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A Genetico-Physiological Study on the Formation of Anthocyanin and Brown Pigments in Plants.

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With Plate I and two Text-figures.

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Introduction.

The workers in genetics have established the fact that in certain cases the formation of anthocyanin pigments is caused by the interaction of a number of definite pigment yielding components which are retained by the separate genetic factors. Neither of these components has the power to produce the pigment unless the complete system is established by their union. We owe much to the labours of BATESON, PUNNETT, Miss SAUNDERS, Miss WHELDALE, Baur and many others on the part of genetics,[1] and WILLSTÄTTER and his collaborators in the field of chemistry who have shown for the first time, the exact chemical constitution and the interrelationship of the colouring matters[2] concerned.

The present paper deals with the result of an investigation carried out in order to discover what relation exists between anthocyanin and brown pigments both of which occur widely in the plant kingdom and what observation can be made with regard to the physiological action of the genes which are analysed by the breeding experiments for the characters in which those pigments are concerned.

I. Physiological Study.

1. The Action of Oxidizing Enzymes on Anthocyanins.

If we accept the view that anthocyanins are formed by the oxidation of flavone instead of by reduction, and the oxidizing enzymes play an essential part in this change in the living plant cells, it is necessary to offer the direct evidence to lend support to the view.

When an alcoholic or aqueous extract of anthocyanin which is slightly

1. See WHELDALE, M., Anthocyanin Pigments of Plants. 1916.
2. See PERKIN, G. A., and EVEREST, A. E., The Natural Organic Colouring Matters. 1918.

acidified to check the formation of the colourless isomer, is mixed with the solution containing active oxidizing enzymes, the characteristic red colour diminishes gradually and finally becomes pale yellow or practically colourless. The aqueous solutions of hydrogen peroxide and certain other inorganic oxidizing agents have the same effect as the enzyme. Such phenomena have been observed by BOUFFARD (1902), KASTLE (1905), KASTLE and HADEN (1911), COMBES (1913), ATKINS (1916) and NAGAI (1917).[1]

The reversible change of flavone to the coloured substance of anthocyanin-like nature by means of reducing and oxidizing agents respectively has been observed by many. ALLEN (1901)[2] stated that when an acidified (by hydrochloric acid) alcoholic solution of quercetin was treated with sodium amalgam the liquid assumed a fine purple colour and on concentration yielded red prisms which dissolved in alcohol and a little alkali forming a green solution, the solution being readily reoxidized with formation of quercetin on exposure to the air. COMBES (1913)[3] observed a similar reversible change in the yellow pigment isolated from the leaf of *Ampelopsis hederacea*.

In the study of anthocyanin in the corn flower, WILLSATÄTTER and EVEREST (1913)[4] found that when an alcoholic solution of cyanidin was warmed with dilute hydrogen peroxide solution, the colour diminished. When it was again warmed on the water bath with the addition of a few drops of dilute hydrochloric acid, the liquid became yellow, and by extracting with ether, beautiful bright yellow crystals were obtained which when treated with alkalies, yielded a deep yellow solution. Miss WHELDALE (1914)[5] attempted

1. BOUFFARD, A., Action de l'acid sulfureux sur l'oxydase et sur la matière colorante du vin rouge. Comp. Rend. Acad. Sci. Paris. 134 : 1380, 1902. KASTLE, J. H., A Method for the Determination of the Affinities of the Acids Colorimetrically by Means of Certain Vegetable Coloring Matters. Am. Chem. Jour. 33 : 46, 1905. KASTLE, J. H. and HADEN, R. L., On the Color Changes Occurring in the Blue Flowers of the Wild Chicory, Cichorium intybus. Am. Chem. Jour. 36 ; 315, 1911. COMBES, R., Passage d'un pigment anthocyanique extrait des feuilles rouges d'automne an pigment jaune contenu dans les feuilles vertes de la meme plante. Comp. Rend. Acad. Sci. Paris, 152 : 1454, 1913. ATKINS, W. R. G., Recent Researches in Plant Physiology. 1916. NAGAI, I, The Aetion of Oxidase on Anthocyanin. Bot. Mag. Tokyo, 31 : 65, 1917.

2. ALLEN, A. F., Commercial Organic Analysis. Vol. III, Part 1, 440, 1901.

3. COMBES, Eoc. cit.

4. WILLSTÄTTER, R. and EVEREST, A. E., Ueber den Farbstoff der Kornblume. Liebig. Ann. 401 : 189, 1913.

5. WHELDALE, M., Our Present Knowledge of the Chemistry of the Mendelian Factors for Flower Colour. Jour. Genet. 4 ; 109, 1914.

to obtain the flavone from the anthocyanin of *Antirrhinum* by the same manner just quoted, but failed. She obtained only a yellowish brown solution.

HARROW and GIES (1919)[1] observed the reversible colour changes in the solution of flavone and anthocyanin isolated from the flower of tulips by means of nascent hydrogen and hydrogen peroxide respectively.

The writer observed that the coloured reduction product of quercetin, myricetin, apigenin, and luteolin yielded a yellow solution when treated with hydrogen peroxide and in the case of the first two, the original colours were resumed by further reduction by means of hydrochloric acid and magnesium powder. If, however, the reduced, coloured solutions were decolourized by an excess of hydrogen peroxide, the yellow solution so obtained failed to recover the reddish hue by reduction.

The aqueous extract of the violet anthocyanin from the perigone leaf of *Iris Kæmpferi* and the purple one from the leaf of *Perilla nankinensis* were also changed to yellow by hydrogen peroxide and they yielded again an orange red colour by reduction but no initial bluish hue.

The red colour of the reduced solution of quercetin, quercitrin, and myricetin were converted to yellow by the addition of an aqueous solution of potassium permanganate, and the further addition finally rendered the solution completely colourless.

The mode of action of hydrogen peroxide and the oxidizing enzyme on anthocyanin was studied colorimetrically. One of the difficulties here met with, was the change in hue as the action proceeded. It is naturally to be expected that yellow colour should increase in depth as the anthocyanin is converted to a flavone like substance and at the point where fifty per cent of anthocyanin is converted to the yellow substance, the colour of the solution becomes about half way between red and yellow, namely orange. When violet anthocyanin is used, the change in hue is so distinct that no direct comparison can be made with the standard colour. With red and orange-red ones, however, the change in hue does not take place in a marked degree up to a certain point, hence the approximate measurement becomes possible.

1. HARROW, B. and GIES, W. J., Experimental Studies on Plant Pigments. Columbia Univ. Proc. Soc. Exp. Biol. Med. 16: 8, 1918, Review in Chem. Abstract. 13: 2695, 1919.

To illustrate, the following results will be mentioned. Ten grams of the fresh petals of scarlet *Papaver Rhoeas*[1] were extracted with 150 cc of distilled water. The colour of the extract was deep red. This was taken as 100, and 50, 25, and 12.5 per cent solutions were prepared. The peroxidase used was prepared from the pressed sap of hypocotyls and rootlets of the soy bean seedlings by precipitating with alcohol. Five cc of the enzyme solution was added to fifty cc of a dilute hydrogen peroxide solution (0.1 cc corresponded to about 0.0003 g oxygen). The mixtures were put in large test tubes of the diameter 4 cm and they were kept in the water bath. The temperature was kept between 17.0 and 17.5 C. At different intervals of time, five cc of the mixture were pipetted out into a test tube and one cc of concentrated hydrochloric acid was added to stop the enzyme activity and to deepen the colour. The standard solution was prepared in the same manner immediately after the enzyme was added. Each portion was then compared with the standard solution so prepared by Dubosq's colorimeter and the intensity of colour of the different portions was expressed as percentages of the initial colour which was taken as 100.[2] They gave the following result.

TABLE 1.

Decolorization of anthocyanin by peroxidase.

A. Relative strength of anthocyanin = 100
B. „ „ „ „ = 50.0
C. „ „ „ „ = 25.0
D. „ „ „ „ = 12.5

A.

Time in minutes (t)	Intensity of colour ($a-x$)	k
0	100.0	0
7.0	100.0	0
40.0	99.06	0.00024
63.5	93.75	0.00102

1. According to WILLSTÄTTER and WEIL (LIEBIG. Ann. 412 : 231, 1916.) one of the pigments of a double purple-scarlet variety is mekocyanin which is a diglucoside of cyanidin.

2. The initial colour corresponded with somewhere between Peach red to Scarlet in RIDGWAY's Color Standard.

B.

Time in minutes (t)	Intensity of colour ($a-x$)	k
0	100.0	0
6.5	97.50	0.00101
15.0	90.00	0.00679
39.0	75 00	0.00738

C.

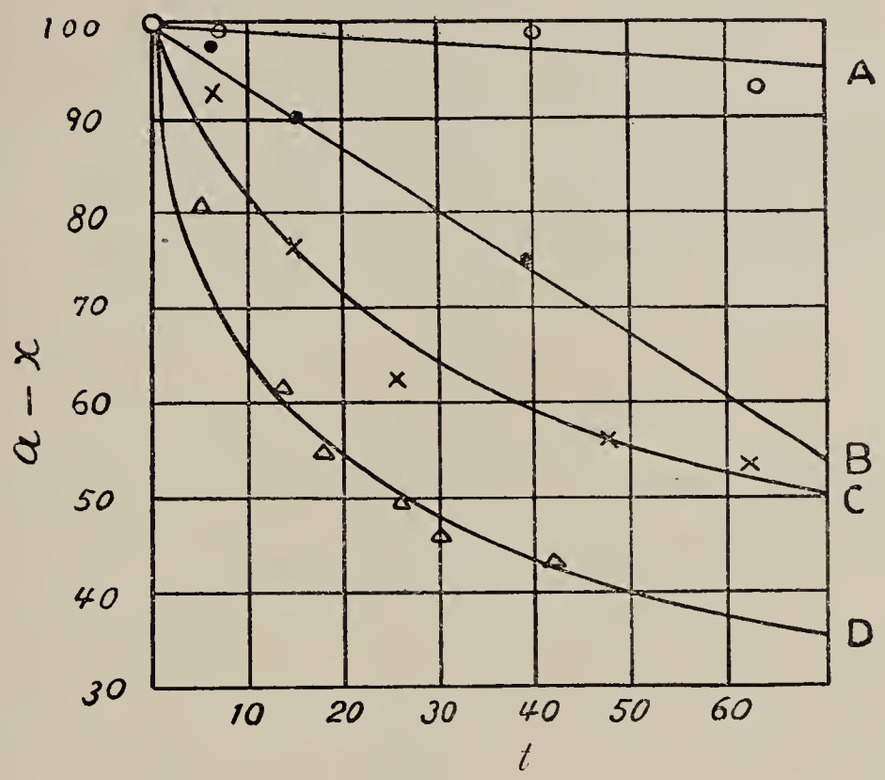
0	100.0	0
6.0	93.75	0.01075
15.0	76.88	0.01752
26.5	62.50	0.01773
48.0	56.25	0 01200
62.0	53.12	0.01020

D.

0	100.0	0
5.0	81.25	0.04154
14.0	62.50	0.03323
18.5	55.00	0.03233
36.0	50.00	0.02666
30.5	46.25	0.02535
41.5	43 75	0.01992

The figures in the third column are the values of the velocity constant calculated as unimolecular reaction, $k=\frac{1}{t}\log.\frac{a}{a-x}$. They show that the rate of decomposition in A was very slow owing to the relative high concentration of anthocyanin and the value of k increased as the reaction advanced. In B, a similar tendency was observed but in D, the value of k rapidly decreased as the reaction advanced. Since the strength of the enzyme was the same in all cases, the different values obtained indicate that the mode of action of peroxidase differed by the relation of the initial concentration of the enzyme to the substrate.

If the values of $a-x$ in A are plotted against time, they show nearly a



Text-fig. 1. Graphs showing the decomposition of anthocyanin by peroxidase. See Table 1.

straight line suggesting that the rate of decomposition was approximately proportional to the time of reaction, hence the value of k calculated for the unimolecular reaction increased as time advanced. If, however, the value of log. $a-x$ are plotted against time, the curves of B, C, and D do not form straight lines, while, the values of log. $a-x$ are plotted against log. time, the curves show more nearly the straight lines. This seems to show that the value of the enzyme was not constant. The active portion of the enzyme decomposed as the reaction proceeded. Two reactions, the decomposition of the enzyme itself and that of anthocyanin by the active portion of the enzyme in the system seem to go on simultaneously, so that the rate of decomposition of the latter cannot be regarded as a simple uni-molecular reaction. The reaction may be uni-molecular only when the specific ratio of the enzyme to the substrate in held and in which the rate of decomposition of the enzyme may be so slow that its value can be considered as nearly constant, while the decomposition of the substance goes on with the constant rate. Hence we are able to consider the value of $a-x$ as the function of time.

Similar observation was made with laccase. Ten cc of a 0.6 per cent

laccase solution[1] which gave the direct oxidase reaction by phenylendiamine, alpha naphtol, and guaiacum were added to fifty cc of the anthocyanin extract which was prepared from the dried powder of the flower of *Lilium tigrinum* by extraction with dilute alcohol. The mixture was kept at the room temperature which varied from 19.0 to 20.0 C.

TABLE 2.

Decolorization of anthocyanin by laccase.

Time in minutes (t)	Intensity of colour ($a-x$)	k
0	100.00	0
12	90.63	0.00820
30	83.75	0.00592
50	73.12	0.00626
74	67.00	0.00533
101	62.50	0.00465
204	42.50	0.00419

TABLE 3.

Same as Table 2, except the concentration of the enzyme which was reduced to half that of the former.

Time in minutes (t)	Intensity of colour ($a-x$)	k
0	100.00	0
2	97.31	0.00094
20	90.77	0.00198
32	84.27	0.00258
100	76.92	0.00262

It was found that certain salts and the sugars retarded the activity of the enzyme. One of the experiments gave the following result. Fifty cc of a weak alcoholic (25 per cent) extract of anthocyanin from the violet coloured perigone of *Iris Kæmpferi* to which was added glucose (MERCK'S pure) to

1. The material was kindly furnished to the writer by Prof. K. SHIBATA.

make up 5 per cent, was mixed with 0.2 cc of a dilute hydrogen peroxide and 0.5 cc of the enzyme solution which was prepared from the pressed juice of the soy bean seedling twice precipitated by alcohol. The mixture was kept at 19.0 to 20.0 C. The change in the hue from violet to red was observed as already mentioned but by an addition of strong acid to the pipetted portion by which the comparison of the colour was made, the colour was red all alike. Thus:

TABLE 4.

Influence of glucose on the decolorization of anthocyanin by peroxidase.

5 per cent glucose			Control (without glucose)		
Time (t)	Intensity of colour ($a-x$)	k	Time (t)	Intensity of colour ($a-x$)	k
0	100.00	0	0	100.00	0
10	97.64	0.00239	10	90.48	0.00999
20	95.24	0.00244	20	83.81	0.00884
32	90.48	0.00313	40	78.09	0.00589
62	88.57	0.00175	69	76.19	0.00396

The above fact seems to bear some physiological importance. The higher the concentration of sugar or salts dissolved in the cell sap, the weaker would be the action of oxidizing enzyme on anthocyanin. Thus the latter may be protected from decomposition.

As already mentioned, hydrogen peroxide alone decolorizes the anthocyanin and if a small amount of finely pulverized animal charcoal is added, the action of hydrogen peroxide is highly accelerated.

The different amount of animal charcoal and 1 c.c. of hydrogen peroxide which was equivalent to 0.014 g. oxygen was added to 5 c.c. of a reduced alcoholic solution of 0.001 mol. quercitrin. Time required for the mixtures to be completely decolorized at 14.0 C.—16.5 C. was as follows:

TABLE 5.

Influence of animal charcoal on the decolorization of anthocyanin by hydrogen peroxide.

Charcoal added in gram. (q)	No. of minutes required for decolorization (t)	qt
0.05	29.0	1.45
0.04	34.5	1.38
0.03	42.0	1.26
0.02	62.0	1.24
0.015	89.0	1.34
0.01	140.0	1.40
Control	ca. 400.0	——

When the concentration of hydrogen peroxide varied and the amount of animal charcoal made constant, at the temperature 20.0 C, the following result was obtained.

TABLE 6.

Same as Table 5.

Charcoal added in gram	Cc of hydrogen peroxide added	Concentration equivalent to oxygen in g.	No. of minutes required for decolor.
0.05	1	1 = 0.014	6.0
0.05	1	1/2	11.5
0.05	1	1/4	18.5
0.05	1	1/8	20 5
0	1	1 = 0.014	ca. 180.0

It seems clear, from the data so far presented, that although the oxidizing enzymes, are universally present in the plant cell, particularly co-existing with anthocyanin, and although the normal oxygen relation is essential to the formation of anthocyanin in the living tissue as we shall see later, yet these enzymes have no direct relation to the formation of anthocyanin from flavone. On the contrary, anthocyanin is converted to a flavone-like yellow substance by the action of the oxidizing enzymes.

2. The Action of Oxidizing Enzymes on Flavones.[1]

When the aqueous or alcoholic extracts of the plant tissues which are rich in flavone, are mixed with the freshly prepared pressed plant-juice containing the active oxidase, a marked brown to reddish brown colour is formed instantly. The more flavone the extract contains, the deeper is the colour produced. On standing, a brownish precipitate is formed and subsides. The production of that colouring matter is produced at the expense of the flavone contained in the extract. It can be proved by testing the intensity of the reduction colour of the extract at the beginning and at the end of the experiment. At the end of the experiment, a marked decrease in the flavone content can be seen by means of its reduction colour, whenever the brown colouring matter is formed.

The extract of leaves, twigs, white flowers, fruits, and other parts of plants of different species were examined and in general, the parallelism in the depth of the brownish colour .produced by the oxidase and that of the reduction colour of the extract was established. The brownish colouring matter thus formed has its colour intensified by the addition of alkali and, on the addition of acid diminishes or changes to yellow. The colour change just mentioned is very sensitive being performed in an indicator like manner.

Pure chemical preparations were then tried and it was found that certain flavones and flavonols yielded a marked oxidation colour by the action of oxidizing enzymes. For example, myricetin, even in a comparatively dilute alcoholic solution, yielded a beautiful red colour immediately after the oxidase was added. The colour, however, was unstable, and changed to brownish red and finally to brown. Quercetin and luteolin yielded also a deep red colour rapidly changing to brown. Kæmperol, apigenin, and tringin on the other hand, showed practically no change. In the former cases, the reduction colour when tested after being acted on by the enzyme, was decidedly less deep than that of the control or that of the initial one, while in the latter cases, practically no difference was observed showing that the flavones remained unchanged.

Glucosides gave less characteristic colour than non glucosides. Myricitrin

1. The full account of the investigation will be published by the author jointly with Prof. K. Shibata.

and quercitrin yielded a less bright red colour than did myricetin and quercetin respectively.

These observations indicate that the oxidation colours of flavones and flavonols are largely influenced by the chemical constitution of the substance especially the number and the position of OH group substituted in beta phenyl group as in the case of the reduction colours.

It is now beyond boubt that the action of oxidases and peroxidases on flavones bears no direct relation to the chemical changes of flavones to anthocyanins ; nevertheless the oxidizing enzymes may play an important part in other metabolic changes in the living tissue.

3. Flavone Derivative as Chromogenic Substances of Reddish Brown Plant Pigments (Phlobaphenes).[1]

The reddish brown and brown pigments are widely distributed in the plant kingdom, namely in the bark, rhizome, seed coat, dead leaves and in others, some of them are known as phlobaphenes which were originally the name given by Stähelin and Hofstetter (1814)[2] to certain brownish red substances of unknown chemical constitution isolated from the bark of *Pinus sylvestris*, *Platanus acerifolia*, *Betula alba*, *Cinchona Calisaya* etc. According to these authors and others,[3] phlobaphenes are considered to be the oxidation product of tannins. Hlasiwetz considered that there are two groups of substances which may respectively give rise to two groups of phlobaphenes. One of the groups of phlobaphenes to which 'china red', 'chinava red', and 'oak red' etc. belong, yields by fusion with alkali, protocatechuic acid alone, and the other group to which 'filix red', 'ratanhia red' and 'chestnut red' belong, yields together with protocatechuic acid, phloroglucin. Along with them,

1. Nagai, I., On some Reddish Brown Plant-Pigments. Bot. Mag. Tokyo, 31 : I, 1917. In Japanese.

2. Stähelin, C. and Hofstetter, J., Chemische Untersuchungen einiger Rinden. Liebig. Ann 51 : 63, 1844.

3. Hesse, O, Ueber die humusartingen Bestandtheile der China-rinden. Liebig. Ann. 109 : 341, 1859. Hlasiwetz, H., Ueber die Beziehungen der Gerbsäure, Glucoside, Phlobaphene und Harze. Liebig. Ann. 143 : 209, 1867. Nierenstein, M., Beitrag zur Kenntnis der Gerbstoffe II. Berich. d.d. chem. Gesel. 42 : 353, 1909. See also Perkin, A. G. and Everest, A. E., Loc. cit. page 436 et seq.

a group of substances such as maclurin, luteolin, catechin, quercetin and scoparin, also yields protocatechuic acid and phloroglucin as their decomposition products. So he supposed that those phlobaphenes which yield protocatechuic acid and phloroglucin are the derivatives of the substance just mentioned. In the epidermis of leaves and the periphery of the bark, those substances may undergo oxidation by the contact with air resulting in the formation of phlobaphene.

WALTER (1890)[1] studied the sklerotic tissue of the rhizome of ferns and came to the conclusion that the brown pigment deposited in the membrane is identical with phlobaphene and its physiological function was considered by him to be the protection of the tissue from the injurious effect of the humidity of the substrate under which these plants grow.

Some of the brown pigments found in nature are hardly soluble in common organic solvents like ether, alcohol, benzine, and acetic ether, but are readily soluble in water and especially in alkaline solutions, yielding a deep brownish red to wine red colour, which by acid is instantly changed to yellow. The brown and reddish brown oxidation products of flavones and flavonols as already stated in the preceding chapter, possess similar properties. Since the flavone derivatives are widely distributed in plants, it seems quite probable to assume that some of them give rise to phlobaphene by oxidation as HLASIWETZ has already supposed. Therefore, we may regard certain flavone and flavonol derivatives as the chromogen of both anthocyanin and phlobaphene pigments.

We assume that certain relations existing among these pigments may be somewhat as follows:

Chromogenic substance	Initial change	Subsequent changes	Product
Certain flavones flavonols and their glucosides	reduction	formation of complex with salts[2]	anthocyanins of different hues.
	oxidation	condensation & polymerization	phlobaphenes

1. WALTER, G., Ueber die braunwandigen sklerotischen Gewebeelemente der Farne, mit besonderer Berücksichtigung der sog. "Stützbündel" Russow's. Bibliotheca Botanica. Heft 18:21, 1890.

2. See SHIBATA, K., SHIBATA, Y. and KASHIWAGI, I., Studies on Anthocyanins. Color Variation in Anthocyanins. Jour, Amer. Chem. Soc. 41:208, 1919.

4. Role of Oxygen in the Development of the Chromogenic Substance and Anthocyanin.

It is already known that the formation of anthocyanin is suppressed when the normal oxygen relation is artificially checked in the living plant.[1]

The writer observed that the hypocotyls of the seedling of the buckwheat remained white as long as they were kept in the dark, but when exposed to the day light, a deep red colour developed. If they were kept in the glass chamber in which the air was replaced by hydrogen gas, they did not form the pigment even when exposed to the day light. The chromogenic substance could be detected from the colourless samples. The alcoholic extract gave a distinct red colour by reduction by means of hydrochloric acid and magnesium powder.[2]

The young seedlings of certain varieties of soy bean form a deep purple anthocyanin pigment together with chlorophyll in the elongated hypocotyls a few days after germination, if they are exposed to strong sun light. If, however, the normal supply of air is checked, the pigment does not develop as in the case with the buckwheat. The following experiment shows clearly the above relation. The young seedlings of a variety of yellow cotyledon which were devoid of both pigments, were placed under the following conditions:

Lot A. A small, tightly fitted glass chamber in which the air was replaced by hydrogen gas which was generated by Kipp's apparatus and washed by the solution of potassium permanganate once. The chamber was dipped in water.

Lot B. Same as A, but the chamber was kept in the air.

Lot C. The chamber was simply closed up.

Lot D. The chamber opened, as control.

All the chambers were kept in a glass case which was placed near the

1. Emery, M., Sur les variations de l' eau dans les perianthes. Bull. Soc. Bot. France. 36: 322, 1889. Katic, D. L., Beitrag zur Kenntnis der Bildung des roten Farbstoffs (Anthocyan) in vegetativen Organen der Phanerogamen. Inaug. Dissert., Halle. 1905. Cited in Wheldale, M., Anthocyanin Pigments of Plants. 1916.

2. See also Miege. E., Recherches sur les principales espèces de Fagopyrum. Thèse de Doctorat de l, Univ. Paris. 1910. Cited in Combes, B., Recherches biochemiques experimentales sur le rôle physiologique des glucosides chez les végétaux. Rev. General. d. Bot. 30: 89, 1918.

window to receive direct sunlight. Three days after the experiment was set up, chlorophyll developed in the seedlings in *C* and *D*; the deep purple pigment was also formed in the latter. None of the pigment were formed in *A*. On fourth day, a slight purple colour was observed in *C* but in *A* and *B*, it failed to appear. In the same day, the chamber *B* was opened to allow normal air. Two days later (on the sixth day) the seedlings in *A* remained still without the pigment. In *B*, chlorophyll was found in the cotyledons but no purple pigment in the shoots. In *C*, the development of the purple pigment was still feeble, whereas in *D* (control), all the seedlings were deeply coloured.

It is a well known fact that the leaf scale of the onion bulb becomes yellow on exposure to light. It is chiefly due to the formation of quercetin.[1] If, however, the bulbs of which already coloured scales were removed, were kept in the closed chamber filled with hydrogen gas, the formation of flavone was inhibited. The bulbs were cut in halves and the coloured scales were removed. The halves were kept in the closed glass chamber in which the air was replaced by hydrogen gas and the other halves were kept for the control experiment. They were kept for sixteen days during which the gas was renewed once. Neither yellow nor green pigment was found except in the control specimens. When the chambers were opened, the bulbs were turgid, but became soft immediately after the air was let in. An equal weight of the scales was taken from the treated and control samples and extraction was made with equal volmes of a weak alcohol. The extracts so prepared, were reduced by means of hydrochloric acid and magnesium powder in the usual manner. They gave the following flavone reactions:

Treated	Trace of pink colour.
Control	Red.

It showed that normal air is essential to the development of flavone in the leaf scale of the bulb of onion even when light is amply supplied.

The bulbs of *Allium Ledebourianum*, a common weed in certain parts of Japan, are white when they are grown in the ground, but they are dug out

1. Perkin, A. G. and Hummel, J. J., Occurrence of Quercetin in the Outer Skin of the Bulb of the Onion (Allium cepa). Chem. Soc. Trans. 69 : 1295, 1896.

and exposed to day light, a deep violet-red colour develops. The coloured scales were removed and the white portions were kept in the same manner as in the previous experiment with the common onion bulbs. No red pigment was formed during the experiment which lasted for two weeks. The bulbs in the control became deep green and the treated ones were white. Alcoholic extracts were prepared, and the flavone content was examined. They showed :

Treated	No reaction
Control	Trace of pink colour

Here the flavone was formed in extremely slight amount even when the light and the air relation was normal.

5. A Group of Substances of Unknown Chemical Nature as the Chromogen of Anthocyanins and Reddish Brown Pigments.

A body of evidences accumulated by the different investigators show[1] that a group of chromogenic substance is present in plants which gives rise to anthocyanin-like pigments as well as the brown pigments.

Wolff and Rouchermann (1915)[2] observed the presence of a chromogenic substance in a number of plants which are sensitive to the action of laccase yielding the brown pigment. The phenomena observed in iodine colour tests are always preceded by the action of a laccase. The authors considered that the chromogens are of the same kind in different plants and the brown pigments which are formed in various plants or organs might be regarded as products of oxidation. Shibata[3] obtained the chromogenic substance from a

1. Malvezin, Ph., Sur 1 'origine de la colour des raisins rouges. Comp. Rend. Acad. Sci. Paris. 147 : 348, 1908 ; Laborde, J., Sur 1 'origine de la matière colorante des raisins rouges et autres organes végétaux. Ibid. 146 : 1411, 1908 ; Dezani, S., Le sostanze cromogene dell' uva bianca. Staz. sper. agr. ital., Modena. 43 : 328, 1910 (cited in Atkins, W. R. G., Researches in Plant Physiology. 1916) (also in Jour. Chem. Soc, Abstract 100 : 223, 1911.) ; Keegan, P. Q, The Chemistry of the Flower Pigments. Chem. News. 107 : 181, 1913 ; Tswett. M., Zur Kenntnis des vegetabilischen Chamæleons. Ber. d. d. bot. Gesell, 32 : 61, 1914 ; Tswett. M., Beiträge zur Kenntnis der Anthocyane. Ueber künstriches Anthocyan. Bioch. Zeit. 58 : 225, 1913.

2. Wolff, J. and Rouchermann, N., Sur les propriétés d'un chromogène universellement répandu dans les végétaux. Comp. Rend. Acad. Sci. Paris, 161 : 399, 1915 : Wolff, J., Phénomènes d' oxidation et de réduction portant sur les chromogènes des végétaux. Ibid. 160 : 716, 1915.

3. Shibata, K., Bot. Mag. Tokyo. 31 : 1919. A brief note in Japanese.

number of plants which yielded a deep red colour on heating with hydrochloric acid and with oxidase a brown to reddish colour. The red colouring matter obtained by heating with the acid is changed to blue by an alkali like anthocyanin. The chromogenic substance is soluble in alcohol and in ether, and with ammonia yields a deep yellow colour as observed with flavone but by reduction yields no colour. It is very sensitive to the action of oxidases forming a brown substance of which the colour is intensified by alkali and changed to yellow or yellowish brown by acid. The plant extract which contains both this substance and certain kinds of flavones shows a characteristic red colour by reduction as well as when heated with hydrochloric acid. SHIBATA considered that the substance might be regarded as a colourless anthocyanin. MOREAUX (1914)[1] considered it proper to rank along with red, violet and blue pigments designed as anthocyanins, the colourless compounds which are inseparable from them and which are always found in the cells as earlier or later products, being closely related to them as regards chemical composition and as having in common with them a mitochondrial origin.

