf-traces. This median ring gives rise to small complexes of four or more bundles, each of which passes out to a flower. The complexes which supply the female (incomplete) flowers consist of somewhat larger bundles and their grouping occurs somewhat earlier than in the case of those which pass into the male. The spike commonly ends in a single terminal incomplete female flower. The two bundles in the axis of the male flower terminate each as a group of spiral elements immediately beneath the anthers. The perianth differs from that of *Ephedra* in that each of its two constituent leaves is traversed by 1–3 vascular bundles; these are reduced to a few spiral elements, but can nevertheless be followed to the upper edge of the leaf. The arrangement of the bundles thus described and figured[2] corresponds closely with that found in *G. scandens* (cf. Text-fig. 2). The anthers dehisce by transverse slits; the structure of the anther wall is similar to that of *Ephedra.* The pollen is oval, often almost round; the exine bears no protuberances, but contains definite thin areas or pores. The same author[3] later states, on the authority of Karsten, that there is no prothallial cell in the pollen-grain and that shortly before the opening of the anther there are present, in some two, in others three, nuclei.

Karsten[4] in 1892 made some reference to the germination of the pollen-grain, but did not deal with the earlier stages until a year later.[5] He then described the development of the anther and microspore from the study of material representing six Malayan species. The youngest node in the inflorescence bears no flowers. There are about nine incomplete female flowers at the top of each axillary ring. The male flowers are much

[1] Strasburger, E. ('72).

[2] Strasburger, l. c., Taf. xxi, Figs. 5 and 14.

[3] Strasburger ('80).

[4] Karsten ('92).

[5] Karsten ('93).

crowded; they are arranged in about eleven regular parastichies, each containing five to seven flowers. These relations obtain in *G. Gnemon*, *G. latifolium*, *G. neglectum*, and *G. funiculare*. In *G. Rumphianum* the number of flowers in each parastichy is smaller. The hairs arise from single superficial cells between the insertions of the flowers; they are particularly abundant between the female flowers and the male which stand immediately beneath them. The axis of the male flower bears two anthers, except in *G. Rumphianum*, in which there is only one. Of the two perianth leaves the upper arises earlier than the lower and overlaps it in aestivation. In the young anther there are two hypodermal tapetal layers clearly derived by periclinal division from a single layer.[1] After the cessation of division in the sporogenous cells, the walls separating them become indistinct and finally invisible. The body of the sporangium then consists of 'lediglich freie Zellen ohne Cellulosewände mit je einem Kerne'; this is surrounded by the two- (sometimes three-) layered tapetum and the epidermis. These 'freie Zellen' are the pollen-mother-cells. A contracted condition of the nuclear thread precedes the first division, which is rarely seen; it is followed immediately by a second division resulting in the presence of four free microspore nuclei in each mother-cell. At maturity the pollen-sac wall consists of a single layer of cells. The cells bordering the transverse line of dehiscence are either smaller or larger than the rest, and their contiguous walls are so rounded off that the area of contact is small. The presence of pores in the exine, as described by Strasburger, is denied; its surface is covered by delicate warty protuberances. Microspores in various stages of germination are found in the same sporangium. The first nuclear division in the microspore was not seen. One of the nuclei of the binucleate spore divides, the spindle in this case being very difficult to see. No more than three nuclei were seen in the pollen-grains; in this condition they appear to be mature. On germination the pollen-grain contains only two nuclei. No distinct generative cell can be seen in the pollen-grain, but it appears in the pollen-tube.

Lotsy[2] finds three free nuclei in the pollen-grain of *G. Gnemon* before its transference to the ovule. He regards one as vegetative, two as generative. He confirms the statement of Karsten that a generative cell occurs in the pollen-tube of *G. mollucanum*.

Coulter[3] found twelve chromosomes in the heterotypic and homotypic divisions in the pollen-mother-cell of *Gnetum Gnemon*.

Gnetum africanum is a climbing species occurring spasmodically in the dense forests of Central Angola, Gaboon, and the Kameruns. Its recorded localities in Angola are about 150 km. from the coast; they are

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[1] Karsten, l. c., Taf. viii, Fig. 14.

[2] Lotsy ('99).

[3] Coulter ('08).

situated at an elevation of about 2,000–2,500 ft. among the hills which separate the low-lying coast-belt from the edge of the central African plateau. *G. Buchholzianum*, also a climber, is known from a few localities in the Kameruns (Abo, Yaunde, Johann-Albrechtshöhe). No other species are at present known to occur in Africa. *G. scandens* is a widely distributed Asiatic species; it extends eastwards from the Bombay Presidency through Sikkim, Assam, the Khasia Hills, Chittagong, Burma, Andaman Islands, and the Malay Peninsula to China.

According to Welwitsch, *G. africanum*[1] is dioecious. The male inflorescence is a slender structure, varying in length from 2 to 2½ inches; the internodes are about 4 mm. long (Pl. LX, Fig. 1, *b*). The apical node bears no flowers (Text-fig. 5). The axis terminates in an elongated, blunt segment which at its base is closely invested by the leaf-cupule.

In *G. Buchholzianum* the male inflorescence in the main features of its form and structure resembles that of *G. africanum*. In the former the average length of the internodes is probably slightly less, and its surface is more strongly curved than in the latter (cf. Pl. LX, Figs. 1, *b*, and 1, *c*). The curvature of the surface of the internode of *G. Buchholzianum* (Pl. LX, Fig. 1, *d*) appears to be a constant character by which the species can at once be distinguished from *G. africanum*. In both, the peduncle bears a pair[2] of reduced connate leaves with strongly acute apices; these are situated usually about ⅓ in. below the first flower-bearing node. The peduncle above and below the insertion of this pair of bracts is approximately circular in section. The cupular leaf-sheath subtending each flower-ring is equally developed all round its edge except the lowest one or two, in which, sometimes in *G. africanum*, commonly in *G. Buchholzianum*, the margin is prolonged into two equal or somewhat unequal teeth which are decussate with those of the sterile peduncular whorl.

The form of the inflorescence of *G. scandens* differs in some important respects from that of the African species. The upper portion of the peduncle is flattened in a plane perpendicular to that of the insertion of the pair of peduncular bracts (Pl. LX, Fig. 1, *a*). The floriferous part of the axis is shorter; its nodes are closely crowded—in these characters contrasting very markedly with the greatly elongated axis of the female inflorescence;[3] its apical segment is very short. Certain structural differences, to be described below, between *G. scandens* and the African species are even more striking. The characteristic features which distinguish *G. scandens* from the latter are found also in other Indo-Malayan species, and it may be that they are common to all the species of this geographical region.

[1] Welwitsch ('71).

[2] Engler ('08) states that there are usually three leaves in this whorl. This is not the case in the material under investigation.

[3] According to Cooke ('07), the fruiting inflorescence may be as much as 10 in. long.

