INTERSPECIFIC HYBRIDIZATION IN NICOTIANA.

IX. FURTHER STUDIES OF THE CYTOLOGY OF THE BACKCROSS PROGENIES OF THE PANICULATA-RUSTICA HYBRID[1]

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INTRODUCTION

As a part of the program of study on interspecific hybridization in progress at the UNIVERSITY OF CALIFORNIA, the *paniculata-rustica* hybrid has been chosen for particular attention on account of certain unique features which it exhibits. Up to the present time it is the only interspecific Nicotiana hybrid from species differing in chromosome number which, although notably deficient in fertility, may yield progeny on selfing, and EAST (1921) has demonstrated that stable recombination products may be established from it. The problem of the manner in which these recombination products come into existence constitutes one of the problems of this study, but of even more interest is the question as to the relationship existing between the parental species.

In a preliminary report GOODSPEED, CLAUSEN and CHIPMAN (1926) demonstrated that the chromosome numbers of the parents were *rustica* $=24_{\mathrm{II}}$ and *paniculata* $=12_{\mathrm{II}}$, and that the F_1 hybrid exhibited conjugation regularly according to the Drosera scheme, $12_{\mathrm{II}}+12_{\mathrm{I}}$. Cytological examination of plants in the backcross progenies demonstrated that the functional gametes of the F_1 hybrid were mostly of the category $12+i$, as respects chromosome number with i having an average value of approximately 6, as is to be expected from random distribution of univalents. In the *paniculata* backcross progeny however, a group of plants reported to have $12_{\mathrm{II}}+12_{\mathrm{I}}$ chromosomes constituted an exceptional class requiring further investigation. The studies reported in this paper demonstrate that this exceptional class occurs, but that instead of having $12_{\mathrm{II}}+12_{\mathrm{I}}$ chromosomes the number is approximately $12_{\mathrm{III}}+12_{\mathrm{I}}$.

These cytological studies disclosed the necessity for parallel studies of chromosome distribution and genetic phenomena as a proper means of elucidating behavior in the progeny of this hybrid. Accordingly a

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[1] The author is very much indebted to Doctor R. E. CLAUSEN, under whose direction this investigation was carried on, especially for suggestions as to method and interpretation of data.

new series of studies has been projected in which it is hoped to make a reinquiry into the cytological phenomena of the F_1 hybrid and to study backcross and selfed progenies in successive generations with a view to determining the extent to which knowledge of the chromosomal situation may aid in an analysis of the genetic phenomena. The present paper, reporting more extended studies of the backcross progenies, contains information as to the chromosome number of the functional female gametes produced by the hybrid.

MATERIAL AND METHODS

Of the many available varieties of *rustica*, *pumila* (U.C.B.G.169/08) was chosen to represent the species, largely because it is a small, early-maturing variety more easily manipulated than the larger varieties. It is our smallest variety of *rustica* and under favorable conditions may mature in 10 to 12 weeks from sowing. The strain of *paniculata* (U.C.B.G. 10/07) used is one typical of this highly stable species. Descriptions and figures of these species are contained in SETCHELL'S (1912) account of the genus.

The F_1 hybrid exceeds both parents in vigor of growth and like other *paniculata-rustica* hybrids is partially fertile. When crossed back as a female parent to the parental species capsules are produced containing an average of approximately 40 seeds each. More difficulty is experienced in producing seed by self fertilization or by backcrossing as the male parent on the parental species and the number of seeds thus produced is always less per capsule.

The seed used in the present investigation was obtained in 1925 by hand pollination in the greenhouse of F_1 *pumila-paniculata* with *paniculata* and *pumila*. These seeds were sown in January 1928 and the plants were grown in six inch pots in the greenhouse. Chromosome numbers were determined by examination of aceto-carmine smears of PMC. In a few instances root-tip counts were made from material fixed in Navaschin's modified solution and stained with iron-haematoxylin. The drawings of chromosome garnitures were made with the aid of a camera lucida; a 2 mm. Leitz apochromatic oil immersion lens and ×8 aplanatic eyepieces were used in working with PMC and gave, at table level, figures magnified about 2700 diameters; a higher magnification was needed with root tips, so ×15 aplanatic eyepieces were used giving figures magnified about 5100 diameters.