We do not know as yet the chemical nature of the substance in question but the similarity in certain properties exhibited by the extract from a number of plants, suggests that a closely allied substance may be present widely in the plant kingdom and it may give rise to certain anthocyanins and the reddish brown pigments. The bearing of the fact on genetics is hardly to be overlooked, for we are now able to locate and to approximate the chromogen in the part of a plant by the test for flavone and the substance under discussion. In the following pages, the former will be named, for the sake of convenience, chromogenic substance F, and the latter chromogenic substance P.

A number of species of plants especially the cultivated plants, were examined for these chromogenic substance. The method employed was as follows. To each gram of the fresh material, ten cc of a weak alcohol were added and extracted on the water bath. Usually three to five grams of the materials were taken. Five cc of the extracts were reduced with one cc of concentrated hydrochloric acid and magnesium powder for the chromogenic substance *F*, and another five cc were simply boiled with the acid which was

1. MOREAUX, F., L' origine et les transformations des produits anthocyaniques. Bull. d. l. oc. Bot. France. 61 : 390, 1914.

added in the same proportion as before, for the chromogenic substance P. After the treated extracts were cool, the colours of the extracts were compared with those of the standard colours and recorded. The standard colours were prepared in the following manner.

Twenty five cc of quercetin dissolved in absolute alcohol, were reduced with five cc of concentrated hydrochloric acid and about 0.5 grams of magnesium powder.

Colour scale	Concentration of quercetin
I	I : 1,000
II	I : 2,000
III	I : 3,000
IV	I : 5,000
V	I : 10,000
VI	I : 20,000

Thus the relative value of the chromogen content in the material was approximately determined. The result of a survey established the following fact.

The chromogenic substance *P* can be detected in different parts of plants, i.e., the leaf, stem, shoot, rhizome, bark, wood, white petals, perigone, seed coat, mesocarp, stigma etc.

It occurs quite independently or in company with the chromogenic substance *F*. Even in the same plant, the distribution of the two chromogens is quite distinct in different organs.

Light seems to have no direct relation to the distribution of the chromogenic substance *P* unlike the case of the chromogenic substance *F* (flavones).[1] For, the underground parts and the interior portions of the upper ground tissues of many plants contain a considerable amount of the chromogenic substance *P*. The bark of young twigs, the seed of immature seeds which ultimately become brown, red or black when fully mature, the young fruits and berries of many of the cultivated fruit trees are especially rich in the chromogenic substance *P*. Thus, for example :

1. Shibata, K. Nagai, I. and Kishida, M., The Occurrence and Physiological Significance of Flavone Derivatives in Plants. Jour. Biol. Chem. 28 : 93, 1916.

TABLE 7.

Chromogen content of the plant extracts.

Name of plant	Part examined	Relative value of chromogens *P*	*F*
Cryptomeria japonica	Green leaf	I	IV
„ „	Green bark	I+++ [1]	IV+
„ „	Wood & pith (young twig)	IV	+
Pinus parvifolia	Green leaf	I+	V
„ „	Bark	I	V
Platanus occidentalis	Bark	I+++	IV−
„ „	Wood	IV	−
Aesculus turbinata	Bark	I+	+
„ „	Wood	IV	−

In the immature green seed of different legumes, the chromogen can readily be detected, so that it is possible to predict whether the seeds may be coloured or not in adult condition. So far the writer has found, the colourless or white seeds show practically no chromogen reaction when they are still young, the relation of the pigment to the chromogen being quite well marked.

TABLE 8.

Chromogen content of the extracts of green seed of the leguminous plants.

Name of plant	Part examined	Colour of seed when ripe	Relative value of chromogens *P*	*F*
Indigofera pseudotinctoria	Seed	Brown	I	−
Vicia sativa	„	„	II+	−
Pisum sativum	„	„	I+	+
Phaseolus vulgaris	„			
"Kotenashi"	„	White	−	−
"Ohtenashi"	„	„	−	−
"Kianeko"	„	Yellow pied	IV	+

1. + sign denotes the deeper colour than that of the colour scale stated. The sign alone denotes the trace of colour or below VI.

Name of plant	Part examined	Colour of seed when ripe	Relative value of chromogens *P*	*F*
"Golden Wax"	Seed	Black	I+	+
"Chosen"	,,	Reddish brown	II	+
"Longfellow"	,,	Brown mottled on light yellow	II	+
"Kumamoto-Ingen"	,,	White	+	–
"Black Valentine"	,,	Black	II	–
"Canadian Wonder"	,,	Reddish brown	II	–
"Birma-Ingen"	,,	Black mottled on brown	I+	–
Phaseolus radiatus var. *aureus*				
Unnamed	,,	White (pale buff)	–	–
"Shiro-Adzuki"	,,	,,	–	–
Unnamed	,,	buff	I+	(0) [1]
,,	,,	Red	I+	IV
"Maru-Ba"	,,	,,	I+	IV
"Wase-Dainagon"	,,	,,	I+	IV
"Madara"	,,	Black flecks on red	I	IV
"Kensaki"	,,	Red	I	IV
"Yogore"	,,	Dark flecks on buff	II	+
"Midori"	,,	Greenish grey	I	(0)
"Wase-Otsubu"	,,	Dark red	I	IV

As we have just seen, the chromogenic substance *P* is plentifully found in the extracts of the immature coloured seed of *Phaseolus vulgaris*, but very scarce in or nearly devoid of the chromogenic substance *F*. In the leaf, the reverse is the case.

TABLE 9.

Chromogen content of the leaf of *Phaseolus vulgaris*.

Name of variety	Colour of flower	Colour of leaf	Chromogen *P*	*F*
"Kotenashi"	White	Yellowish green	–	–
"Ohtenashi"	,,	,,	–	II
"Kianeko"	Cream	,,	–	IV

1. Orange colour.

Name of variety	Colour of flower	Colour of leaf	Chromogen *P*	Chromogen *F*
"Golden Wax"	Pale pink	Greenish yellow	+	IV
"Chosen"	„	Green	−	III
"Longfellow"	White	„	−	III
"Kumamoto-Ingen"	Cream	„	−	IV
"Black Valentine"	Pale pink	„	+	IV
"Canadian Wonder"	Pink	Greenish yellow	−	III
"Biruma-Ingen"	„	„	−	III

Unlike the case of the above, both chromogenic substances co-exist in the leaf of certain fruit trees.

TABLE 10.

Chromogen content of the leaf of certain fruit trees.

Name of plant	Chromogen *P*	Chromogen *F*
Grapes (*Vitis*)		
"Adirondack"	II	I
"Bacon"	I+	I
"Brighton"	I	I−
"Catawba"	I	II
"Champion"	I−	II
"Concord"	I	II
"Hartford Prolific"	II	II
"Hervert"	I+	II
"Koshu"	I+	I
"Ives"	I+	II+
"Lady Washington"	I	II+
"Sweet Water"	I++	I
Sand Pears (*Pirus serotina*)		
"Nijuseiki"	I	I
"Kozo"	II	II
"Chojuro"	II	II
Apples (*Malus sylvestris*)		
"Iwai"	III	III
"Jonathan" ("Kogyoku")	III	I+

Name of plant	Chromogen *P*	Chromogen *F*
"Rawles Janet" ("Kokko")	III	I
"Smith Cider" ("Ryugyoku")	III	II
"King of Tempkins Country ("Hinokoromo")	III	II

If was noted that the reduction colour of the extract of the leaf of the pear and that of the apple was somewhat different; the former was orange red, the latter more scarlet red, suggesting that different flavones might be present in them.

In the leaf of *Morus alba*, which is very important in sericulture, the chromogenic substance *F* was found but not the other, while in the leaf of *Iris Kœmpferi*, the reverse was the case, and in the latter, the chromogen content in the leaf was not correlated with the colour of the perigone, some of which are deeply coloured.

Table 11.

The chromogen content of the leaf of *Morus alba* and *Iris Kœmpferi*.

Name of plant	Chromogen *P*	Chromogen *F*	Remarks
Morus alba			
"Mishima"	—	II	
"Akagi"	—	II	
"Ro-so"	—	III	
"Ro-so" Seedling	—	IV	
"Furisode"	—	IV	
Iris Kœmpferi			
No. 1	I	—	The colour of perigone solid hyacinth purple.[1]
No. 2	II	—	Flecked violet purple
No. 3	III	—	Solid Rood's violet
No. 4	III	—	Flecked violet purple
No. 5	I	(Y)	Self pansy violet
No. 6	I	(Y)	White

1. Nomenclature according to Ridgway, R. Color Standards and Color Nomenclature, 1912.

The skin of the young, green grapes was found to be very rich in the chromogenic substance *P* but it contains only a trace of the chromogenic substance *F*. It is of interest to find that the "white" varieties, so far examined, contained the chromogenic substance as much as that shown by the coloured varieties. Even in the deep black variety, the reduction colour of the extract of the green skin was only faint, so it seems highly probable that the anthocyanin pigment in the skin of the grape may be formed chiefly from the chromogenic substance *P* rather than the chromogenic substance *F*. According to WILLSTÄTTER and ZOLLINGER (1915)[1] the anthocyanin of grape skin (North Italian or hothouse) were ænidin and ænin which are the methyl ethers of delphinidin and delphinin respectively. The chemical investigation of the chromogenic substances in the grape skin is inviting, for it may clear the relation of anthocyanin to the chromogenic substances *P* and *F*.

DEZANI found two kinds of the chromogenic substances in the white grapes of which one only is precipitated by lead acetate. By the action of hydrochloric acid, colouring matters are obtained which are analogous to the ænocyanins. The conversion of these substances into colouring matter is due not to oxidation, but probably to hydrolytic scission with simultanious formation of a reducing substance. In the residue from the chromogenic substances there are other substances which give a red colour with alkali. The result obtained by HEDRICK and ANTHONY[2] shows that (1) "white" is a pure, colour, namely "white" x "white" gives only "white": and (2) "white" is recessive to both black and red.

TABLE 12.

Chromogen content of the skin of green grapes.

Name of variety	Colour of skin when fully ripe	Chromogen *P*.	Chromogen *F*.
"Ives Seedling"	Black	I	VI
"Bacon"	„	I	VI

1. WILLSTÄTTER, R. and ZOLLINGER, E. H., Ueber die Farbstoffe der Weintraube u. der Heiderbeere. Liebig. 408 : 83, 1915, and 412 : 195, 1916. See also DEZANI, S., loc. cit.

2. HEDRICK, U. P. and ANTHONY, R. D., Inheritance of Certain Characters of Grapes. N. Y. Agric. Exp. Station. Technical Bull. 45. 1915, pp. 19.

Name of variety	Colour of skin when fully ripe	Chromogen *P.*	Chromogen *F.*
"Concord"	Black	I+	VI
"Black Hamburg"	,,	I+	VI
"Hervert"	,,	I+	VI
"Bryan"	,,	I+	VI
"Delaware"	,,	I+	VI
"Othelo"	Red	I+	VI
"Bryant"	,,	I	VI
"Lady Washington"	White	I+	VI
"Vergennes"	,,	II+	V
"Moore's Diamond"	,,	I+	VI
"Niagara"	,,	I+	VI
"Golden Champion"	Amber	I	VI

"Bell," "Esther," "Eaton" (whites), "Highland," "Hartford Prolific" (blacks), and "Iowa" (red) showed likewise the marked colour reaction of the chromogenic substance *P.*

Apples, pears, oranges, Kaki fruits, strawberries, bananas and other fruits and vegetables were examined, the results of which were listed and given in Table 13.

In certain plants, the chromogen reaction failed when tested just before the formation of anthocyanin. The leaf scale of the bulb of *Lilium tigrinum* is devoid of anthocyanin when it lies underground. But it becomes purple in exposure to sunlight. Even a few hours exposure causes the purple spots to appear on the surface of the yellowish white leaf scale, and the coloured area extends gradually to the entire scales, within a few days. The alcoholic extract of the suitable material, however, shows practically no reaction of either chromogen.

The potato tuber is another example of this kind. Two kinds of white tubers are known. One is such that the tubers are devoid of anthocyanin as long as they are in the ground but by exposure to sunlight, they become deep purple. The other is such that, even when exposed to the light for a long time, no anthocyanin is produced. The extract of both kinds of white tubers showed a very feeble reaction of the chromogens. It seems that the anthocyanin may be formed so rapidly from the raw material in these instances

that there may be no appreciable amount of the chromogenic substance accumulated to show a definite colour reaction.

The body of evidence so far reported seems to point to the following conclusions.

In a number of plants, anthocyanin and the brown pigment (phlobaphene) can be traced to their respective chromogenic substance previous to the formation of the pigments. Both pigments can be formed from the same chromogenic substance by the action of a number of complementary pigment-yielding agencies. The chromogenic substances can be identified as belonging to two groups of substance with respect to certain colour reactions, one of which is designated as chromogenic substance *F*, (certain flavones and flavonols), and the other as chromogenic substance *P* of which the chemical nature is unknown.

The formation of brown pigment includes at least the following cases.

1. It is chiefly due to the oxidation and subsequent changes of the chromsgenic substance *F*. Example: the awn of *Oryza sativa* as we shall see later.

2. It is chiefly due to the oxidation and subsequent changes of the chromogenic substance *P*. Example: the seed coat of the legumes (*Phascolus vulgaris, Pisum sativum, Glycine soja* etc).

3. It is cheifly due to the oxidation and subsequent changes of the chromogenic substance *F* and *P*. Example: the barks of many trees (*Cryptomeria japonica, Pinus parvifolia, Platanus occidentalis* etc).

Besides those chromogens, tannoids, and carotinoids may play a role in the production of the reddish and yellowish brown pigments.

TABLE 13.

Showing the chromogen content of the plant extracts.

Designations: Peri. = peripheral tissue.

Int. = Internal tissue.

(+) sign without the number of the class in the colour scale, denotes the presence of the colour but below the lowest class in the scale. (+) sign with the numeral denotes the colour somewhat deeper than the scale indicated by the numeral. (−) sign designates likewise the colour below the scale.

(×) sign designates the presence of a distinct colour which differs from that of the colour scale.

R=red, O=orange, OR=orange red, B=blue,
V=violet, M=magenta, Y=yellow, G=green,
YG=yellowish green, Br=brown, BR=brownish red.

Name of plant	Part examined	Chromogen *P*	Chromogen *F*	Remarks.
Gymnosperms.				
Cryptomeria japonica	Lf.	III−	V	
,, ,,	Wood & pith.	IV	+	Young twig.
Pinus densiflora	Lf.	IV	V	
,, ,,	Bark	I	VI	Young twig.
,, ,,	Wood & pith.	VI−	−	
Larix leptolepis	Lf.	III	III	Young twig.
,, ,,	Wood & pith.	V	−	
,, ,,	Bark	I	V+	
Picea ajanensis	Lf.	III	IV	
,, ,,	Bark	I	V	Young twig.
,, ,,	Wood & pith.	V	−	
Thujopsis dolabrata	Lf.	III−	+	
,, ,,	Wood & pith.	V	+	
Angiosperms				
Alismataceæ				
Sagittaria sagitlifolia var. *longiloba f. sinensis*	Bulb. Peri.	VI	VI	The outer skin is blue.
,, ,,	,, Int.	+	−	White tissue.
Araceæ				
Colocasia antiquorum	Rhizome Peri.	VI	−	The outer skin brown.
,, ,,	,, Int.	VI	−	White tissue.
Liliaceæ				
Allium Cepa	Leaf scale	VI	V	
A. fislulosum	Lf.	−	−	Etiolated.
A. Ledebourianum	Lf. scale	+	−	
Erythronium denscanis.	Shoot	−	−	Etiolated part.
,, ,,	Lf.	+	II	Green.
Lilium tigrinum	Lf. scale Peri.	+	−	
,, ,,	,, ,, Int.	+	−	
Amaryllidaceæ				
Narcissus sp.	Lf. scale	−	−	

Name of plant	Part examined	Chromogen *P*	Chromogen *F*	Remarks
Narcissus sp.	Lf.	–	VI–	
,,	Perigone	–	II+	Yellow colour
,,	Corolla	–	II+	Pale yellow
Dioscoreaceæ				
Dioscorea Batata	Rhizome Peri.	VI	+	The skin brown.
,, ,,	,, Int.	V	–	White tissue.
Iridaceæ				
Belamcanda punctata	Fruit	VI	–	Green fruit, black when ripe.
Bromeliaceæ				
Ananas sativus	Fruit Peri.	II+	II	
,, ,,	,, Int.	×(V)	II	
Commelinaceæ				
Commelina communis	Seed	VI	+	Unripe seed, brown when ripe.
Gramineæ				
Agrostis vulgaris	Lf. & culm.	–	VI+[1]	
Avena sativa				
"Tresspass"	,, ,,	–	IV	
"Kohnoen"	,, ,,	–	IV	
"Race Horse"	,, ,,	–	IV	
"Hadaka"	,, ,,	–	IV	
Dactylis glomerata	,, ,,	+	IV	
Hordeum vulgare				
Spring barley A	,, ,,	–	IV	
,, ,, B	,, ,,	–	IV	
,, ,, C	,, ,,	–	IV	
Winter barley A	,, ,,	–	V	
,, ,, B	,, ,,	+	IV	
,, ,, C			IV	
"Golden Melon"	,, ,,	–	IV	
Triticum				
"Gypsy"	,, ,,	–	IV+	
"Red Wave"	,, ,,	–	IV+	
"Silver Sheef Longlessy"	,, ,,	–	IV+	

1. The reduction colour of the extract of most of the grasses examined is tinged with orange red hence the colour can be matched better with the scales which are made by the reduced solution of apigenin or luteolin.

Name of plant	Part examined	Chromogen P	Chromogen F	Remarkes
‘ St. Lous Grand Prize ”	Lf. & culm.	—	IV+	
“ Fulcaster ”	,, ,,	—	III	
“ Castle's Prolific ”	,, ,,	—	IV+	
“ Imperial Amber ”	,, ,,	—	IV+	
“ Eclips ”	,, ,,	—	IV+	
“ Penn. Blue Stem ”	,, ,,	—	IV+	
“ Valley ”	,, ,,	—	IV	
“ Harvest King ”	,, ,,	—	III	
“ Pool ”	,, ,,	—	III	
“ Ruports Grant ”	,, ,,	—	IV	
“ Mortgage Lifter ”	,, ,,	—	III	
“ Rural New Yorker ”	,, ,,	—	IV+	
“ Fultz ”	,, ,,	—	III	
“ Jones Mammoth Amber ”	,, ,,	—	IV+	
“ Giant Square Head ”	,, ,,	—	IV+	
“ Klendyke ”	,, ,,	—	IV+	
“ Dawson's Golden Chaff ”	,, ,,	—	IV	
“ Kanred ”	,, ,,	—	IV+	
“ Turkey ”	,, ,,	—	IV+	
“ Kahrkof ”	,, ,,	—	IV	
“ Rikuu No. 1 ”	,, ,,	—	IV—	
Triticum spertum	,, ,,	×(Br)	III	
Zea Mays				
“ Koshu ”	Seed	×(B)	—	Unripe seed.
Unnamed	,,		—	White dent.
Secale cereale	Lf. & culm.	(Br)	III	
Setaria italica				
“ Honaga ”	,, ,,	+	I	
“ Tsugaru-wase ”	,, ,,	+	I	
“ Akaho ”	,, ,,	+	I	
“ Karasuhashi ”	,, ,,	+	I	
,,	Panicle	×(BG)	IV	
“ Honaga-sasa-awa ”	Lf. & culm.	+	I	
“ Eda-awa ”	,, ,,	+	I	
,,	Panicle	×(B)	IV	Young head
“ Aka-gara ”	Lf. & culm.	+	I	
“ Bukkiri ”	Panicle	×(B)	IV	Young head
Panicum frumentaceum				

Name of plant	Part examined	Chromogen *P*	Chromogen *F*	Remarkes
"Chona"	Lf. & culm.	+	III	
"Nigiri"	,, ,,	+	III	
"Bangoro"	,, ,,	+	II	
"Shindai-naoshi"	,, ,,	+	III	
"Shiro-hie"	,, ,,	+	III	
"Senkoku"	,, ,,	+	II	
"Onaga-hie"	Head	–	IV	
"Shirobana"	,, ,,	–	+	
"Kisen"	,, ,,	–	+	
"Shiro-sangoku"	,, ,,	–	VI	
"Kebie"	,, ,,	–	–	Young head.
"Oso-hie"	,, ,,	III	III	Anthocyanin present.
"Chosen"	,, ,,	VI	VI	Anthocyanin present.
Oryza sativa	(See Table 14)			
"Aikoku"	Lf.	–	V[1]	
"Bungo"	,,	–	V	
"Oba"	,,	–	VI	
"Asaterashi"	,,	–	VI	The awn red.
"Kurafusagi"	,,	–	VI	The awn brown.
"Daikkoto"	,,	–	VI	,, ,, ,,
"Uhei"	,,	–	VI	,, ,, ,,
"Akage"	,,	–	V	,, ,, ,,
"Ono-wase"	,,	–	VI	
"Yamato-chikara"	,,	–	VI	The awn faint yellow.
"Sekiyama"	,,	–	VI	The awn brown.
"Kameno-o"	,,	–	V	Awnless
"Genroku-mochi"	,,	–	V	The awn purple.
"Kawabe-mochi"	,,	–	V	
"Daikoku"	,,	–	VI	Dwarf plant.
"Shiki-shima"	,,	–	IV	
Musaceæ				
Musa sp. (Banana)	Fruit Peri.	VI	VI	Skin, fully riped.
,,	,, Int.	IV	–	Fresh, yellowish

1. The colour scale was prepared by the flavone isolated from the leaf of *Oryza sativa* instead of quercetin.

Name of plant	Part examined	Chromogen *P*	Chromogen *F*	Remarks
Zingiberaceæ				
Zingiber officinalis	Rhizome Peri.	IV	VI	The skin brown.
„ „	„ Int.	V	VI	Yellow tissue.
Cupuliferæ				
Castanea sativa	Cotyledon	+	−	
„ „	Sees coat	IV	VI	Brown, astringent.
Juglandaceæ				
Juglans Sieboldiana	Endosperm	−	−	Oily white tissue.
„ „	Endocarp	VI	−	
Moraceæ				
Morus alba	(See Table II)			
Cannabinaceæ				
Cannabis sativa	Lf.	×(OR)	II	
Polygonaceæ				
Fagopyrum vulgare	Achene	I	IV	Unripe, colourless.
„ „	Lf.	I+	II	
Reynoutria japonica	„	III	I	
Rheum Rhaponticum	„	VI	IV	
Chenopodiaceæ				
Spinacia oleracea	Lf.	−	IV	
Nymphæaceæ				
Nelumbo nucifera	Rhizome. Peri.	III	V	
„ „	„ Int.	III	VI	
Cruciferæ				
Brassica campestris var. *rapifera*	Root. Int.	IV	−	The cortex purple.
B. oleracea var. *viridis*	Lf.	+	III	Yellow lf.
B. oleracea var. *capitata*	Head	IV	+	White lf.
„ „ „ „	Lf.	V	II	Green lf.
B. oleracea var. *botrytis*	Head	VI	+	White lf.
„ „ „ „	Lf.	VI	+	White stem.
Rhaphanus sativus				
"Horyo"	Root	×(Br)	IV	White tissue.
„	Lf.	×(BrY)	III	
Malvaceæ				
Hibiscus syriacus	Seed	I	V	Unripe seed, brown when ripe.
Balsaminaceæ				

Name of plant	Part examined	Chromogen *P*	Chromogen *F*	Remarkes
Impatiens Balsamina	Seed	I	IV	Unripe seed brown when ripe, flower magenta.
,, ,,	,,	I	+	Unripe seed flower white.
Rutaceæ				
Citrus Aurantium Junos	Rind. Peri.	×(O)	VI [1]	Ripe fruit.
,, ,, ,,	,, Int.	×(M)	V	White tissue.
,, ,, ,,	Fruit 'pulp'	×(O)	+	
,, ,, ,,	Seed	×(O)	+	
C. Aurantium amara	Rind. Peri.	×(Br)	III	Ripe fruit.
,, ,,	Fruit 'pulp'	×(O)	+	
,, ,,	Seed	×(O)	+	
C. Limonum	Rind Peri	×(Y)	III	
,,	,, Int.	×(O)	II	
,,	Fruit 'pulp'	×(O)	+	Ripe fruit.
C. Grandis	Rind. Peri.	×(OR)	IV	
,,	,, Int.	×(OR)	III	
,,	Fruit 'pulp'	×(OR)	+	Pink coloured.
,,	Section (carpel)	×	IV	
C. sinensis (Navel)	Rind Peri.	×	III	Ripe fruit.
,,	,, Int.	×	I	White tissue.
,,	Fruit 'pulp'	×	I	
C. nobilis var. *Unshiu*	Rind. Peri.	+	III	Ripe fruit.
,, ,, ,,	,, Int.	−	III	White tissue.
,, ,, ,,	Fruit, 'pulp'	+	VI	
Vitaceæ				
Vitis	(See Tables 10, 12)			
Rhamnaceæ				
Zizyphus vulgaris var. *inermis.*	Fruit	I	VI	Unripe, green fruit.
Saxifragaceæ				
Ribes grossularia	Fruit 'skin'	III	+	Unripe green fruit.
,, ,,	'fresh' & seed.	III−	+	
,, ,,	Lf.	I+	I+	
Ribes sp. (currant)	Fruit	III	+	Green fruit.

1. A beautiful reduction colour is partially due to hesperidin which is present in the rind of oranges and by reduction, yields a marked reduction-colour like flavones. This information, the writer owes to the kindness of Prof. K. Shibata.

Name of plant	Part examined	Chromogen *P*	Chromogen *F*	Remarkes
Ribes sp. (currant)	Lf.	I+	I+	
Oenotheraceæ				
Oe. Lamarckiana	Lf.	+	III	
Umbelliferæ				
Oenanthe stolonifera	Root. Peri.	VI	–	
„ „	Lf. & stem.	VI–	III	
Daucus Carota	Root. Peri.	+	–	
„ „	„ Int.	+	–	
Araliaceæ				
Aralia cordata	Lf.	–	I	
Rosaceæ				
Cydonia vulgaris	Fruit. Peri.	II+	VI	Papillæ removed which are rich in flavone.
„ „	„ Int.	I+	+	
Pseudo-cydonia sinensis	Fruit. Peri.	I	VI	
„ „	„ Int.	I+	+	
Eryobotyria japonica	Fruit. 'skin'	I	III	
„ „	„ 'fresh'	VI	–	
Fragaria chiloensis	Fruit (receptacle) & achenes	II	VI–	Unripe, colourless receptacles.
Malus sylvestris				
"Rawles Janet"	Fruit. Peri.	I++	VI	Unripe fruit.
„ „	„ Int. (cortex of receptacle)	I++	+	
"Jonathan"	Fruit Peri.	I++	VI	
„	„ Int.	I++	VI–	
"Ben Davis"	„ Peri.	I++	VI–	
„ „	„ Int.	I	+	
"Smith Cider"	„ Peri.	I++	VI–	
„ „	„ Int.	I	+	
"Twenty Ounce"	„ Peri.	I+++	VI	
„ „	„ Int.	I++	+	
"Iwai"	„ Peri.	I	+	
„	„ Int.	II	+	
Prunus communis	„ Peri.	III	V	
„ „	„ Int.	III	+	
Pirus serotina	„ Peri.	V	–	
Prunus Mume	„ „	II	+	Fully ripened fruit.
„ „	„ Int.	II	–	
P. triflora (Sumomo)	„ „	II	–	Epicarp deep red.

Name of plant	Part examined	Chromogen *P*	Chromogen *F*	Remarks
Prunus triflora (Hattankyo)	Fruit. Peri.	II+	VI−	
„ „ „	„ Int.	II	+	
Rosa rugosa	Root & rhizome	I	I	
R. multiflora	Lf.	+	I	
„ „	Petals	+	II	White petals.
Papilionaceæ				
Indigofera pseudotinctoria	Seed	I	−	Unripe green seed, dark brown when ripe.
„ „	Pod	I	VI	
Cytisus scoparius	Shoot	×(O)	+	
„ „	Bark	III	IV	
„ „	Wood	+	−	
Phaseolus vulgaris	(See Table 9)			
Ph. radiatus var. *aureus*	(See Table 8)			
Glycine soja	(See Table 20)			
Pisum sativum	Seed	I+	+	Unripe seed, brown when ripe.
Vicia Faba	Seed coat	II	×(O)	
„ „	Cotyledon	−	−	
„ „	Pod	−	V	
Vicia sativa	Lf.	−	I	
„ „	Seed	II+	×(O)	
Trifolium pratense	Lf.	−	II	
T. repens	„	−	V	
Ebenaceæ				
Diospyros Kaki				
"Tsuruno-tomo"	Fruit. Peri (epi.- & meso-carp)	II	VI	Sweet, with brown spots.
„	„ Int.	II	+	„
"Fuyu"	„ Peri.	III	+	„
„	„ Int.	III	−	
"Ama-hyakume"	„ Peri.	I	+	Sweet, no brown spots.
„	„ Int.	I	−	
"Hyakume"	„ Peri.	II	VI	Sweet, brown spotted.
„	„ Int.	III	−	
"Shogetsu"	„ Peri.	II	VI	„
„	„ Int.	III+	−	
"Jiro"	„ Peri.	II	VI	„

Name of plant	Part examined	Chromogen *P*	Chromogen *F*	Remarks
"Jiro"	Fruit. Int.	III	−	
"Hana-gosho"	„ Peri.	II	VI	Sweet, no brown spots.
„ „	„ Int.	III	−	
"Zenji-maru"	„ Peri.	II	V	Sweet, many spots.
„ „	„ Int.	II+	VI−	
"Haku-nyu"	„ Peri.	III	+	Astringent, no spots.
„ „	„ Int.	II+	−	„
"Wase-jisha"	„ Peri.	III	+	„
„ „	„ Int.	II+	−	„
Unnamed	„ Peri.	I+	II	
„	„ Int.	I+	III	„
"Yokono"	„ Peri.	III	VI	„
„	„ Int.	II	+	„
"Sane-nashi"	„ Peri.	II	VI−	„
„ „	„ Int.	II+	−	„
"Mishiradsu"	„ Peri.	II+	−	„
„	„ Int.	II+	+	„
"Fuji"	„ Peri.	II	VI	„
„	„ Int.	II+	VI−	„
"Dojo-hachiya"	„ Peri.	II	VI	„
„ „	„ Int.	II	+	„
"Yotsu-ya"	„ Peri.	I+	VI−	
„	„ Int.	I+	+	„
Inayama"	„ Peri.	II+	V	„
„	„ Int.	II+	VI	„
Convolvulaceæ				
Ipomœa Batatas	Root. Peri.	−	−	The cortex 'white.'
„ „	„ Int.	−	−	Yellow tissue.
Solanaceæ				
Solanum Melongena	Fruit	×(BG)	—	Skin deep purple.
S. tuberosum		+	−	
"Nemuro"	Tuber	+	−	Skin green
" „ Seedling"	Peri.	+	−	Stem green
"White Rose"	Lf.	−	IV+	„ „
"Hayes Kidney"	„	−	IV−	„ „
Nicotiana Tabacum	Lf.	−	III−	Green leaf.
Datura stramonium	„	−	−	
„ „	Flower	×(O)	×(O)	White petal.

