

Polyploidy and Aneuploidy in Guayule¹

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CHROMOSOMAL RANGE IN GUAYULE

In the fall of 1942 LeRoy Powers and W. B. McCallum collected seeds of guayule (*Parthenium argentatum* A. Gray) and mariola (*P. incanum* H. B. K.) in Mexico and Powers and Walter T. Federer collected seeds of these same species in Texas. Early in 1943 many of these seeds, together with those of certain of the commercial strains developed previously through selection by McCallum and probably of Mexican origin, were germinated and planted in fields at Salinas, Calif. Chromosome studies, especially the determination of chromosome numbers, were begun as soon as possible in order to facilitate genetic and breeding experiments.

After a few months of exploratory studies, it was apparent that a polyploid series exists among natural populations and commercial strains of guayule. Stebbins and Kodani (13),² working independently, came to the same conclusion at about the same time.

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²Italic numbers in parentheses refer to Literature Cited, p. 36.

Recognition of this was facilitated by the fact that the whole series was represented in the seeds collected in Mexico. Stebbins and Kodani (13) and Powers and Rollins (10) found a similar series in mariola. From papers written by Russians prior to 1936 (2, 3, 5, 6), it is now evident that they had diploid and tetraploid strains of mariola and also tetraploid strains of guayule but that they failed to recognize a polyploid series in their material.

Up to the present no guayule plants have been found with less than 36 chromosomes. The highest number reported thus far is 144 among offspring of $108 \pm$ -chromosome³ plants (8). Probably every number between 36 and 144 could be found if enough individual plants were examined. Presumably some genic combinations are viable for any particular chromosome number; however, a distinction should be made between the predominant chromosome numbers of populations (stands) of guayule and those of variants which occur in such populations or which were a result of crosses made in greenhouses and field plantings at Salinas. At Salinas are the makings of a great variety of chromosome numbers, because diverse races of guayule which in nature are separated by geographical and ecological barriers have been assembled there. More aneuploids would be found under natural conditions were it not for the fact that facultative apomixis is predominant among the higher chromosome members of the series (4, 10).

POLYPOID SERIES IN NATURAL STANDS AND COMMERCIAL STRAINS

EUPLOID POPULATIONS AND STRAINS

Although Stebbins and Kodani (13) suggested the basic number of x equals 9 for the polyploid series, no plants which have $2x$ equals 18 have been found or reported. Because of the formation of bivalents in $36+$ -chromosome⁴ plants and of trivalents in $54 \pm$ -chromosome plants, x equaling 18 will be used as the basic number in this bulletin. Accordingly, 36 can be considered the diploid condition for naturally occurring, present-day populations of guayule. As reported by Bochantseva (2) this is true for mariola also. The only lower number found in *Parthenium* thus far is for *P. hysterophorus* L., in which $2x$ equals 34 for 2 collections that L. Powers made in Texas.

At Salinas there are 5 diploid collections ($2x=36+$) from seeds harvested in a mountainous area southwest of Mapimí, Durango, Mexico. There are 5 triploid collections ($3x=54 \pm$)—1 from an individual plant growing within the population from which the diploid collection 4255 was obtained and the other 4 from different places near San Bartolo, Durango. Such commercial strains as McCallum's 109, 111, 210, 255, and 258 also are triploid. The majority of the collections at Salinas have $72 \pm$ chromosomes and therefore are tetraploid ($4x=72 \pm$). The seeds came from both natural stands and McCallum's commercial strains. Only 9 of the collections made by Powers and McCallum in Mexico are tetraploid. They came from the

³ \pm after a number indicates a polyploid class, the individual members of which vary in exact number of chromosomes.

⁴ $+$ after 36 indicates the diploid class, the individual members of which may be aneuploids with one or more additional chromosomes.

following locations: One from an individual plant growing within the population from which the collection 4255 was obtained; another from seeds collected near San Juan, Nuevo Leon; a third from Catorce, San Luis Potosi, and the remaining 6 from Majoma in northeast Zacatecas. The trans-Pecos area of Texas is represented by 362 collections. Of these extensive collections cytological work has been done on only 55 and pollen studies on 4 more. All had $72 \pm$ chromosomes. Eleven of the 20 locations in Texas from which the seeds were collected have been sampled. Thus far 25 of McCallum's commercial strains have been found to have $72 \pm$ chromosomes. They are Nos. 38H, 49, 130, 402, 404, 406, 406F, 411, 413, 416, 418, 419, 426, 428, 430, 439, 440, 441, 444, 453, 456, 459, 593, and 735-2 and a Mexican strain (42478 in Powers' collections). Thus far no pentaploid or hexaploid natural stands or commercial strains have been found. Higher chromosome numbers have been limited to certain individuals in triploid and tetraploid populations and strains and to their offspring. All of the Mexican collections have been sampled cytologically and some have been studied intensively, because the whole polyploid series is represented in them and therefore they afford an opportunity to work out the relation among the members of the series.

ANEUPLOID STRAINS

Since reproduction in $3x$ ($54 \pm$ -chromosome) and $4x$ ($72 \pm$ -chromosome) populations is predominantly facultatively apomictic (10), it has led in some instances to the establishment of what are essentially clones of aneuploid $3x$ and $4x$ combinations. For instance, commercial strain 210 seems to have 58 chromosomes, the number that Stebbins and Kodani (13) reported for strain 111. Strains 456 and 735-2 seem to have 74 chromosomes in addition to the pair of miniatures which is present in many $4x$ populations.

"INTERMEDIATE" TYPE

A morphological type called "intermediate" by Powers⁵ and described by Rollins (11) and corresponding to the broadleafed type named *Parthenium lloydii* Bartlett has $72 \pm$ chromosomes. A portion or all of a number of collections from Mexico and Texas as well as several of McCallum's commercial strains are "intermediates." The probable origin involving introgressive hybridization with mariola is discussed by Rollins (11, 12) and Stebbins and Kodani (13). As in the case of aneuploid triploid stands and strains, reproduction by apomixis has led to the establishment of what are essentially clones, which differ somewhat among themselves in morphological appearance because they have arisen more than once and in different places but are sufficiently similar in appearance to warrant classification as "intermediates." They vary in chromosome number, since plants with 71, 72, 73, and 74 chromosomes have been found (see table 4). Regardless of chromosome number or geographical origin many of their pollen mother cells show lagging chromosomes at anaphase I and II and there are many microcytes among

⁵ POWERS, L. SEED COLLECTIONS OF GUAYULE FROM MEXICO AND THE TRANS-PECOS AREA (BIG BEND COUNTRY) OF TEXAS. 1942. [Unpublished.]

mature pollen grains. The only observed difference at metaphase between "intermediates" and the good guayule type was the tendency of the "intermediates" to form more polyvalent associations. This tendency was interpreted as indicating structural hybridity; less homology among the chromosomes is consistent with their hybrid ancestry. Consequently, the presence of lagging chromosomes at anaphase I and II indicates difficulty in separation among the members of these polyvalent associations. The finding of 2 "intermediate" $54\pm$ -chromosome plants among the offspring from non-controlled pollination of a 36-chromosome plant grown at Salinas suggests that $54\pm$ -chromosome "intermediates" are possible and may occur in nature.

MATERIALS AND METHODS

The material used for studying guayule chromosomes consisted mainly of pollen mother cells. It was augmented by tapetal cells occasionally found in division and by root-tip cells. Young flower heads were fixed in a 7:1 absolute-alcohol-acetic-acid mixture. When necessary the material was stored in 70-percent alcohol. Florets from many heads of the same plant were dissected out, pooled, and selected according to size. About a dozen disk florets were used for making each aceto-orcein smear.

Since 4 pollen grains (microspores) normally originate in each pollen mother cell, consequences of the number of chromosomes and of meiotic behavior in pollen mother cells are found in the pollen grains. Therefore additional information concerning the effects of meiotic behavior was sought and found in the examination of pollen for size, percentage of grains aborted (empty or obviously degenerating), presence of microcytes (minute empty grains), and abnormalities of any sort. Pollen-grain preparations were made by placing shed pollen in a drop of cotton blue in lacto-phenol (1-percent cotton blue in equal parts of lactic acid, phenol, glycerin, and water). The diameter of grains was measured from inside wall of intine to inside wall of intine. Usually 100 grains were measured. In order to get the percentage of grains aborted 500 were usually counted.

The primary object in all the chromosome studies was the determination of their number. Observations concerning the meiotic process in pollen mother cells were incidental. Because of paucity of clear metaphase plates due to clumping or stickiness of chromosomes, use was made of any meiotic stage at which chromosome counts could be made or chromosome associations studied. At first anaphase allowance had to be made for the tendency in certain plants for univalents to divide precociously or for the presence of fragments in connection with single or double chromatid bridges. Second telophase counts on the same slide varied because of the presence of such fragments. Occasionally a second anaphase stage could be used. For diploids ($36+$ -chromosome plants), and to a lesser extent for triploids ($54\pm$ -chromosome plants), diakinesis and first metaphase stages were excellent. Because of multivalent formation these stages rarely were satisfactory for determining chromosome number of higher polyvalents; however, they were invaluable for revealing meiotic behavior. For counts among higher polyploids the interphase

or second prophase stage was satisfactory, since the chromosomes were less diffuse and better stained than these stages in $36+$ or $54\pm$ -chromosome plants. Fairly reliable determinations could be made by averaging counts made of several cells in these stages on the same slide. This was shown whenever a check was possible by means of a count of somatic chromosomes in dividing tapetal cells. The interphase and prophase II stages were much more satisfactory than the second metaphase because of the tendency for chromosomes to stick together at metaphase II.

Variation in meiotic chromosome number in the same plant seems to be limited to two sources: (1) Additional fragments resulting from crossing over in inversions and (2) the elimination of chromosomes by aberrant plants (p. 22). There may or may not be a difference in chromosome number between morphologically distinct parts of a plant which is a sectorial chimera.

CHROMOSOME MORPHOLOGY

Because of the relatively small size and compactness of guayule chromosomes during meiosis, little could be learned about the differences in length and the location of the insertion region. No attempt has been made to work out idiograms for the various collections and strains. However, somatic chromosomes in tapetal-cell nuclei (fig. 1, *A* and *B*) show differences in over-all and relative length of each arm; some are J-shaped, but the majority are V-shaped with a median or submedian insertion region. In figure 1, *A*, there seem to be multiples of a few basic types such as a long chromosome with a submedian insertion region, a J-shaped one, a medium equal-armed, a medium unequal-armed, a small equal-armed, and a small unequal-armed, as well as a small type which tapers at one end. The last-named type probably is the satellited chromosome which can be seen attached to the nucleolus in occasional favorable preparations. In root-tip cells fixed and stained according to Meyer's technique (?) a pair of small satellited chromosomes can be seen in $36+$ -chromosome plants. They have a submedian constriction, the small satellite being attached to the shorter arm. In plants having 72 or more chromosomes there usually are several nucleoli of different or equal size.