F_1 *paniculata-rustica* × *paniculata*

In the *paniculata* backcross all of the seeds produced in five capsules were sown. These capsules contained a total of 206 seeds of which 141 germinated and 120 plants were raised to maturity. A few of the seedlings died in the cotyledon stage, and some plants died at various stages, principally from attacks of leaf miner and mosaic. Chromosome numbers of 83 of the plants which reached maturity were determined and are recorded in table 1, under the caption 1928. For comparison the previous

TABLE 1

Frequencies of chromosome numbers in progenies of F_1 rustica-paniculata ♀ × *paniculata* ♂

	CLASS 1, $12_{II}+i_I$													TOTAL	CLASS 2 CA. 48 CHROMOSOMES
Number of univalents	0	1	2	3	4	5	6	7	8	9	10	11	12		
1926	—	—	1	1	4	—	8	—	4	2	—	—	—	20	14
1928	—	5	2	3	13	16	16	6	6	2*	—	—	—	69	14
Total	—	5	3	4	17	16	24	6	10	4	—	—	—	89	28

* Including one plant with $13_{II}+8_I$ chromosomes.

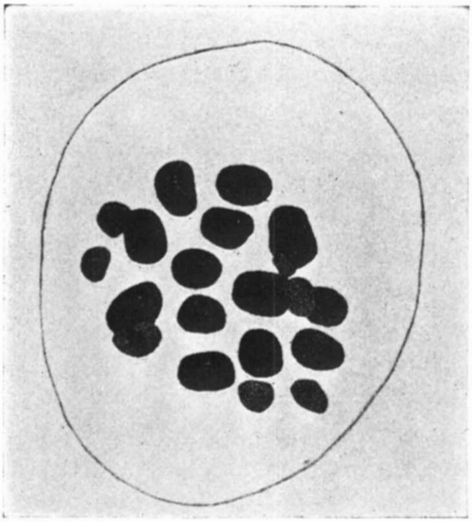


FIGURE 1.—$13_{II}+8_I$ derivative form F_1 *rustica-paniculata* ♀ × *paniculata* ♂ I-M, polar view, univalent chromosomes in gray. ×2700.

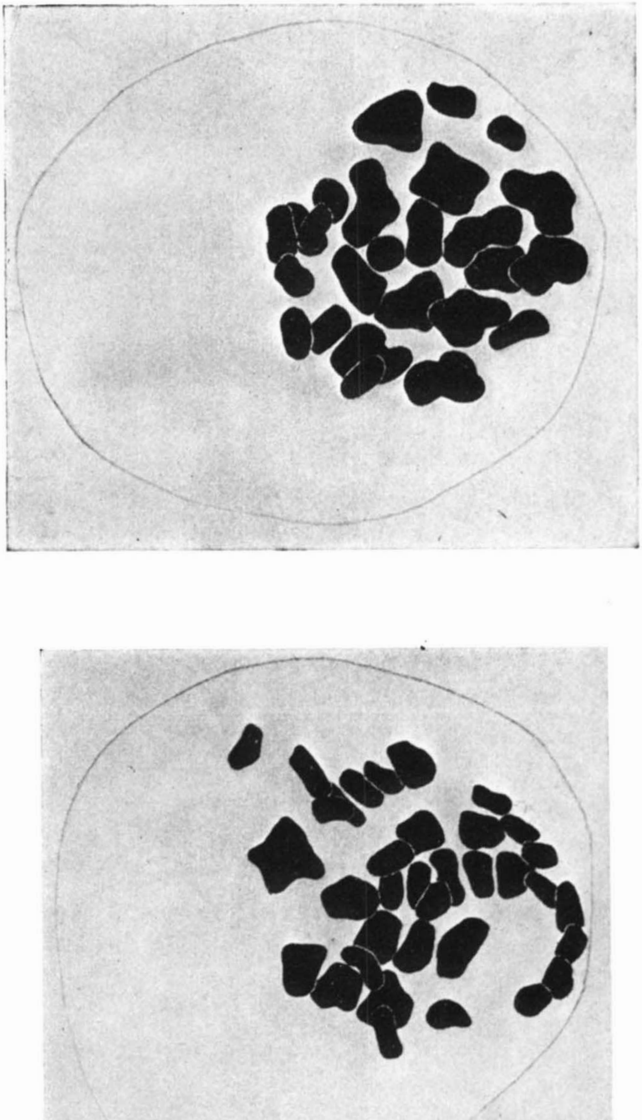
results of GOODSPEED, CLAUSEN, and CHIPMAN (1926) are also included in the table under the caption 1926.