Name of plant	Part examined	Chromogen *P*	Chromogen *F*	Remarks
Labiatæ				
Mentha arvensis var. *piperascens*	Lf.	–	IV	
Cucurbitaceæ				
Cucurbita moschata var. *Toonas*	Fruit. Peri.	+	+	Fully riped, yellow.
„ „	„ Int.	+	–	
Cucumis sativus	„ Peri.	–	VI	
„ „	„ Int.	–	–	
Compositeæ				
Arctium Lappa	Root. Peri.	–	–	
„ „	„ Int.	–	–	
Carthamus tinctoria	Lf.	×(BG)	×(OR)	
„ „	Flower bud	×(BG)	×(OR)	

II. Genetical Study.

1. The Mode of Inheritance of Anthocyanin and Brown Pigment in the Awn and other Parts in *Oryza sativa*.

In the preceding chapters, certain physiological relations in the formation of anthocyanin and the reddish brown pigments to the chromogenic substances are discussed. We shall now consider the genetical factors relating to the formation of these pigments in the awn, paleas, glumes and the grain of *Oryza sativa*.

(a) Colour Types of the Awn.

Among the number of cultivated varieties of *Oryza sativa* var. *utilissima* and *O. s.* var. *glutinosa*, many are awned. They may be grouped under the following types with respect to the colour of the awn.

1. Awn with anthocyanin.
   a. Purple.
   b. Red.
2. Awn without anthocyanin.

a. Brown when fully mature.

b. Faint yellow when fully mature.

The brown and faint yellow awns are green when young and the alcoholic extract of the green material of the brown awn yields a distinct red colour by reduction but that of the faint yellow, only a slightly red tinge. If oxidase is added to the extract, a marked brown colour is produced in the former, while in the latter, practically none. It proves that the colour of the brown awn is due to the pigment produced by the oxidation of the chromogenic substance at the end of the growing period of the plant. The extract of the matured brown awn gives also a distinct reduction colour showing that a part of the chromogenic substance remains without undergoing any serious change. In the matured awn, the brownish yellow substance, sometimes in aggregates, fills the cell, and when treated with ammonia, yields a deep yellow colour. The relative strength of the chromogen content of certain varieties are given below.

TABLE 14.

Chromogen content of the extract of immature green awn of *Oryza sativa*.

Name of variety	Colour when mature.	Chromogen *P*	Chromogen *F*	Oxidation colour [1]
"Sekiyama"	Brown	–	II	Brown
"Uhei"	„	–	II–	
"Kura-fusagi"	„	–	IV	„
"Meirinsen"	„	–	III	„
"Daijo-shiro"	Faint yellow	–	VI	
"Chujo"	„	–	V	
"Koshin-den"	„	–	V	Pale yellow
"Yamato-chikara"	„	–	VI+	
"Tanpo"	„	–	V+	
"Shirahige"	„	–	VI	„
"Nagoya-shiro"	„	–	VI	„

Unlike the awn, the leaf does not vary very widely in the chromogen content. The result of the tests made over 120 varieties at the middle of August, 1919, is given in Table 15.

1. Pressed sap of the radish root and hydrogen peroxide is added.

TABLE 15.

Chromogen content of the extract of leaf in *Oryza sativa*.
The colour scale was made by the flavone isolated from the leaf of *Oryza* instead of quercetin.

Colour scale	Frequency of varieties with respect to the colour of awn and apex of the paleas.				
	Faint yellow	Brown	Red	Purple	Total
III	1				1
IV	3	1		1	5
V	40	8	20	15	83
VI	18	12	3	1	34
Below VI	1				1
	63	21	23	17	124

According to the spectrographic investigation of Y. SHIBATA and KIMOTSUKI (1918)[1], the absorption spectra of the flavone isolated from the leaf of *Oryza sativa* conformed to these of pure luteolin.

(b) The Cross: Brown × Faint Yellow.

The breeding experiments made by the different investigators in Japan, have already shown that the hybrid between certain varieties having the brown awn and the faint yellow, gave in F_1, the red awned plant. The red colour is due to anthocyanin. The segregation in F_2 takes place by the ratio 9 reds, 3 browns and 4 faint yellows, otherwise the ratio is more complicated.[2] The phenomena are in accord with the well known cases of the flower in *Lathyrus* in which the colourless types produce the coloured one in F_1. The writer obtained the similar results by the following crosses.

"Daikkoto" × "Togo"

"Kura-fusagi" × "Nagoya-shiro"

The characters studied in the above crosses are the following:

1. SHIBATA, Y. and KIMOTSUKI, K., Spectro-analysis of the Plant Pigments of Flavone Group. I. Jour. Chem. Soc., Tokyo. 39: 771, 1918. (In Japanese)

2. Some of the results obtained by Dr. KATO are briefly summerized in IKENO, S., Zikken-Idengaku. p. 84, 1918. Also in Botanical Abst. 2: 114, Entry 679, 1919.

	Stigma	Awn	Chromogen in awn [1]	Paleas	Glume
♀ "Daikkoto"	Colourless	Brown	I	Buff	Buff
♂ "Togo"	„	Faint yel.	VI	„	„
F_1 "Dai." × "Togo"	„	Red	I+	Brown	Red
♀ "Kura-fusagi"	„	Brown	I	„	„
♂ "Nagoya-shiro"	„	Faint yel.	V	Buff	Buff
F_1 "Kura." × "Nago."	„	Red	I	„	Red

The red awn changes to brown when fully mature and becomes indistinguishable from the brown one at the time of harvest. The purple is much more stable than the red, hence the purple remains unchanged even at the time of harvest. The segregation observed in the F_2 generation was as follows :

TABLE 16.

Showing the result obtained in F_2.

Kind of eross	Awn red	Awn brown	Awn faint yel.	Totals
"Daik." × "Togo" Pt. I	77	14	24	115
„ II	113	27	42	182
	190	41	66	297
Expect. (9 : 3 : 4)	166.96	55.69	74.25	
Probable errors	± 5.765	± 4.537	±5.033	
Diff. (ob.−expect)	+23.04	−14.69	−8.25	
"Kura." × "Nago." Pt. I	85	25	32	142
„ II	125	43	68	236
	210	68	100	378
Expeet. (9 : 3 : 4)	212.64	70.85	94.50	
Probable errors	±6.506	±5.118	±5.679	
Diff. (ob.−expeet.)	−2.64	−2.88	+5.50	

1. One gram dried awn was extracted with 30 ee of a 45 per eent aleohol. Five ee of the extract were reduced by means of one ee of eoncentrated hydrochlorie aeid and the due amount of magnesium powder.

In the case of the cross "Daikkoto" × "Togo" reds and browns were often difficult to distinguish accurately owing to the faintness of the red pigment and the rapidity in change to the brownish colour. A considerable deviation from the expectation thus arose, though the observed numbers are fairly close to the approximation to a 9 : 3 : 4 ratio.

Thirty six browns and twelve faint yellows were raised in the next year. If a 9 : 3 : 4 ratio is accepted, we should expect among browns which include reds and browns, two groups of families in the F_3 generation. One of them should throw again red and brown, and the other should not throw red. The ratio of two such families should be 3 : 1. We obtained:

	No. of families which threw reds	No. of families which threw no reds	Total
Observed	32	4	36
Expected	27	9	36
Difference	$+5.0 \pm 1.751$	-5.0	

Among the red throwing families, the following families are expected:

Type of families	Segregation
1.	Red constant occurring once in every nine.
2.	Reds, browns and faint yellows in 9 : 3 : 4 ratio occurring four times in every nine.
3.	Reds and faint yellows in 3 : 1 ratio occurring two in every nine.
4.	Reds and browns in 3 : 1 ratio occurring twice in every nine.

Among the families which throw no reds, the following families are expected:

Type of families	Segregation
5.	Brown constant, occurring once in every three.
6.	Browns and faint yellowish 3 : 1 ratio, occurring twice in every three.

We found:

Type of families	No. of families observed	expected	Difference (ob.—exp.)
1.	3	3	0
2.	7	6	+1
3.	10	6	+4
4.	12	12	0
5.	2	3	−1
6.	2	6	−4
	36	36	

The faint yellows are expected to be constant. Twelve families raised were all constant. The actual numbers are given in Table 31.

In the case of the cross "Kurafusagi" × "Nagoya-shiro", the red colour of the awn was more distinct than the former case, hence the agreement of the expected to the observed numbers was close. In the F_3, was obtained:

Type of families	No. of families observed	expected	Difference (ob.—exp.)
1.	8	7.583	+0.417
2.	31	30.332	+0.668
3.	13	15.166	−2.166
4.	18	15.166	+2.834
5.	6	7.583	−1.583
6.	15	15.166	−0.166
	91	90.996	

Thirty two faint yellow gave all constant families. See Table 32.

(c) The Cross: Red × Purple.

The relation of the colour of the stigma, awn, palea, glumes and the leaf-sheath was studied by the cross "Hanbun-nento" × "Genrokumochi". The characters involved in this cross which were subjected to investigation were as follows:

	"Hanbun."	"Genroku."	F_1 "Hanbun." × "Genroku."
Awn	Red	Purple	Purple

	"Hanbun."	"Genroku."	F_1 "Hanbun." × "Genroku."
Stigma	Colourless	Red to purple	Red to purple
Palea	Brown	Purple localized in streaks from the tip to downward on yellow ground colour	Self purple on brown ground colour
Glume	Red	Purple	Purple
Leaf-sheath	Green	Green with purple stripes	Green with purple stripes

Thus we see that the dominant colours are; in the awn purple over red, in the stigma red over colourless, and in the leaf-sheath purple striped over non-striped. A new type of palea was found in the F_1 plant, namely the self purple on the brown ground colour (see Plate I). The brown ground-colour is dominant over yellow, and the self purple is dominant over the above two, being epistatic to brown. A complete linkage is found between the self purple and brown ground colour. The localized purple is always non brown with respect to the ground colour in the paleas. In the F_2 generation, we obtained the following results.

TABLE 17.

Showing the result obtained in F_2.

	Stigma coloured		Stigma colourless		Total
	Pt. I.	Pt. II.	Pt. I.	Pt. II.	
Awn purple	59	49	—	—	108
Awn red	—	—	12	22	34
Total	59	49	12	22	142
Paleas, self purple (deep)	4	6	—	—	10
„ „ (medium)	18	16	—	—	34
„ „ (pale)	14	16	—	—	30
„ purple localized (prominent)	4	3	—	—	7
„ purple (medium)	16	8	—	—	24
„ brown	—	—	8	15	23

	Stigma coloured		Stigma colourless		Total
Paleas, yellow	1 ?	—	4	7	12
Total	57 [1]	49	12	22	140
Glume, purple	59	49	—	—	108
„ red	—	—	8	22	30
„ undetermined	—	—	4	—	4
Total	59	49	12	22	142

By summing up the above figures, we obtain

	Stigma coloured & leaf-sheath striped	Stigma colourless & leaf-sheath non striped
Observed	108.	34
Expected (3 : 1)	106.5	35.5
Diff. (ob.—exp.)	+1.5	−1.5

With respect to the colour of the palea :

	Self purple on brown	Localized purple on yellow	Brown	Yellow
Observed	74	31	23	12
Expect. (9 : 3 : 3 : 1)	78.75	26.25	26.25	8.75
Diff. (ob.−exp.)	−4.75	+4.75	3.25	+3.25
Probable errors	±3.959	±3.115	±3.115	±1.932

The above ratios were confirmed by the result obtained in F_3. The actual numbers are given in Table 33.

The coloured stigma, purple awn and glume, self purple palea, and striped leaf-sheath are inherited together. According to HECTOR (1916),[2] the Indian varieties studied at Ducca, may be classified as follows :

(1) Leaf-sheaths, apiculus of glumes, and stigma coloured.

(2) Leaf-sheaths and apiculus of glumes coloured, but stigma colourless.

1. Two plants died.

2. HECTOR, G. P., Observations on the Inheritance of Anthocyan Pigment in Paddy Varieties. Memoirs Depart. Agri. India. Bot. Series. 8 : No. 2, 89, 1916.

(3) Apiculus of glumes coloured, but leaf-sheaths colourless.

(4) Apiculus of glumes only coloured. He doubts of classes 3 and 4 really exist. Class 1 is the commonest group. Contrary to Indian varieties, the colourless stigma with green leaf-sheath is the most common type found in Japanese varieties. He observed further, that the colour in the leaf-sheaths and apiculus is due to a colour factor *R* acting on a chromogen *C*, and that the purple colour of the stigma is due to a further factor *P* not present in the leaf-sheath and apiculus, and that the simultaneous presence of all three factors *RCP* is necessary for the production of any sort in the stigma. Parnell, Rangaswami, Agyangar, and Raniah (1917)[1] have shown that in certain Indian varieties, the purple lining of the internode is coupled with the purple glumes, the purple stigma with the purple axil (purple-colouring of the epidermis on the inside of the sheath) but the purple lining of the internode and the purple glume do not co-exist with the purple stigma and the purple axil.

Among the Japanese varieties studied by the writer, most of those which have a coloured stigma have a purple awn and leaf-sheaths striped, and only in certain varieties, they are separated. The distribution of anthocyanin in Japanese varieties is shown in the following table.

Table 18.

Distribution of anthocyanin in the varieties of Japanese rice.

Name of typical variety	Stigma	Awn	Palea (tip)	Palea	Glume	Lf. sheath	Lf. blade
"Murasaki"	Coloured	Purple	Purple	Purple	Purple	Purple	Purple
"Tokachi-wase"	„	„	„	Red	Red	Purple striped	Red striped
"Haguro"	„	„	„	Purple	Purple	Red strip.	—
"Edowase"	„	„	„	—	—	„	—
"Bozu-karasu"	„	Awnless	„	—	„	—	—
"Uyejini-shirazu"	„	Purple	Reddish brown	—	„	—	—
"Genroku-mochi"	„	„	—	Purple	Purple	Purple striped	—

1. Parnell, F. R., Rangaswami, Agyangar, G. N, and Raniah, K., The Inheritance of Characters in Rice I. Memoirs Depart. Agri. India. Bot. Series. 9 : 75, 1917.

Name of typical Variety	Stigma	Awn	Palea (tip)	Palea	Glume	Lf. sheath	Lf. blade
" Choja-bozu "	Coloured	Awnless	—	—	—	Purple striped	—
" Kanta-bozu "	,,	,,	—	—	—	—	—
" Isejiro "	,,	Purple	Purple	—	Purple	—	—
" Gorobei "	Colourless	Awnless	Red	—	Red*	—	—
" Hozoroi "	,,	,,	,,	—	,,	—	—
" Homura "	,,	Red	,,	—	,,	—	—
" Asaterashi "	,,	,,	,,	—	,,	—	—

The purple and red awn is green when the panicle is in the leaf-sheath. The red colour begins to develop at first at the tip and the base of the awn, a day or two after the panicle has appeared from the leaf-sheath (see Plate I). The colour gradually extends to the entire portion and at the same time increases in intensity. The purple awn is red in the beginning, but rapidly intensifies in colour and becomes deep purple. In the red awn, on the other hand, the red pigment remains unchanged and sooner or later, it is decomposed. A similar change is observed in the glume.

The development of the pigment in the awn is dependent on the illumination. When the panicle is enclosed in the paper bag to ensure self pollination, the pigment develops only slightly. In the stigma, purple anthocyanin is already present even when the panicle is still in the leaf sheath. Thus the development of anthocyanin is seen to have different physiological requirements even in the different parts of the same floral organs.

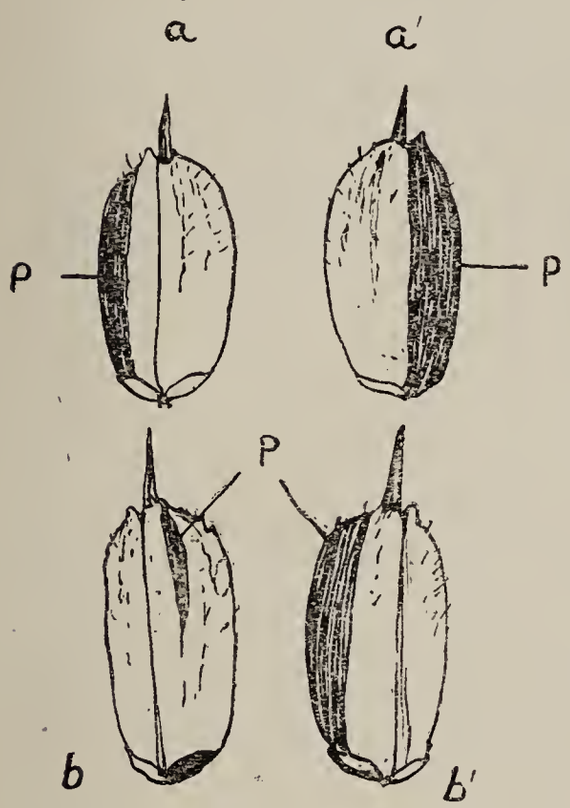
In the cells of the colourless stigma, flavone can be detected by treating them with ammonia which yield a deep yellow colour. It is quite probable, however, that there may be a colourless stigma having no flavone (chromogen). Hector (1916)[1] has shown that the colour of stigma in certain cases, is due to more than three factors.

The anthocyanin pigment in the palea is confined to the epidermis, and the brown pigment to the underlying tissue. The brown pigment is practically insoluble in strong alcohol, but slightly in a weak solution. Fully matured

* The colour indistinct, sometimes obscure.

1. Hector, G. P., Loc. cit.

brown paleas were extracted with alcohol for a long time, and the extract was tested for chromogen. Practically no red colour was found by reducing nor by heating with hydrochloric acid.



Text fig. 2. Showing the anomalous sectorially pigmented paleas. Born on the panicle of which spikelets were self purple. *a*, *a'*, same spikelet; *b*, *b'*, same spikelet seen from the different sides. *p*, purple area.

Among the F_3 plants certain anomalies which are worthy of mention, were found.

Two spikelets born on a panicle of a plant which bore the red awn and yellow paleas, possessed the brown inferior palea.

Two spikelets born on a panicle of a plant having the spikelets which bore a purple awn, paleas and glumes were sectorially pigmented with purple and yellow (see text fig. 2.)

Two grains were found in a single spikelet in one of the spikelets of a normal plant. The spikelet of *Oryza sativa* normally bears only one grain.

In one plant, both paleas, inferior and superior were found to bear awn. The anomalous awns were short and red coloured. Normally, the inferior palea only possess a long awn.

In one of the spikelets born on a panicle of a plant, the lodicules were found to be modified to small palea-like appendages which were narrow and sharply pointed.

(d) The Relation Between the Colour of the Grain and the Paleas.

It is of interest to observe the difference in the kind of chromogenic substance of the brown pigments in the awn and the grain. The colour of the grain of most of the Japanese varieties is pale buff but in few, reddish brown.

The chromogenic substance can be detected in the extract of the milk ripe, green grains of the reddish brown sort but not in that of the pale buff.

TABLE 19.

Chromogen content in the extract of the milk-ripe grain of *Oryza sativa* [1].

Name of variety	Colour of fully matured grain	Chromogen *P*	Chromogen *F*	Colour of extract with peroxidase	Colour of extract with KOH (boiled)
"Uhei"	Pale buff	—	—	—	Yellow
"Kinoshitamochi"	„	+	—	—	„
"Kameno-o"	„	—	—	—	„
"Togo"	„	—	—	—	„
"Seki-yama"	„	—	—	—	„
"Yamato-chikara"	„	—	—	—	„
"Akamoro"	Reddish brown	II	—	Orange brown	Deep blue to red[2]
"Haguro"	„	II	—	„	„

As we have already seen the chromogen in the awn belongs to the group of the chromogenic substance *F*, and in the grain, to the chromogenic substance *P*.

According to KONDO (1917),[3] the reddish brown pigment of the grain is confined to a single cell layer of the integument of the seed coat. In the dark coloured grain of the foreign sort, the writer observed that the pigment was not confined to the seed coat. An anthocyanin-like pigment was found in the pericarp, namely the layer above the tube cells and even in the grain of the brown sort of Japanese rice, a yellowish pigment was found in the cell of the pericarp.

PARNELL et Al[4] have shown that in Indian rice, the red, grey brown, and

1. Three grams of fresh material were extracted with 15 cc of a 40 per cent alcohol.

2. The extract was boiled with caustic potash. A deep blue colour like that produced by the solution of myricetin was found. On cooling, the blue colour changed to red. It may incidentally be mentioned that most of the flavones give a deep yellow colour when treated with caustic potash, but myricetin gives a deep blue instead of yellow.

3. KONDO, M., Untersuchung ueber die Dicke der Reiskleischicht. Bericht. d. Ohara Inst. f. Landwirt. Forsch. 1:219, 1917.

4. See also KIKKAWA, S., On the Classification of Cultivated Rice. Jour. Coll. Agri. Imper. Univ. Tokyo. 3:11, 1912, PARNELL et al. Loc. cit.

white grains segregated by the ratio 9 : 3 : 4 in the descendants of certain natural hybrids, and the red grain plants were purple-pigmented whereas the grey-brown plants were unpigmented. A similar case was found in the cross between two Japanese varieties, "Otsubu" × "Haguro". The grain of the former was pale buff ("white") and the latter was reddish brown. In the F_2 plants the following result was obtained.

TABLE 20.

Showing the result obtained in F_2,
"Otsubu" × "Haguro"

Colour of Awn	Colour of Paleas	Colour of grains: reddish brown	yellow brown	pale buff (white)	Total
Purple	Self purple	119	—	36	155
Purple	Loc. purple	33	—	12	45
Brown	Brown	—	33	11	44
„	Yellow	—	12	4	16
		152	45	63	260
Expect. (9 : 3 : 4)		146.25	48.75	65.00	
Diff. (ob.—exp.)		+5.75	−3.75	−2.00	
Probable errors		±5.375	±4.485	±4.007	

The yellowish brown grains were not found in the purple awn plants. The ratios of coloured to white grains in each awn type showed a normal 3 : 1 ratio (see Table 20), so it is certain that a linkage relation exists between the genes for the purple awn and for the reddish brown grain. With respect to the colour of the awn, purple and brown showed a 3 : 1 ratio, and in the palea, self purple, localized purple, brown and yellow segregated by the ratio 9 : 3 : 3 : 1 (155 : 45 : 44 : 16 observed against 146.25 : 48.75 : 48.75 : 16.25 expected) as in the case with the cross "Hanbunnento" × "Genroku-mochi", already reported in the section (c). These two crosses differ with respect to the colour of paleas, only from the gene groups which enter from the parental plants, but the manner of segregation in the F_2 generation is the same, thus:

Kind of hybrid	Self purple	Loc. purple	Brown	Yellow
"Hanbun." × "Genroku."	—	♂	♀	—
"Otsubu." × "Haguro."	♂	—	—	♀
F_2 "Hanbun." × "Genroku."	9	3	3	1
F_2 "Otsubu." × "Haguro."	9	3	3	1

The section of the reddish brown grain showed that the pigment was chiefly confined to the single cell layer in the testa as KONDO observed, though in the pericarp, a yellowish pigment also occurred. In the yellowish brown grain, the pigment occurred chiefly in the pericarp and the testa was slightly pigmented. We are dealing, therefore, with the pericarp and the seed-coat colour in two types of the grains, but in the fully ripe grain, the two parts are hardly distinguishable unlike the bean in which they are differentiated into the pod and the seed coat.

2. The Mode of Inheritance of Anthocyanin and Brown Pigment in the Seed Coat of *Glycine soja*

Nothing perhaps excels the seed coat of the legumes in diversity of the colour characters exhibited by the plant except the flowers of some ornamental plants. As we have already seen, the seed coat of the coloured bean of the legumes is rich in the chromogenic substance previous to pigmentation. Some data on the genetical behavior of the colour characters in the seed coat of soy bean (*Glycine Soja*) are discussed in this chapter.

(a) Colour Types of the Seed Coat.

The different varieties of Japanese soy beans may be classified under the following types with respect to the colour of the seed coat.

I. Self-colour type.
   1. Black (deep purple).
   2. Reddish brown.
   3. Brown with or without a green tinge including different shades of brown.
   4. Buff.

5. Green.
6. Yellow.

II. Parti-colour type.

1. Black mottled, the ground colour brown with or without a green tinge.
2. Black patch around the hilum. The ground colour is either yellow or green.
3. Dark brown patch around the hilum, the ground colour is either green or yellow.
4. Blue tinged around the hilum. The ground colour either green or yellow. The margin of the blue tinge is not so distinct as in 2 and 3.

The chromogen can be detected in the immature green seed of all the coloured types, except the green and yellow. The chromogenic substance P is abundant but the chromogenic substance *F* is very scarce or absent. In the green and yellow, both are nearly absent. Hence the different types can be distinguished into two groups with respect to the chromogen. One includes those which give a marked chromogen reaction when the seed is still green and the other includes those which give only a slight or no reaction. In this regard, the green and yellow correspond with the white type of the common garden bean and Adzuki-bean (see Tables 8, 9).

Since the reaction of the chromogenic substance *F* is feeble in the seed just before the formation of the pigment, the latter must be formed from the chromogenic substance *P* which is present. In the leaf, however, the chromogenic substance *F* occurs in a considerable amount and can readily be isolated as yellow crystals. The following table will give a general idea of the distribution of the chromogens in the seed coat and the leaf of different varieties of soy-beans.

Table 21.

Chromogen content in the extract of unripe, green seed, and leaf of *Glycine soja*.[1]

Name of variety.	Colour of seed when mature.	Chromogen in seed		oxidation colour[2]	Chromogen in lf.	
		P	*F*		*P*	*F*
"Kurodaidzu-ko"	Solid black	III	—	RBr.	—	III
"Goishi"	,,	III	—	RBr	—	III+
"Goishi" (flower purple)	,,	III	—	,,	—	III+
"Nedzumi-meta"	Brown	IV	—	OBr	—	III—
"Akakzuka"	,,	II	+	,,	—	IV
"Cha"	,,	II	—	,,	—	III+
"Haiiro"	,,	III	+	,,	—	III
"Beni-iro-daidzu"	Red brown	III	—	,,	—	III+
"Akanedzumime"	,,	III	+	,,	—	III
"Madara"	Black mottled brown	III	—	,,	—	III+
"Juseita"	Black patched	IV	—	,,	—	III
"Kura-kake"	,,	III	—	,,	—	III
"Achumuri"	Solid black	III	VI	,,	—	III
"Tanishi"	Blue tinged yellow	V	—	YBr	—	III+
"Tora-mame"	Solid black	IV	—	OBr	—	III+
"Goku-ao"	Green (green)[3]	—	—	—	—	III
"Aobishi"	,, ,,	VI	—	—	—	IV
"Uma-daidzu"	,, ,,	—	—	—	—	III
Goyo"	,, ,,	—	—	—	—	III
"Ao"	,, ,,	—	—	—	—	III
"Toyo-naga"	Green (yellow)	—	—	—	—	III—
"Dateao"	,, ,,	—	—	—	—	III
"Yoshioka"	,, ,,	—	—	—	—	IV
"Shakujo"	Yellow (yellow)	—	—	—	—	III+
"Shiro-sota"	,, ,,	—	—	—	—	III
"Omejiro"	,, ,,	—	—	—	—	III
"Yuki-no-shita"	,, ,,	—	—	—	—	III
"Chogetsu"	,, ,,	—	—	—	—	III
"Kimusume"	,, ,,	—	—	—	—	IV

1. The extraction was made with 5 cc of a 40 per cent. alcohol to each gram of fresh beans and with 10 cc for leaf.

2. Pressed juice of potato tuber and hydrogen peroxide added. The designations of colours are the same as those used in Table 13.

3. The colour of cotyledon.

Name of variety	Colour of seed when mature		Chromogen in seed		oxidation colour	Chromogen in lf.	
			P	*F*		*P*	*F*
"Sennari"	,,	,,	—	—	—	—	III
"Kariha-takiya"	,,	,,	VI	—	—	—	III—
"Shiro-nedzumi"	,,	,,	—	—	—	—	III
"Fukui-shiro"	,,	,,	—	—	—	—	IV
"Shonai-wase"	,,	,,	—	—	—	—	III
"Shiro-hachikoku"	,,	,,	—	—	—	—	IV
"Abura-mame"	,,	,,	—	—	—	—	III

The purple and blue pigments are anthocyanins. Both anthocyanin and brown pigments are confined to the epidermis existing in the same cell. When a common black bean is boiled with an alkaline solution, the colour of the solution becomes blue to green at first, but soon changes to deep wine red owing to the extraction of the brown pigment in alkali.

The peroxidase was tested in a number of varieties at the stages previous to pigmentation of the seed coat. The reaction was very distinct in the seed coat of all the coloured types as well as in the yellow and green ones, but the direct oxidase reaction failed by alpha naphtol, benzidine, myricetin and guaiacum. It was often noted when alpha naphtol was applied, the reaction was very faint in the epidermis even in the presence of hydrogen peroxide. There could be found no definite difference with respect to the peroxidase reaction exhibited by the seed coats of beans which contain the chromogenic substance and those lacking it;

In the black patched bean, however, the part where the anthocyanin colour will appear in a patch, yielded a deep purple or brown colour by immersing the whole bean which was previously treated with alcoholic solution of benzidine or myricetin, in a dilute solution of hydrogen peroxide. The remaining part where anthocyanin will not be formed at all, yielded less distinct colour in the case of benzidine and nearly failed in the case of myricetin. The chromogenic substance was detected in the tissue of the non-black part. The alcoholic extract of the portion gave a slight red colour by heating with hydrochloric acid.