Practically all of the $36+$ -chromosome plants thus far examined and very many of those with higher chromosome numbers have in addition one to several very small chromosomes. The number seems to be constant for individual plants but may vary among members of the same culture. They are heteropycnotic, since they stain more deeply than other chromosomes at diakinesis and less so at metaphase I or later stages. In root-tip cells fixed and stained according to Meyer's technique (?) such differential staining is lacking. If 2 or more cells are present, pairing may be seen at diakinesis or metaphase I. The insertion region if not terminal is nearly so. This is confirmed by observations on root-tip cells. Sometimes when unpaired they are uncoiled and threadlike at metaphase I and later stages. They may divide precociously at anaphase I. They may be of different size, even in the same cell (fig. 1, *C*). Similarity in size and shape to arms of V- or J-shaped chromosomes suggests that they owe their origin to breakage of such chromosomes. They equal the smaller arm of a

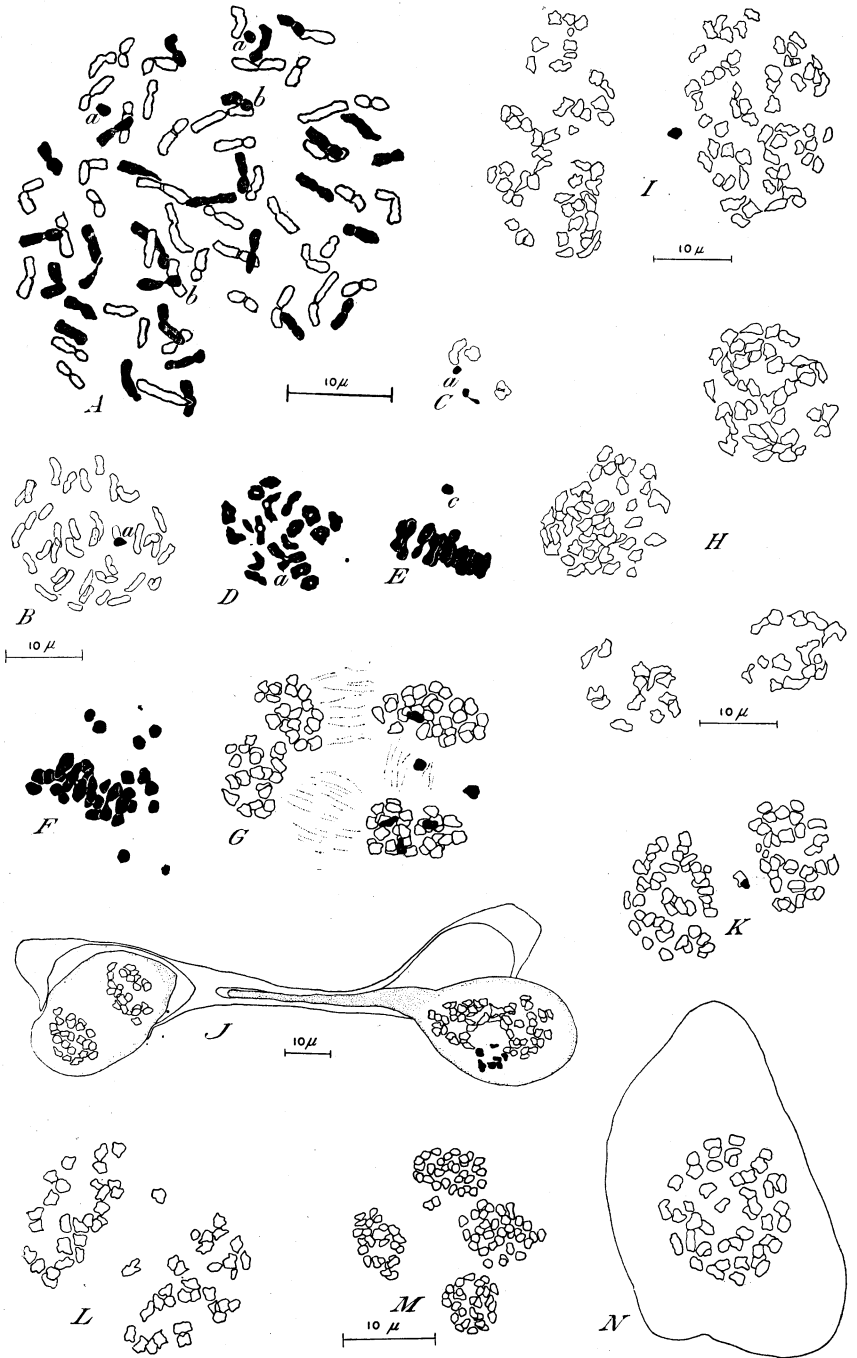


FIGURE 1.—For explanatory legend see opposite page.

medium-sized V-shaped chromosome in length and width (fig. 1, *A*, *a*). Actual attachment to regular chromosomes is rarely seen but is shown in figure 1, *D*, where a small chromosome is attached to the ends of 2 chromosomes belonging to a configuration of 4. Although Stebbins and Kodani (13) referred to such chromosomes as small, they will be called miniature chromosomes⁶ in this bulletin to distinguish them from small bialarmed chromosomes and from occasional fragments resulting from crossing over in inversions. Their function is unknown, but they can be transmitted through the pollen grain as well as through the egg cell.⁷

CHROMOSOME BEHAVIOR IN POLLEN MOTHER CELLS

DIPLOIDS

Information about meiosis in pollen mother cells of diploid plants is based on observations made on plants belonging to the five 36+-chromosome collections from Mexico and on some of the diploid offspring from the noncontrolled pollination of several plants of 1 of these collections (4255). The diploid nature of 36+-chromosome plants is indicated by the presence of 18 bivalents at diakinesis and metaphase I as previously reported (1). Usually the distribution at anaphase I was 18:18, although rarely 17:19 could be found. Stebbins and Kodani (13) also commented on the remarkable meiotic regularity of diploid 36+-chromosome plants.

⁶ Miniature chromosomes are not included in the chromosome number but are represented by a superscript following a + sign.

⁷ Rollins, R. C. Unpublished data.

EXPLANATORY LEGEND FOR FIGURE 1

FIGURE 1.—*A*, The 73⁺² chromosomes from a tapetal cell of a plant of strain 593. Note 2 miniature chromosomes (*a*) and chromosome (*b*) that has 1 arm of the size and shape of the miniature chromosome. *B*, The 37⁺¹ chromosomes from a tapetal cell of a haploid from collection 4285. Note miniature chromosome (*a*). *C*, Two miniature chromosomes (*a*) and 3 bivalents at metaphase I from a pollen mother cell of a 72⁺²-chromosome plant. *D*, Sixteen bivalents, 1 univalent, and a circle of 4 chromosomes with miniature chromosome (*a*) attached at metaphase I from a pollen mother cell of a haploid from collection 42357. *E*, Lateral view of chromosomes at metaphase I, when bivalent formation was almost complete, from a pollen mother cell of a haploid from collection 4267. Note the 1 univalent (*c*). *F*, Six bivalents and 24 univalents at metaphase I from a pollen mother cell of a haploid from collection 42108. *G*, Multipolar spindle with 4 groups of chromosomes at anaphase I from a pollen mother cell of a 106-chromosome aberrant plant. *H* and *I*, Chromosomes in 4 nuclei (*H*) and in 2 (*I*) at prophase II from pollen mother cells of a 107-chromosome plant. *J*, Chromosomes at prophase II in twin pollen mother cells partly squeezed out of a ruptured common exine of a 107-chromosome aberrant plant. Note the 2 nuclei in each cell and the 46 chromosomes in one cell and the 61 in the other. *K*, Chromosomes of 2 nuclei at prophase II from a 78-chromosome pollen mother cell of the 107-chromosome plant that produced those shown in *H* and *I*. *L*, Chromosomes of 2 nuclei at prophase II from a 59-chromosome pollen mother cell of the 107-chromosome plant mentioned in *H*, *I*, and *K*. *M*, Chromosomes of 4 nuclei at telophase II from a 60-chromosome pollen mother cell of the 106-chromosome plant mentioned in *G*. *N*, Tapetal cell containing 46 chromosomes from a disk floret of an aberrant plant with about 108 chromosomes. All drawings except *A* and *J* are of the same magnification. The shading of some chromosomes has no significance except to increase clarity.

Bivalent formation at metaphase I is not invariable, however. One plant showed as many as 3 quadrivalents in some pollen mother cells; 3 others showed one quadrivalent each in some. This multivalent formation indicates either reciprocal translocation or polyploidy and may lead to unequal distribution at anaphase I. The presence of inversions was indicated in plants belonging to 4 of the 36+-chromosome collections by the occasional presence of bridges at anaphase I or II.

Two meiotic irregularities which affected the quality of pollen were found in collection 4255. In a sample of 290 plants 4 had 90 percent or more of the pollen aborted; 3 of the 4 were examined cytologically and found to be asynaptic: at metaphase I there were a few but varying number of bivalents plus univalents. They had 5, 6, and 7 miniature chromosomes, respectively. The other irregularity was partial or complete failure of cytokinesis. This was found in 3 plants which came from noncontrolled pollinations. Only 1 was studied at metaphase I. It showed 16 bivalents plus a chain of 4 (or 18 bivalents) plus 6 miniatures. No accurate determination of aborted pollen could be made. Some of the grains were compound, consisting of a tetrad of incompletely separated spores, frequently of unequal size. Other large grains were obviously the result of diad instead of tetrad formation. Giant grains, the equivalent of pollen mother cells, also were numerous.

TRIPLOIDS

Information about meiosis in pollen mother cells of $3x$ and aneuploid $3x$ plants is based on samples of the five $54\pm$ -chromosome collections from Mexico and of the $54\pm$ -chromosome commercial strains and also on some of the $54\pm$ -chromosome offspring from noncontrolled pollinations of $36+$ -chromosome plants of collection 4255 and noncontrolled pollinations of $54\pm$ -chromosome plants of collection 4263 growing in the field at Salinas. The $3x$ aneuploids which had less than 54 chromosomes and some which had more than 54 came from these noncontrolled pollinations at Salinas. Included also were 2 offtype-normal plants from a $72\pm$ -chromosome Texas collection.

Chromosome behavior characteristic of triploids was observed. At diakinesis and metaphase I trivalents were seen, but the number varied with a corresponding varying number of bivalents and univalents. Whenever the metaphase I stage could be observed, at least a few trivalents were seen. The exceptions were the 2 offtype-normal Texas plants and 1 other, all of which showed partial asynapsis. At anaphase I the majority of pollen mother cells showed lagging chromosomes, some of which divided precociously. Those pollen mother cells of 54 -chromosome plants which showed lagging chromosomes averaged 2.1 laggings; whereas those of aneuploids averaged 4.6, but this higher average was caused mostly by only a few of the cells in which the number of laggings was large. The number of laggings, which is given as a single number, does not indicate how scattered they were in the cytoplasm (table 1). Chromatid bridges and small fragments were seen infrequently. Lagging chromosomes were seen also at anaphase II, as is shown by the distribution 23:23:30:3:27. A few cells showed the effects of lagging at both anaphases, as in the distribution 26:26:2:26:1:25. Laggings form

microcytes, which later can be seen as minute empty cells among mature pollen grains.

TABLE 1.—*Distribution of chromosomes at anaphase I in pollen mother cells of 3x and aneuploid 3x (49- to 56-chromosome) plants of guayule*

[Miniature chromosomes not included; laggards indicated by central figure in series of 3]

Group of plants	Plants	Chromosomes in 2 groups only		Chromosomes in 2 groups plus laggards	
		Pollen mother cells with indicated chromosome distribution	Chromosome distribution	Pollen mother cells with indicated chromosome distribution	Chromosome distribution
49-chromosome.....	Number 1	Number 1	24:25	Number 1	20:4:25
51-chromosome.....	1			1 1 1	19:5:27 20:10:21 23:3:25
52-chromosome.....	5	1 1 1 2	23:29 24:28 25:27 26:26	1 1 2 1 1 1	21:10:21 22:4:26 23:1:28 23:2:27 24:4:24 25:1:26
53-chromosome.....	5	2	26:27	1 1 1 1 1 1 1 1 1	16:16:21 21:1:31 21:3:29 21:9:23 22:7:24 23:6:24 25:3:25 25:2:26
54-chromosome.....	8	1 1 5 3	22:32 23:31 25:29 27:27	1 1 2 2 3 1 1 1 1	23:5:26 24:1:29 24:2:28 24:3:27 25:1:28 25:2:27 25:3:26 26:1:27 26:2:26
56-chromosome.....	5	1 2 1 2	25:31 26:30 27:29 28:28	1 2 1	25:5:26 26:3:27 27:2:27

Miniature chromosomes are less conspicuous as the number of chromosomes increases in the polyploid series because their numbers do not increase. No $3x$ natural populations or commercial strains showed more than 3, and some seem to be entirely free from them. Some of the $3x$ and aneuploid $3x$ offspring from noncontrolled pollination of plants of collection 4255, however, showed as many as 8. Undoubtedly most of them were contributed by the egg nucleus, since the seed parents of these plants had 6 to 10 miniature chromosomes.