In *G. scandens* the uppermost flowers in each ring,[1] as in *G. Brunonianum* and other Indo-Malayan species, are female (incomplete). The occurrence of female flowers in this position seems to be extremely rare in the African species. A single example has been observed in *G. africanum*, and in this case the upper circle of the ring showed only one female flower.[2]

In the young spikes the flowers are completely concealed by the bract-cupule, the edge of which is closely applied to the projecting base of the next younger cupule in *G. scandens*, or to the base of the succeeding internode in the African species. The appearance of the anthers at the surface before they dehisce is due primarily to the elongation of the floral axis, in consequence of which the imbricating leaves of the perianth are pushed apart and the edge of the cupule is forced outwards. It is probable that at the same time there occurs a slight intercalary elongation of the axis of the inflorescence. In *G. Brunonianum*, to which *G. scandens* possesses so high a degree of structural resemblance, the apex of the male spike is commonly occupied by a single female flower.[3] A similar case has not been observed in *G. scandens*, nor in either of the African species.

TEXT-FIG. 1. Diagram of transverse section of peduncle of male spike of *G. africanum*, just below insertion of pair of reduced sterile leaves (cf. Fig. 1, *b.s.*). *l. b.* = leaf-base; *l.t.* = leaf-trace. × 28.

In *G. africanum* and *G. Buchholzianum*, the peduncle immediately below the pair of sterile bracts contains twelve to fifteen bundles in the ring. Four traces of single or double origin arising from these pass into each leaf (Text-fig. 1); the two median pass out direct and in advance of the other two, which, arising from the sides of the ring, pursue a curved path in the cortex through nearly a quarter of a circle. In *G. scandens* each of the corresponding pairs of bracts receives seven traces, of which the median leaves the ring first; the rest follow in pairs, each succeeding pair being separated by a wider angle from the median. In all three species the ring above the insertion of the sterile bracts contains from twelve to fourteen

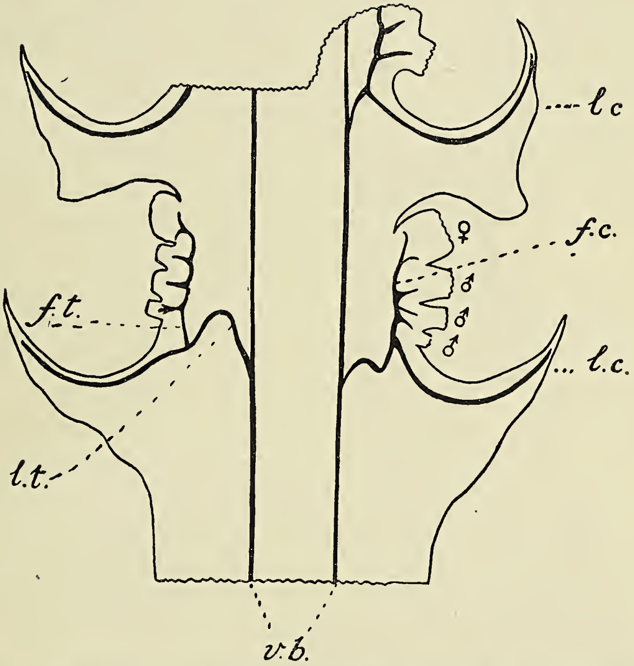
[1] In *G. scandens* the two uppermost nodes bear male flowers only; and occasionally the lowest node is barren and bears only hairs.

[2] This was seen in a hand-section. It is therefore possible that more than one female flower may have been present.

[3] Strasburger ('72).

bundles. These relations correspond fairly closely with those described by Strasburger[1] for *G. Brunonianum.*

In the axis of the inflorescence the bundles of the ring may be somewhat more numerous. A branch from each passes out at each floriferous node to the leaf-cupule. On its way through the cortex it bifurcates once at least in a horizontal plane; on entering the cupule it undergoes further forking so that numerous small bundles pass up towards the free edge of the cupule. From each leaf-trace just before it enters the cupule in *G. scandens*, there arises an inverted strand which ascends until it becomes lost in a complex network from the nodes of which the bundles supplying the flowers are derived (Text-fig. 2).[2] These branches from the leaf-traces furnish the whole of the vascular supply to the flowers in *G. scandens*, as is also the case in *G. Brunonianum.*[3]



TEXT-FIG. 2. Diagram of longitudinal section through node and internode of male spike of *G. scandens*, to show course of vascular bundles. *v.b.* = bundles of the ring; *l.c.* = leaf-cupule; *f.c.* = flower bundle-complex; *f.t.* = flower-trace. × 15.

In both the African species the origin of the vascular network from which the flower-strands are derived is less simple. In these there is an outer series of normally orientated bundles arising from the leaf-traces at some distance from their origin from the ring-bundles, and proceeding upwards to the level of the lowest flowers, where they enter the network

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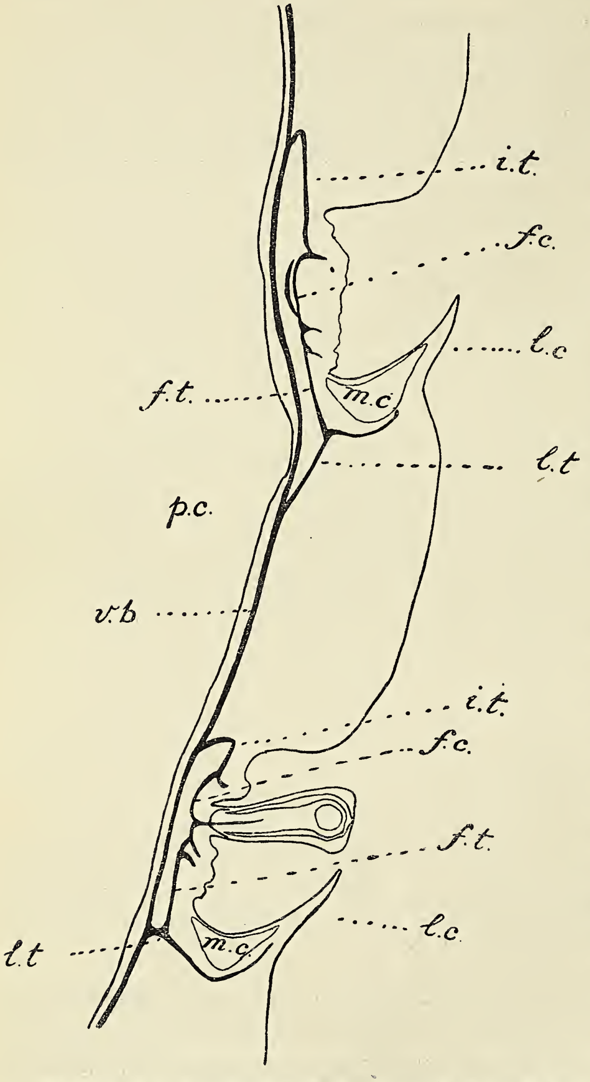
[1] Strasburger ('72).

[2] Cf. Strasburger ('72), Taf. xxi, Fig. 5.