The above observation seems to point out the fact that at the time of pigment formation, the black patched portion of the seed coat contains more

active peroxidase system than the rest of the portion where the development of the anthocyanin pigment is inhibited, in spite that the chromogenic substance can be detected in a slight extent.

The above observation agrees with the findings of KEEBLE and ARMSTRONG (1912)[1] who have shown the parallelism extisting in the distribution of anthocyanin and peroxidase in the corolla of *Primula sinensis* and in others.

When the seed coat is still green just previous to the formation of anthocyanin, a distinct red colour develops at the portion of black patch by treating the bean with hydrochloric acid in cold. It shows that the anthocyanin pigment is already present in a colourless state.

Oxidation seemed to accelerate the development of anthocyanin in the seed coat of the soy bean. Slightly coloured beans rapidly deepened in colour if the pod was opened and exposed to air. Injury also accelerated the development of the pigment. The portion near the injury became purple at first on exposure to air[2]. Conversely the exclusion of air retarded the development of the pigment. The slightly red-coloured beans ceased to develop the purple pigment when kept in a closed chamber in which the air was replaced by hydrogen gas. Similar phenomena were observed in ripening grapes. In both cases, light had no influence.

(b) The Cross : Blue Tinged Yellow × Brown and the Reciprocal.

The colour of the seed coat of the parental plants are the following :

"Tanishi." Yellow with a tinge of blue colour which is most prominent around the hilum, fading gradually further beyond.

"Haiiro." Brown with a tinge of greyish green colour.

The F_2 seeds produced on the F_1 plant were green with a pale blue tinge as in "Tanishi" (see Plate I). In F_2 solid blacks, and non-tinged greens and yellows were found, together with the parental type, by the following numbers.

1. KEEBLE, F. and ARMSTRONG, E. F., The Role of Oxydases in the Formation of the Anthocyan Pigment of Plants. Jour. Gent. 2 : 277, 1912.

2. KEEBLE, F. and ARMSTRONG, E. F., Loc. cit.

Table 22.

Showing the results obtained in F_2.

"Tanishi" × "Haiiro."

	Blue-tinged		Non-tinged		Black	Brown	Total
	Green	Yelllow	Green	Yellow			
Ob.	19	6	6	0	7	3	41
Exp.	17.30	5.77	5.77	1.92	7.69	2.56	41
Diff.	+1.70	+0.23	+0.23	−1.92	+0.69	+0.44	

"Haiiro" × "Tanishi."

	Blue-tinged		Non-tinged		Black	Brown	Total
	Green	Yellow	Green	Yellow			
Ob.	23	13	4	2	3	3	48
Exp.	20.25	6.75	6.75	2.25	9.00	3.00	48
Diff.	+2.75	+6.25	−2.25	−0.25	−6.00	0	

The calculated ratio is 27 : 9 : 9 : 3 : 12 : 4. A relative small number in the F_2 plants made the determination of the exact ratio somewhat uncertain but by the F_3 plants, the above ratio was confirmed.

Table 23.

Showing the segregation of the offspring of the heterozygous blue-tinged green F_2 plants of the cross.

"Tanishi" × "Haiiro."

F_2 family no.	Blue-tinged		Non-tinged		Black	Brown	Total
	Green	Yellow	Green	Yellow			
4	18	5	4	3	5	3	38
15	16	6	2	1	9	3	37
30	32	8	3	2	11	2	58
31	30	6	19	2	20	7	84
Total	96	25	28	8	45	15	217
Expect.	91.55	30.52	30.52	10.17	40.69	13.56	
Probable errors	±4.906	±3.454	±3.454	±2.111	±3.816	±2.405	
Diff. (ob.-exp.)	+4.45	−5.52	−2.52	−2.17	+4.31	+1.44	

The rest of the F_3 families agreed with the expectation except three which were apparently contaminated by accidental crossing; hence they were discarded. The actual numbers are given in Table 34. See also Table 29.

(c). The Cross : Buff × Black.

The colour of the seed coat of a variety "Ware-mame" is buff, and that of a variety "Achumuri" is solid black with peculiar mesh-like, white markings which are due to the small breaks of the epidermal tissue exposing the colourless, underlying tissue of the seed coat (see Plate I). The F_1 seeds born on the F_1 plant of the hybrid between them were self black. In F_2 self blacks, imperfect blacks, and browns were found with the following numbers.

TABLE 24.

Showing the result obtained in the F_2 generation of the cross "Waremame" × "Achumuri."

	Self black	Imperfect black	Brown	Bluff	Total
Ob.	33	9	6	0	48
Exp.	27	9	9	3	48
Diff.	+6.0	0	−3.0	−3.0	

The expected ratio is 9 : 3 : 3 : 1. The buff was not found in the F_2 plants owing to the small number of individuals. In F_3, families derived from the heterozygous self blacks, buffs were found in the expected ratio.

TABLE 25.

Showing the result obtained in F_3 families derived from the heterozygous self black F_2 plants.

F_3 family no.	Self black	Imperfect black	Brown	Buff	Total
3	21	9	2	1	33
4	31	4	10	4	49
6	46	18	19	4	87

F_3 family no.	Self black	Imperfect black	Brown	Buff	Total
	10	4	1	1	16
12	12	4	1	5	22
18	26	8	10	2	46
19	35	13	13	6	67
23	21	7	9	4	41
25	23	9	7	3	42
26	24	15	14	3	56
27	26	10	3	3	42
33	40	14	9	7	70
Total	315	115	98	43	571
Expected	322.18	107.06	107.06	35.69	
Probable errors	±7.481	±6.291	±6.291	±3.962	
Diff. (ob.-exp.)	−6.18	+7.94	−9.06	+7.31	

What is designated as imperfect black is a new type. It differs from the self black by the incomplete development of the anthocyanin pigment, resulting in the brown ground-colour being made visible (see Plate I). The ground colour of the imperfect black appeared to be brown instead of buff, but in the F_3 generation, they threw only imperfect black and buff but no brown. It seems therefore that the buff colour was deepened by the action of the gene which develops the anthocyanin in the same epidermal cell. The present instance resembles that of the palea colour observed in the cross "Hanbun-nento" × "Genroku-mochi" in which the self purple was completely linked with the brown ground-colour and the localized purple excluded the latter. In both cases, the full development of anthocyanin is in some way associated with the brown pigment.

Table 26.

Showing the segregation of the offsprings of imperfect black F_2 plants.

F_3 family no.	Black imperfect	Brown	Buff	Totals
17	26	—	—	26
35	27	—	—	27
34	14	—	3	17
36	35	—	9	44
38	26	—	5	31
39	39	—	15	54
40	4	—	2	6
41	5	—	1	6
	176		35	211

An approximation to a dihybrid ratio was confirmed by forty six families raised in the F_3 generation. The details are given in Tables 30 and 35.

The buff is found to be the most recessive character to any other one in the colour characters so far studied. Since the chromogenic substance can be detected in the unripe, green seed of buffs, browns, and blacks, but is nearly absent in yellows and greens, and further that yellows and greens are dominant over the former colours, it must be concluded that an inhibitor for the development of the pigment is present in the green and yellow.

A mention may be made regarding the brown character. We can distinguish several browns which differ more or less in hue and shade but exact discrimination is very difficult. If it is made among the segregates, the lighter shade appears to be domniant over the deeper one. When the distinctly reddish brown such as we see in the seed coat of a variety "Aka-nedzumime" is crossed with the brown like that we have already dealt with ("Haiiro"), the reddish brown behaves as a single recessive to brown. The F_2 seed of the cross "Aka-nedzumime" × "Haiiro" was brown like that of the father which is brown with a greyish green tinge. In F_2, reddish brown and brown, regardless of the green tinge, segregated in the following number:

	Reddish brown	Brown	Total
Observed	69	19	88
Expected	66	22	88
Diff. (ob.-exp.)	+3.0	−3.0	

The hybrid between "Kari-mame" and "Akadzuka" in which the yellow and reddish brown were crossed, produced the yellow in the F_1 plant, and in F_2, the following segregation was observed.

TABLE 27.

Showing the result obtained in the F_2 of the cross "Kari-mame" × "Akadzuka."

Plant No.	Green	Yellow	Black	Brown	Reddish brown	Total
I	57	29	14	6	4	110
II	21	11	7	—	4	43
	78	40	21	6	8	153
Expect.	86.062	28.687	28.687	7.172	2.391	
Probable error	±4.139	±3 256	±3.256	±1.763	±1.014	
Diff. (ob.-exp.)	−8.062	+11.313	−7.687	−1.172	+5.609	

The expected ratio is 36 : 12 : 12 : 3 : 1. Here again the deeper brown is shown to be recessive to lighter brown. A more detailed analysis of the brown characters is under way, and the result will be reported as data become available.

Speaking in general, the inhibited colour of the seed coat can be guessed by the colour of the hilum and that of the narrow ring around it. In the present communication, the colour of the hilum and the ring is not considered.

3. AN INTERPRETATION OF THE RESULTS.

It is a well known fact that there are at least two groups of genes

present in relation to the formation of anthocyanin pigments in plants. One of them includes those which are known as the chromogen factors, and the other includes those which are complementary to the former. A complete system provided by the union of these genes produces the plant in which the formation of anthocyanin pigment is realized. To designate those genes *C* is often used for the chromogen, and *R* for the complementary one. The most simple case is the counterpart of two genes *C* and *R*. We may denote those genes which are related to the formation of the chromogenic substance in plants by 'chromogens' (*C*) and those which are related to the formation of any biochemical agency, by means of which the chromogenic substance is converted to a coloured anthocyanin or brown pigment, by 'chromopheleins' (*R*, *O*, etc.). According to the view put forward by Miss WHELDALE, the chromogen factors in the flower of *Antirrhinum* are related to the formation of certain flavone glucosides (glucoside of apigenin and luteolin) and the chromopheleins are related to certain oxidizing agencies probably the peroxidases.

It must clearly be understood that the phenomena of inheritance and development are of different kinds, and the data of the latter should not be confused in interpreting the genetical data. The factor is such an entity of the organism that by its means certain groups of biochemical reactions are set free to build up the character which is the phenotypic expression of the gene. The biochemical reactions and their products alone are dealt with as physiological and developmental data. Some of them can well be regarded as the clue to the difference in the genetical units, but these phenomena themselves are nothing to do with those of inheritance. A well marked, different biochemical phenomena may not necessarily correspond with the difference in their genetical potency.

It is an impossible task to know all the biochemical changes which are governed by a given gene; all we can attempt, if at all, is to find certain correlations between the known biochemical facts and the genetical data, by which the chief function of the gene may be inferred. Such an attempt may be useless or may fall short of the aim. But, as the writer believes, genetics aims to discover not only the laws of the mechanism of distribution of hereditary units, but also the links between the gene and the actual

biochemical or physiological processes in the somatic cells that are set free by the corresponding genetical make-up.

In the case of the colour characters in the awn of *Oryza sativa*, we are apparently dealing with instances similar to those that were observed by Miss WHELDALE in *Antirrhinum* and BATESON in *Lathyrus*. Suppose a pair of genes *C* and *c* are concerned. The gene *C* produces the chromogenic substance in the brown awn to such an amount that it can readly be detected in the extract and by *c*, the production of the same substance is as much as ten to twenty times less than that produced by *C*. Consequently the faint yellow awn appears to be devoid of chromogen.

The oxidation and subsequent changes of the chromogenic substance leading to the formation of brown pigment may be due entirely to post mortem changes and may have no relation to the action of a gene whatsoever. If any gene is concerned, we may suppose the following possibilities.

The gene *C* has the simultaneous action of converting the chromogenic substance to the brown pigment in which the oxidation plays an important role.

Or we may suppose that another gene *O*, a chromophelein, which converts the chromogenic substance to the brown pigment, and the genes *C* and *O* are so linked each other that they may be considered as a single gene complex. In the awn of *Oryza*, and in the seed coat of *Glycine* as we shall see later, the chromogenic substance and certain brown pigments which are the oxidation product of the former, appear to be due to the action of a single gene. Wherever the chromogenic substance is produced, it is invariably converted to the pigment of phlobaphene nature, unless the inhibitory gene enters into the system. In this connection, it is of interest to refer the findings of WOLFF, WOLFF and ROUCHERMANN[1] and Mrs. WHELDALE ONSLOW (1919),[2] who have shown in a number of cases that the reaction of direct oxidase is invariably associated with the presence of the chromogenic substance.

If the latter assumption is adopted, the genes concerning the formation of brown awn may be designated by $\widehat{CO}$ and the faint yellow awn by $\widehat{co}$.

1. WOLLF, J., Loc. cit.
   WOLFF, J., and ROUCHERMANN, N. Loc. cit.
2. WHELDALE ONSLOW, M., Oxidizing Enzymes. I. The nature of the "peroxidase" naturally associated with certain direct oxidizing systems in plants. Bioch. Jour. 13 : 1, 1919.

Further we admit that the gene R is present in the faint yellow awn plant, and by the completion of the system provided by the genes CR ($\widehat{CO}R$), the chromogenic substance is converted to red anthocyanin, but by cR ($\widehat{co}R$) and cor ($\widehat{co}r$) the system is incomplete. The parental faint yellow plant may therefore be designated by cR ($\widehat{co}R$) and the brown plant by Cr ($\widehat{CO}r$). The red awn F_1 plant is $Cc\ Rr$ ($\widehat{CO}\widehat{co}Rr$) and by selfing, the following zygotic series would arise in F_2 by the ratio 9 : 3 : 3 : 1, viz., CR ($\widehat{CO}R$), Cr ($\widehat{CO}r$), cR ($\widehat{co}R$), and cor ($\widehat{co}r$), and in which the last four would be faint yellows giving rise io 9 reds, 3 browns and 4 faint yellows. The assumption covers the numerical ratio observed in the F_2 and F_3 generations.

Let us suppose in another way that the brown awn may have the genetic composition COr and the faint yellow cOR, in which O is the gene common to both of the parental plants. It is necessary to suppose that no red anthocyanin should be formed by cOR in which the chromogenic substance produced is only in such a small amount that no anthocyanin is formed from it even in the presence of OR. If however, the reduction processes set working by the gene complex here concerned are just as powerful as we provide in vitro by means of hydrochloric acid and magnesium powder, even a trace of the chromogenic substance should be converted to the coloured anthocyanin, for, we can readily detect even a trace of the chromogenic substance (1 : 20,000) by a distinct pink colour by reduction.

In the case of the cross "Hanbun-nento" × "Genroku-mochi", in which the purple and red awn are concerned, the basal system of anthocyanin formation CR is complete in both of the parental plants. If we let the gene R' convert the red anthocyanin to purple, the purple awn may be designated by CRR' and the red by CRr'. The designation CRR' may be substituted by a single letter, say P, and CRr' by p. Since we observe the stages of the change, chromogen → red pigment → purple pigment in the plant, CRR' seems to represent the actual phenomena occurring in the sporophyte.

In a number of cases reported, the purple colour is dominant over the red.[1] In *Antirrhinum* Miss. Wheldale (1914)[2] found that orange anthocyanin

1. See summary in Bateson, W., Mendel's Principles of Heredity. Third edition. 1913. Wheldale, M., Anthocyan Pigments of Plants. 1916.

2. Wheldale, M., Our Present Knowledge of the Chemistry of the Mendelian Factors for Flower Colour. Jour. Genet. 4 : 8, 1914.

was the derivative of apigenin and rose doré and magenta from luteolin. Two factors are necessary to convert the chromogen (luteolin) to magenta but only one is essential to rose doré. Thus

yyiiRRbb	*yyiiRRBB*	White
YYIIrrbb		Ivory (apigenin)
YYiirrbb		Yellow (luteolin)
YyiiRrbb		Orange
YyiiRrBb		Crimson
YyIiRrbb		Rose doré
YyIiRrBB		Magenta

in which *I* is a dominant ivory factor, inhibiting the formation of luteolin. *Y* is the factor for yellow which is due to apigenin, *R* and *B* are the factors which convert the chromogen to anthocyanin.

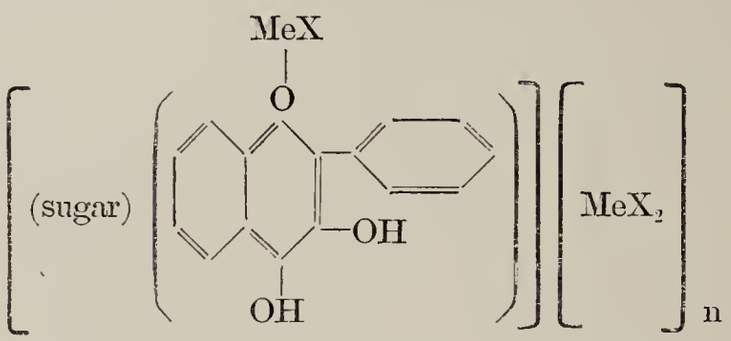
The case of *Linaria maroccana*, studied by CORRENS (1912)[1] was as follows: Red is dominant over white, and purple over red. At present his interpretation of the above case is hardly to be maintained. He dwelt upon the fact that anthocyanin is red in acid and blue in alkali and tried to interpret the factor difference of purple and red by that of acidity in the cell sap. But such seems to be unlike the usual case. HAAS (1916)[2] found that the cell sap containing blue anthocyanin is acid or neutral but rarely alkaline if acidity is determined by the hydrogen gas chain.

WILLSTÄTTER attempted to explain the variation in the colour of cyanidin from red to blue by the quinonoid structure of the molecule. The phelolic character of the benzene ring allows the formation of salt with alkali, the red is the acid salt (oxonium salt) the blue is the potassium or metallic salt (alkali phenolate) and the violet is the anhydride of the pigment. But his hypothesis hardly explanis how the deep blue anthocyanin can exist in the cell sap of the plant which is acid in reaction and further that an addition of alkali destroys the blue anthocyanin rather than deepening the colour.

A new explanation of the causes of variation in the colour of flowers has been put forward by K. SHIBATA, Y. SHIBATA, and I. KASHIWAGI (1919)[3] based

1. CORRENS, C., Die Neue Vererbungsgesetze. 1912.
2. HAAS, A. R., The Acidity of Plant Cells as shown by Natural Indicators. Jour. Biol. Chem. 27 : 233, 1916.
3. SHIBATA, K., SHIBATA, Y., and KASHIWAGI, I., Loc. cit.

upon experimental evidence, that the metal organic or complex compounds of reduced flavonol glucoside



are the most important factor in the production of flower colours. The blue anthocyanins are complex compounds of reduced flavonol glucosides, which possess several hydroxyl groups belonging to the flavonol nucleus besides those of sugar molecules, and the metal with which they are co-ordinated is probably calcium or magnesium, for salts of these metals are always present in the plant cells. The violet, violescent red or red pigments are either the analogous metallic complex compounds of flavonol glucosides, which contain fewer of the auxochrome hydroxyl groups or are a mixture of the blue pigments and their decomposition products by excess of acids, i.e., the red oxonium salts of R. WILLSTÄTTER.

It is likely to be inferred then that the purple and red anthocyanins formed in the awn and in other parts of *Oryza* are the derivatives of the same chromogenic flavonol glucoside and the purple is the complex salt of the red anthocyanin which is formed at first in the cell by the reduction of the chromogenic substance. The latter part of the changes may be due to the action of gene *R*. Indeed, it can be observed that the extract of the slightly red coloured awn yielded a more intense red colour by reduction than that produced by a simple addition of hydrochloric acid, and the faintly coloured extract of red and purple anthocyanins that is due to isomerization, attained a bluish hue by the addition of $ZnCl_2$ and a reddish hue by $CaCl_2$. The gene *R'* may therefore be referred to the agencies which set free the reactions leading to the formation of a purple complex salt from red anthocyanin with the existing metallic salts in the cell.

With respect to the colour of the paleas, the following hypothesis may be provided. Let *B* be the gene for the brown ground-colour, *b* for the non

brown. The chromogenic substance of the brown pigment in the paleas was tested at the time previous to pigmentation but failed. Therefore it is evident that the chromogenic substance of the brown pigment in the palea is different from that of the awn which gives rise to the red anthocyanin by the action of the *R* gene. But *B* has a specific relation to the formation of purple pigment in the epidermis, inasmuch as the full colour in the purple palea is always associated with the brown ground-colour, and the localized purple excludes the latter. It appears therefore that either *B* has a simultaneous action on extending the purple pigment to the entire surface of the palea or that the phenomenon is due to another gene which is completely linked with *B*.

The formation of any purple pigment in the palea appears to be due to another group of genes which are similar in kind to that which affected the awn, inasmuch as the purple awn is completely linked with any purple present in the palea but no red ever occurs in the latter. Let *P'* be the gene for the presence of purple pigment in the palea, and *p'* for the absence of the same. Further admit that *P'* and *P* are linked. Thus

"Hanbun-nento" *ppp'p'BB* Awn red, paleas brown.
"Genroku-mochi" *PPP'P'bb* Awn purple, paleas purple localized.
F_1 "Hanb." × "Genrok." *PPP'p'Bb* Awn purple, paleas purple.

The F_1 plant is heterozygous to *P'* and *B*, since *P'* and *P* are linked. In F_2 we expect the following genotypes to occur;

Genotype	Phenotype		Designation
	Awn	Paleas	
PP'B	Purple	Self purple with brown	*PP*
PP'b	„	Localized purple without brown.	*P*
pp'B	Red	Brown	*B*
pp'b	„	Yellow	*Y*

We should expect therefore the following segregation in F_3 with respect to the colour of paleas.

Genotype of F_2 plant		Segregation in F_3	Ratio.
P'P'BB	1	*PP*	Const.

Genotype of F_2 plant		Segregation in F_3	Ratio.
$P'P'Bb$	2	$PP : P$	3 : 1
$P'p'BB$	2	$PP : B$	3 : 1
$P'p'Bb$	4	$PP : P : B : Y$	9 : 3 : 3 : 1
$P'P'bb$	1	P	Const.
$P'p'bb$	2	$P : Y$	3 : 1
$p'p'BB$	1	B	Const.
$p'p'Bb$	2	$B : Y$	3 : 1
$p'p'bb$	1	Y	Const.

The actual result obtained is given in the following table.

TABLE 28.

Showing the result obtained in F_3 of the cross
"Hanbun-nento" × "Genroku-mochi".
See also Table 33.

Segregation	Ratio	No. of families observed	Expected	Diff. (ob.—ex.)
PP	Const.	1	4.1875	−3.1875
$PP : P : B : Y$	9 : 3 : 3 : 1	15	16.7500	−1.7500
$PP : B$	3 : 1	9	8.3750	+0.6250
$PP : P$	3 : 1	10	8.3750	+1.6250
P	Const.	7	4.1875	+2.8125
$P : Y$	3 : 1	13	8.3750	+4.6250
B	Const.	3	4.1875	−1.1875
$B : Y$	3 : 1	5	8.3750	−3.3750
Y	Const.	4	4.1875	−0.1875

If we regard the gene $P = CRR'$, $p = CRr'$ as already discussed, P' may also be regarded as a complex of genes CRR' but p' must differ from p, inasmuch as in the paleas, purple and non purple are allelomorphic, but no red ever occurs, whereas in the awn, purple and red are allelomorphic to each other. It can be imagined that in p', crr' behave as a single unit, allelomorphic to the complex CRR'. Else P' and p' may differ only in C but contain R and R' providing that cRR' forms no anthocyanin.

With respect to the colour of the grain, let two pairs of genes O' o' and C' c' be responsible. The chromogen C' produces the chromogenic substance P in the testa and in pericarp, and c' produces practically none. The gene O' converts the chromogenic substance to a reddish brown pigment especially in the testa and o' to a less extent. Thus by $C'O'$ the reddish brown grain is formed and by $C'o'$ the yellowish brown grain. The parental reddish brown-grain plant may be assumed to be $C'O'$ and the "white"-grain plant $c'o'$. The 9 : 3 : 4 ratio would arise in F_2 by selfing the F_1 plant $C'c'O'o'$. Thus

$C'O'$	9	Reddish brown
$C'o'$	3	Yellowish brown
$c'O'$	3	"White"
$c'o'$	1	,,

With respect to the colour of the paleas, the segregation in F_2 was similar to that of the cross "Hanbun-nento" × "Genroku-mochi", so we may assume that the analogous genes are concerned in the cross "Otsubu" × "Haguro" in which the grain colours are studied. But one of the genes for the grain colours probably O' is completely linked with the gene for the purple colour of the palea. Suppose O' is linked with P' but C' is independent of the latter, we expect the following segregation in F_2.

$BP'O'C'$	27	Awn purple,	palea self purple,	grain reddish brown.
$BP'O'c'$	9	,,	,,	"white"
$bP'O'C'$	9	,,	localized purple	reddish brown.
$bP'O'c'$	3	,,	,,	"white"
$Bp'o'C'$	9	brown	brown	yellow. brown.
$Bp'o'c'$	3	,,	,,	"white"
$bp'o'C'$	3	,,	yellow	yellow. brown.
$bp'o'c'$	1	,,	,,	"white"

If reddish browns, yellowish browns and "whites" are added together irrespective of the colour of the paleas, we obtain 36 : 12 : 16 or 9 : 3 : 4 ratio. The ratio of coloured to non coloured grains in each colour types of the paleas is 3 : 1 showing that the gene C' is independent of P' and O'.

As we have already seen, green and yellow seed coat of the soy beans are dominant over black, brown and buff. The green and yellow contain

practically no chromogenic substance whereas the rest of them is prominent. This is to indicate that a dominant inhibitor is present in the green and yellow. Let *G* be the gene for the green colour and *g* for the yellow. Further we assume that *C* and *c* are the chromogens. The amount of chromogenic substance produced by *c* is less than that produced by *C*. Let *O* be the chromophelein which converts the chromogenic substance to brown pigment and *o* to reddish brown. It is assumed that the same chromogenic substance is converted to the purple anthocyanin by *R* but not by *r*. The gene *I* inhibits the full development of the pigment in the seed coat and by *i* no such effect is done. The inhibitory action of the gene *I* seems to extend to the action of *C* and *R*. Accordingly the different colour types may be designated as follows:

COGRI	Blue tinged green
COgRI	„ „ yellow
COGrI	Non tinged green
COgrI	„ „ yellow
COGRi	Black (green hypostatic)
COgRi	„ (yellow hypostatic)
COGri	Brown (with green tinge)
COgri	„ (without green tinge)
Cogri	Reddish brown
cOgri	Buff

The genetic composition of the parental plants of the cross between "Haiiro" and "Tanishi" would be *CCOOGGrrii* and *CCOOggRRII* respectively. The F_1 plant is therefore heterozygous to three genes *Gg Rr* and *Ii* but homozygous to *C* and *O*. The different phenotypes appeared in F_2 are due to the recombination of these genes. Thus:

	Blue tinged	Black	Non tinged	Brown	Total
Yellow	*COgRI* 9	*COgRi* 3	*COgrI* 3	*COgri* 1	16
Green	*COGRI* 27	*COGRi* 9	*COGrI* 9	*COGri* 3	48
	Anthocyanin present 48		Anthocyanin absent 16		64

In blacks, yellow, green or brown is completely covered, but in brown green colour is visible, hence blue tinged greens, blue tinged yellows, non tinged greens, non tinged yellows, blacks and browns arise by the ratio 27 : 9 : 9 : 3 : 12 : 4. If we denote above phenotypes by *B.T.G.*, *B.T.Y.*, *G*, *Y*, *Bl*, and *Br*. respectively, the following segregation is expected in the F_3 generation.

	F_2 plant.		Segregation in F_3	Ratio.
B.T.G.	*GGRRII*	1	*B.T.G.*	Const.
,,	*GGRRIi*	2	*B.T.G.* : *Bl.*	3 : 1
,,	*GGRrII*	2	*B.T.G.* : *G.*	3 : 1
,,	*GGRrIi*	4	*B.T.G.* : *G* : *Bl* : *Br.*	9 : 3 : 3 : 1
,,	*GgRRII*	2	*B.T.G.* : *B.T.Y.*	3 : 1
,,	*GgRRIi*	4	*B.T.G.* : *B.T.Y.* : *Bl.*	9 : 3 : 4
,,	*GgRrII*	4	*B.T.G.* : *B.T.Y.* : *Y*	9 : 3 : 3 : 1
,,	*GgRrIi*	8	*B.T.G.* : *G.* : *B.T.Y.* : *Y* : *Bl* : *Br.*	27 : 9 : 9 : 3 : 12 : 4
Bl.	*GGRRii*	1	*Bl.*	Const.
,,	*GGRrii*	2	*Bl* : *Br.*	3 : 1
,,	*GgRRii*	2	*Bl.*	Const.
,,	*GgRrii*	4	*Bl.* : *Br.*	3 : 1 (12 : 4)
,,	*ggRRii*	1	*Bl.*	Const.
,,	*ggRrii*	2	*Bl.* : *Br.*	3 : 1
G.	*GGrrII*	1	*G.*	Const.
,,	*GGrrIi*	2	*G.* : *Br.*	3 : 1
,,	*GgrrII*	2	*G* : *Y*	3 : 1
,,	*GgrrIi*	4	*G* : *Y* : *Br.*	9 : 3 : 4
B.T.Y.	*ggRRII*	1	*B.T.Y.*	Const.
,,	*ggRRIi*	2	*B.T.Y.* : *Bl.*	3 : 1
,,	*ggRrII*	2	*B.T.Y.* : *Y*	3 : 1
,,	*ggRrIi*	4	*B.T.Y.* : *Bl.* : *Y.* : *Br.*	9 : 3 : 3 : 1
Br.	*GGrrii*	1	*Br.*	Const.
,,	*Ggrrii*	2	*Br* (greenish) : *Br.*	3 : 1
,,	*ggrrii*	1	*Br.*	Const.
Y.	*ggrrII*	1	*Y.*	Const.
,,	*ggrrIi*	2	*Y.* : *Br.*	3 : 1

The observed data showed a close approximation to the above expectation. See the table below.

Table 29.

Showing the result obtained in F_3 of the cross "Tanishi" × "Haiiro." See also Table 34.