In the majority of cases determination of chromosome number depended upon chromosome counts at anaphase I, prophase II, and metaphase II stages. Since the number of chromosomes in the two groups at prophase II and metaphase II, plus any lagging chromosomes present, reflects the distribution that occurred at the preceding anaphase I stage, the prophase II and metaphase II records were pooled

with the anaphase I records and the total was examined to learn the nature of the anaphase I distribution.

The distribution of chromosomes at anaphase I in pollen mother cells of $3x$ and aneuploid $3x$ plants was observed in plants the chromosome numbers of which ranged from 49 to 56 (table 1). For pollen mother cells showing no lagging chromosomes in 54-chromosome plants the theoretical distribution, calculated according to the random distribution of 18 trivalents, includes 10 classes from 18:36 to 27:27. Only 10 pollen mother cells were observed. Of these 1 showed 22:32; 1, 23:31; 5, 25:29; and 3, 27:27. The percentages of observed frequencies were compared with those of calculated frequencies. Obviously the observed number of cells was too small for the comparison to have any meaning; however, there was no disagreement between calculated and observed frequencies. In aneuploids with less than 54 chromosomes there were only 2 instead of 3 of 1 or more of the 18 kinds of chromosomes ($x=18$). These 2 chromosomes probably form a bivalent and separate to opposite poles of the spindle at anaphase I and thereby contribute toward an equal distribution of all the chromosomes to the 2 daughter nuclei. More equality in number of chromosomes in the 2 daughter nuclei is therefore expected. Although the number of pollen mother cells is small, this is indicated. This is true also for the 56-chromosome plants, which have 2 extra chromosomes, 3 being the greatest deviation from equality (25:31 instead of 28:28). This, too, is consistent with expectation. These 2 additional chromosomes either convert 2 of the trivalents into quadrivalents (4 of a kind) or constitute an additional pair (2 of a kind). In either case they decrease the amount of odd balance and increase that of even balance, thus influencing the tendency toward equal distribution.

Among the pollen mother cells from 54-chromosome plants that showed lagging chromosomes, the distribution at anaphase I fell within the range shown by cells without laggings. There were fewer chromosome groups with more than 27 chromosomes but not more groups with less than 24 chromosomes, although the latter would be expected. This held true also for the 56-chromosome plants. For the 49- to 53-chromosome plants the presence of more laggings per pollen mother cell not only decreased the number of chromosome groups with more than half of the somatic chromosome number but also increased the number which had less than half.

If the chromosome groups from all of the pollen mother cells are considered individually, only 11 consisted of less than 22 chromosomes. For the 54-chromosome plants 89 percent contained 24 to 29 chromosomes, inclusive. For the 49- to 53-chromosome plants 71 percent contained 23 to 28 chromosomes. For the 56-chromosome plants, 80 percent contained 25 to 28 chromosomes. Therefore, most of the pollen grains probably contained more than 22 and less than 30 chromosomes, even after allowing for additional lagging of chromosomes at anaphase II. Not all produced viable pollen, and probably very few could fertilize an egg nucleus. Powers and Rollins⁸ rated $54 \pm$ -chromosome plants poor as pollen parents.

Some data indicating the chromosome number of functional pollen grains produced by $3x$ plants were obtained by determining the

⁸ Powers, L., and Rollins, R. C. Unpublished data.

chromosome numbers of aberrants belonging to the triploid collections 4257 and 4264 and aberrant offspring from noncontrolled pollinations of field-grown plants belonging to the triploid collection 4263. Since aberrants arise from the fertilization of eggs which have the unreduced chromosome number, the probable number of chromosomes contributed by the pollen could be determined by subtracting the number contributed by the eggs from the total (table 2). For collections 4257 and 4264 the probable chromosome numbers of effective pollen ranged from 26 to 33. Pollen from both $54 \pm$ - and $72 \pm$ -chromosome plants probably fertilized plants belonging to 4263, as indicated by the range, which extended to 36. Also 1 aberrant offspring from a 54-chromosome species hybrid had 85 to 87 chromosomes, indicating fertilization by a 31- to 33-chromosome pollen grain.

TABLE 2.—*Estimated number of chromosomes contributed by pollen grains involved in production of aberrant plants in three 3x stands of guayule*

Collection No.	Aberrant plants		Chromosomes in plants	Estimated chromosomes in effective pollen
	Number	Number	Number	Number
4257.....	6	80-86	26-32	
4264.....	3	81-87	27-33	
4263.....	10	81-90	27-36	

In order to learn whether any pollen grains that are produced by triploids and have less than 26 chromosomes can effect fertilization, a controlled cross was made by using plants belonging to collection 4255 ($36+$ chromosomes) as the seed parent and plants belonging to collection 4262 ($54 \pm$ chromosomes) as the pollen parent. The chromosome numbers of a few of the offspring were determined. Their numbers (43, 45, 46, 48, and 49) indicated fertilization by pollen grains which had 25 to 31 chromosomes. Therefore, on the basis of controlled and noncontrolled pollinations it appears that triploids produced functional pollen grains which contained 25 to 33 chromosomes, whereas the prediction made from a study of anaphase distributions was 22 to 30. Apparently grains which contained 22, 23, or 24 chromosomes were nonfunctional.

TABLE 3.—*Distribution of chromosomes at anaphase I in pollen mother cells of 43- and 45-chromosome plants of guayule*

[Miniature chromosomes not included; laggards indicated by central figure in series of 3]

Group of plants	Plants	Chromosomes in 2 groups only		Chromosomes in 2 groups plus laggards	
		Pollen mother cells with indicated chromosome distribution	Chromosome distribution	Pollen mother cells with indicated chromosome distribution	Chromosome distribution
	Number	Number		Number	
43-chromosome.....	1	{ 1 1	19:24 21:22	1 1	19:1:23 20:2:21
45-chromosome.....	2	{ 1 3	21:24 22:23	1	20:3:22

Meiosis was observed in one 43-chromosome plant and two 45-chromosome ones. At metaphase I, 1 of the latter showed 19 bivalents plus 7 univalents in a pollen mother cell whereas the other showed 16 bivalents plus 7 univalents plus 2 trivalents. The approximation to equal distribution in 9 pollen mother cells at anaphase I is rather good (table 3).

TETRAPLOIDS

Information about meiosis in pollen mother cells of $4x$ and aneuploid $4x$ plants is based on samples of the 9 collections from Mexico, 15 percent of the extensive Texas collections, 25 of McCallum's commercial strains, and nonapomictic progeny of controlled pollinations in experiments. This sampling of tetraploids included 2 morphological types, the so-called good guayule type, which gives no evidence of previous admixture with mariola, and the "intermediate" type, which does show some of the characteristics of mariola and for the origin of which introgressive hybridization with mariola has been proposed by Rollins (11) and Stebbins and Kodani (13).

From such wide sampling a generalized, over-all picture of meiosis in tetraploids was obtained. In apomictically produced $4x$ plants of both "intermediates" and good guayule types quadrivalents were seen but the number of quadrivalents and trivalents varied among collections; some showed very few of them and others more. Possibly as many as 6 chromosomes were involved in occasional configurations. In general, at metaphase I plants which had 72, 73, or 74 chromosomes showed mostly bivalents plus 1 or a few quadrivalents, possibly trivalents, and a few univalents. The "intermediate" type seemed to have more quadrivalents and polyvalents than the good guayule type and correspondingly fewer bivalents. In view of the origin of "intermediates" involving introgressive hybridization with mariola, some of these polyvalents probably are catenations of chromosomes formed as a consequence of structural hybridity. Many pollen mother cells showed lagging chromosomes at anaphase I. All of the "intermediates" averaged higher than the good guayule type in the number of cells having lagging chromosomes, and the 72- and 73-chromosome "intermediates" averaged more laggings per pollen mother cell than the good guayule type of the same chromosome number. The good guayule type (72, 73, and 74 chromosomes) had fewer cells showing laggings than the 54-chromosome plants; but the average number of laggings per cell was about the same for both. However, in proportion to the total chromosome complement the number of laggings per cell among the $72\pm$ -chromosome plants was less than among the $54\pm$ -chromosome (tables 1 and 4). Chromatid bridges with or without fragments were not common, but they were seen occasionally in many plants of diverse origin. Some lagging at anaphase II were observed also.

Meiosis in some of the plants classified morphologically as offtype normals was indistinguishable from normals, whereas others showed meiotic irregularities. Two of the irregularities observed in a few diploids were found also among aneuploid $4x$ plants. They were partial asynapsis seen in some aneuploids, the chromosome numbers of which ranged from 65 to 71, and less frequently failure of cytokinesis seen in a few aneuploids, the chromosome numbers of which

TABLE 4.—*Distribution of chromosomes at anaphase I in pollen mother cells of 4x and aneuploid 4x (67- to 74-chromosome) plants of guayule*

[Miniature chromosomes not included; lagers indicated by central figure in series of 3]

Group of plants	Plants	Chromosomes in 2 groups only		Chromosomes in 2 groups plus lagers	
		Pollen mother cells with indicated chromosome distribution	Chromosome distribution	Pollen mother cells with indicated chromosome distribution	Chromosome distribution
	<i>Number</i>	<i>Number</i>		<i>Number</i>	
67-chromosome.....	1	2	33:34	1	31:3:33 33:1:33
69-chromosome.....	3	1	33:36	1	28:11:30 31:7:31
70-chromosome.....	5	3	33:37	1	31:4:35
		2	34:36	1	33:2:35
		2	35:35	1	34:1:35
71-chromosome.....	4	5	35:36	1	29:7:35
Do.....	1	1	35:36	1	31:3:37
		7	34:38	1	33:3:38
		12	35:37	1	33:3:36
72-chromosome.....	20	19	36:36	1	34:1:37
				1	34:2:36
				1	34:3:35
				7	35:1:36
Do.....	1	1	32:40	1	32:7:33
		1	33:39	1	33:1:38
		2	34:38	2	33:3:36
	10	10	35:37	1	33:5:34
		5	36:36	2	34:2:36
				1	34:3:35
				2	35:1:36
73-chromosome.....	3	2	34:39	1	35:3:35
		2	35:38	2	36:1:36
		4	36:37		
Do.....	1	1	35:38	1	32:5:36
		4	36:37	1	33:2:38
				1	33:4:36
	8			2	34:1:38
				1	35:1:37
				1	35:2:36
				1	35:3:35
				1	36:1:36
74-chromosome.....	4	1	34:40	1	33:1:40
		1	35:39	1	35:4:35
		8	36:38	1	36:1:37
		6	37:37		
Do.....	1			2	34:2:38
				1	34:3:37
				1	35:1:38
	2			2	35:2:37
				1	35:3:36
				1	36:1:37

1 "Intermediate" type.

ranged from 68 to 72 chromosomes. Two offtype normals belonging to the good guayule type showed many lagers at anaphase I and II but not many microcytes among mature pollen grains. However, the one with less than 72 chromosomes showed 50 percent of pollen aborted and the other, with 72 chromosomes, about 98 percent. Male steril-

ity, as evidenced by scant sporogenous tissue, was found in several plants (69- to 72-chromosome). Restitution nuclei (semiheterotypic or pseudohomeotypic) were seen, but they were confined largely to plants with less than 70 chromosomes and usually associated with partial asynapsis. Often 2 or more irregularities were found in the same plant, especially among the 65- to 69-chromosome plants.