[3] Strasburger, l. c.

from the nodes of which the flower-traces are derived. The bundles of this outer series are apparently homologous with the outer series of the female spike in *G. scandens*[1] and *G. africanum*,[2] and, in that case, are not represented in the male spike of the former species (Text-fig. 2). In addition to the outer series there is in the male spike of *G. africanum* and of *G. Buchholzianum*, an inner series of flower-traces, inversely orientated through the whole or the greater part of their course. Arising either from the leaf-traces in close proximity to their insertion on their parent-bundles, or directly from the parent-bundles themselves, either at some point within the node or in the lower half of the next higher internode, they pass upwards within the cortex for a longer or shorter distance before turning sharply downwards to join the floral complex at the level of the uppermost flowers of the node (Text-fig. 3, *i.t.*). After separating from the leaf-traces or ring-bundles the ascending traces frequently run up so close to the ring-bundles that their phloem groups fuse; a transverse section through such a combination shows a more or less complete amphivasal structure. Quite exceptionally such a case as that shown in Text-fig. 4 is found. Here the main trunk of the leaf-trace leaves the ring-bundle at the



TEXT-FIG. 3. Longitudinal section through two nodes and an internode of *G. Buchholzianum* (one half only shown; the letters *p.c.* stand in the pith cavity). *i.t.* = internodal flower-trace; *m.c.* = mucilage cavity in the leaf cupule. Other lettering as in preceding figures. × 15.

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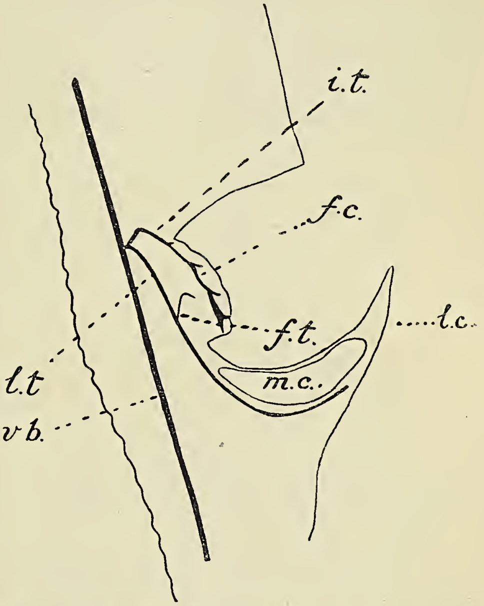
[1] Thoday ('11), Text-fig. 2, OB.

[2] l. c., Text-fig. 3, OB.

top of the node; the descending bundle (of the inner series) arises from it close to its point of origin, and the outer flower-trace is given off on its way down to the leaf. This is probably to be regarded as a case in which the main trunk of the leaf-trace has remained adnate to the ring-bundle through the length of the node. And it is not improbable that in the more normal case shown in Text-fig. 3, the bundles of the inner series really arise from the leaf-traces or from the ring-bundles in the neighbourhood of the point of departure of the leaf-traces, and are adnate to the latter until they reach the level of the top of the node or some higher point in the lower half of the next internode.

The inner series of bundles now described for the male spikes of *G. africanum* and *G. Buchholzianum* are without doubt to be regarded as the homologues of the inner series of the female spike of *G. africanum*,[1] which they resemble in their origin, their course, and their orientation, and also of the inner series of the female spike,[2] and probably the single series in the male spike of *G. scandens* (Text-fig. 2), which are orientated in the same manner. The 'augmenting' bundles which appear to be a constant feature of the more advanced female spike in *G. scandens* and *G. africanum*[3] are not found in the male spike of these species nor of *G. Buchholzianum*.



TEXT-FIG. 4. Longitudinal section through a node and parts of two internodes of *G. africanum* (an exceptional case). Lettering as in preceding figures. × 15.

The course of these ascending, later descending, bundles of the inner series in the male and female spikes of *G. africanum* and in the male of *G. Buchholzianum* bears a resemblance to that of the sporangiophore trace in *Palaeostachya vera*.[4] This arises from the ring immediately above the bract-bundle,[5] and it 'does not separate from the main bundle until it has ascended through almost exactly half the internode'. It then becomes reflexed as in the African *Gneta*. In the case of *Palaeostachya* there are grounds for the opinion that the course of the sporangiophore trace is to some extent determined by the distribution of sclerized tissue. In *Gnetum*

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[1] l. c., Text-fig. 3, BIV.

[2] l. c., Text-fig. 2, BIV.

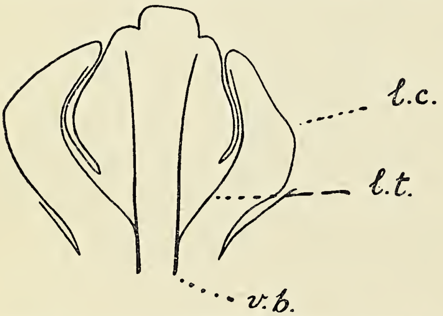
[3] l. c., Text-fig. 2, MIV.; Text-fig. 3, OM, LOM.

[4] Hickling ('07).

[5] l. c., Text-fig. 1.

no such explanation is possible, for although the ground tissue contains a great development of sclerenchyma, its elements are for the most part isolated and its distribution is quite independent of the arrangement of the bundles (Text-fig. 4). It is stated that one of the outstanding differences between *Calamostachys* and *Palaeostachya* is the absence of any considerable reflection of the sporangiophore trace in the former. Hickling's conclusion that the two forms need not be remotely separated on this account receives some support from the fact that we have in *Gnetum* an even greater difference of a like character between living species of the same genus.

From the nodes of the network formed by the branching and anastomosis of the traces described above, short branches arise and proceed directly outwards towards the insertions of the flowers. These are more or less concentric or distinctly collateral; in the latter case the phloem may be situated on either the upper or the lower side of the xylem.[1] Each of these upon entering the floral axis gives off two branches in a vertical plane which pass, the one into the upper, the other into the lower, perianth leaf of the male flower (Text-fig. 3); the main trunk almost immediately forks in a horizontal plane and becomes resolved into two xylem-traces (Pl. LX, Fig. 3), which pass up the axis of the flower and end blindly, one beneath each anther, as described by Strasburger.



TEXT-FIG. 5. Longitudinal section through apex of inflorescence of *G. scandens*. × 15.

In still another character of the male spike, the African species differ from *G. scandens* and from other Indo-Malayan species whose structure has been investigated. In *G. scandens* the bundles of the leaf-cupule lie not far beneath the upper epidermis, with which they are united by continuous tissue. In both the African species a large mucilage canal intervenes between the leaf-bundle and the upper epidermis[2] (cf. Text-figs. 3 and 4; Pl. LX, Fig. 2). This canal is continuous all round the cupule. The early stages of its development have not been seen, but it is clearly lysigenous in origin. Its roof usually consists merely of the epidermal cells, to which, however, the remains of underlying tissue are frequently attached. Its occurrence is not limited to the flower-bearing nodes, for it attains complete development in the youngest node, which bears no flowers (Text-fig. 6). In the African species also the pith tissue of the older internodes of the inflorescence is always disorganized and its cavity filled with a mass of mucilage. In herbarium specimens the internodes are shrunk to such an extent that they have the form of stout threads connecting the nodes, which

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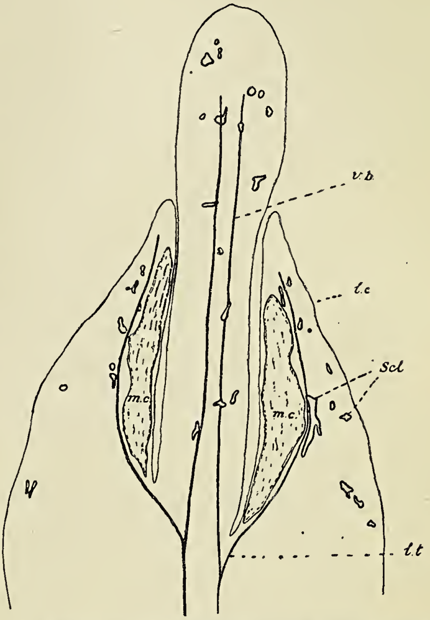
[1] Cf. Thoday ('11), p. 1106.