An examination of the data contained in table 1 reveals the existence of two main classes as respects chromosome number: (1) 89 plants belonging to the series $12_{II}+i_{I}$; values of i ranging from 1 to 9 and (2) 28 plants having approximately $12_{II}+24_{I}$ or 48 chromosomes. The average value of i in class 1 was 5.2 as compared with an expected value of 6, based on purely random distribution. Included among these plants is an exceptional individual which uniformly exhibited $13_{II}+8_{I}$ in I-M. (figure 1). Not only was this plant unusual in having 13 bivalents but one of the univalents was distinctly smaller than the others. Equational non-disjunction in one of the univalents in the formation of the female gamete may possibly account for its production.

The 28 plants of class 2 require special comment. Accurate chromosome counts of these plants from PMC are impossible because of their variable behavior, but as a class they are easily recognized. Within a given acete-carmine mount the number of chromatin elements may vary from cell to cell from a minimum of about 24 to a maximum of approximately 36. A cell like that illustrated in figure 2 might possibly be interpreted as $12_{II}+12_{I}$, but closer examination showed that the larger chromatin elements were really not bivalents comparable to those shown in figure 1, but were more or less distinctly revealed, on focusing with the fine adjustment, as trivalents. The other extreme is represented in figure 3, depicting a cell from the same slide as the previous one. The range of variation in these cells, therefore, apparently lies between $12_{III}+12_{I}$ and $12_{II}+24_{I}$. Intermediate conditions are more usual, indicating either variation in the original formation of trivalents or successive stages in the separation of loosely attached univalents from the more closely associated bivalents.

In the previous paper of GOODSPEED, CLAUSEN, and CHIPMAN (1926), the 14 plants included in class 2 were recorded as $12_{II}+12_{I}$. There seems to be no doubt that this determination was incorrect, probably resulting from selection of cells approaching the condition illustrated in figure 2 of this paper, that is, $12_{III}+12_{I}$, and not $12_{II}+12_{I}$. As respects morphological features, these plants were identical with those assigned to class 2 in these studies.

Inasmuch, as accurate counts of chromosome number in plants of class 2 cannot be made by examination of aceto-carmine smears of PMC attempts were made to make counts from root-tip preparations. In making



FIGURES 2 and 3.—Class 2 derivative from F_1 *rustica-paniculata* ♀ × *paniculata* ♂. Figure 2: I-M, polar view showing apparently 25 chromatin elements (really $12_{III}+12_{I}\pm$). Figure 3: I-M, polar view from same anther showing other extreme condition, that is about 35 chromatin elements. ×2700.

FIGURE 4.—Somatic plate of a class 2 derivative from F$_1$ *rustica-paniculata* ♀ × *paniculata* ♂ showing 46 chromosomes. × 5100.