Miniature chromosomes were seen in some $4x$ and aneuploid $4x$ plants. At most they were limited to 2. The Mexican material except collection 4258 seemed to be without any; the seeds for 4258 came from an individual plant growing within the diploid population from which collection 4255 was obtained. (In collection 4255 the number of miniatures ranged from 2 to 10.) Most of the plants from the commercial strains had as many as 2; however, some plants with only 1 were found in strains 406 and 444.

The distribution of chromosomes at anaphase I in pollen mother cells of $4x$ and aneuploid $4x$ plants was observed in plants the chromosome numbers of which ranged from 67 to 74 (table 4). For cells showing no lagging chromosomes in 72-chromosome plants belonging to the good guayule type, the observed range of distribution at anaphase I was from 34:38 to 36:36 with the following frequencies: 7 showed 34:38; 12, 35:37; and 19, 36:36. For those belonging to the "intermediate" type it was from 32:40 to 36:36 with the following frequencies: 1 showed 32:40; 1, 33:39; 2, 34:38; 10, 35:37; and 5, 36:36. A χ^2 test showed that these 2 distributions were not significantly different. The percentages of observed frequencies in these classes were compared with the percentages of expected ones calculated according to the random distribution of 18 quadrivalents. For the good guayule type all of the observed frequencies were in 3 classes in which, according to calculations, only 59.5 percent should be; for the "intermediate" type 89.4 percent were in these same 3 classes. This lack of close agreement between observed distribution frequencies at metaphase I and those calculated according to the random distribution of 18 quadrivalents is the result of an obvious tendency toward equal distribution of chromosomes to the 2 poles of the spindle at anaphase I. If 72-chromosome plants formed only 36 bivalents at metaphase I, a 36:36 distribution would be expected at anaphase I. If they formed only 18 quadrivalents at metaphase I, close agreement would be expected with distributions calculated according to the random distribution of 18 quadrivalents. Actually all showed some bivalents at metaphase I, the good guayule type in general showing more than the "intermediates." Therefore the observed distributions are consistent with the observed tendency toward bivalent formation at the $4x$ level in the polyploid series.

For cells showing no lagging chromosomes in aneuploids with more or less than 72 chromosomes, the tendency toward an even distribution of chromosomes to each pole is evident and nowhere does the distribution fit one based on quadrivalent formation.

For pollen mother cells showing lagging chromosomes in 72-chromosome plants, the range of the anaphase I distribution closely approximated that of pollen mother cells without laggings, although there were fewer chromosome groups with more than 36 and more with less than 34 chromosomes. The same was true for the aneuploid

4x plants, namely fewer groups with more than half of the somatic chromosome number and more with less than half.

If the chromosome groups from all of the pollen mother cells which showed lagging chromosomes are considered individually, only 11 consisted of less than 33 chromosomes. For the 72-chromosome plants 88.6 percent contained from 33 to 36 chromosomes, inclusive. Even the 67- to 71-chromosome plants had 54 percent of groups with 33 to 36 chromosomes. The 73-chromosome plants had 62.5 percent of groups with 35 to 36 chromosomes, and the 74-chromosome plants had 63.6 percent of groups with 35 to 37 chromosomes. Therefore, even after additional lagging of chromosomes at anaphase II, most of the pollen grains probably contained not less than 33 or more than 36 chromosomes.

Some data indicating the chromosome number in functional pollen grains was obtained by determining the chromosome number of 50 aberrant plants belonging to 4x collections and then subtracting the probable number of chromosomes contributed by the egg. The range extended from 28 to 40, usually from 31 to 38. Also 7 aberrants obtained from controlled pollinations, where the chromosome number of the parents also had been determined, showed that the functional pollen grains contained 31 to 39 chromosomes. These data on aberrants, therefore, indicated a wider range of chromosome numbers contained in functional pollen grains than was estimated from the anaphase data in the preceding paragraph.

PLANTS WITH HIGHER CHROMOSOME NUMBERS

Information about meiosis in pollen mother cells of plants having 81 or more chromosomes is based almost entirely on aberrant plants which occurred in natural populations and commercial strains and among progeny of controlled and noncontrolled pollinations in experiments.

Aberrant plants occurring in $54 \pm$ -chromosome populations and strains and among progeny of 3x seed parents ranged in chromosome number from 80 to 93. Observations of the metaphase I stage were confined mostly to plants belonging to collection 4257. Some trivalents and polyvalents and a few univalents were seen, but bivalents predominated; therefore, the tendency toward bivalent formation which was evident in 4x plants continued on up through the polyploid series. The frequencies of chromatid bridges and chromosome lagging at anaphase I and II were about the same as for normal plants in the same cultures. As many as 3 miniature chromosomes were seen in some plants; some had fewer, and others had none. Two plants with about 85 chromosomes had scant sporogenous tissue and therefore were practically male sterile.

The tendency toward equal distribution of chromosomes to the 2 poles of the spindle at anaphase I is apparent in pollen mother cells of 81- to 93-chromosome plants (table 5). It was evident even in a 122-chromosome offspring from a self-pollinated, 83-chromosome plant where 2 cells showed 59:63 and 60:62 distributions at anaphase I. Among pollen mother cells without lagging chromosomes the extreme deviation from equal distribution was 36:47 (instead of 41:42) and among those with laggings it was 33:14:39 in an 86-chromosome

plant. As in the case of 3x and 4x plants, cells with lagging chromosomes had fewer groups of chromosomes consisting of more than a half of the somatic number as compared with those which do not have laggings.

If the chromosome groups from all of the pollen mother cells are considered individually, only 1 consisted of less than 35 chromosomes. For the 81-chromosome plants 75 percent of the groups consisted of 39 to 43 chromosomes, inclusive. Because of more lagging chromosomes in the 86-chromosome plants, they had practically the same number of groups (71 percent) consisting of 39 to 43 chromosomes. For all of the cells 69 percent of the groups consisted of 39 to 43 chromosomes and 85 percent 39 to 46 chromosomes. Probably most of the pollen grains contained at least 38 chromosomes but not more than 43.

TABLE 5.—*Distribution of chromosomes at anaphase I in pollen mother cells of 81- to 93-chromosome plants from 3x (54±-chromosome) seed plants of guayule*

[Miniature chromosomes not included; laggings indicated by central figure in series of 3]

Group of plants	Plants	Chromosomes in 2 groups only		Chromosomes in 2 groups plus laggings	
		Pollen mother cells with indicated chromosome distribution	Chromosome distribution	Pollen mother cells with indicated chromosome distribution	Chromosome distribution
	<i>Number</i>	<i>Number</i>		<i>Number</i>	
81-chromosome.....	5	1 1 1 2 5	36:45 37:44 38:43 39:42 40:41	1 1	38:1:42 39:2:40
82-chromosome.....	1	1	41:41	1	39:2:41
83-chromosome.....	2	1 1	36:47 41:42	1	39:2:42
86-chromosome.....	6	4 1 1	40:46 42:44 43:43	1 1 1 1 1	33:14:39 35:9:42 39:4:43 39:5:42 39:7:40 42:1:43
88-chromosome.....	1	1 1	42:46 44:44		
90-chromosome.....	1	1 1	41:49 42:48		
93-chromosome.....	1			1	40:8:45

Aberrant plants occurring in 72±-chromosome populations and strains and among progeny of 4x seed parents ranged in chromosome number from 99 to 120. Observations of the metaphase I stage showed a preponderance of bivalents; however, some plants showed many trivalents and quadrivalents and others many univalents. This varied behavior may depend upon the fact that some of the aberrants in populations of the good guayule type had mariola as their pollen parent and some were from "intermediate" populations. Chromatid bridges at anaphase I were seen only infrequently. Not more than 3 miniature chromosomes were counted in 1 plant. Chro-

mosome lagging at anaphase I was frequent but limited to only a few chromosomes so that, in proportion to the total, it did not materially reduce the number of chromosomes in the 2 groups in each pollen mother cell. Lagging at anaphase II was seen only infrequently. Male sterility in the form of scant sporogenous tissue was observed in a 100-chromosome plant.

The tendency toward equal distribution of chromosomes in pollen mother cells of 99- to 109-chromosome plants to the 2 poles of the spindle at anaphase I is evident (table 6). Among cells without lagging chromosomes the extreme deviation from equal distribution was 49:58 (instead of 53:54) and among those with lagers it was 47:4:57 in a 108-chromosome plant. Cells which had lagging chromosomes had somewhat fewer groups consisting of more than a half of the somatic number than those which did not have lagers.

TABLE 6.—*Distribution of chromosomes at anaphase I in pollen mother cells of 99- to 109-chromosome plants from 4x (72±-chromosome) seed plants of guayule*

[Miniature chromosomes not included; lagers indicated by central figure in series of 3]

Group of plants	Plants	Chromosomes in 2 groups only		Chromosomes in 2 groups plus lagers	
		Pollen mother cells with indicated chromosome distribution	Chromosome distribution	Pollen mother cells with indicated chromosome distribution	Chromosome distribution
	<i>Number</i>	<i>Number</i>		<i>Number</i>	
99-chromosome	1			1 1	46:3:50 47:2:50
100-chromosome	2			1 1	48:2:50 49:2:49
104-chromosome	1			1 1 1	48:1:55 49:1:54 51:1:52
105-chromosome	2	2	50:55	1 1 1	48:1:56 49:2:54 51:1:53
106-chromosome	2	1	52:54	1 1	49:2:55 51:3:52
107-chromosome	5	1 1 1 3	49:58 51:56 52:55 53:54	1 1 1 1 2 1 2	48:2:57 50:1:56 50:2:55 51:3:53 52:1:54 52:2:53 53:1:53
108-chromosome	7	1 1 6	50:58 51:57 53:55	1 1 3	47:4:57 51:5:52 52:1:55
109-chromosome	3	2 1	52:57 54:55	2 1 1	53:1:55 53:2:54 54:1:54

If the chromosome groups from all of the pollen mother cells are considered individually, only 3 consisted of less than 48 chromosomes. For the 107- to 109-chromosome plants 73 percent of the groups consisted of 52 to 55 chromosomes, inclusive. For all of the cells, 62 percent of the groups consisted of 52 to 55 chromosomes and 83 per-

cent 49 to 55. Probably most of the pollen grains contained at least 48 chromosomes but not more than 55.

Among the progeny of aberrant plants belonging to collection 42163 there were some plants that showed only a few of the morphological characteristics of aberrants, although the chromosome numbers ranged from 74 to 93. They were called offtype normals from aberrants. Pollen mother cells of 6 plants, whose chromosome numbers ranged from 85 to 92, were studied. Lagging at anaphase I was limited to a relatively few chromosomes. Although the data for anaphase I distribution are scant (table 7), they show the same tendency toward equal distribution to the 2 poles of the spindle that was evident in the aberrant plants which had approximately the same number of chromosomes (table 5). The extremes in deviation from equality came within the limits of deviation shown by the aberrants. From the standpoint of meiosis in pollen mother cells, therefore, this group did not differ from other plants having high chromosome numbers.