F_2 phenotype	F_2 gonotype	No. of families observed	expected	difference
B.T.B.	*GGRRII*	—	0.594	
	GGRRIi	2	1.187	
	GGRrII	1	1.187	
	GGRrIi	3	2.375	
	GgRRII	3	1.187	
	GgRRIi	3	2.375	
	GgRrII	1	2.375	
	GgRrIi	4	4.751	
		17	16.031	+0.969
Bl.	*GGRRii*, *GgRRii*, *ggRRii*	1	2.375	
	GGRrii, *GgRrii*, *ggRrii*	6	4.751	
		7	7.126	−0.126
G.	*GGrrII*	—	0.594	
	GGrrIi	2	1.187	
	GgrrII	1	1.187	
	GgrrIi	3	2.375	
		6	5.343	+0.657
B.T.Y.	*ggRRII*	1	0.594	
	ggRRIi	1	1.187	
	ggRrII	1	1.187	
	ggRrIi	3	2.375	
		6	5.343	+0.657
Y.	*ggrrII*	—	0.594	
	ggrrIi	—	1.187	
		—	1.1781	−1.178

F_2 phenotype	F_2 genotype	no. of families observed	expected	difference
Br.	*GGrrii*	1	0.594	
	Ggrrii	1	1.187	
	ggrrii	—	0.594	
		2	2.375	−0.375

In the case of the cross "Waremame" × "Achumuri" in which buff and black are crossed, two pairs of genes are concerned in the formation of four types of seed coat, i.e., self black, imperfect black, brown and buff. If we let a pair of genes *C* and *c* stand for the chromogens, imperfect black was found to carry no *C*, in spite of the fact that the ground-colour appeared to be brown, as already mentioned, hence we may assume that the full development of the deep purple anthocyanin pigment is only possible by the presence of *C* and *R*. The gene for the formation of the chromogenic substance may therefore have a simultaneous action on the formation of brown and self black from the same chromogenic substance. Here the gene *G* is not concerned. The parental plants may have the following genotypic composition with respect to the colour of the seed coat:

Self black	*CCOORRiigg*
Buff	*ccOOrriigg*

The F_1 plant is heterozygous to *C* and *R*, and in F_3 we should expect the following families:

Self black	*CCRR*	1	Black	const.
,,	*CCRr*	2	Black : brown	3 : 1
,,	*CcRR*	2	Black : imperf. black	3 : 1
Self black	*CcRr*	4	Black : imperf. black : brown : buff.	9 : 3 : 3 : 1
Imperfect black	*ccRR*	1	Imperf. black	Const.
,,	*ccRr*	2	Imperf. black : buff	3 : 1
Brown	*CCrr*	1	Brown	Const.
,,	*Ccrr*	2	Brown : buff	3 : 1
Buff	*ccrr*	1	Buff	Const.

The actual numbers observed are as follows.

TABLE 30.

Showing the result obtained in F_3 of the cross "Waremame" × "Achumuri"
See also Table 35.

F_2 plant phenotype	genotype	no. of families observed	no. of families expected	diff. (ob.—exp.)
Black	*CCRR*	3	2.875	+0.125
,,	*CcRR*	8	5.750	+2.250
,,	*CCRr*	6	5.750	+0.250
,,	*CcRr*	15	11.500	+3.500
Black imp.	*ccRR*	2	2.875	−0.875
,,	*ccRr*	6	5.750	+0·250
Brown	*CCrr*	3	2.875	+0.125
,,	*Ccrr*	3	5.750	−2.750
Buff	*ccrr*	0	2.875	−2.875

The different colour types of the soy bean seed coat so far concerned constitute the following series when they are arranged according to dominancy: blue tinged green>blue tinged yellow>green>yellow>black>imperfect black>browns (lighter brown>deeper brown)>buff.

It is of interest to compare the case of the soy bean to that of the Adzuki bean which have been investigated by TAKAHASHI and FUKUYAMA (1917)[1]. They have shown that the different colour types behaved strictly in accordance with Mendelian principle as in the case with the soy bean. The different types can be arranged, according to dominancy, as follows: Blue black>black>black flecked ("Yogore")>black flecked red>greenish grey>deep buff ("Cha")>red (self)>red-eyed white>white.

The test for the chromogenic substances already remarked (see Table 8) shows that the chromogenic substance *P* and *F* are present in the green, unripe beans of all the coloured types but absent in white which is a "warm buff" according to the nomenclature by RIDGWAY. White is the most recessive character in the series. In the case of the soy bean, the types which show verp little chromogen content are green and yellow which are dominant over the types rich in the chromogenic substance. The difference in the genetical

1. TAKAHASHI, Y. and FUKUYAMA, J. Morphological and Genetic Studies on the Adzuki-bean. Hokkaido Agric. Exp. Station, Japan. Report 7, pp. 161 (in Japanese).

behavior of the chromogen containing types in the two species of plants is due to the presence and absence of an inhibitor. The authors showed that in the cross between buff and white, the F_2 seed was buff and segregated in F_2 buffs, reds and whites by a rate 9 : 3 : 4. While the cross between deep buff and white gave deep buff in F_1 (F_2 seed) and deep buffs, buffs, reds and whites in the F_2 generation by the ratio 27 : 9 : 12 : 16. Thus we see that the deep buff differs from buff by a single factor-difference and buff and red also by another factor pair. We see therefore that the inhabitor is also present in that case but differs from that of the soy bean in such a way that the inhibitor in the Adzuki-bean inhibits the formation of reddish brown pigment from the chromogenic substance but the action does not seem to extend over the formation of the chromogenic substance, while in the case of the soy bean, the inhibitor inhibits the formation of the chromogenic substance, so that no chromogenic reaction can be observed in the green and yellow. In the buffs, the chromogenic substance can readily be directed and if another gene, a chromophelein is added to it, the deep buff is produced.

The writer was able to test the chromogenic substance by the material which was kindly furnished to him through the courtesy of Mr. FUKUYAMA. Fully ripened deep buff and buff were found to be rich in the chromogenic substance *P*. The green unripe beans born on the plants raised from the same material in the next year also gave the similar result.

In order to compare the genotypic compositions of the types of Adzuki and those of the soy beans, it is convenient to change the designations used by the authors to those proposed in the present paper. They gave *RHf* for buff, *Rhf* for red, and *rhf* for white in which *R* is the gene for red, *H* an inhibitor and *F* the gene for buff. We assume that the genes for the red pigment are *C* and *o* and by the action of an inhibitor *I* results in the formation of buff. The gene *I* only inhibits the action of *o* but that of *C* is left free. We have some data for believing that the reddish brown pigment of the Adzuki-bean is the oxdidation product of the chromogenic substance. The pigment is insoluble in strong acids, but readily soluble in water and especially in weak alkalies yielding a deep wine red colour which becomes yellow by acid. The alkaline pigment is insoluble in ether and acetic ether but in a weak acid solution, sparingly soluble in ether, and by evaporating

the solvent yields an amorphous reddish brown pigment. The pigment may be precipitated from aqueous solution by lead acetate.

Consequently the gene *I* seems to inhibit the action of oxidizing agency acting on the chromogenic substance. The above mentioned relation which exists in buff and red seems to be analogous to that of the case of the dominant white in *Primula sinensis*. According to KEEBLE and ARMSTRONG (1912)[1], KEEBLE, ARMSTRONG and JONES (1913)[2] and KEEBLE and Miss PELLOW (1910)[3] certain dominant whites contain chromogen, which occurs in the recessive white in an extremely slight amount and the inhibitory substance which obscures the peroxidase reaction is present in the former. The buff coloured seed coat of the Adzuki-bean can be considered somewhat analogous to the dominant white in the flower of *Primula* and the white to the recessive white. The colour of buff and white in the seed coat differ slightly from each other. The peroxidase reaction in the seed coat was also examined and an indication to the similar relation that was observed in the flower of *Primula sinensis* was obtained. In the epidermis of the seed coat in which the pigment is confined, the peroxidase reaction was extremely slight in the unripe green seed of buff and deep buff whereas in the white, very distinct. The observation was repeatedly made with the material taken at the different stage of maturity. The section was placed under the cover glass with the alcoholic solution of benzidine or alpha naphtol with a dilute solution of hydrogen peroxide. In this manner, the direct oxidase has failed to be detected in all cases.

The reddish brown in the soy bean (such as "Aka-nedzumime") corresponds with red in the Adzuki-bean. The gene *O* which modifies the reddish brown to brown in the soy bean corresponds with the gene *I* in the other, inasmuch as they suppress the formation of reddish-brown, oxidation product of the chromogenic substance, though they differ in the manner toward the formation of the chromogenic substance as already mentioned. The genetic composition of the different self coloured types in both species can be expressed by the same designations in the following manner:

1, KEEBLE, F. and ARMSTRONG, E. F., Loc. cit.

2. KEEBLE, F., ARMSTRONG, E. F., and JONES, W. N., The Formation of the Anthocyan Pigments of Plants. 6. Proc. Roy. Soc. London, B. 87 : 113, 1813.

3. KEEBLE F. and PELLOW, C., White Flowered Varieties of Primula sinensus. Jour. Genetics. 1 : 1, 1910.

	Soy bean		Adzuki-bean
Buff	*cOrig*	White	*corig* (*rfh*)
Reddish brown	*Corig*	Red	*Corig* (*Rfh*)
Brown	*COrig COriG*	Buff	*CorIg* (*RfH*)
		Deep buff	*COrIg* (*RFH*)
Black	*CORig* etc.	Black	*CorIg* (*RFhMC*)
Yellow	*COrIg* etc.		
Green	*COrIG* etc.	Green	*COrIG* etc.

Also, in the seed coat of *Phaseolus vulgaris*, *Phaseolus multiflorus*[1] *Pisum sativum*[2], *Vigna unguiculta* and *Vigna sinensis*[3], the coloured types which are due to anthocyanin pigments are dominant over those which are due to phlobaphene pigments and the latter are dominant over white types. In *Pisum*, the well known work of MENDEL has already shown that the brown coloured pea is a simple dominant over the colourless one. LOCK enumerated the genes concerning the colour of the testa as follows; (1) greyish or brownish pigmentation as opposed to the absent (white) (*C*) (*c*), (2) purple spotted of bright purple spots as opposed to very faint or absence of the character (*S*) (*s*), (3) the presence of maple character. Mapling or mottling of a rich brown colour as opposed to the absence of the character (*M*) (*m*).

1. LOCK, R. H., Studies in Plant Breeding in the Tropics. Ann. Roy. Bot. Gard. Peradeniya. 3 : 95, 1906. SHULL, G. H., Some Latent Characters in White Bean. Science. N. S. 25 : 828, 1907. —SHULL. A New Mendelian Ratio and Several Types of Latency. Amer. Nat. 42 : 433, 1908.—TSCHERMAK, v. E., Weitere Beiträge ueber Verschiedenwertigkeit der Merkmarle bei Kreuzung von Erbsen u. Bohnen. Zeit f. d. l. Versuch. Osterreich, 1901, 641.—TSCHERMAK, Weitere Kreutzungsstudien an Erbsen, Levkojen und Bohnen. Ibid. 1904, 533.—TSCHERMAK, Bastardierungsversuche an Levokojen, Erbsen, und Bohnen mit Rücksicht auf der Faktorenlehre. Zeit.f. induk. u. Vererb. 7 : 81. 1912. EMERSON, R. A., Inheritance of Color in the Seeds of the Common Bean, Phaseolus vulgaris. Ann. Report Nebraska Exp. Station 22 ; 67; 1909.—LUNDBERG, J. and AKERMAN, A., The Colour of the Seed in the Descendants of A Natural Hybrid of Two Varieties of Phaseolus vulgaries. Sveriges Utsadesforeinge Tidskrift. 27 : 115, 1917. (cited in Internat. Review of Sc. and Pract. of Agric. 8 : Entry 1013, 1917.)

2. LOCK, R. H., The Present Stage of Knowledge of Heredity in Pisum. Ann. Roy. Bot. Peradeniya. 4 : 93, 1908. MENDEL. G., Versuche under Pflanzenhybriden. Verhand. Naturforsch. Verein in Brünn. 10 : 1865.—WHITE, O. F., Researches on the 35 Factors Determining the various Characters of the Genus Pisum, Jour. Agric. Research. 11 : 166, 1917.

3. SPILLMAN, W. J., Inheritance of the "Eye" in Vigna. Amer. Nat. 45 : 513. 1911.—SPILLMAN, Color Correlation in Cowpeas. Science N. S. 38 : 302, 1913.—HARLAND, S. C., Inheritance of certain characters in the Cowpas (Vigna Sinensis). Jour. Genet. 8 . 101, 1919.

In certain varieties of *Phaseolus vulgaris*, TSCHERMAK (1912) showed that the coloured seed coat was dominant over white, and among coloured types the relation, black>violet>brown was established. SHULL (1908) also found in the same plant, purple, brown, yellowish-brown and yellow were dominant over white. He proposed the following genetic composition for the different self coloured types :

Brown and yellow	*Pbm*
Black	*PBm*
White	*pBM*

in which *P* is a gene for the pigment, *B* the modifier of the pigment, and *M* the mottling gene. *P* may correspond with *CO* or *C* and *B* with *R* in our case.

In dealing with the colour of patterns of the seed coat of *Vigna unguiculata* and *Vigna sinensis* SPILLMAN (1913) and HARLAND (1919) respectively found that the solid coloured types were dominant over the mottled and less coloured ones. The latter author showed also that black was dominant over brown, and brown over red. The brown was completely dominant over red in F_1 and brown, maroon, and red arose in the F_2 by the ratio 12 : 3 : 1. These colours are of the phlobaphene nature and no anthocyanin is concerned except black. The genetical behaviour of the phlobaphene colour types in this plant is quite similar to that which we have seen in Adzuki and soy beans. In all these cases the more intense reddish-brown is recessive to less intensely coloured types.

In *Zea Mays*, EAST and HAYES (1911)[1] showed that the dark-red pericarp was a simple dominant over white. The colour of the pericarp is due to the pigment belonging to the phlobaphene group. The purple and red aleurone colours are due to anthocyanins. The formation of the anthocyanin pigment in the aleurone cells is in certain cases, governed by the genes (*C. R, P* of EAST and HAYES, *C, R, A, Pr.* of EMERSON) which are apparently similar in kind as those met in the case of *Lathyrus, Antirrhinum* and *Oryza.*

1. EAST, E. M. and HAYES, H. K., Inheritance in Maize. Bull. Conn. Agric. Exp. Station. 167, 1911.—EMERSON, R. A., A Fifth Pair of Factors, *A a*, for Aleurone Color in Maize, and Its Relation to the *C c* and *R r* Pairs. Cornell University Agric. Exp. Station Memoir 16, 1918.

Thus we see that the pigment yielding mechanism in the seed coat of different species of plant falls in general under a similar category, particularly in the seed coat of the legumes.

4. Discussion.

To a gene we imply a specific protoplasmic entity which sets up the biochemical apparatus in the sporophytic cells and to the end product of the reaction performed by the mechanism so set up, we refer a character, morphological and physiological. Therefore, even we infer a gene to a character, that gene itself may have no direct relation to the character. A catalyst does not appear in the final product of a chemical reaction, but may alter the velocity of the reaction and sometimes change the position of equilibrium to be attained.

When such agencies or genes are paired forming an allelomorph, and they segregate in a normal way, we can deduce the relation between the character and the gene by the numerical ratio of character that is required by the supposed genetic entities. We disregard the biochemical processes involved in the changes which are set up by the gene to bring about the equilibrim, of which state we perceive the character. Dynamically viewed, however, the possibility is not excluded even in such a case in which a single allelomorphic character-difference is due to more than a factor-difference. Supposing the change $A \rightarrow O$ in which the substance A undergoes certain changes to form the substance O which may be regarded as a single character in the Mendelian sense, such as a purple pigment in a certain organ in the plant. $A \rightarrow O$ reaction would appear to be a single change when the initial and the end product alone is considered, but it may involve the catenary changes $A \rightarrow B \rightarrow C \rightarrow D \rightarrow O$. Such complex changes are likely to occur in most of the biochemical processes like respiration and photosynthesis which seem comparatively simple when the initial substance and the final product alone are considered.

If we consider an imaginary instance in which $A \rightarrow B$, $B \rightarrow C$, $C \rightarrow D$, and $D \rightarrow O$ reactions are involved in a whole change $A \rightarrow O$, and these separate changes are governed by the respective genetic entities, yet they are not

separable at the time of synapsis. The character which is due to the end product of the final change, therefore would appear as due also to a single genetic entity. Supposing that the purple anthocyanin is produced by $C \rightarrow R \rightarrow P$ changes and three separate genes are actually taking part to bring about complex chemical changes. But if these genes are linked, or so to say, form a complex, and do not separate in gametogenesis, they may well be considered as a single entity and can be substituted by a single designation to express the genetic entities to a given character. When they separate from the complex by any cause, a supposed single unit character would appear to be constituted by more than a single gene.

The separation of genes from the complex may take place either by hybridization or by unknown internal causes, and of the latter cases, we call mutation.

It is a comparatively simple matter to determine the number of genes concerned with given characters by hybridization experiments when the contrasting characters are distinct and the segregation in the offspring of the hybrid is sharply defined. But it is extremely difficult to interpret those genes in terms of biochemistry or physiology. We are likely to fall into the danger of providing a superficial analogy and drawing sweeping conclusion by confusion of the genetical data to those of physiology.

In the case of the formation of anthocyanin and phlobaphene pigments in the plants studied, the genes *C*, *O*, *R* and *P* appear to govern certain groups of biochemical reactions in the sporophytic cells more or less in a distinct manner, yet we must have great reserve in referring these genes to any physiological factors. It is true that the peroxidase coexists with the pigment, and the normal oxygen relation is essential to the formation of the pigment, but these facts prove in no way to allow us in interpreting the complementary gene of the colour producing system in plant is exclusively relating to peroxidase. Even in the case of the formation of brown and reddish brown pigments, in which the oxidation of the chromogenic substance is an essential change, the direct inference of the gene to peroxidase or oxidase may deserve serious consideration.

The formation of brown plant-pigments (phlobaphenes) resembles, as we have already seen in the preceding pages, that of melanin pigments in animals

in some respects. Certain authors go so far as to regard the brownish pigments in the seed coat of the legumes as a sort of plant melaninic pigment.[1]

WRIGHT (1917)[2], proposed an hypothesis regarding the colour inheritance in Mammals. He proposes first, that melanin is produced by the oxidation of certain products of protein metabolism by the action of specific enzymes; second, that this reaction takes place in the cytoplasm of cells probably by enzymes secreted by the nucleus; third, that various chromogens are used, the particular ones oxidized depending on the characters of the enzymes present, and finally that hereditary difference in colour are due to hereditary differences in the enzyme element of the reaction. It is supposed that color depends on the rates of production or of potency of two enzymes. Enzyme I is essential to the production of any colour, but by itself only produces yellow. Enzyme II is supplementary to enzyme I, producing no effect by itself. The compound enzyme I—II is also more efficient than enzyme I in another way. It produces sepia pigment even when enzyme I is at too low a potency to produce any yellow by itself. Above the level at which enzyme I produces effects, the enzyme I and I—II, complete the oxidation of chromogen.

Regarding the place of the enzyme reaction to the chromogenic substance, his hypothesis may be referred to the view of UNNA (1913)[3] in which he maintained that in the tissue of the animal skin, the plasma is the reduction place ("Reduktionsort") and the nucleus, the oxidation place ("Sauerstoffort"). In plants, however, SCHNEIDER (1914)[4] could not establish UNNA's view.[5]

If the mitochondria is the seat of the pigment synthesis as GUILLIERMOND

1. MANN, A., Coloration of the Seed Coat of Cowpeas. Jour. Agric. Research. 2:33, 1914. The substances known as "Phytomelan" are, however, different from phlobaphenes. See DAFERT, F. W. and MIKLAUZ, R., Untersuchungen ueber die kohleähnliche Masse der Kompositen. I. Denkschr. d. Kais. Akad. d. Wien. Bd. 87, 1911. Cited in MOLISCH, H., Mikrochemie der Pflanze. p. 319, 1913.

2. WRIGHT, S., Color Inheritance in Mammals. Jour. of Heredity. 8:224, 1917.

3. UNNA, P. G., Biochemie der Haut. Jena. 1913.

4. SCHNEIDER, H., Ueber die Unnaschen Methoden zur Feststellung von Sauerstoff-und Reduktion-Orten u. ihre Anwendung auf Pflanzliche Objekte-Benzidin als Reagens auf Verholzung. Zeitsch. f. wiss. Mikro. Tech. 31:51, 1914, a.—SCHNEIDER. Neue Studien zur Darstellung der Reduktions u. Sauerstofforte der Pflanzenzelle. Ibid. 478. 1914. b.

5. Cf. OSTERHAUT, W. J. V., The Role of the Nucleus in Oxidation. Science. N. S. 46:367, 1917.

and others[1] have reported, the reaction place seems to be chiefly located in the cytoplasm. All the genes must be retained in the nuclear substance of the sporophytic cell in some latent state, and the reaction done by them in the cell to produce the pigment must be realized by some sort of substances derived from the nucleus. The actual relation between the substance of genes in the nucleus and the mechanism in the cytoplasm conditioned by the former, is known to none of us. It seems therefore altogether premature to speculate, as certain biologists might propose, that the gene itself is the enzyme. Even in the pure chemical field, we do not know as yet the exact chemical nature and the mode of action of enzymes.

III Summary and Conclusion.

In a number of species of plants examined, two groups of pigments anthocyanins and the reddish brown pigments (phlobaphenes) can be traced to the chromogenic substances, previous to their formation. In certain cases, both of the pigments can be formed from the same chromogenic substance by the action of various complementary pigment-yielding agencies.

The chromogenic substances can be identified to two groups of allied substances, one of which is designated as the chromogenic substance *F* which includes the glucoside of certain flavones and flavonols, and the other, the chromogenic substance *P* of which the chemical nature is yet unknown.

Evidence is given to show that certain brown and reddish brown pigments (phlobaphenes) are the oxidation products of the chromogenic substance *P* and *F*.

Certain anthocyanins are completely decolorized by the action of oxidizing enzymes.

Certain flavones, flavonols and their glucosides yield a characteristic oxidation colour by the action of oxidizing enzymes.

1. Guilliermond, A., Sur la formation de l'anthocyane au sein des mitochondries. Comp. Rend. Acad. Sci. Paris. 156 : 1924. 1913.—Guilliermond, Nouvelles recherches cytologiques sur la formation des pigments anthocyaniques. Ibid, 157 : 1000, 1913.—Guilliermond, Quelques observations cytologiques sur la mode de formation des pigments anthocyaniques dans les fleurs. Ibid. 161 : 494, 1915.—Guilliermond, Recherches cytologiques sur la formation des pigments anthocyaniques. Rev. Gene. Bot. France. 25 : 295, 1914.—Moreaux, F., Loc. cit.—Mirande, M., Observation sur le vivant de la formation cytologique de l' anthocyanine. Comp. Rend. Acad. Sci. Paris. 163 : 368, 1916.

The anthocyanin pigment is the reduction product of the chromogenic substance when the chromogenic substance *F* alone is concerned, but the other possibilities are not excluded when the chromogenic substance *P* is concerned.

When the complete system is laid down in the sporophytic cells by the combination of the separate components which are retained by the specific genetic entities, anthocyanin pigment is formed in the awn, and glumes of *Oryza sativa*. Hence by a proper crossing, the awn of the hybrid plant between two races which lack the pigment, forms anthocyanin.

A linkage relation was observed between the purple colour in the awn and the reddish brown colour in the testa in the varieties of *Oryza sativa* studied.

The brown pigment of the awn of *Oryza* is due chiefly to the oxidation product of the chromogenic substance *F*, and that of the testa is due chiefly to that of the chromogenic substance *P*.

The coloured stigma, purple awn, paleas, and striped leaf-sheath which are due to the presence of anthocyanin are inherited in a group, and in the paleas, the solid purple is linked with the brown pigment which is formed at the underlying tissue of the same organ, while the localized purple repells the latter.

The brown and reddish brown pigments and the purple anthocyanin formed in the seed coat of *Glycine soja* are derived chiefly from the same chromogenic substance belonging to the group of the chromogenic substance *P*.

The formation of these pigments as well as the chromogenic substance is entirely or partially suppressed by the action of dominant inhibitors.

Certain genetic phenomena relating to the colours of the seed coat are studied. The following is the list of characters studied, arranged according to dominancy in the ascending order. Blue tinged green > blue tinged yellow > green > yellow > black > brown (lighter brown > reddish brown) > buff.

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TABLE 31.

Showing the result obtained in F_3 of the cross "Daikkoto" × "Togo."

F_3 family no.	Awn of F_2 plant	Red	Brown	Faint yellow	Total
14	Brown	36	—	—	36
29	„	96	—	—	96
38	„	106	—	—	106
4	„	15	—	9	24
9	„	21	—	10	31
19	„	15	—	7	22
24	„	46	—	21	67
33	„	49	—	11	60
35	„	76	—	27	103
31	„	57	—	21	78
2	„	37	20	—	57
6	„	19	2	—	21
10	„	63	24	—	87
12	„	47	18	—	65
16	„	65	31	—	96
17	„	46	20	—	66
30	„	20	9	(1)	30
32	„	75	22	—	97
35	„	16	10	—	26
34	„	48	22	—	70
3	„	35	15	14	64
7	„	49	15	32	96
8	„	52	17	23	92

F_3 family no.	Awn of F_2 plant	Red	Brown	Faint yellow	Total
11	Brown	45	14	16	75
15	,,	33	15	16	64
18	,,	33	10	15	58
20	,,	10	11	4	25
22	,,	23	15	20	63
23	,,	30	19	13	62
25	,,	19	10	5	34
26	,,	58	18	22	98
28	,,	44	6	16	66
1	,,	—	47	17	64
13	,,	—	43	18	61
21	,,	—	64	—	64
37	,,	—	69	—	69
39	Faint Yellow	—	—	101	101
40	,,	—	—	63	63
41	,,	—	—	89	89
42	,,	—	—	63	63
43	,,	—	—	66	66
44	,,	—	—	67	67
45	,,	—	—	33	33
46	,,	—	—	98	98
47	,,	—	—	98	98
48	,,	—	—	101	101
49	,,	—	—	97	97
50	,,	—	—	31	31

Table 32.

Showing the result obtained in F_3 of the cross
"Kurafusagi" × "Nagoyashiro"

F_3 family no.	Awn of F_2 plant	Red	Brown	Faint Yellow	Total
31	Brown	61	—	—	61
42	,,	94	—	—	94
44	,,	95	—	—	95
45	,,	62	—	—	62

F_3 family no.	Awn of F_2 plant	Red	Brown	Faint yellow	Total
80	Brown	57	—	—	57
82	,,	100	—	—	100
84	,,	96	—	—	96
88	,,	63	—	—	63
2	,,	67	29	(1)	97
3	,,	48	13	(1)	62
22	,,	49	16	—	65
23	,,	67	28	(1)	96
36	,,	67	30	—	97
49	,,	67	32	—	99
57	,,	66	27	—	93
58	,,	51	21	—	72
60	,,	75	25	—	100
62	,,	56	13	—	69
64	,,	45	15	—	60
69	,,	66	31	—	97
73	,,	65	23	—	88
74	,,	75	18	—	93
77	,,	68	29	—	97
83	,,	74	17	—	91
86	,,	33	13	—	46
21	,,	23	8	—	31
15	,,	44	(1)	15	60
32	,,	46	—	16	62
63	,,	19	—	11	30
85	,,	47	—	18	65
89	,,	51	—	12	63
91	,,	28	—	4	32
93	,,	28	—	5	33
94	,,	30	—	4	34
97	,,	25	—	11	36
98	,,	50	—	12	62
102	,,	71	—	13	84
103	,,	67	—	26	93
105	,,	72	—	25	97
1	,,	27	22	11	60
5	,,	32	6	24	62
10	,,	38	22	18	78

F_3 family no.	Awn of F_2 plant	Red	Brown	Faint yellow	Total
13	Brown	46	21	33	100
18	,,	50	18	28	96
19	,,	55	13	31	99
24	,,	10	5	3	18
25	,,	49	23	26	98
27	,,	9	2	5	16
29	,,	25	12	24	61
33	,,	43	8	16	67
34	,,	19	23	29	101
35	,,	62	12	22	96
39	,,	42	21	24	87
41	,,	43	24	26	93
43	,,	33	14	16	63
50	,,	56	19	17	92
52	,,	19	8	6	33
53	,,	40	14	17	71
59	,,	43	20	25	78
65	,,	29	14	22	65
75	,,	34	10	22	66
76	,,	34	16	15	65
79	,,	32	15	14	61
87	,,	48	21	26	95
101	,,	58	19	24	101
92	,,	64	8	19	91
107	,,	42	11	16	69
108	,,	56	13	32	101
109	,,	54	18	24	96
110	,,	27	13	25	65
4	,,	—	44	14	58
6	,,	—	62	22	84
7	,,	—	72	18	90
9	,,	—	26	10	36
28	,,	—	68	25	93
30	,,	—	73	20	93
37	,,	—	77	21	98
38	,,	—	76	23	99
44	,,	—	29	11	40
70	,,	—	67	28	95

F_3 family no.	Awn of F_2 plant	Red	Brown	Faint yellow	Total
71	Brown	—	78	23	101
78	,,	—	41	20	61
81	,,	—	38	15	53
90	,,	—	63	33	96
56	,,	—	39	9	45
8	,,	—	96	—	96
12	,,	—	13	—	13
14	,,	—	28	—	28
40	,,	—	63	—	63
47	,,	—	57	—	57
71	,,	—	91	—	91
111	Faint yellow	—	—	64	64
112	,,	—	—	33	33
113	,,	—	—	63	63
114	,,	—	—	99	99
115	,,	—	—	98	98
116	,,	—	—	101	101
117	,,	—	—	99	99
118	,,	—	—	98	98
119	,,	—	—	64	64
120	,,	—	—	99	99
121	,,	—	—	87	87
122	,,	—	—	100	100
123	,,	—	—	94	94
124	,,	—	—	33	33
125	,,	—	—	92	92
126	,,	—	—	85	85
127	,,	—	—	61	61
128	,,	—	—	97	97
129	,,	—	—	95	95
130	,,	—	—	92	92
131	,,	—	—	97	97
132	,,	—	—	86	86
133	,,	—	—	96	96
134	,,	—	—	64	64
135	,,	—	—	65	65
136	,,	—	—	62	62
137	,,	—	—	89	89

1 2 3 4

5 6

7 8 9 10

11 12 13 14 15 16 17

N. Mizushima del.

F_3 family no.	Awn of F_2 plant	Red	Brown	Faint yellow	Total
138	Faint yellow	—	—	61	61
139	,,	—	—	33	33
140	,,	—	—	34	34
141	,,	—	—	34	34
142	,,	—	—	38	38

Table 33.