[2] l. c., p. 1104.

by comparison appear to be greatly swollen. This disorganization of the axial parenchyma is absent from all the material of *G. scandens* that is available for examination. It appears, therefore, that the tendency to undergo mucilaginous degeneration is much greater in the African species than in others that have been studied. But a somewhat similar disintegration of the tissue of the leaf-base in contact with the axillary bud occurs in the seedling of *G. Gnemon.*[1]

Apart from the remarkable behaviour of the vascular strands supplying the flowers in the African species the most peculiar feature of the male spike is the basipetal development of the flowers in each ring; this character is common to all three species, and, so far as is known, no species has been described in which it does not occur. In *G. scandens* the bundles which supply the flowers arise as branches from the leaf-traces just before these enter the leaf. Such an origin of the vascular supply of an axillary bud seems to be somewhat rare among the cases investigated,[2] but it occurs in *G. africanum.*[3] The stem of this species is heterophyllous. So far as is shown by the material available, branches never arise from the axils of the ordinary foliage-leaves, but are subtended by reduced leaves which bear a close resemblance to the sterile leaves on the peduncle; their leaf-traces, from which those of their axillary branches arise, are as small and frequently almost as numerous as those which pass into the cupules of the inflorescence. The narrow space between the reduced leaf and its axillary shoot is occupied by a dense growth of hairs similar in form and origin to those which occur among the flowers of each ring. The flower-ring as a whole is, therefore, undoubtedly axillary in position. But the basipetal order of development of its members is not paralleled in the vegetative organs. A few examples of supra-axillary shoots have



TEXT-FIG. 6. Longitudinal section through apex of male inflorescence of *G. africanum*. *Scl.* = sclerenchyma. × 28.

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[1] Bower ('82), p. 283; Pl. XXV, Fig. 19.

[2] The two bundles from the axillary branch unite at once with two of the leaf-traces in Clematis (de Bary ('84), p. 244).

[3] Material of the vegetative parts of *G. scandens* is not available.

been seen, but the succession of these is strictly acropetal. It appears that the basipetal development of lateral organs is very frequently, if not always, associated with arrested apical growth.[1] In the *Gnetum* male inflorescence the same association is found, for although the rings follow one another in acropetal succession, the growth of the axis is definite. The number of rings in each spike is almost constant within narrow limits, 14–17 in *G. scandens*, 8–10 in *G. africanum*, 7–9 in *G. Buchholzianum*; exceptionally the axis becomes arrested early, and the rings produced are fewer—two in one example of *G. Buchholzianum*. But whether the number of floral rings is normal or less, the axis at length terminates in a segment which differs from those beneath it. This last segment bears no flower-primordia, and the bundles which should supply the flowers are not represented even by procambial strands (Text-figs. 5 and 6). Such a segment as this puts an end to the normal apical development of the axis as effectively as if it were a flower. And in *G. Brunonianum* ' der Scheitel der Inflorescenzaxe gipfelt auch hier häufig, wie in den rein weiblichen Inflorescenzen, in einer einzigen weiblichen Blüthe '.[2] The axis then is of limited growth. This being the case, and since the inflorescence may consist of as few as two flowering nodes and a terminal segment (which in some cases is an ovule with appendages), it is conceivable that the ancestral type of inflorescence was an axis bearing a single lateral ring of male flowers, and a terminal female flower. In any case it seems at present impossible to refer the male spike of *Gnetum* and either the spike or flower of *Welwitschia* to a common type that is not very remote from both.

The structural characters of the male spike in which *G. scandens* and other Indo-Malayan species differ from the African species may be summarized thus:

Indo-Malayan species.[3]	*African species.*
Internodes suppressed.	Internodes longer than nodes.
A ring of imperfect female flowers at the top of every node except one or two nearest the apex.	In nearly all cases female flowers entirely absent.[4]
No mucilage canal in the cupule.	Large mucilage canal continuous round the cupule between upper epidermis and vascular bundle.
Vascular complex supplying the flowers derived entirely from a single series of strands ascending from the leaf-traces.	Vascular complex supplying the flowers derived from a double set of strands, one as in *G. scandens*, the other descending from the top of the node or from the internode above.

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[1] Goebel ('00, '05.)

[2] Strasburger ('72), p. 158.

[3] Hitherto described.

[4] A single exception seen in *G. africanum*.

Until more complete information as to the structure of the axis of the spike in other species and in earlier stages of development is available, it is useless to seek for an explanation of the remarkable differences now shown to exist between *G. scandens* and some of its allies on the one hand, and the African species on the other. It is, however, worthy of emphasis that the most remarkable fact established in this investigation—viz. the presence of a series of strands descending to the nodal flower-complex—is associated with elongated internodes. It would be of great interest to know whether this association is constant. The structural differences found between the male and the female axes of the same species are such as might be anticipated in view of the much longer duration of the female spike and the greater demands made upon its vascular system. Speaking generally, these are (1) a tendency to lignification of the medullary tissues of the female and to mucilaginous degeneration of the same tissues in the male; (2) the appearance of adventitious bundles 'augmenting' the inner series, and of transfusion tracheides associated with the cupular traces in the female and their absence from the male.

The similarity of structure of the male spikes of the two African species, and the differences which separate them from those of the Indo-Malayan species whose anatomy is known, are of phylogenetic interest. If the internal characters of the female spike of *G. Buchholzianum* bear a close resemblance to those of *G. africanum*, as may be expected, the near affinity of these two will be more firmly established. But while nothing is known of the internal structure of the vegetative and reproductive organs of any of the eleven American species, nor of the majority of those which occur in the Indo-Malayan region, no definite conclusions can be drawn as to the phylogenetic position of the African species with regard to the rest of the genus.

The hairs which occur among the flowers are usually of the character which has been described by previous writers. Occasionally, however, they assume the form of cell-plates, which are of interest owing to their resemblance to ramenta (Fig. 4). In the younger inflorescences the cells of the hairs contain starch.

In the youngest flowers available the anther wall consists of an epidermis and two layers of tapetum (Fig. 5); these latter are clearly derived by tangential divisions from an initial layer.[1] As development proceeds the outer of these becomes exhausted and flattened, while the cells of the inner undergo considerable enlargement (Fig. 6), and at length many of them become binucleate (Fig. 9). These stages are closely matched both in *Ephedra*[2] and in *Welwitschia*.[3] By the time that the pollen-grains have reached the 3-nucleate condition, the outer tapetal cells are hardly recognizable, and those of the inner layer have preserved nothing of their

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[1] Karsten ('93), Taf. viii, Fig. 14.

[2] Land ('04), Figs. 5–7, 14.