TABLE 7.—*Distribution of chromosomes at anaphase I in pollen mother cells of offtype normals from aberrant 85- to 92-chromosome plants of guayule*

[Miniature chromosomes not included; laggards indicated by central figure in series of 3]

Group of plants,	Plants	Chromosomes in 2 groups only		Chromosomes in 2 groups plus laggards	
		Pollen mother cells with indicated chromosome distribution	Chromosome distribution	Pollen mother cells with indicated chromosome distribution	Chromosome distribution
	<i>Number</i>	<i>Number</i>		<i>Number</i>	
85-chromosome.....	2	2 1 2	40:45 41:44 42:43		
89-chromosome.....	1			1 1	39:5:45 44:1:44
91-chromosome.....	2	1 2	42:49 44:47	1 2	42:1:48 45:1:45
92-chromosome.....	1			1 1	40:2:50 44:1:47

HAPLOIDS

As previously reported (1) a few plants with 36+ chromosomes were found in 72±-chromosome populations. Information about meiosis in pollen mother cells of such haploid plants is based on observations made on 9 of them which were studied cytologically; 3 had 36⁺¹ chromosomes, 5 had 37⁺¹, and 1 had 37⁺². In a tapetal cell of the haploid from collection 4285, 37⁺¹ chromosomes were counted (fig. 1, B). All the haploids had at least 1 miniature chromosome. Sporogenous tissue was scant, and the walls of pollen mother cells were extremely thin. The metaphase I stage was observed in 8 plants, all of which showed some bivalents; 2 plants showed only a few and 4 practically all (fig. 1, D, E, and F). A circle of 4 chromosomes was seen in some pollen mother cells of the haploid from Tesnus, Tex., (fig. 1, D) and a chain of 4 in cells of the haploid from strain 130. These configurations of 4 chromosomes may indicate reciprocal

translocation. In 1 cell the miniature chromosome was attached to the circle of 4 (fig. 1, *D*). Distribution of chromosomes at anaphase I in 7 haploid plants (table 8) was less regular than the distribution in diploid 36+-chromosome plants. One pollen mother cell at anaphase II was observed; the distribution, 18 : 18 : 17 : 19, indicated an irregularity at this stage also. Practically all of these haploids produced very little pollen, or the pollen grains were mostly empty.

TABLE 8.—*Distribution of chromosomes in pollen mother cells at anaphase I of haploids from 4x (36- and 37-chromosome) seed plants of guayule*

[Miniature chromosomes not included; laggards indicated by central figure in series of 3]

Group of plants	Plants	Chromosomes in 2 groups only		Chromosomes in 2 groups plus laggards	
		Pollen mother cells with indicated chromosome distribution	Chromosome distribution	Pollen mother cells with indicated chromosome distribution	Chromosome distribution
	Number	Number		Number	
36-chromosome.....	2	{ 3 2 2	16:20 17:19 18:18	}	-----
37-chromosome.....	5	{ 1 9	17:20 18:19	1 1	17:2:18 18:1:18

Bivalent formation in these haploids demonstrates that there is considerable homology among the 4 sets of chromosomes in the 72±-chromosome plants from which they arose. However, such homology ought not be used as evidence for autopolyploidy as opposed to allopolyploidy, since it is known that some pairing occurs even between guayule and mariola chromosomes in hybrids between these 2 species. For instance, two 54-chromosome natural hybrids were the result of fertilization of 18-chromosome guayule eggs by 36-chromosome mariola pollen grains. In 1 hybrid a maximum of only 10 univalents was seen, whereas in the other, where the maximum of univalents was only 6, from 1 to several trivalents were observed.

These studies of chromosome behavior have centered around associations at metaphase I, distribution at anaphase I, and, for plants having 54± and 72± chromosomes, the effects of this distribution on the production of functional pollen grains. Throughout this series from 36 (both diploids and haploids from tetraploids) to 120± it is evident that meiosis in pollen mother cells in most guayule plants at all levels in the polyploid series is complete; reduction of chromosome number is effected, and a quartet of microspores is formed within the cell by division of the cytoplasm after telophase II. In only a few variants have exceptions to this normal behavior been found (such as lack of sporogenous tissue, abortion of all pollen, or other meiotic irregularity).

In general there was a tendency toward equal distribution of chromosomes at anaphase I, throughout the polyploid series, regardless of whether the plants were of amphimictic or apomictic origin and regardless of the number of chromosomes. Among 36+-chromosome diploids anaphase I distribution was predominantly 18:18.

Among 36- and 37-chromosome haploids from $72\pm$ -chromosome populations the distribution was less regular. Among triploids anaphase I distribution was not in disagreement with calculations based on the random distribution of 18 trivalents, although the number of observations was too small for statistical analysis. Among tetraploids at anaphase I there was an obvious tendency toward equal distribution. Among plants with more than 81 chromosomes there was a pronounced tendency toward equal distribution. This tendency was evident also in those cells from aberrants which contained fewer chromosomes than the number typical of the aberrants in which they were produced as well as in the amphimictically produced offspring of aberrants. The inequality of distribution in the higher polyploids was no greater than that in triploids and in proportion to the total number of chromosomes involved was much less. This tendency toward equal distribution of chromosomes at anaphase I was interpreted as being the result of the tendency toward bivalent formation at metaphase I. Usually there were 18 bivalents in pollen mother cells of $36+$ -chromosome diploids. Among 36- and 37-chromosome haploids, however, the number of bivalents was variable. The number of bivalents shown by tetraploids varied among the collections, the number being greater for the good guayule type. Among plants with higher chromosome numbers, where polyvalents were expected, bivalent formation was pronounced.

POLLEN

Examination of shed pollen in many instances preceded a study of meiosis in pollen mother cells because of the ease and speed with which pollen can be observed. For instance, microcytes were observed in the pollen of "intermediate" plants before studies of meiosis in pollen mother cells of these plants were begun and a causal relation between lagging chromosomes at the anaphases and microcytes in pollen was suggested. The conclusion that the diameter of pollen grains is proportional to the number of chromosomes within the grain was reached after the parallel determination of chromosome number and anaphase distributions in pollen mother cells and size of pollen grains of many individual plants throughout the polyploid series (8, table 3).

The five diploid collections differed in average amount of aborted pollen (9). To what extent aneuploidy, inversions, and nondisjunction were reflected in the amount of aborted pollen cannot be stated. As noted on page 8, three plants which produced mostly aborted grains showed asynapsis at metaphase I. Certainly there was no correlation between amount of abortion and number of miniature chromosomes (table 9). Microcytes were entirely absent or rare in all diploid plants examined.

Pollen of $3x$ plants may be distinguished from that of $2x$ plants by the presence of microcytes and by the variable size of the good grains rather than by the percentage of pollen grains aborted. The average percentage of the latter is somewhat higher than in diploids (9, table 1). In pollen from plants belonging to the five collections from Mexico and four commercial strains the writer found varying percentages of grains aborted, ranging from 5 to 66 percent. Aneuploid $3x$ plants averaged higher; the range extended from 50 to 98 percent,

the latter being a consequence of partial asynapsis at metaphase I. The variable size of good grains is shown statistically by Powers (8, table 3). Some grains are small like those produced by $2x$ plants, others are larger like those produced by $4x$ plants, and still others are intermediate in size.

TABLE 9.—Occurrence of aborted pollen grains and miniature chromosomes in 38 plants of five $36+$ -chromosome (diploid) collections

Collection No.	Pollen grains aborted	Miniature chromosomes	Collection No.	Pollen grains aborted	Miniature chromosomes
	<i>Percent</i>	<i>Number</i>		<i>Percent</i>	<i>Number</i>
4250	53	3	4255	5	3
	56	2		5	6
4251	4	2		6	8
	11	2		9	8
	16	0		12	10
	17	0		14	6
4253	25	2		15	2
	16	1		15	4
	31	1		15	8
	43	1		15	10
	46	1		16	3
4254	70	1		21	6
	3	0		24	2
	5	2		31	2
	10	2		54	1
	12	0		54	4
	14	3		58	6
	30	0		95	7
	42	2			
	59	2			

¹ Based on only 100 grains each; determinations by E. J. Gardner.

Pollen of $4x$ plants may be distinguished from that of $2x$ plants by the larger size of the good grains (8) and from that of $3x$ plants by their more uniform size. The "intermediate" type shows more microcytes than the $3x$, but the good guayule type shows fewer. For the collections included in the study made by Powers and Gardner (9) the range of percentage of grains aborted by the good guayule type coincided with that of the $36+$ -chromosome collections. The range for the Texas "intermediate" type was somewhat in excess of that of the $54\pm$ -chromosome collections. The "intermediates" averaged 2 to 5 times the number of microcytes in the good guayule type. The range of the 4 most commonly grown commercial strains was between that of the good guayule type and the "intermediate" Texas type. Only 1 Mexican collection was included in Powers and Gardner's study.

Pollen samples of all nine of the Mexican collections were examined by the writer. Only one of the good guayule type averaged higher in percentage of pollen aborted than the collection included in Powers and Gardner's study (9), but four averaged lower. The amount of aborted pollen of "intermediates" was consistently higher than that of the good guayule type in those collections where both types occurred. The averages of number of microcytes for the Mexican collections came within the range of the averages for the Texas collections. In every instance the "intermediates" could be distinguished by their many microcytes.

Some of the less known commercial strains were sampled by the writer. Five strains of the good guayule type produced very little

aborted pollen, whereas five others produced plenty of pollen practically all of which was aborted. This sort of male sterility is different from the lack of sporogenous tissue shown by several aneuploid off-type normals from the Texas collections. For four of the "intermediate" commercial strains about 15 percent of the grains was aborted. Again the average number of microcytes ranged from two to five times that for the good guayule type.

The amount of aborted pollen produced by plants classified as off-type normals (53 to 75 chromosomes) ranged from 15 to 99 percent. The pollen produced by 5.5 percent of these plants consisted of less than 25 percent of aborted grains, whereas the pollen produced by 59.3 percent of these plants consisted of 50 percent or more of aborted grains. In general, those plants the pollen of which consisted of 75 percent or more aborted grains had 70 or less chromosomes. The most striking exception was a 72-chromosome plant the pollen of which consisted of about 98 percent of aborted grains.

Pollen of plants with high chromosome number may be recognized by the large size of the good grains. Some grains are round, but most of them are elliptical in shape. As shown by Powers (8, table 3), there is a greater range in size than for plants with 72 or less chromosomes. This is partly due to the varying number of chromosomes within the grains and partly to the fact that the diameter was measured in the line of the ocular micrometer regardless of the orientation of the elliptical grains with reference to the micrometer. The percentages of grains aborted varied with the collections; in general, they fell within the range of the normals of the same populations. This was especially true for the aberrants from $3x$ plants. For the aberrants from $4x$ plants there was a tendency toward increased percentage of abortion as compared with the normals. The number of microcytes produced by aberrants from $3x$ plants fell within the range characteristic of the normals of the same collections. Among aberrants from $4x$ plants those belonging to the good guayule type, regardless of source, showed a slight increase in number of microcytes as compared with the number produced by comparable normals. In general the so-called rough aberrants averaged high in number of microcytes. In one of the Mexican "intermediate" collections (4242), the aberrants averaged less than half as many microcytes as the normals of that collection; in others they averaged about the same.

LOSS OF CHROMOSOMES BY ABERRANTS

A phenomenon which seems to be rather widespread among aberrant plants is the loss of chromosomes, both meiotically and mitotically. In addition to the loss of a few chromosomes in pollen mother cells through lagging at anaphase I and II, such as was seen in $54\pm$ - and $72\pm$ -chromosome plants, especially those of the "intermediate" type, loss of large groups of chromosomes was observed occasionally during meiosis in pollen mother cells of aberrant plants. This tendency has been observed in collections from Texas as well as Mexico and in commercial strains, although it seems to be more common in some plants than in others. That the spindle mechanism may be inadequate for so many chromosomes is suggested by the formation of a multipolar spindle (fig. 1, G) where the 106 chromosomes are in 4

instead of 2 groups at anaphase I. Four groups instead of the usual 2 at second prophase (fig. 1, *H*) are the result probably of a multipolar spindle during the first division. For comparison, the usual 2 groups at this stage are shown in another pollen mother cell from the same plant (fig. 1, *I*). Another way by which the number of chromosomes can be decreased is by the formation of twin pollen mother cells. For example (fig. 1, *J*), twin pollen mother cells, incompletely separated, contained 107 chromosomes between them; however, these chromosomes were unequally distributed. One half had 46 chromosomes in 2 groups of 22 and 24, respectively, whereas the other half had 7 chromosomes in 1 group and 54 in the other.