Showing the result obtained in F_3 of the cross
"Hanbun-nento" × "Genroku-mochi"

F_3 family no.	Paleas of F_2 plant	Awn purple		Awn red		Totals
		paleas self *P*	paleas loc. *P*	paleas brown	paleas f. yellow	
2	Self purple	58	16	—	—	74
4	,,	70	21	—	—	91
18	,,	75	21	—	—	96
24	,,	72	22	—	—	94
25	,,	54	11	—	—	65
30	,,	74	20	—	—	94
42	,,	51	12	—	—	63
63	,,	6	2	—	—	8
66	,,	11	4	—	—	15
68	,,	26	8	—	—	34
10	,,	74	—	21	—	95
13	,,	62	—	34	—	96
15	,,	55	—	12	—	67
22	,,	78	—	20	—	98
37	,,	61	—	21	1	82
45	,,	65	—	21	—	86
47	,,	64	—	32	—	96
48	,,	71	—	27	—	98
56	,,	25	—	9	—	34
16	Local. purple	—	48	—	15	63
20	,,	—	69	—	19	88
23	,,	—	62	—	37 (2)*	101

* Not recorded.

F_3 family no.	Paleas of F_2 Plant	Awn purple		Awn red		Totals
		Paleas self *P.*	Paleas loc. *P.*	paleas brown	paleas f. yellow	
27	Local. Purple	—	44	—	17	61
31	,,	—	57	—	32	89
39	,,	—	70	—	26	96
44	,,	—	77	—	27	104
49	,,	—	77	—	23	100
53	,,	—	25	—	8	33
57	,,	—	73	—	5	78
58	,,	—	20	—	14	34
59	,,	—	59	—	14	73
64	,,	—	40	—	20	60
21	,,	—	34	—	—	34
36	,,	—	89	—	—	89
41	,,	—	95	—	—	95
51	,,	—	97	—	—	97
61	,,	—	19	—	—	19
67	,,	—	30	—	—	30
6	,,	—	34	—	—	34
3	Self purple	64	5	12	2	83
5	,,	57	19	14	5	95
11	,,	54	17	22	5	98
19	,,	53	16	27	6	102
26	,,	58	19	15	10	102
28	,,	54	15	19	6	94
29	,,	65	14	17	3	99
34	,,	58	16	14	8	96
35	,,	62	13	19	5	99
46	,,	46	22	22	5	95
50	,,	34	8	16	4	62
54	,,	37	16	14	1	68
55	,,	39	11	7	6	63
65	,,	19	4	5	6	34
69	,,	17	10	7	—	34
8	Brown	—	—	72	23	95
9	,,	—	—	42	13	55
32	,,	—	—	58	39	97
33	,,	—	—	69	27	96
43	,,	—	—	59	32	91

F_3 family no.	Paleas of F_2 plant	Awn purple		Awn red		Totals
		paleas self. *P.*	paleas loc. *P.*	paleas brown	paleas f yellow	
62	Self purple	23	—	—	—	23
12	Brown	—	—	102	—	102
17	,,	—	—	78	—	78
40	,,	—	—	31	—	31
1	Faint yellow	—	—	—	104	104
7	,,	—	—	—	81	81
60	,,	—	—	—	78	78
38	,,	—	—	—	68	68

TABLE 34.

Showing the result obtained in F_3 of the cross
"Haiiro" × "Tanishi".

F_3 family no.	Colour of seed coat of F_3 seed	Blue tinged		Non tinged		Black	Brown	Totals
		green	yellow	green	yellow			
14	Blue tin. gr.	1	—	—	—	1	—	2
20	,,	5	—	—	—	1	—	6
		6	—	—	—	2	—	8
		6.0	—			*2.0*		
19	,,	59	—	17	—	—	—	76
		59		17				76
		57.0		*19.0*				
23	,,	18	—	12	—	2	2	34
1	,,	36	—	13	—	—	1	50
11	,,	33	—	8	—	1	—	42
		87		33		3	3	126
		70.875		*23.625*		*23.625*	*7.875*	
2	,,	20	7	—	—	—	—	27
22	,,	58	19	—	—	—	—	77
24	,,	45	11	—	—	—	—	56
		123	37					160
		120.0	*40.0*					
28	,,	41	8	12	6	—	—	67

F_3 family no.	Colour of seed coat of F_3 Seed	Blue tinged		Non tinged		Black	Brown	Totals
		green	yellow	green	yellow			
		41	8	12	6			
		37.688	*12.563*	*12.563*	*4.181*			
9	Blue tin. gr.	10	4	—	—	4	—	18
21	„	29	2	—	—	11	—	42
32	„	6	4	—	—	8	—	18
		45	10			23		78
		43.675	*14.625*			*19 500*		
4	„	18	5	4	3	5	3	38
15	„	16	6	2	1	9	3	37
30	„	32	8	3	2	11	2	58
31	„	30	6	19	2	20	7	84
		96	25	28	8	45	15	217
		91.546	*30.515*	*30.515*	*10.172*	*40 687*	*13.562*	
7	„	40	—	—	—	—	—	40
		40						40
25	„	19	—	—	8	—	—	27
		19			8			
		20.25			*6.75*			
18	„	35	—	12	—	—	—	47
		35		12				
		35.25		*11.75*				
3	Blue tin. y.	—	58	—	15	16	5	94
5	„	—	35	—	2	14	1	52
8	„	—	26	—	6	4	2	38
			119		23	34	8	184
			103.50		*34.50*	*34 50*	*11.50*	
6	Black	—	—	—	—	5	—	5
						5		5
12	„	—	—	—	—	42	12	54
10	„	—	—	—	—	45	17	62
26	„	—	—	—	—	29	9	38
17	„	—	—	—	—	24	10	34
27	„	—	—	—	—	1	1	2
29	„	—	—	—	—	13	7	20

F_3 family no.	Colour of seed coat of F_3 seed	Blue tinged		Non tinged		Black	Brown	Totals
		green	yellow	green	yellow			
						154	56	210
						157.50	*52.50*	
35	Green	—	—	9	—	—	9	18
40	„	—	—	20	—	—	5	25
				29			14	43
				32.25			*10.75*	
38	„	—	—	15	2	—	—	17
				15	2			17
				12.75	*4.25*			
34	Green	—	—	29	8	—	6	43
41	„	—	—	38	14	—	19	71
33	„	—	—	35	10	—	14	59
				102	32		39	173
				97.308	*32.436*		*43.248*	
36	Brown	—	—	—	—	—	57*	57
37	„	—	—	—	—	—	24	24

Table 35.

Showing the result obtained in F_3 of the cross
"Waremame" × "Achumuri".

F_3 family no.	Colour of seed coat of F_3 seed.	Black self	Black imperfect	Brown	Buff	Totals
5	Self black	19	—	—	—	19
22	„	2	—	—	—	2
31	„	51	—	—	—	51
2	„	33	9	—	—	42
7	„	25	9	—	—	34
10	„	12	5	—	—	17
14	„	26	15	—	—	41
15	„	18	9	—	—	27
29	„	4	1	—	—	5
30	„	14	4	—	—	18

* 19 with green tinge.

F_3 family no.	Colour of seed coat of F_3 seed.	Black self	Black imperfect	Brown	Buff	Totals
1	Self black	20	7	—	—	27
2	,,	33	—	9	—	42
9	,,	40	—	16	—	56
16	,,	11	—	2	—	13
21	,,	58	—	16	—	74
24	,,	3	—	1	—	4
32	,,	1	—	2	—	3
3	,,	21	9	2	1	33
4	,,	31	4	10	4	49
6	,,	46	18	19	4	87
8	,,	10	4	1	1	16
11	,,	20	3	5	2	30
12	,,	12	4	1	5	22
13	,,	20	4	10	—	34
18	,,	26	8	10	2	46
19	,,	35	13	13	6	67
20	,,	3	1	2	—	6
23	,,	1	7	9	4	41
25	,,	23	9	7	3	42
26	,,	24	15	14	3	66
27	,,	26	10	3	3	42
33	,,	33	14	9	7	63
17	Black imperfect	—	26	—	—	26
35	,,	—	27	—	—	27
34	,,	—	14	—	3	17
36	,,	—	35	—	9	44
38	,,	—	26	—	5	31
39	,,	—	39	—	15	54
40	,,	—	4	—	2	6
41	,,	—	5	—	1	6
44	Brown	—	—	34	—	34
46	,,	—	—	6	—	6
47	,,	—	—	7	—	7
28	,,	—	—	1	1	2
43	,,	—	—	29	10	39
45	,,	—	—	20	12	32

EXPLANATION OF PLATE I.

Figures 1–10. The seeds of *Glycine soja* showing the colour of the seed coat. Figures 11–17. The spikelets of *Oryza sativa* showing the colour of the awn, paleas and glume.

Fig. 1. "Waremame", buff.

Fig. 2. "Achumuri", solid black with the characteristic local breakings in the epidermis, through which the colourless underlying tissue is shown.

Fig. 3. F_3 seed "Waremame" × "Achumuri", solid black without breakings in the coloured epidermis.

Fig. 4. F_3 seed "Waremame" × "Achumuri", "imperfect black", the ground colour brown.

Fig. 5. F_3 seed "Waremame" × "Achumuri", brown.

Fig. 6. "Haiiro", brown with green tinge. The greyish green tinge shown in this figure changes to brown as shown in Fig. 10 when the seed is kept long.

Fig. 7. "Tanishi", blue tinged yellow.

Fig. 8. F_3 seed "Haiiro" × "Tanishi", non-tinged green.

Fig. 9. F_3 seed "Haiiro" × "Tanishi" non-tinged yellow.

Fig. 10. F_3 seed "Haiiro" × "Tanishi", brown.

Fig. 11. "Hanbun-nento". Early stage in the development of the pigment. The awn red, palaes brown, and glume red.

Fig. 12. Same as Fig. 11. Later stage showing the development of the brown colour in the paleas. The fully riped one is similar to that shown in Fig. 14.

Fig. 13. "Genroku-mochi", purple localized in the paleas.

Fig. 14. F_3 "Hanbun-nento" × "Genroku-mochi". Awn red, paleas brown.

Fig. 15–17. F_1 "Hanbun-nento" × "Genroku-mochi" showing the stages in the development of purple pigment in the awn, paleas, and glume. The fully developed stage is shown in Fig. 17.

POSTSCRIPT.

The reference should be made to the following papers which have been received after the manuscript left the writer's hand.

Takahashi, Y. and Fukuyama. J., (Morphological and Genetic Studies on the Soy Bean. Hokkaido Agric. Exp. Station Report No. 10, 1919.) reported the results of a number of crosses made between the different varieties grown in Hokkaido, in which certain colour characters of the seed coat are treated. Unfortunately the material is presented without genetical analysis owing to the incompleteness of the data. The following is the main results.

Pale yellow × green gave in F_1, a green seed and segregated in F_2, green and pale yellow by the ratio 3 : 1. In the subsequent generation, however, browns and reds appeared. Brown × pale yellow produced in F_1, a pale yellow seed, and segregated in F_2, 29 pale yellows and 2

reddish brown. Pale yellow × black produced in F_1, a pale yollowish green seed and in F_2, 20 greens, 20 pale greens, 10 pale yellows, 9 blacks, 2 browns and 4 reddish brown were found. Among greens and pale yellows, 17 and 7 were tinged with black of different shades respectively. Pale green × brown produced a pale green seed in F_1, and in the next generation pale green, yellow, black, and brown appeared by the following numbers: 27, 9, 15, and 5. Among the first two classes, six of them in each were tinged with blackish shade. Black × green produced in F_1, a pale yellowish green seed, and in F_2, pale green, green, reddish brown, and black were found.

O. Rosenheim (Observations on Anthocyanins. I. The Anthocyanins of the Young Leaves of the Grape Vine. Biochemical Jour. 14 : 178, 1920.) found certain chromogenic substances in the young leaves of the grape vine which gave an anthocyanin-like coloured substance by heating with hydrochloric acid. He called them "leuco-anthocyanin".

June, 1920.

Studies on the Genetics of Flower-Colours in *Portulaca grandiflora*.

By

S. Ikeno.

With Plate II.

CONTENTS.

ERRATA.

Page	Line	
125	22	insert *or successive* after *simultaneous*
126	14	insert *or successive* after *simultaneous*
129	7	read white-II × *orange* instead of white-II × *magenta*.

its flowering begins in June and continues till the end of October. Flowers are beautiful and pretty large; they begin generally to open at about 8 o'clock morning under sunshine, and wither at about the noon of the same day, though in dull weather or in late summer it may continue to open much longer. Each flower is provided with two sepals and five to six petals; there are very numerous slender filaments derived from five stamens by copious ramification; there is one ovary with one style and five to six radiating stigmas, which ripens into a pyxis containing a large number of tiny kidney-shaped seeds. In respect to the colour of the petals there are, so far as I know, five different kinds: they are, namely, white, yellow, orange, flesh-coloured, red, and magenta (s. Pl II), naturally with some fluctuations in their tone. Petals striped in various ways are also found. It may here be remarked that the flower-colour of *Portulaca* is always due to the cell-sap, and never to the chromoplasts.

Materials and Methods.

The breeding experiments of several colour-varieties of this plant were begun in 1915 to study the genetical behaviour of flower-colours. First of all, I have done the self-fertilisation of varieties cultivated in our Botanical Garden in Komaba as well as those obtained from other sources, including white, yellow, orange, red and magenta. Next year seeds got by such selfing were sown. Some of the varieties thus examined were found to segregate, proving themselves to be hybrids; all such ones were rejected, and only those which were found to breed true to their respective types were further cultivated, seeds being taken of course every generation on selfed flowers.[1]

From 1915 I have begun to cross these varieties in various ways, and are now concerned in studying the behaviour of their offspring. Many of these crosses were repeated, and also their offspring were also studied. A variety which bears flesh-coloured flowers was got first in 1917 through the kindness of Dr. I. NAGAI in the Agricultural Experiment Station in Oomagari near Akita; its hybridisation with some other colour-varieties was made in 1918, so that we had in 1920 their F_2-offspring.

[1] On account of poor germination of seeds I have lost the red parent in 1917 and the white-I one in 1918, but I have recovered each of them afterwards by extracting them from their respective hybrids made some years ago, and continue to cultivate them till now.

What makes the breeding of our plant very difficult is the poor germination of seeds. Though each pyxis contains a large number of them many do not come very often to germination; frequently I have met with the cases where even no one from one pyxis had germinated, so that some experiments described in this paper are based on rather few individuals. Many experiments have been carried on to overcome this difficulty. Since, according to my view, the poor germination of seeds would be chiefly due to the difficulty of water passage through seed-coats, one of my methods was to rub out seeds lightly with coarse quartz sand, so as to injure slightly seed-coats and make them easily permeable to water; this treatment has however given no good results whatever. Then I have tried the method of high pressure adopted by DE VRIES:[1] I have used an autoclave-like apparatus specially made for the purpose, and obtained the high pressure by the use of an iron receptacle containing oxygen under high pressure (150 atmospheres when full) instead of an automobile pump, because when the receptacle is connected with the apparatus, the high pressure is attainable instantaneously in the latter. Seeds soaked in water during one night and placed in it were subjected by this means to the pressure of 8 atmospheres or less during 24 hours, and then sown as usual. The following method was made use of in some cases: seeds were first treated with 60% or less concentrated sulphuric acid during 30 minutes, and after repeated washings, they were soaked in water during one night and then sown. The two latter methods above described seem to promote the germination to a certain degree, but not so much as might be wished for.

Seeds from different plants, and later those from different flowers of one individual were sown separately. Since seeds of *Portulaca* are very fine, if we pour down water to seed-pans from above as usual, seeds might often be thrown from one pan to another, thus causing the mixture of seeds from different parents. To avoid this possibility, no water has been given from above: a number of seed-pans were placed in a rectangular vessel of wood, 180 cm. long to 90 cm. wide and 10 cm. deep, partly filled with water, so that the latter may go up gradually.

My study on some of the crosses came already to a certain definite end, and I like to publish here its results, because they will, as I think, enable

[1] *Bot. Gaz.* Vol. 59, 1915, pp. 192–193.

us clearly to understand the genotypic constitution of all colour-varieties used in my experiments, except some few races. The behaviour of the various hybrids of flesh-coloured race was studied till F_2, and I think I am now able to make a certain conclusion about its genotypic constitution, but since my experimental results concerning this race are not yet complete, I will defer their publication to a future paper.

Flower-Colours.

The following colour-varieties were used in my experiments, viz. white, yellow, orange, flesh-coloured, red, and magenta (Pl. II). Besides, I could distinguish clearly two kinds of white, which are almost alike in external appearance, and yet genotypically different; they are called here *white-I* and *white-II* respectively (Pl. II, fig. 7–8).[1]

The colours of all parent varieties as well as their F_1-hybrids were recorded by means of "KLINCKSIECK et VALETTE, Code des Couleurs" (Paris, 1908); the results are indicated in the following Table:—

TABLE OF COLOURS.

Varieties and Crosses.	No. in the "Code."
Orange	176
Yellow	246
Flesh-colour	53C
Red	6
Magenta	between 551–556
Orange × white-I	191 or between 181–186
Orange × red	76
Magenta × white-I	566
Yellow × white-I	between 166–171
White-II × orange	516
White-II × magenta	551
Orange × magenta	536

[1] I think that I have discovered still another race of white, which is externally quite similar to white-I, and yet genotypically different from it. I will call it *white-III*; its behaviour will be described below (p. 120).

Flower-colours on one and the same individual are often more or less different in early and late summer, and also at different hours of one day. Colours in the above Table are those recorded in July and generally soon after the opening of flowers.

Factors.

Before proceeding further I will mention the factors concerned in the colour production of varieties used by me. Of course the action of all these factors has been discovered only after many breeding experiments have been carried on, but in my description I will take the opposite course, because I will mention the action of the factors at first, and then go on to the crossing experiments.

The factors producing the flower-colours of *Portulaca* are as follows:—

1. ***C***, fundamental factor for the production of any colour, ***cc***-plant being *white*; ***C*** alone, either in one or double dose, makes flower *orange*;

2. ***G*** (*gilvus*), changes the orange colour produced by ***C*** into *yellow*;

3. ***R*** changes the orange colour produced by ***C*** into *red*;

4. ***B*** which I will call *blueing factor* changes the red colour produced by the co-operation of ***C*** and ***R*** into *magenta*. ***B***, without ***R***, even in presence of ***C***, has no blueing action.

The factors specially concerned in the genotypic constitution of flesh-coloured and white-III races will not be considered in the present paper.

All my breeding experiments given below lead us to the conclusion that the varieties used by me may be expressed in respect to the colours of petals by the following genetical formulæ, if we adopt the presence-and-absence hypothesis:—

1.	Orange	***CCggrrbb***	(Pl II, fig. 3).
2.	Yellow	***CCGGrrbb***	(,, ,, ,, 4).
3.	Red	***CCggRRbb***	(,, ,, ,, 2).
4.	Magenta	***CCggRRBB***	(,, ,, ,, 1).
5.	White-I	***ccggrrbb***	(,, ,, ,, 8).
6.	White-II	***ccggRRBB***	(,, ,, ,, 7).

In describing the cross experiments below the letters ***gg*** are omitted in genetical formulæ, except in the Cross II.

Crossing Experiments.

Cross I. *White-I* × *orange* and *vice versa*. (Pl II, fig. 8 and 3).

ccrrbb* × *CCrrbb F_1 = ***Ccrrbb***

The white variety designated here by the name *white-I* has greenish stems and leaves, white petals, filaments, stigmas, and lightly greenish styles; while the orange variety has reddish stems and leaves, orange petals, red filaments, stigmas, and styles. The following crosses were made: orange × white-I in 1915, white-I × orange in 1916 and 1917. The F_1-plants have paler orange flowers than in the orange parent (s. the Table of Colours, p. 96). The offspring in F_2-and F_3-generation are indicated in the Table I; it may here be remarked that the difference of homo-and heterozygous orange plants in these generations is so slight in respect to their colour that we could

TABLE I.

F_2-generation (1916, 1917, 1918).[1]

F_1-parent.		No. of F_2-offspring. Orange	White.	Totals.
Orange × white-I.		42	14	56
White-I × orange, No. I.		17	7	24
„ „ „ II.		22	8	30
Total	Actual	81	29	110
	Expected	82·5±4·5†	27·5±4·5	110

F_3-generation (1917, 1918).

Colour of F_2-parent.	No. of selfed plants.		No. of F_3-offspring. Orange	White.	Totals.
Orange	4		131	0	131
		Expected	131	0	131
Orange	7		190	62	252
		Expected	189±6·9	63±6·9	252
White	4		0	28	28
		Expected	0	28	28

[1] The results of F_2-, F_3-generations, etc. in all my crosses were recorded in my field-book separately for each flower, but in the present paper the offspring derived from one cross are collected together for brevity's sake, except in some special cases.

† The figures affixed to the expected numbers denote always their respective standard errors calculated by the well-known formula $\sigma = \sqrt{npq}$.

hardly distinguish them exactly by their external appearance.

Thus we have in F_2 4 homo- and 7 heterozygous plants of orange colour, which accords, in spite of their small number, fairly well with the calculated numbers 3·7 and 7·3 respectively.

From all above described we see that the difference between orange and white-I varieties is due to one factor, and that this factor which we call ***C*** produces orange colour.

Though I have also raised the F_4 generation of the above cross it will not be perhaps worth while to describe here its details, and it may suffice simply to say that it has fully confirmed the results of the former generations.

During some generations of the above cross certain peculiar phenomena were often met with, which might somewhat confuse the Mendelian results obtained; thus, for instance, few *magenta* and *red* progeny are produced from seeds taken on orange or even white plants, and also few *orange* ones from those taken on white plants, etc. (naturally seeds being taken on selfed flowers). These phenomena will, according to my view, chiefly, though not all, belong to the so-called "reverse mutations," and since they were observed in many other cases they will be pointed out each time in the course of my description, and discussed together later in a separate chapter (s.p. 121 ff).

Cross II. *Yellow* × *white-I.* (Pl II, fig. 4 and 8).

CCGGrrbb* × *ccggrrbb F_1 = ***CcGgrrbb.***

In order to study the genotypic constitution of the yellow variety I have done, firstly, its cross by the white-I (1915), and secondly, the two reciprocal crosses between it and the orange (1917). The F_1-hybrid produced by the latter crosses has borne flowers where yellow and orange patches of various size are irregularly scattered on each petal; in F_2 we were however impossible to exactly determine yellow and orange individuals, which we might have expected to have been produced by segregation, and the experiment was abandoned, at least for a time. As to the first of the crosses above cited, I was much more fortunate, though on account of the poor germination of seeds the behaviour of the F_3-generation could not be so fully investigated as might be wished for.

The F_1-hybrid made by crossing it by white-I has borne yellow flowers whose colour is nearly the same, or even more intense than in the yellow parent (s.

the Table of Colours, p. 96). The F_2-offspring were composed as follows :—

TABLE II.

F_2-generation (1916).

	Yellow	Orange	White	Total.
Actual	71	23	37	131
Expected	73·69±5·68	24·56±4·47	32·75±4·95	on 9 : 3 : 4 basis
	65·50±5·72	32·75±4·95	32·75±4·95	on 2 : 1 : 1 „

Thus the actual numbers of the three classes of the F_2-offspring agree with the expected ones, either on 9 : 3 : 4 or on 2 : 1 : 1 basis, though the agreement is much closer in the former than in the latter case. The final decision which alternative will be here realised, would however be possible only after the examination of the F_3-offspring ; thus if the segregation under discussion will belong to the 2 : 1 : 1 type all yellows should be heterozygous, and all oranges homozygous, whilst if it will belong to the 9 : 3 : 4 type some yellows should be homozygous, and some oranges heterozygous. Unfortunately on account of the poor germination of seeds the F_3-generation contains very few individuals, and the results are consequently rather imperfect, because I was able to examine the F_3-offspring of only 2 oranges, 3 whites and 3 yellows in all of which generally very few seeds came to germination, viz. :—

1 orange has thrown almost exclusively oranges (22 in all),[1]

1 orange has segregated into 9 oranges and 2 whites,

3 whites have thrown whites only (19 in all),

1 yellow has thrown only 1 yellow,

1 yellow has segregated into 5 yellows and 1 white,

1 yellow has segregated into 6 oranges and 1 white.

The fact that the ratio of segregation in our case should be 9 : 3 : 4 may be concluded from the presence of one heterozygous orange just cited, because in the 2 : 1 : 1 type no orange should be heterozygous. This orange has undergone

[1] Besides 22 oranges 1 magenta was found among the progeny. As discussed in Chapter "Mutations, etc.", I (p. 121 ff) this 1 magenta is regarded to have been produced by the reverse mutation, and consequently the orange parent is considered to be of the composition ***CCggrrbb*** (s. also Table VIII, No. 9 ; discussion, p. 125).

the segregation into 9 oranges and 2 whites, which agree fairly well with the calculated numbers, 8·25±1·43 and 2·75±1·43 respectively, and may be represented by ***Ccggrrbb,*** whilst another orange is homozygous and has the constitution ***CCggrrbb.*** —As above stated, of three yellows of which I could have examined the offspring one has thrown only 1 yellow, and is quite useless for our experiment. The second has segregated into 6 oranges and 1 white, and since there will be no yellow which will show such segregation without throwing any yellow at all, it seems to me to be probable that the ratio is here really 9 yellows : 3 oranges : 4 whites, of which no yellow did germinate (i.e. ***CcGgrrbb***). The third has produced 5 yellows and 1 white: it has segregated either in the ratio of 3 yellows and 1 white (i. e. ***CcGGrrbb***), or in that of 9 yellows, 3 oranges, and 4 whites like the second, of which no orange did germinate. No homozygous yellow (***CCGGrrbb***) came under my observation, and this is not to be astonished, because we should have only 1 such out of 9 yellows. Our conclusion is therefore that we have in our case in F_2 the 9 : 3 : 4 type of segregation, that the yellow variety has in respect to its flower-colour one more factor than the orange, and that consequently the cross between yellow and white-I varieties is based on the two factors difference, viz. ***C*** and ***G***.

Cross III. *Red* × *orange* and *vice versa* (Pl II, fig. 2 and 3).

CCRRbb × ***CCrrbb*** F_1 = ***CCRrbb***

The F_1-hybrid has red flowers whose colour intensity is almost similar to that of the red parent (s. the Table of Colours, p. 96). The F_2-and F_3-offspring are composed as in Table III (s. p. 102) :—

As will be seen from this Table, though 6 out of 8 oranges have given rise in F_3 exclusively to oranges (=70), one of the remaining two has thrown 3 oranges and 1 red, and another 5 oranges and 20 reds. Despite all such facts these two orange parents (i. e. F_2 plants) are considered to be homozygous like all others, so that all 8 oranges in F_2 are to be expressed by the formula ***CCrrbb*** (s. Table VIII, Nos. 19 and 11, and also discussions about them in Chapter "Mutations, etc.," IV and II respectively, p. 128 and p. 127).

As regards 11 reds in F_2 whose behaviour in F_3 I could have examined 6 were proven to be homo-, and 5 to be heterozygous (expected, 3·65

Table III.

F_2-generation (1917).[1]

F_1-parent.	No. of F_2-offspring.		
	Red	Orange	Totals
Red × orange	21	6	27
Orange × red	27	11	38
Totals	48	17	65
Expected	48·75±3·5	16·25±3·5	65

F_3-generation (1918).

Colour of F_2-parent	No. of selfed plants	No. of F_3-offspring.		
		Red	Orange	Totals.
Red	6	35	0	35
		Expected 35	0	35
Red	5	85	23	108
		Expected 81±4·5	27±4·5	108
Orange	6	0	70	70
		Expected 0	70	70
*Orange †	1	1	3	4
		Expected 0	4	4
*Orange	1	20	5	25
		Expected 18·75±2·2	6·25±2·2	25

homo: 7·30 hetero) (s. Table III), so that the ratio of individuals in these two classes of reds does not well agree with what we might expect theoretically, but much importance should not be laid on this fact in view of the small number of individuals.

From the above experiments we see that the difference between orange and red is due to one factor which we call ***R*** and which changes the orange caused by ***C*** into red.

[1] Though, as may be seen from the results in F_3-generation, there are two kinds of reds (homo- and heterozygous), they are collected in F_2 under the class *red*, because they are not exactly distinguishable from each other by their external appearance.

† The parent prefixed with an * denotes one which has produced some unexpected individuals among its offspring. Thus, for instance, in this No. we find besides 3 oranges 1 red which was quite unexpected. For the discussion of these phenomena s. pp. 124.

Cross IV. *White-I* × *white-II* and *vice versa*, etc. (Pl II, fig. 8 and 7).

$$ccrrbb \times ccRRBB \qquad F_1 = ccRrBb.$$

At the beginning of my breeding experiments I have conducted the crosses between the various white varieties, because it was thought not to be impossible that the cross of two certain whites might produce the progeny with coloured flowers, as in the classical example of Sweet Pea. For instance, the cross between white-I and white-II and its reciprocal have been done in 1915; all F_1-offspring were found to bear white flowers in both cases, and it was the same in F_2-progeny. The crosses between all white varieties, including white-III also, made in various ways agree in the fact that they never give rise to coloured plants, both in F_1 as well as F_2, and evidently in any further generation. The treatment of flowers of all these varieties by ammonia vapour according to Miss WHELDALE[1] and SHIBATA[2] does not give yellow reaction, indicating that they do not contain flavones. All white plants are *cc*, because the presence of *C* produces the orange colour, as above stated.