[3] Pearson ('06), Fig. 4; ('09), Fig. 2.

original structure save a shrunken, delicate, and interrupted outline (Fig. 7). The cells of the epidermis which now constitute the anther wall are somewhat radially elongated, the outer wall being slightly thickened and cutinized. Running vertically from the summit of the anther for some distance down each side is a double row of cells smaller than the rest, and whose walls, at this stage, are less thickened. These are doubtless the rows described by Karsten between which dehiscence occurs. In all cases in *G. africanum* they are smaller[1] than the rest of the cells of the wall.

The anthers, of which in all three species under investigation there are two on each flower-axis, are normally quite free from one another. Rarely, however, they cohere in the lower half. One case has been seen in which the septum separating them for more than two-thirds of their height was reduced to a cell-wall.

In the stage of Fig. 5 the walls of the sporogenous cells are delicate, but in no way difficult to observe. Later, as the nuclei approach the condition known as synapsis, and while the tissue connexions are still maintained, the cell-walls become extremely thin and indistinct (Fig. 8). At this time the walls stain very faintly in Delafield's haematoxylin or *Lichtgrün*; but in unstained or badly stained preparations they are practically invisible. This must be the stage described by Karsten in *G. Gnemon* in which the sporogenous mass is said to consist of 'lediglich freie Zellen ohne Cellulosewände'.[2] During synapsis, when perhaps the wall is more difficult to distinguish than at any other time, the most careful observation of good preparations is necessary to convince one that Karsten's description does not apply to *G. africanum*. Here, however, there is no doubt that throughout this and the stages which immediately follow it, a wall is always present. After synapsis it undergoes a gradual thickening, but it does not become conspicuous until the condition of the 'equatorial plate' is approached. The supply of material has not been sufficient for a detailed investigation of this remarkable behaviour of the wall of the mother-cell. There can, however, be no doubt that the substance of the wall on both sides of the middle lamella is actually removed, for the reticulum of the cytoplasm can be seen to be in contact with the delicate separating membrane (Fig. 8). A similar series of changes has not been described for *Ephedra* nor for *Welwitschia*.

Soon after the distinct reappearance of the wall it undergoes a considerable degree of mucilaginous thickening, which, in early stages, is marked by the irregularity of its distribution (Fig. 9). At this stage many of the cells are partially separated from their neighbours (Fig. 9), though their isolation is not usually complete until after the conclusion of the heterotypic division (Fig. 10). When the mother-cells are quite free from one another the wall is thickened all round, but not uniformly so (Figs. 11–15). The

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[1] Cf. Karsten ('93).

[2] Karsten ('93), p. 345.

number of chromosomes appearing in the heterotypic division is probably twelve, as stated by Coulter for *G. Gnemon.*[1] No cell-plate is recognizable in the course of this division.

The homotypic division usually occurs after the isolation of the mother-cells. The two spindles sometimes lie approximately parallel to one another (Fig. 11); in other cases they are inclined at any angle up to 90° (Fig. 12). Stages intermediate between these and that of Fig. 13 have not been seen. In the last figure, spindle-fibres are still visible, and although two of the three cell-plates are not continuous with the wall of the mother-cell (probably on account of contraction in preparation), there can be no doubt that the mother-cell becomes four-chambered in the same way as in *Pinus.*[2] The chambers are frequently all traversed by the same section; such an arrangement would follow the nuclear division shown in Fig. 11. The walls separating the microspores remain thin (Fig. 14) until these acquire their own cell-walls. Eventually they become almost as thick as the outer wall of the mother-cell (Fig. 15). There is nothing to suggest that the thickening is a second wall and not merely an addition to the primary cell-wall or a uniform swelling of its substance. As a rule it appears perfectly homogeneous in optical section. It stains faintly in Delafield's haematoxylin, but it can hardly consist of unaltered cellulose.[3] Apparently ruptured mother-cells from which one or more of the microspores have escaped have been met with. But the setting free of the microspores appears to be more generally due to a gradual solution of the wall of the mother-cell, which loses its definite outline and becomes almost invisible while the microspores are still held in position. This suggests that the thickening of the wall, at least in part, and its final disorganization, are the result of mucilaginous degeneration. This chambering of the mother-cell occurs also in *G. scandens*, but it has not been described for *Ephedra*, nor for *Welwitschia*, nor for any other species of *Gnetum.*

Before the disorganization of the mother-cell wall is complete, the wall of the microspore commences to thicken, and exine and intine are quickly organized. As stated by Karsten, in opposition to Strasburger, the exine contains no distinct pores, but is covered by minute, somewhat irregular protuberances (Figs. 16–20). Also, as in the species studied by Karsten, the microspores in a particular sporangium are not all in the same stage of germination at the same time. A greater degree of uniformity prevails in the stages preceding the organization of the microspore, but even here the development does not proceed *pari passu* in the two sporangia of the same flower.

The germination of the microspore commences after it has been set free from the mother-cell. The spindle in the first mitosis is exceedingly

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[1] Coulter ('08).

[2] Ferguson ('04), p. 35, Pl. IV, Fig. 45.

[3] Cf. Ferguson ('04), pp. 35, 36.

delicate (Fig. 17) ; an evanescent cell-plate is formed ; the two resulting nuclei lie quite free in the cytoplasm (Fig. 18). One of the two quickly divides again, the spindle being perhaps less indistinct than in the preceding division. An evanescent cell-plate is formed in this case also (Fig. 19). After the completion of this division, the cytoplasm contains three free nuclei, of which two are usually a little larger than the third. They are most frequently arranged as shown in Fig. 20, but sometimes they lie in a row one above the other. This is the most advanced condition that has been found, and in anthers which are already protruding beyond the edge of the cupule all the pollen-grains are in this state. This is true also for *G. Buchholzianum*. There can be little doubt that these are adult pollen-grains, a conclusion which is in agreement with Karsten's account. As is pointed out above, Karsten states that at the time of germination the pollen-grain contains only two free nuclei. One of the three must therefore disappear; presumably this is the one which does not participate in the second mitosis, and which is to be regarded as 'prothallial' in character and homologous with one of the three free nuclei in the mature pollen-grain of *Welwitschia*.[1] The other two nuclei are probably the one a tube nucleus, the second a generative nucleus.[2] No indication of the organization of a generative 'cell' has been observed. If such a cell is developed later, as is probable,[3] then in the germination of the microspore and the structure of the adult pollen-grain *Gnetum* differs in no important respect from *Welwitschia*. Between *Gnetum* and *Ephedra*, on the other hand, there is a wide gap.

SUMMARY.

1. *G. scandens*, *G. africanum*, and *G. Buchholzianum* have been investigated. The study of the microsporangium and microspore has been mainly carried out on inflorescences of *G. africanum*.

2. The male inflorescence is definite ; it bears a number of axillary rings of basipetally developed flowers.

3. Other characters of the inflorescences are summarized on p. 614.

4. The structure of the anther wall and the tapetum is similar to that described by Karsten.

5. The pollen mother-cells are not free naked cells during the early stages of their development. But immediately before and after the condition of the nucleus known as synapsis, the cell-wall is extremely delicate and difficult to observe.

6. Later the wall thickens irregularly and the mother-cells are not usually free from one another until the homotypic division is completed.