The loss of chromosomes from somatic cells has not been seen but may be inferred. Because Meyer's technique (7) was developed only recently, observations on somatic divisions in root-tip cells are still meager. However, it has been observed in root-tip cells from aberrant plants that intact cells from the same plant may vary in chromosome number. That such losses may occur some time prior to the formation of pollen mother cells is shown by the fact that intact pollen mother cells which contained fewer than the usual number of chromosomes have been seen. Differences in the size of the pollen mother cells were discernible, since there is a correlation between their size and the number of contained chromosomes. In the same plant which produced the pollen mother cells whose chromosome contents are shown in figure 1, *H* and *I*, other pollen mother cells which contained fewer chromosomes were found. One had 78 (fig. 1, *K*), while another had 59 (fig. 1, *L*). Another pollen mother cell had 85 chromosomes. Another example is given in figure 1, *M*, where a cell at telophase II contains only 120 chromosomes in the 4 nuclei. This cell must have contained only 60 chromosomes before meiosis began. Another cell from this same plant had 63 chromosomes. Other chromosome numbers, such as 40, 45, 49, 52, 55, 56, 57, 58, 65, 67, 68, 76, 77, 80, 81, 84, and 93, were counted in exceptional pollen mother cells from 14 other aberrant plants whose chromosome numbers ranged from 102 to 120 and which came from 10 different collections.

That such pollen mother cells may go through both meiotic divisions is known because chromosomes have been counted at telophase II. One 60-chromosome cell showed only 1 bivalent at metaphase I, the remaining chromosomes being univalent. An 80-chromosome cell from another plant showed both bivalents and univalents at metaphase I, and a 76-chromosome cell showed only univalents. Distribution of chromosomes at anaphase I in other cells whose chromosome numbers are listed in the preceding paragraph follows:

Chromosomes in pollen mother cell:	Distribution ¹	Chromosomes in pollen mother cell:	Distribution ¹
40.....	18:22	59.....	27:2:30
45.....	21:3:21	65.....	30:1:34
49.....	24:25	68.....	32:36
52.....	22:1:29	77.....	33:7:37
55.....	27:28	78.....	33:2:43
56.....	25:1:30	79.....	35:1:43
57.....	24:33	81.....	39:42
58.....	27:1:30	84.....	39:1:44
58.....	27:3:28	93.....	44:3:46

¹ Laggards indicated by the central figure in the series of 3.

Although these are exceptional cells from aberrant plants, the distribution is not essentially different from that observed in those plants with comparable chromosome number (tables 1, 3, 4, and 5).

Reduction in chromosome number can precede the development of a tapetal cell. For example, a tapetal cell containing only 46 chromosomes was found (fig. 1, *N*) on the same slide with pollen mother cells containing about 108.

Among aberrants in $54 \pm$ -chromosome populations very few pollen mother cells which contained less than the usual number were found, although the ratio of 81 to 54 is the same as that of 108 to 72. Two cells showed 54 and 57 chromosomes, respectively, in an 86-chromosome plant and 1 showed 73 chromosomes in an 88-chromosome plant. Apparently it is the high chromosome number (100 or more) among aberrants in $72 \pm$ -chromosome populations which leads to loss of chromosomes. The loss of large groups of chromosomes by aberrant plants is of interest to the plant breeder if embryo sacs are produced by chimeras which have fewer chromosomes. Apomictic production of seeds by such chimeras could lead to the establishment of clones having chromosome numbers different from those of either the aberrant or the usual types in the collections to which they belong.

CHROMOSOMAL VARIANTS WITHIN THE POLYPLOID SERIES

ORIGIN

One of the characteristics of $36+$ -chromosome populations of guayule is the evident morphological diversity within the different collections. This is the consequence of amphimixis and cross-pollination, since they are largely self-incompatible (10). In contrast to this diversity among diploids the various triploid and tetraploid stands are fairly uniform among themselves although differing from one another in morphological characteristics. This uniformity is a consequence of apomictic reproduction. However, there are individual plants which deviate from the usual type characteristic of the $3x$ and $4x$ stands and commercial strains in which they occur. Three types of morphological variants have been distinguished: Haploids, the so-called offtype normals, and aberrants. Most of these plants are the result of amphimixis, since apomixis is only facultative (10). Moreover, Esau (4) has shown that normal meiosis may occur in plants which are predominantly aposporous. Therefore, some eggs with the reduced number of chromosomes are produced. If they develop without fertilization (parthenogenesis), they give rise to haploids, a number of which have been found in $72 \pm$ -chromosome populations. If eggs which have the reduced number of chromosomes are fertilized (amphimixis), they give rise to plants which may or may not have the same number of chromosomes as apomictically produced plants in the same cultures, the variability in chromosome number being the result of the varying number contained in the egg nucleus plus the varying number brought in by the pollen grain. Probably most of the offtype normals are amphimicts. In addition, eggs which have the unreduced number of chromosomes may be fertilized also. Aberrants are produced in this way. Consequently morphological variants may have the same number of chromosomes as the apomicts of the populations in which they occur or they may have more or fewer chromosomes.

HAPLOIDS FROM TETRAPLOIDS

Plants which result from the development of an egg which has the reduced number of chromosomes without fertilization have been called haploids. Unfortunately, the word "haploid" has been used in 2 senses, namely as being equal to one-half of the diploid number of chromosomes (monoploid) and also as being equal to one-half of the somatic number. According to the first definition a haploid guayule should have 18 chromosomes. No such plant has been found as yet. According to the second definition a series of haploids is theoretically possible, since there is a polyploid series in guayule. Regardless of the sense in which the word is used, however, a haploid owes its origin to the development of an unfertilized egg nucleus which has the reduced number of chromosomes. As previously reported (1), a few haploids were found in populations which have $72 \pm$ chromosomes. Since then more have been obtained from seeds collected in Texas and also from commercial strains by Powers and Rollins (10), by Rollins,⁹ by Stebbins and Kodani (13), and by the writer. In general the plants were slow growers. They flowered profusely, but the flower heads were small.

ANEUPLOIDS FROM DIPLOIDS

How much aneuploidy exists among the diploid collections cannot be stated exactly because of the additional miniature chromosomes. Since there is but slight difference in length between the smallest biarmed chromosomes and miniatures, a trisomic univalent might be counted as a miniature. Fortunately in only 1 of the collections are miniatures more numerous than 3. If the postulation that miniatures owe their origin to the breakage of biarmed chromosomes is correct, they are duplicate portions of chromosomes and the plants in which they occur are aneuploid to that extent. A small offtype-normal plant with rudimentary disk floret bracts was found to have 37 chromosomes.

Since diploid $36+$ -chromosome plants reproduce amphimictically, there is a profusion of aneuploid triploids among their progenies when they are used as seed parents in crosses with $54 \pm$ - and $72 \pm$ -chromosome plants. The range of the sample studied was 43 to 56 chromosomes, inclusive. The plants chosen for cytological study from noncontrolled pollinations showed at least 50 percent of the pollen aborted and mostly more than 75 percent. Their chromosome numbers ranged from 49 to 56. Morphologically these plants differed from one another, but their diversity did not exceed the morphological diversity characteristic of $36+$ -chromosome populations. The only small plant studied was a 53-chromosome plant which had thick leaves. All the others were vigorous and medium-sized or large, irrespective of chromosome number, and could not be distinguished from other plants in the same cultures. From controlled pollination with $54 \pm$ -chromosome plants, the plants studied ranged in chromosome number from 43 to 49. The 48- and 49-chromosome plants were of average vigor, but the 43- and 45-chromosome ones grew slowly and were less vigorous.

⁹Rollins, R. C. Unpublished data.

OFFTYPE NORMALS FROM TRIPLOIDS AND TETRAPLOIDS

A few of the variants classified as offtype normals in apomictic $3x$ and $4x$ populations had exactly 54 and 72 chromosomes, respectively; most of them were aneuploids. For the $54\pm$ -chromosome populations an example of aneuploids may be cited. As a check in an experiment, seeds obtained from noncontrolled pollination of plants in collection 4263 were sown. Among the progeny there were 4 offtype normals which had 54, 56, 64, and about 66 chromosomes, respectively. The 56-chromosome plant was larger than the other 3, but all were smaller than the apomicts in the same culture. The plant with about 66 chromosomes had a loose growth habit. The 64-chromosome plant had large, gray, thickened leaves and large flower heads. At least 75 percent of the pollen produced by all 4 was aborted.

Most of the offtype normals whose chromosome number was determined occurred in cultures of $72\pm$ -chromosome Texas populations and commercial strains. They do not constitute a random sample. Among plants characterized by slower growth, less vigor, or unusual morphological characteristics, representatives were selected by L. Powers for cytological study in the hope that their chromosome number and meiotic behavior would indicate their mode of origin. In general, after a year's growth few of these selected plants were equal in size to the apomicts in the same cultures and not many were even medium-sized; most of them were small. Many tended toward compactness or low, spreading habit indicative of shortened internodes. There were variations in leaf size, shape, and thickness. At least 2 of the plants were sectorial chimeras. There was no apparent correlation between chromosome number and plant size. Several 72-chromosome plants were either small or very small, whereas one 67-chromosome plant was medium-sized. The chromosome numbers of the large plants ranged from 70 to 72, about the same range as shown by the very small ones. The few plants which had 73 to 75 chromosomes were small or medium-sized; 2 had thickened leaves.

The lack of correlation between chromosome number and plant size is not surprising, since it is not chromosome number but interactions among the genes that determine the vigor of a plant. A study limited to total chromosome number fails to show how many of each kind of chromosome are present. Even a 72-chromosome plant, for instance, may be an aneuploid if, instead of having 4 each of 18 kinds of chromosomes (assuming that x equals 18), it has 5 of one or more kinds and correspondingly 3 of others. The simplest alteration would be 4 each of 16 kinds plus 5 of the seventeenth plus only 3 of the eighteenth. In plants having more or less than 72 chromosomes, the balance is further upset by the absence or addition of whole chromosomes. The same sort of chromosomal unbalance may exist in 54-chromosome plants and in those having more or less than 54.

Of the offtype normals whose chromosome number was determined to be $4x$ or aneuploid $4x$, 33.9 percent had 65 to 70 chromosomes; 38.9 percent, 70 or 71; 18.6 percent, 72; and 8.5 percent, 73 to 75. In addition there were 2 which had 53 and 56 to 59 chromosomes, respectively. Thus, it is evident that most of these offtype normals were aneuploids and that three-fourths of them had less than 72 chromosomes. Moreover, the chromosomal deficiency was limited to only 1

or 2 chromosomes in the majority of these plants with less than 72 chromosomes. It is possible that plants with more than 72 chromosomes were sufficiently vigorous and normal in appearance to escape being classified as offtype normals. However, the presence of additional chromosomes may be deleterious, since none of the large offtype normals had more than 72 chromosomes. Analysis of anaphase I distribution of chromosomes of tetraploids (p. 15) showed that pollen grains produced by 72-chromosome plants contained a varying number of chromosomes, mostly 33 to 36. If an equivalent variability in the reduced chromosome number in egg nuclei is assumed, then a $4x$ population should produce amphimicts ranging in chromosome number from 66 to 72. This range includes a deviation of at least 6 from 72, since 66 will be the total produced by the union of 2 gametes, each of which contains 33 chromosomes. The union of gametes which contain more than 36 chromosomes each produces plants with more than 72 chromosomes. The observed range of 65 to 75 chromosomes among offtype normals in $4x$ populations is in close agreement with the calculated range based on a study of anaphase I distribution of chromosomes. However, since the majority of the offtype normals were deficient in only 1 or 2 chromosomes, most of the combinations which involved the union of gametes which contained less than 34 chromosomes must have been inviable because of too greatly disturbed genetic balance.

Those plants which had the lowest chromosome number may be mentioned separately. Two of the Texas offtype normals had 65 chromosomes. This aneuploidy may be represented as $4x-7$. Both plants were small but unlike, and both were partially asynaptic and showed restitution nuclei at metaphase II. Sixty-five percent of the pollen of one was aborted, and the diameter of the good grains indicated that they contained the unreduced number of chromosomes. The other produced a scant amount of normal-sized grains, 50 percent of which were aborted.