Cross V. *White-I* × *magenta* and *vice versa*. (Pl II, fig. 8 and 1).

$$ccrrbb \times CCRRBB \qquad F_1 = CcRrBb$$

The cross, white-I × magenta and its reciprocal were made in 1916 and 1917 respectively. In both F_1-hybrids we see that magenta is almost perfectly dominant to white (s. the Table of Colours, p. 96). On account of very poor germination of seeds the number of individuals is rather small, especially in F_2, but the actual results agree fairly well with the theoretical expectation. The Table IV indicates the results of the F_2- and F_3-generation. (s. p. 104).

We have thus in F_2 homozygous magentas (*CCRRBB*): magentas segregating into 3 magentas and 1 orange (*CCRrBb*): magentas segregating into 3 magentas and 1 white (*CcRRBB*): magentas segregating into 9 magentas, 3 oranges, and 4 whites (*CcRrBb*) in the ratio 1 : 1 : 1 : 4, their expected numbers being 0·8 : 1·6 : 1·6 : 3·1 respectively. Furthermore, we have 5 homo- and 6 heterozygous oranges, while their theoretical numbers are 3·65 and 7·30 respectively. Of 7 whites 5 have produced only whites.

[1] *Journ. of Genetics*, Vol. 4, 1915, p. 113.

[2] *Bot. Mag.*, Tôkyô, Vol. 29, 1915, pp. 121–122.

Of the remaining 2 one has produced 1 orange besides 13 whites, and another nothing but 1 orange; as discussed in Chapter ' Mutations, etc.", I, I consider both, in spite of such facts, to be the whites of the constitution ***ccrrbb*** (s. Table VIII, Nos. 12 and 13; and discussions about them, p. 124), so that all 7 white parents here are regarded to be genotypically equivalent, viz. ***ccrrbb***.

TABLE IV.

F_2-generation (1916, 1917).

F_1-parent	Magenta	Orange	White.	Totals
Magenta × white-I	126	51	55	232
White-I × magenta	16	2	10	28
Totals	142	53	65	260
Expected	146·25±7·99	48·75±6·29	65±6·98	260

F_3-generation from white-I × magenta (1917).

Colour of F_2-parent	No. of selfed plants		No. of F_3-offspring: Magenta	Orange	White	Totals
Magenta†	1		2	0	0	2
		Expected	2	0	0	2
Magenta	1		3	0	1	4
		Expected	3	0	1	4
Magenta	1		5	4	0	9
		Expected	6·75±1·3	2·25±1·3	—	9
Magenta	4		84	27	28	139
		Expected	78·19±5 8	26·06±4·6	34·75±5·1	139
Orange	5		0	48	0	48
		Expected	0	48	0	48
Orange	6		0	101	24	125
		Expected	0	93·75±4·8	31·25±4·8	125
White	5		0	0	78	78
		Expected	0	0	78	78
*White	1		0	1	13	14
		Expected	0	0	14	14
*White	1		0	1	0	1
		Expected	0	0	1	0

† The number of segregates from this magenta parent is so small that it is considered to be homozygous only provisionally.

From Table IV as well as what I have just stated we will see that we have here apparently to deal with a typical case of dihybrid segregation.

In the various cases studied till now by several authors it was found that for the production of bluish-red colour (magenta, purple, etc.) the factor for producing the red anthocyanin and that for changing the latter into bluish-red one participate; thus, for instance, in flowers of *Lathyrus odoratus* (***R*** and ***B***, BATESON)[1], and of *Antirrhinum majus* (***F, R, D,*** BAUR;[2] ***R*** or ***L, T*** and ***B,*** Miss WHELDALE),[3] in the aleurone of Maize (***R*** and ***P,*** EAST and HAYES;[4] ***R*** and ***Pr,*** EMERSON),[5] etc. The question naturally arises whether the magenta colour of flowers of *Portulaca* is not also due to the combined action of such factors. The results of our present cross do furnish, as will be seen from what was stated above, no positive evidence towards such a conclusion, but some other breeding experiments, especially the Cross VIII (p. 112), prove beyond all doubts that the magenta colour in our case is due, quite similarly as in all cases above cited, to the action of the two factors which I call ***R*** (reddening) and ***B*** (blueing) respectively. We have therefore in the hybrid white-I × magenta or its reciprocal a trihybrid instead of a dihybrid, inasmuch as it may be expressed by the genetical formula ***CcRrBb***, as above given. If the three factors ***C, R, B*** contained in this hybrid will make free assortment, we should have eight kinds of male and female gametes, i.e. ***CRB, cRB, CrB, crB, CRb, cRb, Crb, crb***, and consequently the ratio of 27 magentas : 9 reds : 12 oranges : 16 whites in F_2.[6] The reason why notwithstanding this we have in our case the ratio of 9 magentas : 3 oranges : 4 whites in F_2 will be seen, when we think that of the three factors ***C, R, B*** contained in magenta the two latter are in the state of complete "coupling" or "linkage" (to use the word more frequently adopted recently), and act just like one single factor, so that we have here simply four kinds of male and female gametes, i.e. ***CRB, cRB, Crb, crb.*** If ***R***

1 MENDEL'S *Principles of Heredity*, p. 91.

2 *Zeitsch. f. ind. Abstamm. u. Vererbungs'ehre*, Bd. 3, 1910, pp. 41–43.

3 Ibid., p. 326; also *Journ. of Genetics*, Vol. 4, 1915, p. 110.

4 *Conn. Agric. Exp. Stat., Bull.* No. 167, 1911.

5 *Cornell Univ. Agric. Exp. Stat.,Memoirs* 16, 1918.

6 This is somewhat similar to the F_2-generation of the classical example of Sweet Pea (s. BATESON, l. c., p. 91), though we have in the latter 28 whites instead of 12 oranges and 16 whites, since the factor ***C*** does not produce any colour in Sweet Pea.

and ***B*** were always absolutely linked to each other, we will have naturally no means of discerning the composite nature of this factor-complex, but sometimes the linkage is broken down, at least partially, and we are then enabled to disclose its real nature (s. the Cross VIII, p. 112 ff.)

Cross VI. *White-II* × *magenta*. (Pl. II. fig. 7 and 1).

ccRRBB × ***CCRRBB*** $F_1 = $ ***CcRRBB***.

Though white-II is externally very similar to white-I, it may differ sometimes from the latter in certain respects. The white-II bears white petals like the white-I; but sometimes (not always) they have few magenta stripes or spots; filaments are white, but often some few ones are magenta (s. p. 120). The F_1-hybrids (1918) bear magenta flowers, whose colour intensity is almost perfectly similar to that of the magenta parent (s. the Table of Colours, p. 96). As the white-I and white-II are genotypically different from each other, the composition of the F_2-offspring produced ex white-II × magenta is quite different from that of those ex white-I × magenta (Cross V). Thus we have the results shown in the Table V (s. p. 107.)

In this Table we see both in F_2 and F_3 the production of a certain number of unexpected individuals. Thus 8 out of 10 F_1-plants have segregated in F_2 into magentas and whites, as was just expected, whereas each of the remaining two has produced besides these two classes of the segregates 1 orange which is quite unexpected. The formation of these two oranges is very difficult to be accounted for, and it might be due to the contamination from other families, though utmost care was taken for avoiding such. Till the contrary to the latter assumption will be definitely established these two oranges will not be taken into account, and then we see clearly that each of these F_1 parents is of the constitution ***CcRRBB***.

In F_3 generation three magentas have produced among others 1 flesh-coloured, 1 orange and 1 pseudo-white (Table V, F_3, Nos. 2, 3, 4), and two other magentas 3 flesh-coloured and 5 red plants (Table V, F_3, Nos. 6 and 7). Since the genotypic constitution of flesh-coloured and pseudo-white races is not yet exactly known it is naturally impossible to make any surmise about the mode of their production (though probably by mutation), and these unexpected plants must here be left out of account. The production of the orange and

Table V.

F_2-generation (1919).

F_1-parent	No. of plants selfed	No. of F_2-offspring			
		Magenta	White	Orange	Totals
White-II × magenta	8	509	163	0	672
* „ „ „	1	107	48	1	156
* „ „ „	1	10	2	1	13
Totals	10	626	213	2	841
Expected		630·75±12·6	210·75±12·6	0	841

F_3-generation (1920).

Colour of F_2parent	No. of plants selfed.	No. of F_3-offspring						
		Magenta	White	Red	Flesh	Orange	Pseudo-white[1]	Totals
Magenta, No. 1	3	113	42	—	—	—	—	155
* „ „ 2	1	28	13	—	—	1	—	42
* „ „ 3	1	40	23	—	1	—	—	64
* „ „ 4	1	40	3	—	—	—	1	26
Totals	6	203	81	—	1	1	1	287
Expected		215·25±7·3	71·75±7·3		0	0	0	287
Magenta, No. 5	2	36	—	—	—	—	—	36
* „ „ 6	1	35	—	—	1	1	—	37
* „ „ 7	1	12	—	5	2	—	—	19
Totals	4	83	—	5	3	1	—	92
Expected		92	—	0	0	0	—	92
White, No. 1	4	—	72	—	—	—	—	72
* „ „ 2	1	2	42	—	—	—	—	44
* „ „ 3	1	5	77	—	—	—	—	82
Totals	6	7	181	—	—	—	—	188
Expected		0	188	—	—	—	—	188

the red in this case might be due to the so-called "loss-mutation," (s.p.127). If what is above stated will be taken into consideration we have clearly to think the first three magenta parents to be of the composition ***CcRRBB***,

[1] For pseudo-white s. p. 119.

and the remaining two of that ***CCRRBB***. Of 6 whites 2 (Table V, F_3, Nos. 2 and 3) have produced besides white offspring 7 magenta ones. That each of these two white parents is to be regarded to have the constitution ***ccRRBB*** will be discussed later in this paper (s. Table VIII, Nos. 21 and 22, discussions in "Mutations, etc." I).

From the results in F_3 above indicated we see that we have in F_2' 4 homo- and 6 heterozygous magentas (expected, 3·3 and 6·6), and that all whites are homozygous, whence we may conclude that the actual and the expected results agree fairly well to each other. Hence it is evident that this cross is based upon one single factor difference. We can also easily understand the reason why this cross will produce quite different results from those in the Cross V where white-I is used instead of white-II, because the latter contains both ***R*** and ***B*** like our magenta variety, while white-I has none.

Cross VII. *White-II* × *orange* and *vice versa*. (Pl. II, fig. 7 and 3),

ccRRBB × ***CCrrbb*** F_1 = ***CcRrBb***

The cross between white-I and orange has been described before (s. p. 98 ff). That white-II is genotypically different from white-I in spite of their external resemblance is especially clear, when we make the cross between the former and the orange, because we get then quite different results: the F_1-hybrids, whether from white-II × orange or its reciprocal, bear always magenta flowers, whose colour intensity is almost equal to that in our magenta variety (s. the Table of Colours, p. 96). As indeed white-II (***ccRRBB***) may be considered to be a magenta variety which remains colourless on account of the absence of ***C***, it is quite natural that its mating with the orange will produce the magenta, ***C*** being introduced from the latter parent. The genotypic constitution of the F_1-hybrid in the present case is therefore perfectly equal to that in the Cross VI (p. 106), so that the composition of the F_2- and F_3-offspring should be naturally quite the same as in those derived from the same cross. This fact could be perfectly confirmed experimentally, as we will see from the F_2 and F_3 offspring presented in the Table VI, A and B.

TABLE VI, A.

F_2-generation (1916, 1917, 1920).

F_1-parent.	No. of the F_2-offspring				Totals.
	Magenta	Orange	White	Red	
White-II × orange, No. 1	35	13	17		65
* „ „ „ „ 2	81	20	34	1†	136
Orange × white-II	96	50	43		189
Totals	212	83	94	1	390
Expected {	219·37±9·8	73·12±7·7	97·50±8·6	0	390††
	195·00±9·9	97·50±8·6	97·50±8·6	0	390†††

As we see from the above Table, the actual results in F_2 agree with the theoretical, either on 2 : 1 : 1 or 9 : 3 : 4 ratio, just as in the Cross II, though somewhat better on the latter basis, and the question which possibility will here occur in reality was decided by raising the F_3-generation ex white-II × orange, No. 1 and orange × white-II cited in the Table VI, A. The results of this cultivation are shown in Table VI B (s. p. 110):—

As we see in this Table magenta No. 2 has produced 75 magentas and 42 reds, and yet it is to be regarded as having the constitution ***CCRRBB*** (s. Table VIII, No. 23; discussion in Chapter "Mutations, etc.," III, p. 127). In respect to the fact that magenta No. 6 should be considered to be ***CcRrBb*** s. Table VIII, No. 24, and discussion, p. 129. — The orange No. 2 is considered to be ***CCrrbb,*** and the orange Nos. 4 and 5 to be ***Ccrrbb*** (s. Table VIII, Nos. 25, 26, and 27; discussions, in "Mutations, etc." I).— White No. 2 composed of 4 plants has produced together besides whites some magentas and reds; each of these 4 is despite this fact regarded to be ***ccrrbb*** (Table VIII, No. 28; discussion in Chapter "Mutations, etc.," III). From the Table VI as well as from what was just stated the following conclusion may be drawn:—we have in F_2 homozygous magentas (***CCRRBB***): those segregating into 3 magentas and 1 orange (***CCRrBb***): those segregating into 3 magentas and 1 white (***CcRRBB***): those segregating into 9 magentas, 3 oranges, and 4 whites (***CcRrBb***), in the ratio 3 : 9 : 7 : 7,

† This 1 red is not taken here into consideration; s. the foot-note p. 110.
†† On 9:3:4 basis.
††† On 2:1:1 basis.

Table VI, B.

F_3-generation (1917, 1918).

Colour of F_2-parent	No. of selfed plants		No. of F_3-offspring: Magenta	Red	Orange	White	Totals
Magenta, No. 1	2		36	——	——	——	36
		Expected	36	——	——	——	36
* „ „ 2	1		75	42	——	——	117
		Expected	117	0	——	——	117
„ „ 3	9		109	——	37	——	146
		Expected	109·5±5·3	——	36·5±5·3	——	146
„ „ 4	7		61	——	——	26	87
		Expected	65·25±4·04	——	——	21·75±4·04	87
„ „ 5	6		40	——	21	24	85
		Expected	47·8±4·8	——	15·9±3·6	21·2±4·0	85
„ „ 6†	1		28	2	3	11	44
		Expected	s.p. 129	——	——	——	——
Orange, „ 1	7		——	——	108	——	108
		Expected	——	——	108	——	108
* „ „ 2	1		——	1	1	——	2
		Expected	——	0	2	——	2
„ „ 3	9		——	——	190	73	263
		Expected	——	——	197·25±7	65·75±7	263
* „ „ 4	1		12	2	45	30	89
		Expected	0	0	66·75±4·1	22·25±4·1	89
* „ „ 5	1		1	——	4	2	7
		Expected	0	——	5·25±1·1	1·75±1·1	7
White „ 1	18		——	——	——	215	215
		Expected	——	——	——	215	215
* „ „ 2	4		7	——	5	42	54
		Expected	0	——	0	54	54

theoretically 2·9 : 5·8 : 5·8 : 11·6 respectively. Furthermore, we have 8 homo- (***CCrrbb***) and 11 heterozygous oranges (***Ccrrbb***), theoretically 6·3 and 12·6 respectively. Besides, all whites are considered to be homozygous, as above

† That the appearance of 2 reds in the offspring of this No. which is considered to be of the genotypic constitution ***CcRrBb*** is due to the formation of a certain number of the gametes ***CrB, CRb, crB, cRb,*** i.e. to the change of the complete "coupling" between ***R*** and ***B*** into a partial, will be discussed p. 129. The appearance of 1 red in F_2 (s. Table VI, A, white-II × orange, No. 2) may also be due to the same cause.

stated. We have therefore proven that we have in F_2 the ratio 9 magentas : 3 oranges : 4 whites, and we see that in this generation we have the complete linkage of ***R*** and ***B*** just as in Cross V.

That the F_1-hybrid under discussion produces the gametes ***CRB***, ***cRB***, ***Crb***, ***crb***, but not ***CrB***, ***crB***, ***CRb***, ***CRb*** (except very rare cases, s. Table VI, B), will be seen also by crossing it back by white-I (***ccrrbb***), i. e. that variety which contains neither ***C*** nor ***R*** nor ***B***, thus, for instance, this crossing was done in 1919, and we had in 1920 :—

	Magenta	Orange	White	Total
(White-I × orange) × white-I	27	21	36	84
Expected	21±4·0	21±4·0	42±4·6	84

This cross indicates us clearly what kinds of gametes are produced by the F_1-hybrid, and from the results we see that ***B*** and ***R*** are completely linked to each other, because if the free assortment were the case, we should have the ratio 1 magenta : 1 red : 2 oranges : 4 whites instead of that 1 magenta : 1 orange ; 2 whites, as it was actually the case.

The experiment of Miss YASUI on the crossing of white and pale yellow, giving rise to magenta F_1-plants,[1] seems to agree with my present cross in several respects, though she has used pale yellow instead of orange. Though her experiment ends with F_2, the actual numbers of magentas, yellows and whites in the latter generation accords pretty well with the expected, and there will be perhaps no doubt that she had to deal in this generation with the 9 : 3 : 4 segregation. Her explanation in respect to the appearance of magentas in F_1 agrees also with what I have above stated about the same phenomenon ; the difference between her view and mine lies however in the fact that while she considers the magenta colour to be due to one single factor ***R*** (in co-operation with ***C***), I regard the same colour to be due to the factor-complex ***RB*** (naturally in co-operation with ***C***). Though from the analogy with several cases studied till now by many authors the combined action of some such factors for the production of the magenta colour seemed to me *a*

[1] *Bot. Mag.*, Tôkyô, Vol. 34, 1920, pp. 59–63.

priori very probable, I could find no such indication at the beginning of my experiment. On studying, however, the F_3-generation of the present cross, I have found some reds in the offspring of one magenta F_2-parent (Table VI, B: Magenta, No. 6). This points out naturally towards the composite nature of the magenta factor, and I was able to establish the fact by the Cross-experiment VIII.

Cross VIII. *Magenta* × *orange*. (Pl. II, fig. 1 and 3).

$$\boldsymbol{CCRRBB \times CCrrbb} \qquad F_1 = \boldsymbol{CCRrBb}.$$

The F_1-hybrid bears magenta flowers (s. the Table of Colours, p. 96). The F_2-offspring are composed as in the following Table :—

TABLE VII, A.

F_2-generation (1917).

No. of F_1-plants selfed	No. of F_2-offspring		
	Magenta	Orange	Total
20	151	73	224
Expected	168±6·5	56±6·5	224

The deviation of the actual results from the theoretical calculated on the 3 : 1 basis is 17, and consequently 2·6 times the standard error (=6·5). Thus the actual results are not in very good agreement with the expected; the chief cause of this discrepancy is in all probability to be sought in the poor germination of seeds, and I think that we have here in spite of discrepancy a case of segregation into 3 magentas and 1 orange.[1] The lack of reds in this case is evidently due to the absolute linkage of ***R*** and ***B***, as it was the case in the Cross V, VI and VII, or at least to the linkage of very high intensity where so few reds are expected that they will not appear at all unless an enormous number of plants are in cultivation.

Now what is very remarkable about the F_3-offspring derived from the F_2-plants under discussion is the fact that some of the magentas have under-

[1] We may have 1 such case out of about 112 trials as the result of random sampling.

gone the segregation into *magentas*, *reds* and *oranges* instead of that into magentas and oranges simply, as will be seen from the following Table:—

Table VII, B.

In this Table the offspring derived from each magenta individual are exceptionally recorded separately.

F_3-generation (1918).

Colour of F_2-parent	No. of selfed plants	No. of F_3-offspring			
		Magenta	Red	Orange	Totals
Magenta, No. 1	1	1†			1
„ „ 2	1	6			6
„ „ 3	1	16			16
„ „ 4	1	33			33
Totals	3	55			55
Expected		55			55
„ „ 5	1	7		1	8
„ „ 6	1	6		4	10
„ „ 7	1	14		1	15
„ „ 8	1	3		2	5
„ „ 9	1	3		1	4
„ „ 10	1	6		4	10
„ „ 11	1	15		2	17
„ „ 12	1	4		1	5
Totals	8	58		16	74
Expected		55·5±3·7		18·5±3·7	74
„ „ 13	1	54	5	16	75
„ „ 14	1	6	2	3	11
„ „ 15	1	3	1	1	5
„ „ 16	1	7	2	0	9
„ „ 17	1	0	1	1	2
„ „ 18	1	12	2	6	20
„ „ 19	1	18	3	6	27
„ „ 20	1	17	1	9	27
„ „ 21	1	12	1	4	17
„ „ 22	1	20	1	10	31
„ „ 23	1	32	1	8	41
Totals	11	181	20	64	265
Expected	s. below(p.114)				
Orange	12			201	201
Expected				201	201

† This is not entered in the total.

From the results given in the Table VII, A we expect to have 1 ***CCRRBB***, 2 ***CCRrBb*** and 1 ***CCrrbb*** in the F_2-generation. In the Table VII, B showing the F_3-offspring we have the F_2-parents of magenta colour, No. 1–23. Of these No. 1 has produced only 1 magenta plant and is not entered in the total, because it indicates naturally nothing for our experiment. Nos. 2–4 belong to ***CCRRBB***, and Nos. 5–23 to ***CCRrBb*** whilst each of 12 oranges belongs to ***CCrrbb***, because it has produced, nothing but oranges. We have thus 3 ***CCRRBB***: 19 ***CCRrBb***: 12 ***CCrrbb***, theoretically 8·5 : 17·0 : 8·5. Of the heterozygous magentas ***CCRrBb*** (i. e. Nos. 5–23) each of Nos. 5–12 has segregated into magentas and oranges, just as did the F_1-hybrid in F_2, their approximate ratio being 3 : 1 in total, and evidently this is so, because in these magentas the factors ***R*** and ***B*** remain in absolute linkage. On the contrary, in each of magentas, Nos. 13–23, we observe the appearance of the *reds*, and this is clearly due to the breaking down of the complete linkage between these factors. Since we have in all 181 magentas, 20 reds and 64 oranges, i.e. 7·6% reds, there are too few reds to consider that the free assortment has taken place between ***R*** and ***B***, because we should have in the latter case nearly 149 magentas, 50 reds and 66 oranges (9 : 3 : 4), i.e. 18·9% reds. It is quite evident that in our present case the linkage has been *changed from complete to partial*, or at least has changed its intensity from very high to low. What is then the ratio of "coupling" or "linkage" in the latter case? For its determination the following calculations were made:—

	Magenta	Red	Orange	Totals	
	(***CRB***)	(***CRb***)	(***CrB*** + ***Crb***)		
Actual	181	20	64	265	
Expected	178·5±7·6	20·2±4·5	66·2±7·1	265	on 5 : 1 : 1 : 5 basis
	181·2±7·6	17·6±4·2	66·2±7·0	265	„ 6 : 1 : 1 : 6 „

If we calculate the closeness of fit by the method of PEARSON we find for the first and the second case $\chi^2 = 0{\cdot}1099$ and $0{\cdot}4006$ respectively, each of which should indicate an almost perfect agreement of the actual number with the

theoretical.[1] So it is clear that we have here to deal with a case of linkage belonging to either one of the two above series or at least to some series similar to it.

To sum up: the cross magenta × orange has segregated in F_2 in the ratio of 3 magentas and 1 orange, because ***R*** and ***B*** are completely linked together.; in F_3 the linkage became partial in some magentas, and consequently their F_3-offspring contain *reds* besides magentas and oranges.

If we will adopt the chromosome theory of MORGAN we have, for instance, the gametes ***CRB, CRb, CrB, Crb*** in the ratio 5·5 : 1 : 1 : 5·5 (5·5 being the average of 5 and 6 in the two series of linkage above cited), and consequently 15·4% cross-overs and 84·6% non-cross-overs respectively.[2] The following consideration may then be made. The factors ***R*** and ***B*** are located very near in the same chromosome, and consequently closely linked together as the rule. But sometimes the crossing-over takes place, so that they come to lie in two different, though homologous, chromosomes, the ratio of cross-overs against non-cross-overs being found to be 15·4 : 84·6.

The facts which point out towards the variation of the linkage ratio of certain factors have been sometimes observed till now. To cite some instances, GREGORY in his researches on *Primula sinensis*, has found in the F_2-offspring ex ***Ms*** × ***mS*** (magenta and long-styled by red and short-styled) the complete linkage between ***M*** and ***S***, whereas in those ex ***MS*** × ***ms*** (magenta and short by red and long) the partial one belonging to the series 7 : 1 : 1 : 7 has been observed between the same factors.[3] BAUR, in his experiments in *Antirrhinum majus*, has found in the F_2-offspring ex ***FFGG*** × ***ffgg*** (red and *picturatum* flower by non-red and *non-picturatum*) that the linkage ratio between ***F*** (red) and ***G*** (*picturatum*) is variable in different cases (3 : 1 : 1 : 3, 4 : 1 : 1 : 4, 7 : 1 : 1 : 7, or even 1 : 1 : 1 : 1, i. e. normal).[4] In *Lathyrus odoratus* BATESON

[1] S. PEARSON, *Tables for Statisticians and Biometricians*, Cambridge, 1914, Table XII.

[2] If we calculate the linkage ratio according to the formulæ of EMERSON (Amer. Naturalist, Vol. 50, 1916, pp. 1411-1429) we have from the corrected phænotypic ratios ***CRB*** : ***CRb*** : ***CrB*** : ***Crb*** = 178·75 : 20 : 20 : 46·25 (from the actual 181 : 20 : 20 : 64-20 = 181 : 20 : 20 : 44) $r = 6{\cdot}80$ and $s = 1{\cdot}34$ and consequently the linkage ratio = $r/s = 5{\cdot}0 : 1$.

[3] *Journ. of Genetics*, Vol. 1, 1911, p. 129.

[4] *Zeitsch. f. ind. Abstamm. u. Vererbungslehre*, Bd. 6, 1912, p. 204 ff; and *Einleitung in die*

and PUNNETT have found in F_2 ex $BL \times bl$ (purple flower and long pollen by red and round) the linkage of the series 7 : 1 : 1 : 7 between B and L, whilst in further generations of the same cross that of the series 15 : 1 : 1 : 15 has been observed;[1] also in the same plant and in the same kind of the cross PUNNETT has observed sometimes the 7 : 1 : 1 : 7 linkage, and sometimes the 10 : 1 : 1 : 10 one.[2] Again, in the progeny of one of his Maize hybrids (endosperm horny-waxy, coloured-white) KEMPTON has generally detected the linkage belonging to the series 3 : 1 : 1 : 3, whilst in the progeny derived from the same parents he could find that of the series 3 : 2 : 2 : 3, though exceptionally.[3] Recently experiments to modify artificially the amount of linkage in *Drosophila* by the influence of varying temperatures have been carried on with success by PLOUGH.[4] The variation of the linkage ratio in the cross under discussion is thus no unprecedented fact; whether it is due to a certain change in the internal mechanism, or to the influence of the environment, or to certain other reasons is however yet unsettled, and will be the interesting subject of future studies. Furthermore, though in our present case we have seen the complete linkage of R and B in F_2 and its change into a partial in F_3, it is very doubtful whether such would be always the case. It seems to me very probable that there might be several modifications. Thus, for instance, the absolute linkage of R and B seen in F_2 might remain as such in F_3; on the contrary, their partial linkage or even their free combination might occur already in F_2, etc., etc. Still further, some other important questions will await the answer. Thus the fact should be determined whether the ratio of linkage will remain constant in all cases. Also it will be necessary to investigate the fact whether or not this ratio will be equal on the male as well as the female side of one and the same plant. As one of the extreme cases of the inequality of the linkage ratio on the two sides we might have, for instance, that where the linkage is complete on one side and partial on the other; nor would it be not impossible that on the one side we

experimentelle Vererbungslehre, 3. u. 4. Aufl., 1919, p. 172 ff.

[1] *Report to the Evolution Committee R. S.*, Report 4, 1909, p. 11 ff.

[2] *Journ. of Genetics*, Vol. 3, 1913, pp. 78–79; also ibid., Vol. 6, 1917, p. 187.

[3] *U. S. Department of Agric., Bull.* 754, 1919, p. 78 ff.

[4] *Journ. of experimental Zool.*, Vol. 24, 1917, pp. 147-209.

see some form of linkage, and on the other the free assortment of the gametes. Experiments for determining all such important points were already begun.

Some further breeding has been performed in respect to the present cross, but I will here simply state shortly what I have found about the reds in F_3. Of 20 reds in all which have appeared in F_3, only two could be selfed, and their offspring ($=F_4$) were examined; the results were as follows:—

	Magenta	Red	Orange	Totals
No. 1	1†	32	9	42
„ 2	——	24	14	38
Totals	1	56	23	80
Expected	0	60±3·8	20±3·8	80

Hence we see that both reds examined are heterozygous in respect to ***R***, i. e. ***CCRrbb***. When ***R*** and ***B*** are linked in the ratio 5 : 1 : 1 : 5 or 6 : 1 : 1 : 6 we should have ***CCRrbb : CCRRbb*** in the ratio 10 : 1 or 12 : 1 respectively, and thus there is no wonder that we have met with no homozygous reds at all, because only two reds were examined.

Multiple Allelomorphism.

One of my objects of investigation of the flower-colours of *Portulaca* was to pursue, if we have there a case of the so-called "multiple allelomorphism" of MORGAN and his school: thus white, orange, flesh-colour, yellow, red and magenta in our varieties might be a series of multiple allelomorphs which occupy the corresponding loci in certain homologous chromosomes. We have however above seen that the results of all crosses in *Portulaca* executed by me are very well explainable by means of usual unit factors. The following remarks might also be of some interest. If my experiments in *Portulaca* had ended with the F_2-generation, we might perhaps be led in certain cases at least to conclude that we have then to deal with a case of multiple allelomorphism. For instance, I have found that the F_2-offspring ex red ×

† This magenta came to development, because at least one gamete ***CRB*** was produced by reversion. Similar facts were observed in respect to No. 24 (Table VIII), F_4-generation (discussion, p. 129) and No. 29 (same Table, discussion p. 127).

magenta contain 126 magentas and 30 reds, theoretically $117 \pm 5{\cdot}4$ and $39 \pm 5{\cdot}4$ respectively on 3 : 1 basis; further, we have seen that the F_2-generation ex red × orange (or orange × red) (p. 101) as well as that ex magenta × orange (p. 112) segregate each into 3 : 1 respectively, i. e.