7. The reduced number of chromosomes is probably twelve.

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[1] Pearson ('06), Pl. XVIII, Figs. 13–16.

[2] Cf. Lotsy ('99), p. 94.

[3] Karsten ('93) ; Lotsy, l. c.

8. The nuclei formed in the heterotypic division are free.

9. The spindles in the homotypic division are arranged either bilaterally or tetrahedrally.

10. Cell-plates appear after the homotypic division, dividing the cavity of the mother-cell into four loculi, each containing one microspore.

11. While the wall of the microspore is being laid down, the septa of the mother-cell become almost as thick as its outer wall. Before the microspores are set free, exine and intine are differentiated.

12. The outer wall and septa of the mother-cell are probably mucilaginous in character. They usually disappear gradually as if in process of solution.

13. The microspore nucleus undergoes mitosis; one of the two daughter nuclei divides again. There result three free nuclei which may show a radial or a linear arrangement.

14. This appears to be the condition of the pollen when dehiscence of the anther occurs.

15. The three nuclei are probably to be identified as one prothallial, one vegetative (tube), and one generative.

16. The germination of the microspore and the structure of the pollen-grain point to a much closer degree of affinity with *Welwitschia* than with *Ephedra*.

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[1] I am indebted to Dr. B. Daydon Jackson, Sec. L. S., for his kindness in furnishing me with a copy of this paper.

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EXPLANATION OF PLATES LX AND LXI.

Illustrating Prof. Pearson's paper on the Microsporangium and Microspore of *Gnetum*.

Figs. 5–20 = *G. africanum*.

Fig. 1. Male spikes of *Gnetum*. *a*, *G. scandens*; *b*, *G. africanum*; *c*, *d*, *G. Buchholzianum*. (*a*, *b*, *c* = natural size; *d* = enlarged).

Fig. 2. Tangential longitudinal section through a node of the male spike of *G. africanum*. The stages of development shown by the anthers are as follows:

1, 1′, 2 = free microspores (uninucleate).
3′ = microspores almost free of mother-cell wall.
3 = ,, enclosed in mother-cell wall.
4 = spireme condition of mother-cell nuclei.
4′ = synapsis ,, ,, ,,
5, 5′, 6 = sporogenous cells earlier than mother-cell condition. × 28.

Fig. 3. Transverse section of the axis of the male flower of *G. africanum* above the insertion of the perianth, showing the two xylem strands. × 305.

Fig. 4. An exceptional type of hair inserted on the axis of the inflorescence among the male flowers (*G. Buchholzianum*). × 305.

Fig. 5. Transverse section through part of a young anther. × 600.

Fig. 6. A later stage showing the enlargement of the cells of the inner tapetum and the exhaustion of those of the outer. × 600.

Fig. 7. Section through part of the anther wall passing through line of dehiscence. × 305.

Fig. 8. Spore mother-cells a little before the 'synapsis' condition. The cell-walls at this stage are very delicate and with difficulty to be distinguished. × 940.

Fig. 9. Spore mother-cells at a later stage. The wall is everywhere distinct and much thickened in places. The separation of the cells is nearly complete. One of the two cells of the inner tapetum is binucleate. × 1,000.

Fig. 10. Spore mother-cells before the commencement of the homotypic division, showing a stage in the separation of the cells. × 1,000.

Figs. 11 and 12. Spore mother-cells with their nuclei in course of the homotypic division. × 1,000.

Figs. 13, 14 and 15. Stages in the septation of the mother-cell. × 1,000.

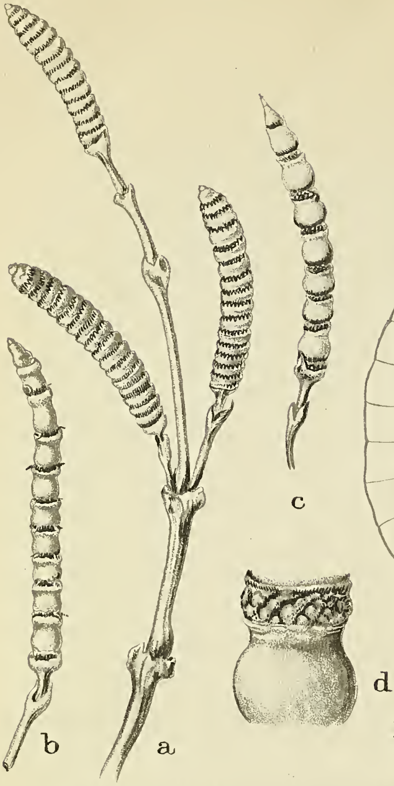
Fig. 16. A mature microspore. × 1,000.

Fig. 17. Germination of the microspore; first mitosis. × 1,000.

Fig. 18. Germination of the microspore; binucleate stage. × 1,000.

Fig. 19. Germination of the microspore; second mitosis. × 1,000.

Fig. 20. Pollen-grain. × 1,000.



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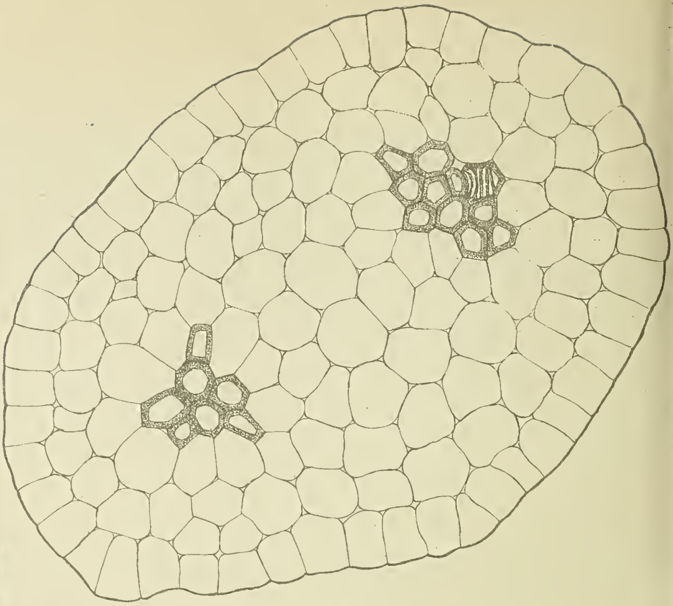