There were 2 exceptional plants from Tesnus. A very small plant which had 56 to 59 chromosomes came from a nonselected collection of seed. Meiosis was characterized by partial asynapsis and lagging chromosomes. About 98 percent of the pollen was aborted, and the good grains probably had the unreduced number of chromosomes. The plant flowered profusely. The other plant, which was small and compact, had only 53 chromosomes. (Both it and a 71-chromosome offtype normal came from seeds collected from an individual plant of the good guayule type.) At metaphase I some univalents and many bivalents were observed. There were lagging chromosomes at anaphase I. About 85 percent of the pollen was aborted, and there were many microcytes. The existence of plants with such low chromosome numbers among $72 \pm$ -chromosome populations requires an explanation. It was concluded that, since haploids have been found among Tesnus collections by both Rollins¹⁰ and the writer, these 2 plants, which had 53 and 56 to 59 chromosomes, respectively, must have come from the fertilization of eggs which had the reduced number of chromosomes by pollen from haploids.

¹⁰ Rollins, R. C. Unpublished data.

Some offtype normals from $72\pm$ -chromosome populations showed normal meiosis, and the percentage of pollen aborted was not greater than that produced by apomicts in the same cultures, although no plants with less than 68 chromosomes were free from meiotic irregularities. With a few exceptions plants showing these irregularities were of small size.

Most of these offtype normals came from seeds from noncontrolled pollinations. However, 11 came from controlled self- and cross-pollinations made by Powers and Rollins (10). The range of their chromosome numbers was 66 to 74 and is practically identical with that of the whole group of offtype normals. Of these plants 5 came from cross-pollinations; since they showed both paternal and maternal characteristics, they must have come from the fertilization of eggs which had the reduced number of chromosomes.

It has been assumed that all of these offtype normals were the result of amphimixis. However, from a controlled cross-pollination there was 1 exceptional, small plant which, although it showed no characteristics of the pollen parent, was distinctly different from the apomicts in the same culture (10). It had 72 chromosomes and lacked the additional miniature chromosome which the seed parent had. It had many lagging chromosomes at anaphase I and II and about 98 percent of its pollen was aborted. Powers and Rollins (10) suggested diplospory to account for the origin of this plant. Powers has furnished evidence by means of breeding tests that diplospory is occurring in 1 of the Texas collections (8), and it can be presumed to be occurring in other collections also. How many offtype normals are the result of pseudogamous diplospory is not known. It is possible that some of the $54\pm$ - and $72\pm$ -chromosome offtype normals arose in this way. It is more probable that most of them resulted from normal amphimixis and that the morphological variances were the consequence of new combinations of genes brought about through amphimixis.

ABERRANTS FROM TRIPLOIDS AND TETRAPLOIDS

The morphological variant called aberrant may be described as follows: Growth generally is slower than that of the normals of the same cultures; leaves are thickened and have an irregular pattern; pollen grains and flower heads are larger; and the peduncle is thicker. Some plants show this syndrome of characteristics in more pronounced fashion than others; some are fairly vigorous, whereas others fail to live through the first year. Another characteristic is their high chromosome number, which also is variable, the range extending from 81 to 122. The extent to which this syndrome is expressed is not necessarily correlated with chromosome number. For example, of 11 aberrants obtained from the open pollination of $54\pm$ -chromosome plants belonging to collection 4263, 8 had 85 to 90 chromosomes. The 2 most extreme plants, however, had only 81 and 83 chromosomes, respectively. Also, among offspring from a 72-chromosome plant (42163) a 99-chromosome plant was more extreme in type than $108\pm$ -chromosome plants in the same culture.

As Esau (4, pp. 353-354) has shown, where apomixis is the predominant form of reproduction, "the megaspore mother cell does not form

tetrads but directly develops into a uninucleate embryo sac with a diploid number of chromosomes." Powers and Rollins (10) have shown that pollination is necessary for the production of apomictic progeny from embryo sacs which have the unreduced number of chromosomes (pseudogamous diplospory). Aberrants which occur in apomictic stands and have a higher chromosome number than the apomicts in the same stands are the result of the fertilization of eggs which have the unreduced number of chromosomes. That aberrants occur after cross- as well as self-pollination has been shown by Powers and Rollins (10, table 3). The total chromosome number of an aberrant plant then is the sum of the unreduced number in the egg nucleus and the number of chromosomes in the pollen grain. The variability in total chromosome number depends largely upon the varying number of chromosomes in the pollen grain.

Aberrants which were found among $54\pm$ -chromosome populations had 81 to 89 chromosomes. From noncontrolled pollination of $54\pm$ -chromosome field plants at Salinas aberrants were obtained which had 81 to 90 chromosomes. Among $54\pm$ -chromosome commercial strains plants were found which had 81 to 93 chromosomes. Since the egg nuclei before fertilization contained $54\pm$ chromosomes, chromosome numbers between 81 and 84 suggest fertilization by pollen from $54\pm$ -chromosome plants; numbers above 90 suggest fertilization by pollen from $72\pm$ -chromosome plants. Plants whose chromosome numbers ranged from 84 to 90 could have resulted from fertilization by pollen from either $54\pm$ - or $72\pm$ -chromosome plants.

Aberrants found among $72\pm$ -chromosome populations had mostly 103 to 112 chromosomes, although infrequently such extremes as 99 and 120 were found. They were obtained from all of the $72\pm$ -chromosome collections from Mexico. Included also in the sample which was examined cytologically were aberrants from 12 of the Texas collections, from 9 of the commercial strains, and from a cross between 2 of the last-named. Both the good guayule and "intermediate" types were included. Numbers around 99 suggest fertilization by pollen from $54\pm$ -chromosome plants or from $72\pm$ -chromosome plants which contained less than 30 chromosomes.

Many of the aberrants were called rough aberrant by Rollins (11, 12). They were found in stands of the good guayule type as well as the "intermediate" type. Since Rollins has shown experimentally that one way to produce rough aberrants is through the fertilization of guayule eggs which have the unreduced number of chromosomes by pollen from mariola, the rough aberrants found in stands of the good guayule type probably are a type of species hybrid produced by the fertilization by mariola pollen of guayule eggs which have the unreduced number of chromosomes. The 120-chromosome plant referred to was a rough aberrant and probably was the result of fertilization by pollen from a 90-chromosome mariola which, according to Stebbins and Kodani (13), occurs in the same general region with guayule.

Stebbins and Kodani (13, p. 168) referred to aberrants as "auto-triploid with respect to their immediate parents." They had no proof whether the egg or the pollen grain contributed the unreduced number of chromosomes. That aberrants might be the result of fertilization of an egg which has the reduced number of chromosomes

by a pollen grain which has the unreduced number was considered as a possibility by Stebbins and Kodani (13), who cited indirect evidence by showing the prevalence of triads instead of tetrads of microspores within pollen mother cells. The writer was not able to confirm such observations on her material. Moreover, breeding evidence does not support this possibility. As shown by Rollins (11) experimentally produced aberrant hybrids resembled the maternal parent more closely than the paternal parent and also more closely than the hybrids which had come from eggs having the reduced chromosome number in the same cultures.

OFFTYPE NORMALS FROM ABERRANTS

Information concerning the methods of reproduction by aberrant plants is limited at the present time to Powers' study of aberrants in collection 42163 (8). The chromosome numbers of a few were determined. The range extended from 99 to 109. Among the offspring of aberrants there were a small number of offtype normals which showed only a few of the characteristics of aberrants. No 2 plants were alike. The chromosome numbers of 12 of these plants from 9 aberrants were determined; they ranged from 74 to 93. Meiosis in pollen mother cells was analogous to that of aberrants having approximately the same number of chromosomes. There was no correlation between leaf characteristics and chromosome number as shown as follows:

Leaf characteristics:	<i>Chromosome number</i>
Much thickened, slightly irregular pattern.....	93
Thickened:	
Slightly irregular pattern.....	91, 92
Regular pattern.....	74, 82
Slightly thickened, regular pattern.....	85, 89, 91
Not thickened:	
Irregular pattern.....	85
Slightly irregular pattern.....	91
Regular pattern.....	85, 88

None of these plants had thickened peduncles. Since all of the chromosome numbers were less than 95, they probably were the result of fertilization of eggs which had the reduced number of chromosomes and represented the best of the genic recombinations that resulted from amphimixis.

SECTORIAL CHIMERAS

Among the offtype normals which were examined cytologically, at least 2 were sectorial chimeras; 1 of these consisted of 2 morphologically distinct parts and the other of 3. These plants had 72 chromosomes. No clear differences in chromosome number among the sectors could be discerned.

Among aberrants sectorial chimeras are fairly common, particularly after the first year of growth. Affected portions of plants of recognizable size vary from sectors of leaves to whole branches. Two sectorial chimeras among aberrant plants have been studied cytologically. One had 99 chromosomes in the small sector which had greenish malformed leaves and 106 in the rest of the plant which had the usual gray leaves. The other plant probably started with 91 to 94 chromosomes. At present only a small part of it has this number because the part which has 86 chromosomes and greenish smooth leaves has overgrown the

91+-chromosome part which has gray malformed leaves. It is possible that some of the irregular growth pattern of aberrants is the result of different growth rates among cells which vary in chromosome number if somatic loss of chromosomes occurs frequently in tissues.

Further evidence in support of a causal relation between high chromosome number and the occurrence of chimeras may be cited. Powers (8) obtained hyperaberrant offspring from aberrants in collection 42163. Of these plants, some of which had as many as 144 chromosomes and all of which probably had at least 120, many obviously were sectorial chimeras by the time they were 8 months old and past the first flowering stage. In some of the plants 2, 3, or even 4 distinct portions of varying size could be distinguished. A few of the less extreme portions of several of these plants were examined cytologically and found to have less than 95 chromosomes.

FACTORS LIMITING ANEUPLOIDY AND POLYPLOIDY IN GUAYULE

Because meiosis in pollen mother cells of guayule plants is complete, a study of this normal meiotic behavior clearly and quickly revealed the polyploid nature of the series of collections examined. The triploid nature of the $54\pm$ -chromosome plants was indicated by the presence of trivalents at metaphase I and the tetraploid nature of $72\pm$ -chromosome plants by the presence of quadrivalents. In general there was a tendency toward equal distribution of chromosomes at anaphase I throughout the series. Pollen grains contained the reduced number of chromosomes. This choice of pollen mother cells in which to study chromosome behavior was fortunate because these same plants, beginning at the $54\pm$ -chromosome level and continuing throughout the series, do not show normal meiosis in their megaspore mother cells. Instead there is apomeiosis of the type of generative apospory (4).

According to Powers and Rollins (10) nonsegregating apomixis is the predominant but not the sole method of seed production in plants which have 54 or more chromosomes. Apomixis actually plays a dual role. It maintains a uniformity among $3x$ and $4x$ stands which could not exist if amphimixis were common. Probably if it were common, $3x$ euploids would be restricted to individual plants and even $4x$ plants would be quite variable. Moreover, by means of apomixis the rare favorable aneuploid combinations that arise through amphimixis, since apomixis is facultative, or diplospory have a chance to survive, to increase in number of plants, and therefore to establish what amounts to a clone. In effect nonsegregating apomixis is equivalent to vegetative reproduction. Because most aneuploids found in $3x$ and $4x$ apomictic populations are not as vigorous as the apomicts and produce fewer or inferior seeds, apparently not many have given rise to clones and therefore to aneuploid stands or strains. Furthermore, because apomixis is the predominant method of reproduction in $3x$ and $4x$ populations, in these apomictic stands there is now only limited opportunity for the creation of new types by means of amphimixis. For these 2 reasons aneuploid strains are uncommon.