1. ***CCRRbb* × *CCrrbb***
   (red) × (orange)
2. ***CCRRBB* × *CCRRbb***
   (magenta) × (red)
3. ***CCRRBB* × *CCrrbb*** ;
   (magenta) × (orange)

consequently we should have in (3) the segregation 9 : 3 : 4 instead of 3 : 1, were it not for the complete linkage between ***R*** and ***B***, as was stated before (p. 114 ff.) Our present case is very similar to that of *Aquilegia* leaves, green, *chlorina* and *variegata*, the well-known so-called "Dreieck" of BAUR[1] or to that of the Rabbit, self-coloured, Himalayan and albino studied by PUNNETT.[2] Thus, for instance, we have in *Aquilegia*

1. ***aaBB* × *aabb***
   (green) × (*chlorina*)
2. ***AAbb* × *aabb***
   (*variegata*) × (*chlorina*)
3. ***aaBB* × *AAbb***
   (green) × (*variegata*)

and in the Rabbit

1. ***CCSS* × *CCss***
   (self-coloured) × (Himalayan)
2. ***CCss* × *ccss***
   (Himalayan) × (albino)
3. ***CCSS* × *ccss***
   (self-coloured) × (albino)

[1] *Zeitsch. f. ind. Abstamm. u. Vererbungslehre*, Bd. 6, 1912, pp. 215–216.
[2] *Journ. of Genetics*, Vol. 2, 1912, pp. 236–237; also ibid., Vol. 5, 1915, pp. 45–46.

In each of these crosses their F_2-offspring are composed approximately of 3 dominants and 1 recessive, and the fact that in each of the above (3) we have a monohybrid instead of a dihybrid segregation is explained by the assumption that in *Aquilegia* ***A*** and ***B***, and in the Rabbit ***C*** and ***S*** are in absolute linkage. This explanation, though yet hypothetical, seems to me not improbable in view of my results in the cross magenta × orange just mentioned, because in the latter case the absolute linkage exactly similar to that assumed in *Aquilegia* and the Rabbit has been, not merely assumed, but adequately proven.

MORGAN and his school are inclined to explain the case of *Aquilegia* and the Rabbit above given by means of their theory of multiple allelomorphism.[1] I will not enter here into the discussion which of the two alternative hypotheses will better explain the above cases, but I will simply state that what I have observed in respect to the cross in *Portulaca* corresponds exactly to what the hypothesis advanced by BAUR and PUNNETT demands.

Note on the so-called "pseudo-white" Race.

Pseudo-white is the name given to a peculiar race of white colour which has newly arisen in my culture. In this race leaves and stems are reddish as in coloured varieties, but the corolla is white, though slightly flashed with magenta, especially in its periphery, and each petal is generally furnished with a magenta spot at its basal part ("Herzfleck" of German authors); filaments, styles and stigmas are reddish (Pl. II, fig. 6). This race may belong either to that type in which the production of anthocyanin is inhibited, or to that which Miss WHELDALE calls "partial albino."[2] One pseudo-white was produced in the F_3-offspring ex white-II × magenta (s. the Table V), in all probability by mutation, but it may be produced regularly in the offspring of certain crosses of white-III, and very probably according to Mendelian rule: thus, for instance, the cross orange × white-III has been followed up till F_3, and this fact has been made probable, though the details of the results obtained by me will not now be published, because they are yet far from complete. Below I

[1] *The Mechanism of Mendelian Heredity.* New-York, 1915, pp. 157; also, STURTEVANT, *Amer. Naturalist*, Vol. 47, 1913. pp. 234–238.

[2] *The Anthocyanin Pigments of Plants*, Cambridge 1916, p. 153.

will simply compare the results of the crosses of three kinds of white by orange :—

	F_1	F_2
1. Orange × white-I	Orange	3 oranges : 1 white. (Cross I).
2. „ „ „ II	Magenta	9 magentas : 3 oranges : 4 whites. (Cross VI)
3. „ „ „ III	Orange	9 oranges : 3 pseudo-whites : 4 whites

Colours of Vegetative Organs and Floral Parts.

In all varieties of *Portulaca* the colour of vegetative organs, as stems and leaves on the one hand, and that of floral parts, as petals, filaments, styles, stigmas on the other, are intimately correlated to each other. In white-I stems, leaves, and styles are green ; and petals, filaments, styles, and stigmas are white. In white-II stems and leaves are green, and whilst filaments, styles, stigmas, and petals are also white, petals have sometimes a few broad or narrow magenta stripes or spots, and there may be few magenta filaments mingled with white ones. In all coloured varieties stems and leaves are reddish green ; filaments and styles are red or magenta, and so are also stigmas, though less intensely than in the latter. Ovaries are green, because their wall contains chloroplasts, and in coloured varieties they are somewhat reddish, but so slightly as to easily escape the notice of casual observers. When a coloured flower is produced on a white plant (*bud-mutation*) the branchlet bearing such a flower as well as leaves on it are more or less reddish, whilst other branchlets remain green. The pseudo-white race seems to deviate from this rule, because while leaves and stems are reddish, the corolla is white, but in reality the latter is not perfectly white, being tinged with magenta.

From the facts above given we may conclude that the factor ***C*** either alone or in conjunction with ***R*** (with or without ***B***) is able to give colour to stems, leaves, petals, filaments, styles and stigmas. In white-II ***R*** and ***B*** are unaccompanied by ***C***, and consequently are able to give colour, neither to stems and leaves, nor to petals, filaments, styles and stigmas, but sometimes

able to produce colour in a small segment of a petal, or in certain few filaments.

The peculiar condition in pseudo-white seems, as far as my observation goes, to be due to a special factor; the discussion on the latter will be reserved for a future paper.

Mutations, etc.

As the readers must have often noticed in the course of my description of the various crossing experiments we find not unfrequently a number of unexpected individuals among the offspring of certain crosses: thus, for instance, few magenta or orange plants are often seen among the progeny derived from seeds taken on selfed flowers of white parents, etc. At the beginning of my experiments it was thought that since seeds of *Portulaca* are very fine we might then have the chance admixtures from coloured plants, though not very probable in view of the utmost care taken for avoiding such. As the experiments progressed on the cases where unexpected individuals are detected have increased to such an extent that we came finally to the conclusion that they are clearly no chance admixtures, but normal products. Though seed-pans were placed near each other they were never watered from above, and so we must have avoided the danger of hurling down seeds of one pan to the neighbouring by this process. Nor would it be very probable that seeds were blown down from one pan to another by wind, since the earth in pans was held constantly moist by keeping them in a vessel partly full of water. If some coloured individuals detected among the progeny from white parents were really derived from pans containing seeds taken on coloured parents (by wind, etc.) no such fact will certainly occur, were pans containing seeds of both kinds kept distantly from each other. The following experiment made in 1920 may be of some interest: pans which contain seeds of whites on the one hand and those which contain seeds of coloured plants on the other were kept in some spots of our Botanical Garden where *Portulaca* was never cultivated before (thus avoiding the invasion of seeds of the former cultivation) and which are nearly 30 metres distant from each other and separated by fourfold high fences. In spite of all these treatments I have found as usual several coloured plants among the progeny of white parents.

Unexpected individuals are mostly dominant forms derived from recessive ones. These phenomena are, as I think, to be explained chiefly by the so-called "reverse mutations" or simply "reversions," by which I mean the return of a form to its original form from which it has been derived by mutation.[1] As the magenta variety of *Portulaca* will in all probability be the original wild form, from which other colour-varieties have been derived by mutation (so-called "loss-mutation") by one or several steps, such process, as the production of magentas or reds from whites is to be called a "reverse mutation." As I am just beginning to study such phenomena in *Portulaca* the explanations given below which are yet largely hypothetical and provisional are merely trials for indicating some possible ways of such changes. Many breeding experiments would of course be necessary in order to settle the question definitely.

Though in the course of my description all such cases met with were generally denoted with an *, they are collected below in the Table VIII. Each of them is prefixed with an *; those without an * are presented here for the first time.

[1] Reversions in the meaning here employed have been studied in *Antirrhinum majus* (DE VRIES, *die Mutationstheorie*, Bd. 1, p. 494), *Mirabilis Jalapa* (CORRENS, *Ber. d. Deutsch. Bot. Ges.*, Bd. 28; 1910, pp. 418–434), *Zea Mays* (EMERSON, *Amer. Naturalist*, Vol. 51, 1914, pp. 87–115; *Genetics*, Vol. 2, 1917, pp. 1–35), *Oryza sativa* (TERAO, *Amer. Naturalist*, Vol. 51, 1917, pp. 690–698), and *Plantago major variegata* and *contracta* (IKENO, *Genetics*, Vol. 2, 1917, p. 413; *Revue générale de Botanique*, Tome 32, 1920, pp. 49–56).

TABLE VIII.[1]

The following abbreviations are used in this Table :—M = magenta, R = red, O = orange, F = flesh-coloured, P = pseudo-white, W = white. The genetical formula placed between parentheses is that of the respective parent plant which I think to be probable.

No.	Cross	F_1	F_2	F_3	F_4
* 1	White-I × orange		O(*CCrrbb*)	1M+1R+52O	
2	„ „ „		O(*CCrrbb*)	R(*CCRrbb*)	2M+35R
3	White-I × orange			W(*ccrrbb*)	1M+15W
4	„ „ „			W(„)	2R+14W
5	„ „ „		W(*ccrrbb*)	M(*CCRRBB*)	32M
6	„ „ „		W(„)	O(*CcrrbbO*)	3O+3W
7	„ „ „		W(„)	O(„)	1M+1R+ 10O
8	„ „ „		W(„)	M(*CCRRBb*)	103M+22R
* 9	Yellow × white-I		O(*CCggrrbb*]	1M+22O	
*10	Red × orange		O(*CCrrbb*)	1R+3O	
*11	„ „ „		O(„)	20R+5W	
*12	White-I × magenta		W(*ccrrbb*)	1O+13W	
*13	„ „ „		W(„)	1O	
*14	White-II × magenta	M(*CcRrBb*)	107M+1O+48W		
*15	„ „ „	M(„)	10M+1O+2W		
*16	„ „ „		M(*CcRRBB*)	28M+13W+1O	
*17	„ „ „		M(„)	40M+23W+1F	
*18	„ „ „		M(„)	22M+3W+1P	
*19	„ „ „		M(*CCRRBB*)	35M+1F+1O	
*20	„ „ „		M(*CCRRBB*)	12M+5R+2F	
*21	„ „ „		W(*ccRRBB*)	2M+42W	
*22	„ „ „		W(„)	5M+77W	
*23	White-II × orange		M(*CCRRBB*)	75M+42R	
*24	„ „ „		M(*CcRrBb*)	28M+2R+3O+11W	
*25	„ „ „		O(*CCrrbb*)	1R+1O	
*26	„ „ „		O(*Ccrrbb*)	1M+4O+2W	
*27	„ „ „		O(*Ccrrbb*)	12M+2R+45O+30W	
*28	„ „ „		W(*ccrrbb*)	7M+5O+42W	
29	Magenta × orange			O(*CCrrbb*)	1M+118O+58W
*30	„ „ „			R(*CCRrbb*)	1M+32R+9O

[1] The production of oranges in white-II × magenta F_1 is enumerated in this Table (Nos. 14-15), but not discussed, inasmuch, as already spoken, they might be due to the contamination from other families (s. p. 106).

All cases mentioned in the above Table (with some exceptions) may be explained according to one of several ways discussed below, either alone or combined.[1]

1. *Reversion during the Formation of Gametes*—Nos. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 21, 22, 25, 26, 27, 28 and 30 in the above Table may be included here. All of them are explainable when we will assume a certain reversional change of allelomorphs in cells concerned in the formation of gametes, either male or female. The question, in what stage of development such a change will take place cannot be yet exactly answered, but its occurrence might be perhaps sought in the reducing division leading to their formation, especially in synapsis stage. In some cases the reversion of one single allelomorph into its corresponding suffices to explain the phenomenon, but in others that of two or even three allelomorphs must be assumed.

To begin with the simplest case: the white of the constitution ***ccrrbb*** produces normally the gametes ***crb***; suppose that the reverse mutation of the allelomorph ***c*** into ***C*** takes place in some cells concerned in the reducing division, then the gametes ***Crb*** will be produced besides the normal ones ***crb***. Since the number of the mutated gametes is certainly very small as compared to that of normal ones the gametes ***Crb*** will meet in fertilisation most commonly with ***crb***, though very exceptionally the meeting of the mutated male and female gametes ***Crb*** might occur; the resulting zygotes are phænotypically the same in both cases, viz. orange, but genotypically different, viz. ***Ccrrbb*** in the first and ***CCrrbb*** in the second case. The production of oranges from whites, as seen in Nos. 6 (F_3), 7 (F_3), 12, and 13 may be due to such a reverse mutation. For instance, in No. 6 one orange has been derived in F_3 from the seed taken on a F_2 white plant, and that orange was found to segregate in F_4 into 3 oranges: 3 whites, proving thus itself to be of the constitution ***Ccrrbb***, though here the actual numbers of the two kinds of segregates in F_4 do not very well agree with the calculated (4·5 : 1·5), evidently on account of the small number of individuals. No. 12 might

[1] What are given below are, as above stated, mere trials to explain the appearance of unexpected individuals, and would contain necessarily some defects and mistakes, especially as the very poor germination of seeds makes the explanation difficult, and in certain cases even almost impossible. It would be quite possible that in future some of them might be replaced by much better ones.

perhaps belong to this category, though the behaviour of the F_3-orange in F_4 was not yet examined; so will be perhaps also No. 13, where however no seed of white came to germination.—The production of some reds from homozygous oranges, as seen in Nos. 1, 2 (F_3), 10, and 25 is also explainable by assuming the reversion of one allelomorph, viz. the formation of some gametes ***CRb*** besides normal ***Crb***, and the occurrence of the fertilisation ***CRb*** × ***Crb*** or ***CRb*** × ***CRb*** (or their reciprocal), giving rise to red zygotes of the composition ***CCRrbb*** or ***CCRRbb***.—The production of magentas from whites, as seen in Nos. 21 and 22 might also be due to the reversional change of one single allelomorph; since the F_2-whites in these two Nos. are derived from the cross white-II × magenta, and consequently should possess the genotypic composition ***ccRRBB*** its normal gametes are ***cRB***, and thus the formation of few gametes ***CRB*** by means of the reversion of ***c*** into ***C***, and the mating of the latter, either with normal gametes ***cRB*** or the mutated ***CRB*** themselves will give rise to magenta zygotes, hetero- in the first and homozygous in the second case.—In No. 2 (F_4) we see the production of magenta from red, and this is also evidently a simple process consisting in the reversion of one ***b*** into ***B***, the development of few gametes ***CRB*** as the consequence, and the fertilisation ***CRB*** × ***CRb*** or its reciprocal.

In the production of magentas from oranges, as seen in Nos. 1 and 9, as well as that of reds from whites, as seen in No. 4, the occurrence of a simultaneous reversion of two allelomorphs should be necessarily assumed. Thus in the first of two cases just cited the orange of the composition ***CCrrbb*** must produce the gametes ***Crb*** (normal) and ***CRB*** (mutated), and in the second the white ***ccrrbb***, the gametes ***crb*** (normal) and ***CRb*** (mutated)[1]

The reversion process in No. 27 may be explained as follows:—the orange parent is here heterozygous, i.e. of the composition ***Ccrrbb***; since normally the gametes ***Crb*** and ***crb*** (either male or female) are produced in equal numbers their meeting will give rise to 3 oranges: 1 white, so that if magentas and reds segregated out in this case are considered to be oranges

[1] The formation of the gametes of two kinds, viz. ***CRb*** and ***CrB*** in the first case, and that of ***Crb*** and ***cRb*** in the second will lead to the same results, but then the fertilisation between the two mutated gametes, viz. ***CRb*** × ***CrB*** as well as ***Crb*** × ***cRb*** (or their reciprocal) must be assumed to have occurded.

we have $12+2+45=59$ oranges and 30 whites (expected, $66{\cdot}75\pm4 : 22{\cdot}25\pm4$). The production of some magentas and reds in this case may be explained if we will assume that a certain number of the gametes of the composition ***CRB***, and ***CRb*** have arisen on account of the reversion of one or two allelomorphs, and the fertilisation, such as ***CRB*** × ***Crb***, ***CRB*** × ***crb***, ***CRb*** × ***Crb*** ***CRb*** × ***crb*** have taken place. Almost the same might perhaps be said in respect to No. 26, though it contains too few individuals to lead us to a somewhat probable conclusion (compare Table VIII, that No.) The result of No. 30 may be explained in an almost similar way; its normal gametes are ***CRb*** and ***Crb***, and we have 33 reds and 9 oranges, expected $31{\cdot}5\pm2{\cdot}8$ and $10{\cdot}5\pm2{\cdot}8$, if we will reckon 1 magenta among the reds; this magenta was produced because at least one gamete ***CRB*** has arisen by reversion.

In respect to the production of magentas from whites, as we see in Nos. 3, 5 (F_3), 8 (F_3), and 28 we must assume the simultaneous reversion of three allelomorphs ***c***, ***r*** and ***b*** into ***C***, ***R*** and ***B*** respectirely, though here on account of the complete linkage of ***R*** and ***B*** the whole process will be reduced to the reversion of one factor and one factor-complex. Suppose that on account of this process few gametes ***CRB*** are produced besides normal ones ***crb***; the fertilisation between ***CRB*** and ***crb*** which will be the most prevailing one will give magenta of the constitution ***CcRrBb***, whilst very rarely the fertilisation ***CRB*** × ***CRB***, i.e. that between two mutated male and female gametes might take place. To the latter category may belong No. 5, because here the magenta produced in F_3 from a seed taken on 1 white F_2 plant seems to breed true in F_4, the offspring in the latter generation consisting wholly of magentas (32 in all), though it would not be impossible that all other segregates did not come to germination.

In No. 28 few oranges and magentas were produced from whites of the composition ***ccrrbb***. Here it must be assumed that a small number of gametes of two kinds, viz. ***Crb*** and ***CRB*** were simultaneously produced besides normal ones ***crb***, and that the fertilisations ***Crb*** × ***crb*** as well as ***CRB*** × ***crb*** (or their reciprocal) have take place.

In No. 8 1 magenta derived in F_3 from 1 F_2 white was found to segregate into 103 magentas and 22 reds, whence it may be inferred that this magenta parent might be of the constitution ***CCRRBb***, though the

actual number of the two kinds of segregates does not very well agree with the expected (expectation on 3 : 1 basis, 93·75±4·8 : 31·25±4·8). How the magenta of such composition has arisen from the white cannot yet be determined, and though its origin might be perhaps inferred this is merely a matter of conjecture, and it would not be worth while to state here such an inference.

II. *Reversion in the Somatic Cell.*—To this class belongs No. 11 in Table VIII. Suppose that the reverse mutation has occurred in a certain somatic cell of the composition ***CCrrbb***, at least one cell-generation before the reducing division, and suppose further that this cell has got the composition ***CCRrbb*** in consequence of this process. From the latter cell the male and the female gametes ***CRb*** and ***Crb*** will be derived after one or more cell-generations according to different cases. The fertilisation between them will give rise to zygotes consisting of 1 ***CCRRbb***, 2 ***CCRrbb*** and 1 ***CCrrbb***; the production of 20 reds and 5 oranges in No. 11 may be due to such a process (expected 18·75±2 and 6·25±2).

III. The mutations described in I and II are, as already stated, the reverse ones, i.e. those from recessive to dominant condition. The mutations in the opposite sense, i.e. those from dominant to recessive condition (so-called "loss-mutation") have been also observed sometimes, though not frequently, to which might belong Nos. 23 and 29 in Table VIII. In No. 29 one orange which should be theoretically homozygous, i.e. ***CCrrbb***, has produced 1 magenta, 118 oranges, and 58 whites. According to our hypothesis a certain somatic cell having the genotypic composition ***CCrrbb*** has undergone a "loss-mutation," and changed into ***Ccrrbb***; the gametes ***Crb*** and ***crb*** are derived from it after a number of cell-generations, and their free assortment has given rise to oranges and whites in the approximate ratio 3 : 1 (119 oranges : 58 whites, theoretically 132·75±5·8 : 44·25±5·8, if we will count 1 magenta as orange); 1 magenta was produced at the same time, perhaps because 1 gamete ***CRB*** has arisen by reversion of ***b*** into ***B***. Thus the whole process in No. 29 consists in loss-mutation and reversion combined.

No. 23 (magenta) was found to segregate in F_3 into magentas and reds, whence we might at once be led to the assumption that that magenta parent should have the composition ***CCRRBb*** (75 magentas : 42 reds, expected 87·75±4·7 : 29·25±4·7). Since however this parent has been originally derived

from white-II × orange, and since we see there the complete linkage between the factors ***R*** and ***B***, it would not be probable that it has had that composition from the very beginning; in all probability it has been at first of the constitution ***CCRRBB***, changed into ***CCRRBb*** by a "loss-mutation", and then undergone the segregation above stated.

In all cases under I above enumerated (and in some cases under III) I have assumed that the reversion takes place during the formation of gametes, but it would be equally possible that this process of mutation occurs, not during the gametic formation, but some time before in the somatic cell. To cite one instance for illustrative purpose, in No. 6 (Table VIII) where white-I (***ccrrbb***) gives rise to orange of the constitution ***Ccrrbb*** the process may be as follows:—a somatic cell of the constitution ***ccrrbb*** gets that of ***Ccrrbb*** by reversion; such cell produces during each succeeding cell-division the cells ***Ccrrbb***, so that in the reducing division the gametes of the constitution ***Crb*** and ***crb*** are formed. (Compare the discussion in p. 124). If what is above described be true the whole process of reversion in I (and also in some cases under III) is essentially identical with that in II. To determine exactly in each individual case which alternative will be realised, i.e. whether the reversion occurs in the somatic cell or first in the formation of gametes would be an almost impossible task in the present state of our knowledge.

IV. Each of the F_2 magenta parents in Nos. 16, 17 and 18, derived from the cross white-II × magenta has segregated into magentas and whites, and produced in addition 1 orange, 1 flesh-coloured and 1 pseudo-white. As this segregation has given rise to 90 magentas and 42 whites in total, i. e. $99 \pm 4{\cdot}97$ and $33 \pm 4{\cdot}97$ respectively on 3 : 1 expectation we may in all probability regard each magenta parent in our case to be of the constitution ***CcRRBB***. The question, however, to what kind of mutation will be due the production of flesh-coloured and pseudo-white plant in this case would be quite unexplainable, especially as the exact genotypic composition of the two latter is yet unknown.

In respect to Nos. 19 and 20 we may consider that the F_2 magenta parent is homozygous, i.e. of the constitution ***CCRRBB*** and has bred true

in F_3 (35 and 12 magentas produced respectively). The orange and the red found in addition have probably been produced by the "loss-mutation", but as to the mode of production of flesh-coloured plants we are in the same position as in respect to Nos. 17 and 18 just cited, and consequently we are not able to make any surmise about it.

V. *Change of the Linkage Ratio.* In No. 24 we see that one magenta ex white-II × magenta segregates into 28 magentas, 2 reds, 3 oranges and 11 whites. We may consider this magenta parent to have had the composition ***CcRrBb***, and the appearance of 2 reds which have never been met with in F_2-generation (with one exception, s. the Table VI, A) is, as I think, due to the change of the *complete* linkage between ***R*** and ***B*** into a *partial*, as we have seen in the Cross VIII. Suppose that ***R*** and ***B*** are linked according to the series $n:1:1:n$; since no such relation exists between ***C*** and ***R*** or between ***C*** and ***B***, we should have the eight following classes of gametes in the ratios indicated, viz.:—

$$n\boldsymbol{CRB}+n\boldsymbol{cRB}+1\boldsymbol{CrB}+1\boldsymbol{crB}+1\boldsymbol{CRb}+1\boldsymbol{cRb}+n\boldsymbol{Crb}+n\boldsymbol{crb}.$$

The mating of male and female gametes of such constitutions should give rise to the four following kinds of zygotes in the ratios indicated, viz.:—

Magenta	Red	Orange	White
$9n^2+12n+6$	$6n+3$	$3n^2+6n+3$	$4n^2+8n+4$

Though the number of individuals in No. 24 is rather small, the following calculations were made. Since we have observed in the Cross VIII the linkage between ***R*** and ***B*** belonging to the series $5:1:1:5$ or $6:1:1:6$, I have put $n=5$ or 6, and then we have in respect to the expected number of individuals for each kind of zygotes.

	Magenta	Red	Orange	White	Totals
$n=5$	22·23±3·3	2·52±1·5	8·25±2·6	11·00±2·9	44
$n=6$	22·56±3·3	2·19±1·4	8·25±2·6	11·00±2·9	44
Actual	28	2	3	11	44

The agreement between the theoretical and the actual numbers is not very bad in both cases in view of the small number of plants, except in respect to the orange where the number of individuals is much smaller than might be expected theoretically; this may be due possibly to the fact that a comparatively large proportion of seeds of this class of zygotes failed to germinate. The further behaviour of one of the two reds segregated out in F_3 was ascertained, because I could get seeds on it by selfing. It has produced in 1919 the F_4-offspring containing 1 magenta, 28 reds, 12 oranges and 17 whites, thus proving itself to be heterozygous. We may consider the red examined to have been of the constitution ***CCRrbb***,[1] and that it has changed into ***CcRrbb*** before the formation of gametes; the male and the female gametes ***CRb***, ***cRb***, ***Crb***, ***crb*** are formed, and their mating has given rise to 29 reds: 12 oranges: 17 whites, theoretically $32{\cdot}6\pm3{\cdot}8 : 10{\cdot}9\pm 3{\cdot}0 : 14{\cdot}5\pm3{\cdot}8$ on 9 : 3 : 4 basis, if we will count 1 magenta as 1 red; 1 magenta was formed because at least one gamete ***CRB*** was produced by reversion, so that the whole process consists, exactly as in No. 29 (Table VIII, discussion p. 127), in the "loss-mutation" combined with reversional change.

In the Table VI, A (p. 109) we have seen that the F_2-offspring ex white-II × orange No. 2 contains 81 magentas, 20 oranges, 34 whites and 1 red, 136 in all. They are the total of all the offspring derived from 12 F_1-plants by selfing. Of these 12 families of the F_2-offspring 11 contain no red at all, so that the one red under discussion belongs to one family composed of only 7 plants, viz. 5 magentas, 1 red and 1 orange.[2] The occurrence of 1 red in this family might perhaps be due to the partial linkage similar to that in the case just stated above, but no sure conclusion can be drawn here on account of the small number of individuals.

Bud-mutations have been observed, though not frequently. They are below simply described, but no discussion will be made, this being impossible

[1] Since the factors ***R*** and ***B*** were linked according to the series 5 : 1 : 1 : 5 or 6 : 1 : 1 : 6 in the preceding generation, we should have in the present case the reds of the composition ***CCRrbb*** and ***CCRRbb*** in the ratio 10 : 1 or 12 : 1 respectively, and it is quite natural that we have now ***CCRrbb***, because only two reds were examined.

[2] Whites are wanting in this small family, perhaps because all seeds which will give rise to whites failed to germinate.

on account of the small number of the cases which I have encountered till now.

1. One white plant derived ex orange × white-I (Table I, p. 98) has produced besides white flowers some orange-coloured ones. Two of the latter were selfed, and seeds thus obtained have given rise in the next year to 14 plants which have borne, not orange flowers as was expected, but white ones simply. Some white flowers on the original plant were selfed, but seeds got from them did not germinate. As the second generation did not bear orange flowers, the phenomenon will not belong properly to bud-mutation.

2. One white F_3-plant derived ex white-I × orange has produced one branchlet bearing orange flowers. These as well as one white flower were selfed. Only two seeds from the former came to germination, and one of the plants thus produced has so far developed as to bear one flower which was *magenta*; three seeds taken on the white flower had germinated and developed to 2 *magentas* and 1 *orange*.

3. One white plant among the F_3-offspring ex red × white-I has borne 2 white flowers and 1 magenta one. No seeds could be obtained from the former, but those got on the latter has produced in the next year 3 plants, viz. 2 magentas and 1 white, thus the magenta flower was proven to have been heterozygous in its genotypic constitution.

4. One white plant among the F_3-progeny ex white-II × magenta has borne besides white flowers one magenta one in 1920. Whether the latter is homo- or heterozygous will be examined in this year, because I have got seeds on this flower.

Summary.

1. All coloured varieties of *Portulaca grandiflora* are characterised by possessing the factor ***C***.

2. ***C*** alone gives rise to *orange* flower-colour. ***C*** and ***G*** produce together *yellow* flower-colour, ***C*** and ***R*** together red; whilst the *magenta* colour is due to the co-operation of the three factors, ***C***, ***R*** and ***B***.

3. As to the genetics of *flesh-coloured* and "*pseudo-white*" races my experiments are not so far advanced as to be able to fully establish their respective genotypic constitution.

4. All ***cc***-plants are white. There are three kinds of whites, which I call *I, II* and *III* respectively. The *white-I* (***ccrrbb***) possesses perfectly white floral parts, while the *white-II* (***ccRRBB***) may produce few magenta stripes or spots on petals and few magenta filaments. The *white-III* is externally perfectly similar to the *white-I*, but is characterised by producing in certain crosses a number of the so-called " pseudo-white," in all probability according to Mendelian rule; its genotypic constitution is yet unsettled.

5. The factors ***R*** and ***B*** are generally in *complete* linkage or " coupling," and act like one single factor, but sometimes it changes into a *partial*, causing the production of a certain number of unexpected *red* individuals.

6. The colour of vegetative organs and floral parts are correlated to each other.

7. A small number of unexpected individuals of several kinds may often be produced, thus for instance, magenta or red, etc. is found among the offspring of orange or white parent (selfed!) I have tried to explain such phenomena chiefly (but not all) by means of " reverse mutations."

8. Bud-mutations were also observed.

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EXPLANATION OF PLATE II.

All figures are from water-colour drawings from nature.

Fig. 1. Magenta.

Fig. 2. Red.

Fig. 3. Orange.

Fig. 4. Yellow.

Fig. 5. Flesh-coloured.

Fig. 6. Pseudo-white.

Fig. 7. White-II. Magenta stripes on some petals!

Fig. 8. White-I.

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