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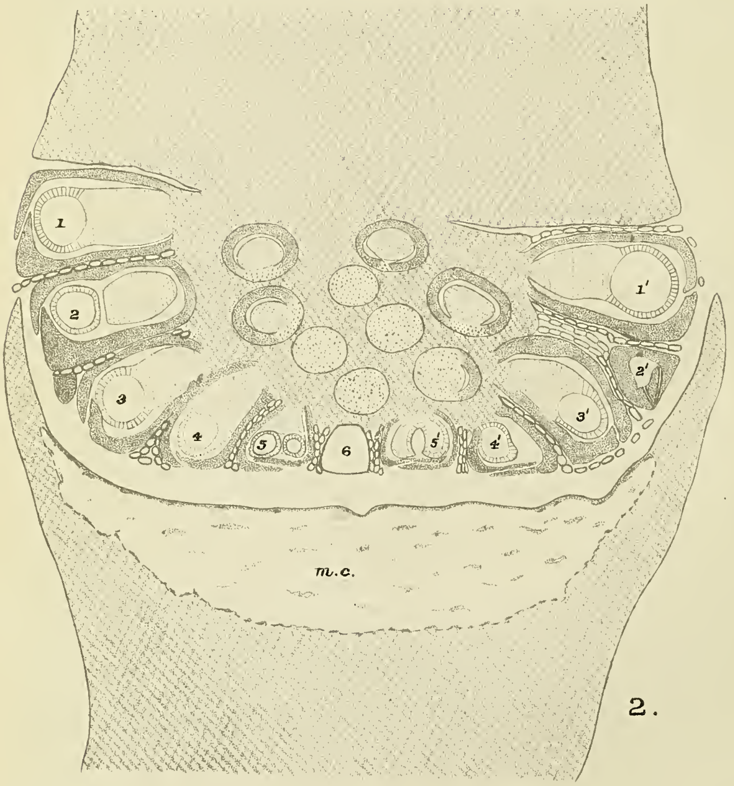

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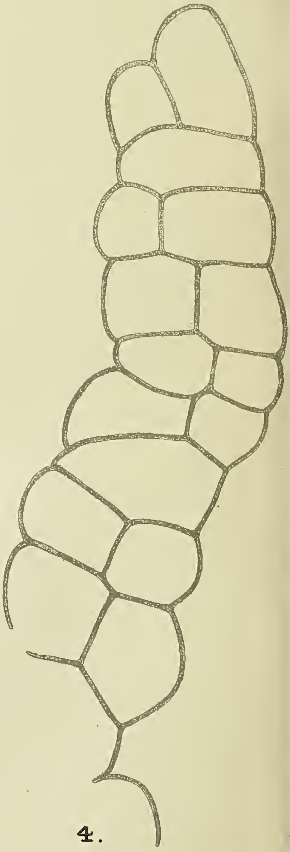

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PEARSON—GNETUM.

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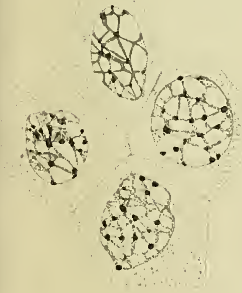

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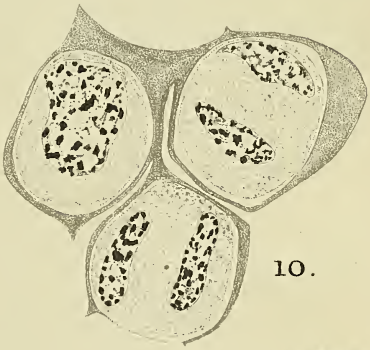

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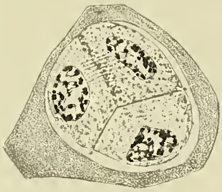

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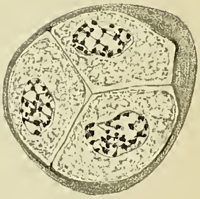

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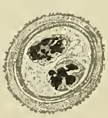

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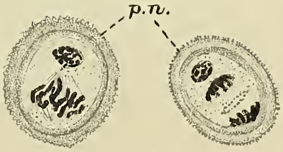

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Huth, lith. et imp.

NOTE ON THE INFLORESCENCE AXIS IN GNETUM.—Professor Pearson's investigation of the male inflorescence has made it advisable to add some comment to the short description of the male axis in *G. scandens* made on p. 1106 of my recent paper (Annals, 1911), and Professor Pearson has kindly allowed me to add a note here. On comparison with his full account I find that my description of the male cone, which was based on a small quantity of material and given only for the purposes of comparison, refers to a case which in one respect is not normal. It was stated that the bundle supply of the male floral complex originates in practically the same way as that supplying the ovule. It is now clear that in the male inflorescence axis all the bundles supplying the flowers correspond normally with the *inner* inverse series alone of the female flower, and it is not normal to find any of the bundles of the male floral complex inserted further out on the bract-bundles in a position corresponding to the *outer* series supplying the ovule. This difference between the male and female axes did not become evident to me till I saw Professor Pearson's paper (and consequently made a re-examination of my material), owing to the fact that I first examined an exceptional case in which a few of the lowest floral bundles were inserted in this position.

A comparison between the female cones of the two species investigated by Professor Pearson and myself throws some light on the great difference in the vascular system of the male axes, i. e. the presence of 'descending' bundles in the one species and their absence in the other. This is not accompanied by as great a difference in the female axes, traces of a descending system being present in the female axis of *G. scandens* (ibid., p. 1106). It appears to me probable that the greater or less development of the descending system of bundles in the inflorescence axes of this genus is conditioned by the length of the internode and position of insertion of the flowers characteristic in the species, and by the stage of development of the particular strobilus in question. I have already stated in my paper that in the young cone axis of *G. scandens* no descending bundles were present, but that as the ovules increased in size new bundles were laid down, some of which, on entering the stem, instead of turning downwards to join the crowded ovular bundles at their insertion on the bract-bundles, ran upwards to join the main bundles.

In the axis of *G. Africanum* the descending series was far better developed, even in the young cone, and the greater the age of the cone the greater the development and complication of this series. These facts are probably due to the relatively short internode in *G. Africanum*, and the consequent crowding of the cupules and flowers in this species. In *G. scandens* the young female flowers are at first situated closely in the axil of the cupule, but are afterwards carried out on a stalk so that they are never inserted by a very broad surface, and above their level of insertion there is a long stretch of bare internode beneath the cupule at the base of the next node. But in *G. Africanum* the ovules, sessile at all stages, are inserted by their whole base on the stem, above the cupule, and as the ovules develop and their base widens, they take up an ever-increasing part of the short internode. Thus it is obviously much more convenient for the later formed vascular bundles in the upper part of the ovule to be inserted on the main bundles higher up in the node or even in the internode above, than to run downwards towards the already complicated mass of vascular tissue at the base of the node: on entering the stem they therefore continue their course through the cortex towards the main bundles, forming the 'descending' series.

In *G. Gnemon* and *G. funiculare*, two other species which I have recently examined, both of which have long internodes and sessile ovules, there is hardly any trace of the descending series, except that in the older ovules a few bundles are found to originate from the main bundles a little above the origin of the bract bundles and to run downwards from these to join the mass of vascular tissue which supplies both bract and flowers.

M. G. THODAY (SYKES).

MANCHESTER, *Jan.* 1912.

NOTE.

TELOSYNAPSIS AND PARASYNAPSIS.—There exists at the present time a considerable amount of confusion as to the actual nature of the controversial points which cluster, or are supposed to cluster, around the manner of pairing of the somatic chromosomes at meiosis, whereby the well-known heterotype chromosomes are produced. This confusion is due in great part to the attempts which have been made to crystallize the essential differences between two divergent schools of interpretation by the introduction of the terms Telosynapsis and Parasynapsis respectively.

But it must have become evident to every one at all conversant with the current work on mitosis, that however appropriate these terms may be to express a special mode of chromosome union, they have now become rather misleading to any one not familiar with the details of cytological advance within the last few years. The fact is, that by emphasizing a point of comparative unimportance, they have led to a misconception on the part of many people, as to the really fundamental differences which still divide the two schools of investigators.