In contrast to the relative paucity of aneuploid individuals in apomictic $3x$ and $4x$ stands and commercial strains, there was an

abundance of aneuploids with varied chromosome numbers among the offspring of plants which reproduced amphimictically. For instance, a segregating culture was obtained from the seeds of a Texas mariola plant which had some guayule characteristics and probably had 90 chromosomes. The chromosome numbers of 23 of the plants in this culture were determined. They ranged from 70 to 95. No 2 of the plants were morphologically alike. In size they varied from very large to very small. Also, by using diploid $36+$ -chromosome plants, which are amphimictic, as seed parents, aneuploids were obtained which varied in chromosome number from 43 to 49 when pollen from $54\pm$ -chromosome plants was used and from 49 to 56 when pollination was not controlled.

One-third of the $4x\pm$ offtype normals studied had less than 70 chromosomes. These plants were deficient for 3 or more chromosomes. Other evidence shows that considerable chromosomal unbalance existed among viable combinations. From $72\pm$ -chromosome populations an unbalance which may be represented as $4x-7$ was found in plants which had as few as 65 chromosomes. From noncontrolled pollination of $54\pm$ -chromosome plants a $64-$ and a 66 -chromosome plant were obtained. From noncontrolled pollination of $2x$ plants aneuploid $3x$ plants which varied in chromosome number from 49 to 56 were obtained. There was even greater chromosomal unbalance in some of the offspring from crosses between $36+$ - and $54\pm$ -chromosome plants, such as those which had 43, 45, 46, and 48 chromosomes. Because such unbalance between the $2x$ and $3x$ levels is possible, the wide deviations from euploid multiples of 18 which were found in aneuploids with high chromosome numbers are not surprising and actually involve less chromosomal unbalance because multiples of each of the 18 chromosomes were present. Therefore, if amphimictic reproduction were more common in $3x$ and $4x$ populations, not only would more aneuploids be expected but the chromosome numbers of many of them would deviate considerably from the euploid numbers.

In attempting to account for the relative paucity of aneuploid individuals in apomictic $3x$ and $4x$ stands and commercial strains, one is conscious of the fact that most of those studied cytologically were less vigorous and had a lower survival rate before reaching maturity. In contrast, most of the aneuploid $3x$ offspring obtained from crosses in which the seed parents were the amphimictic $2x$ plants were vigorous, in spite of the fact that in some of them there was even greater chromosomal unbalance, in proportion to the total number of chromosomes involved, than the $4x-7$ chromosomal unbalance in the 65 -chromosome plants found in $4x$ populations. Therefore, the lessened vigor of most of the aneuploids found in $3x$ and $4x$ apomictic populations must be attributed to genic unbalance following amphimixis rather than to variation in chromosome number per se.

A form of amphimixis, fertilization of egg which has the unreduced number of chromosomes, led to the formation of those plants with high chromosome numbers which were found in $3x$ and $4x$ populations. Aneuploidy was more common than euploidy. The chromosome numbers of plants examined thus far extend in unbroken sequence from 80 through 93 and in unbroken sequence from 99 through 110, 112, 120, and 122. The chromosome number depended upon the number of chromosomes in the egg ($54\pm$ or $72\pm$) plus the number

brought in by the pollen grain. Since cross- as well as self-pollination occurs, the number of chromosomes contributed by the pollen grain may be quite variable. It has been shown from breeding data that pollen from triploids contains about 25 to 33 chromosomes and that from tetraploids about 31 to 39. In a mixed stand of $54 \pm$ - and $72 \pm$ -chromosome plants, therefore, pollen grains would contain about 25 to 39 chromosomes. Consequently, if the egg had exactly 54 chromosomes, the expected range of chromosome numbers of the aberrants would be 79 to 93 and if it had exactly 72, the range would be 97 to 111. The expected range would be less if most of the plants in a stand had the same chromosome number: out of $54 \pm$ -chromosome stands it would be 79 to 87 if the egg had exactly 54 chromosomes, and out of $72 \pm$ -chromosome stands it would be 103 to 111 if the egg had exactly 72.

One may arbitrarily call 90-chromosome plants pentaploids (5 times 18) and 108-chromosome plants hexaploids (6 times 18), but since aneuploidy is more common than euploidy at these high levels in the polyploid series the terminology has little meaning; and the terms "pentaploid" and "hexaploid" indicate only that the total number of chromosomes equals 5 or 6 times x , which equals 18. If chromosome association at metaphase I were used as a criterion for determining polyploidy, then these plants would be considered partial tetraploids at most. Thus far no natural stands or commercial strains which are pentaploids or hexaploids have been found, and none were reported by the Russians. Chromosome numbers higher than 72 have been found only in individual plants in $3x$ and $4x$ populations and in their offspring.

Cytological studies of plants with high chromosome number have been restricted almost entirely to plants classified as morphologically aberrant. All aberrants which exhibited the whole syndrome of characteristics have had a high chromosome number. A few sectors of chimeras must be excepted, but such plants obviously began with a high chromosome number. How many nonaberrant plants have more than $72 \pm$ chromosomes is not known. One 80-chromosome plant in a $54 \pm$ -chromosome population was of type normal but not aberrant.

That the syndrome of characteristics by which aberrants are distinguished from other morphological types cannot be attributed to high chromosome number alone was shown by the offtype normals (nonapomictic offspring) from aberrant plants. Although 11 of the 12 plants had 82 to 93 chromosomes, which is the same range of chromosome numbers shown by aberrants from $54 \pm$ -chromosome populations, none could be classified as real aberrants. These plants differed, however, in mode of origin from aberrants in $3x$ populations. Since their chromosome numbers were considerably lower than those of their seed parents, they came from the fertilization of eggs which had the reduced number of chromosomes. They constituted only a small fraction of the total number of offspring from aberrants and represented the best of the recombinations of genes that resulted from amphimixis.

In attempting to understand why most plants which had a high chromosome number were aberrant, it should be remembered that the aberrants studied occurred in $3x$ and $4x$ apomictic populations.

An aberrant results from the fertilization of an egg which of itself, without fertilization, is adequate for producing a viable plant (apomict). This aposporous egg is genically balanced in itself. Apparently an unbalance results when such an egg is fertilized, the degree of unbalance depending upon the total genic complex. The more vigorous of the aberrants probably indicate a less disturbed genic balance.

Insofar as high chromosome numbers in guayule are limited to plants which are morphologically aberrant, the establishment of natural stands which have high chromosome numbers, such as pentaploids and hexaploids, will be limited to whatever success aberrant plants have in establishing themselves as natural stands. Unfortunately, most aberrants grow more slowly than normal plants in the same cultures. Also, Powers (8) obtained a very low percentage of germination of seeds produced by aberrants. Moreover, the frequent occurrence of sectorial chimeras in older aberrants and the loss of chromosomes observed in individual cells indicate instability of chromosome number among these plants. The combination of slow growth, few offspring, and instability of chromosome number probably explains why no stands of aberrants, and incidentally of pentaploids and hexaploids, have been found in nature. For the time being at least, tetraploidy seems to be the upper limit in the polyploid series for natural stands and will continue to be until, perhaps by some internal adjustment, the deleterious aspects of aberrancy are overcome. However, for individual plants tetraploidy is not the upper limit, since plants with high chromosome number are continually produced *de novo* in the $3x$ and $4x$ populations through the fertilization of eggs which have the unreduced number of chromosomes.

SUMMARY

The sample of guayule plants used for the determination of chromosome numbers included Texan and Mexican populations, some of McCallum's commercial strains, and offspring from both controlled and noncontrolled pollinations of selected plants. The Mexican material included $36+$ -chromosome (diploid), $54\pm$ -chromosome (triploid), and $72\pm$ -chromosome (tetraploid) stands. Both $54\pm$ - and $72\pm$ -chromosome commercial strains were studied. Only $72\pm$ -chromosome stands were found in the material from Texas. Both the "intermediate" and good guayule $72\pm$ -chromosome types from Mexico and Texas were included. There were no stands with higher chromosome numbers. However, individual plants with higher chromosome numbers, mostly so-called aberrants, were found among the $3x$ and $4x$ stands and strains.

Meiosis in pollen mother cells was complete at all levels in the polyploid series. In diploids, bivalents were seen at metaphase I and the anaphase I distribution of chromosomes was equal. In triploids, trivalents or bivalents plus univalents were seen at metaphase I; in tetraploids, quadrivalents, trivalents, bivalents, and univalents were found. The tendency toward equal distribution of chromosomes at anaphase I, which was suggested in triploids, was more pronounced in tetraploids. In plants with higher chromosome numbers, there was an excess of bivalents at metaphase I. Anaphase I distribution tended toward equality. Hence, the inequality in anaphase I distri-

bution among higher polyploids was less than the inequality in triploids in spite of the larger number of chromosomes. Haploids from tetraploids showed some bivalents at metaphase I. Anaphase I distribution was less regular than in diploids. Such meiotic irregularities as asynapsis and failure of cytokinesis were observed infrequently in diploids and in some aneuploid $4x$ offtype normals. Male sterility due to lack of sporogenous tissue and restitution nuclei also were observed among aneuploid tetraploids.

"Intermediates" were characterized cytologically by more polyvalents at metaphase I than the good guayule type, more of their pollen mother cells showing lagging chromosomes at anaphase I and II, and by the presence of many microcytes among the pollen grains.

The number of chromosomes in functional pollen grains produced by $3x$, $4x$, and aberrant plants was estimated from the numbers in the chromosome groups in pollen mother cells after anaphase I. For the $3x$ it was 22 to 30; for the $4x$, 33 to 36; for aberrants from the $3x$, 38 to 43; and for aberrants from the $4x$, 48 to 55. As a check on these estimates, calculations based on breeding data showed that for the $3x$ plants the range was 25 to 33 and for the $4x$, 31 to 39.

Instability of chromosome number among aberrants was suggested by the loss of groups of chromosomes in pollen mother cells through such mechanisms as multipolar spindles and twin cells. Loss of chromosomes previous to the formation of pollen mother cells was inferred from the finding of cells which contained fewer chromosomes among those which had the high number produced by aberrants in $4x$ populations and less frequently in $3x$; the actual number of chromosomes was variable. Considerable chromosomal unbalance is possible in guayule. From both controlled and noncontrolled pollinations of $2x$ plants growing at Salinas were obtained offspring whose chromosome number ranged from 43 to 56.

Because apomixis is the predominant form of reproduction among triploids and tetraploids, $3x$ and $4x$ stands are fairly uniform. A few of the commercial strains are aneuploid $3x$ and $4x$. Because of limited amounts of amphimixis and normal meiosis in megaspore mother cells, however, there were individual variants among the $3x$ and $4x$ populations. These morphologically distinct types included haploids, off-type normals, and aberrants. Nine haploids from Texas collections and commercial strains were studied; 3 had 36 and 6 had 37 chromosomes. Most of the offtype normals were aneuploids. The chromosome numbers of the $3x$ offtype normals ranged from 54 to 66. The numbers of the $4x$ and aneuploid $4x$ offtype normals ranged from 65 to 75, the distribution being as follows: 33.9 percent had less than 70 chromosomes; 38.9 percent, 70 or 71; 18.6 percent, 72; and 8.5 percent, 73 to 75. Aberrants from triploids had 80 to 93 chromosomes; those from tetraploids, 99 to 120.

All aberrants had a high chromosome number which equalled the unreduced number of the seed parent plus the number contributed by the pollen grain which effected fertilization. They occurred after either self- or cross-pollination in both $3x$ and $4x$ populations. The pollen parent was either guayule or mariola. Sectorial chimeras were fairly common among older aberrants in $4x$ populations. Differences in chromosome numbers were involved in the few chimeras examined.

Aberrancy seemed to be restricted to those plants which arise in $3x$

and 4x populations through abnormal amphimixis (nonreduction followed by fertilization) and to their apomictically produced offspring. Twelve offtype normals from aberrants which varied in chromosome number from 74 to 93 probably arose through amphimixis. Since these latter plants showed only a few of the phenotypic characteristics of aberrants, they probably indicated more successful genic balances in spite of high chromosome number.

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