Montgomery in America, and Farmer and Moore in England, working independently on very different material, came to the conclusion that the heterotype chromosome arose as the result of an end-to-end union—or a lack of disjunction—of a pair of somatic chromosomes. These paired chromosomes are arranged more or less in the form of an open loop, the sides of which they constitute. The familiar figures observed at diakinesis, and at still earlier stages, depend on the various ways in which the limbs of a loop behave as they approximate towards, or coil round, each other.

In formulating this explanation, the investigators above named were mainly influenced by the very common occurrence of loop-like figures at an earlier stage, which were also traced through succeeding phases; the turn of the loop was supposed to coincide with the point of union between the two somatic chromosomes.

The closer approximation of the sides of a loop was believed by the English authors to take effect at the stage called 'second contraction', a stage which, where it was discerned, appeared to be one of very short duration, and consequently is easily missed. It is obvious, however, that there is no essential difference between a lateral approximation achieved by the twisting together of the sides of such a loop, and an approximation produced by the coming together in pairs of chromosomes hitherto disunited, nor is it a matter of any importance whether the approximation occurs at a somewhat earlier or later period in mitosis.

The really vital question at issue between the two schools does not, as a matter of fact, consist in Telosynapsis *v.* Parasynapsis as etymologically understood, *but upon the interpretation to be placed on the much earlier stages of prophase* in the heterotype mitosis.

Investigators, represented in the first instance by Grégoire and his pupils, who hold the 'Parasynaptist' view, believe that the early so-called *longitudinal fission of the chromosomes* marks the union of the pairs of somatic chromosomes. Furthermore, they attach no importance to *second contraction*, which is interpreted by their oppo-

[Annals of Botany, Vol. XXVI. No. CII. April, 1912.]

nents as concerned in bringing about this union. The 'Telosynaptists', on the other hand, regard this early longitudinal fission as a reality, and as indicating *precisely the same fission as is responsible for the bipartition of the chromosomes of a normal premeiotic or postmeiotic nuclear division.*

I endeavoured to put forward the main features of the whole position as plainly as I could in 1905:

> 'Thus the essential peculiarities of the meiotic[1] phase can be explained as follows: They are due to the coherence in pairs of premeiotic chromosomes and to the intercalation of a special form of chromosome-distribution during the course of what would not differ materially from an ordinary premeiotic mitosis. In the first of the two divisions, a distribution of entire premeiotic chromosomes is secured, and thus the number of these bodies is really halved. In the second division, the longitudinal division begun, but temporarily arrested, in the preceding prophase takes effect.'

I have never receded from this position, because it has always appeared to me that, in spite of the many differences in detail exhibited during meiosis in the various members of the animal and vegetable kingdoms, this interpretation harmonizes best with the observed facts, and also with what we have ascertained as the result of comparisons with the other mitoses in the same organisms. It has also materially gained in strength of late years, since improved technique has made it evident that in *somatic* mitoses the chromosomes are actually longitudinally split during the late telophase of the *preceding* division, and that in the succeeding early prophase each differentiating chromosome can be distinctly recognized as already consisting of two longitudinally arranged halves. This duality commonly becomes apparently, but only apparently, lost during the following period of rapid growth and change of form before the chromosome becomes arrayed, along with its fellows, in the equatorial plate. At this latter stage, as every one knows, the split appears, or rather reappears, and results in the separation of the respective pairs of daughter chromosomes. The proof that features precisely similar in this respect are present in the *postmeiotic* as well as in the premeiotic mitoses (first given by Dr. H. Fraser) effectively disposes of the suggestion that this 'early fission' might represent an abortive 'pairing of homologous chromosomes' in the vegetative mitosis. Furthermore, the results obtained by Miss Digby and others, showing that the 'early fission' at the heterotype mitosis is exactly similar to the 'early fission' of the preceding archesporial divisions, render it extremely improbable that a fundamentally different interpretation is to be placed on the two cases.

I have tried in this brief note to point out as clearly as possible what are the really outstanding differences which at the present time constitute the main points at issue between the 'Telosynaptists' and the 'Parasynaptists', inasmuch as they have been greatly obscured by the rather unfortunate names under which the divergent views are now so commonly classified.

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LONDON.

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[1] In the original (Quart. Journ. Micr. Sci., vol. xlviii) the word was printed 'maiotic'.

FLORAL MECHANISM

By A. H. CHURCH, M.A., D.Sc.

LECTURER IN BOTANY IN THE UNIVERSITY OF OXFORD

The following statement has been drawn up by Professor Sydney H. Vines

THE object of this work is to provide the botanical student with a complete description of the development, morphology and mechanism of the principal types of flowers. Whilst giving the kind of information that is to be found in Payer's *Organogénie de la Fleur*, and in the late Professor Eichler's well-known *Blüthendiagramme*, it supplements this with an account of the ecology of the flower, including pollination and the formation of fruit and seed. Hence, when complete, it will be the most comprehensive treatise on the flower that has yet been published.

The general plan of the work may be gathered from Part I, which was published in 1908 as a royal 4to volume of 211 pages. In it are described the following twelve types of floral structure, selected from familiar garden flowers that bloom in the early part of the year (January–April):—

Helleborus niger . .	Christmas Rose.	*Viola odorata*	Sweet Violet.
Galanthus nivalis . .	Snowdrop.	*Narcissus Pseudo-Narcissus*	Daffodil.
Jasminum nudiflorum	White Jasmine.	*Erica carnea*	Heath.
Crocus vernus . . .	Blue Crocus.	*Ribes sanguineum*	Flowering Currant.
Richardia africana .	White Arum Lily.	*Cydonia japonica*	Scarlet Cydonia.
Daphne Mezereum .	Mezereon.	*Vinca major*	Greater Periwinkle.

In connexion with each type, two or three allied species are described for purposes of comparison.

The description of each type is illustrated by a full-page coloured plate, giving an accurate longitudinal section of the flower, and by a black-and-white plate giving the inflorescence, the floral diagram, and other structural details. As each subsidiary species has also a coloured plate allotted to it, the volume contains no less than forty coloured and fourteen uncoloured plates, in addition to a large number of figures, chiefly developmental, included in the text. It can be obtained at the original price of £1 1*s*. net by subscribers to Part II.

It was hoped that the reception of so striking a volume as Part I would have been such as to justify the Delegates of the Press in proceeding forthwith to publish Part II, the material for which is in readiness. Inasmuch as this anticipation has unfortunately not been realized so far, the Delegates are not disposed to undertake the publication of Part II without some assurance that the necessarily large expenditure involved will meet with the general support of those who, in one way or another, are interested in flowers. But the University Press has received such warm commendations of the work from Botanists who desire to push on the study of Botany in the English-speaking countries that they desire, if possible, to continue publication. They propose, therefore, to ask for subscriptions for copies of Part II at One Guinea each, on the understanding that Part II will, like Part I, consist of descriptions of twelve types of flowers, with allied forms, and be similarly illustrated, though it may be found necessary to reduce somewhat the number of coloured plates. Any copies not subscribed for will not be sold at less than thirty shillings each.

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