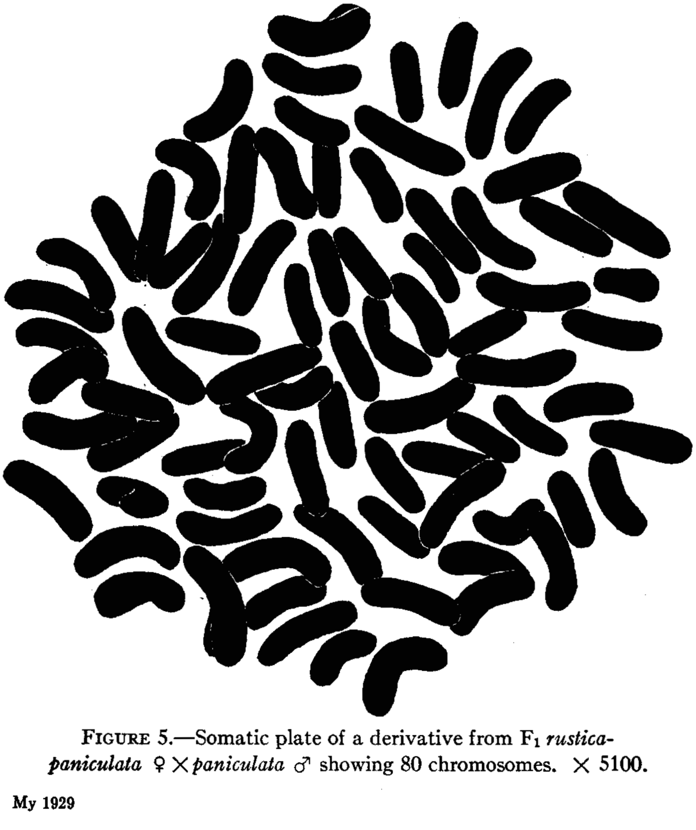


FIGURE 5.—Somatic plate of a derivative from F$_1$ *rustica-paniculata* ♀ × *paniculata* ♂ showing 80 chromosomes. × 5100.

these determinations advantage was taken of the fact that division figures in older roots exhibit shorter and thicker chromosomes than those of younger roots. The two plants determined had 46 chromosomes. A plate from one of them is illustrated in figure 4.

In addition to the above plants a single plant was found which exhibited a very high chromosome number. Attempts to determine its chromosome number from PMC were unsuccessful but root-tip counts gave 80 as its somatic number (figure 5).

As regards morphological features, the plants of this backcross progeny varied to such an extent that no two of them were identical, as was also reported for the previous studies of GOODSPEED, CLAUSEN, and CHIPMAN (1926). This is no doubt to be expected for individuals belonging to class 1, but those belonging to class 2 appeared to be no less variable, as may be seen by comparison of figures 6 and 7. In view of the fact that these plants arise through union of a somatic gamete produced by the F_1 hybrid with a normal *paniculata* gamete they should be identical in constitution and in morphological features. Their diversity evidently arises from the fact that the production of somatic gametes by the F_1 hybrid is usually accompanied by irregularities in chromosomal distribution, particularly by loss of a few chromosomes, so that the "somatic" gametes do not possess the entire somatic complement of chromosomes as is shown by the root-tip counts reported above. Preliminary observations on fertility failed to disclose any obvious relations with chromosome number. Fertility was uniformly low in class 2, to such an extent that no seed was obtained

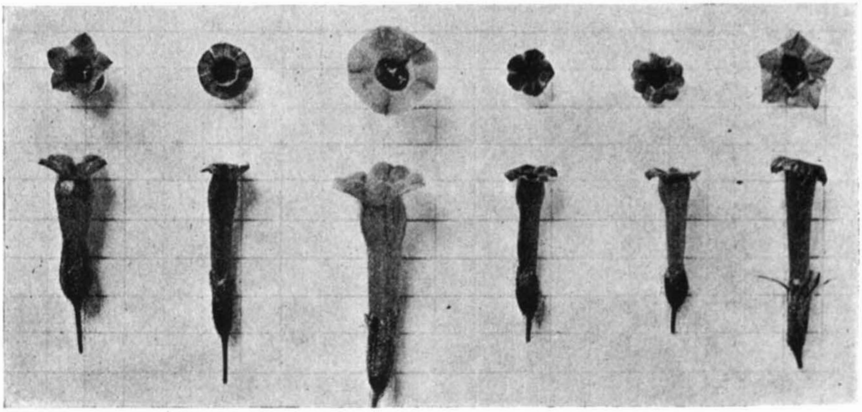


FIGURE 6.—Flowers from six different class 1 derivatives of F_1 *paniculata-rustica* ♀ × *paniculata* ♂. The scale in these figures is indicated by the background which is ruled in 5 mm. squares.

from selfing or backcrossing to *paniculata*, but strangely enough some seed was secured from the backcross to *rustica*. In class 1 fertility was more variable and a limited amount of seed was secured by backcrossing to *paniculata*. On the whole fertility in this group of plants appeared to be lower than that of the F_1.

The above results demonstrate that the viable female gametes of F_1 *paniculata-rustica*, as shown by the chromosome counts of *paniculata* backcross progenies, are apparently produced in two distinct ways: (1) through normal separation of bivalents and approximately random distribution of the univalents and (2) through equational division of the entire chromosomal complement, accompanied by minor irregularities, resulting in the production of "somatic" gametes having approximately the full somatic complement of chromosomes of the F_1 hybrid. Rarely other types of gamete formation may occur, as is evidenced by the production of an 80 chromosome plant in this progeny which probably arose through the production of an F_1 gamete having approximately double the somatic complement of chromosomes.

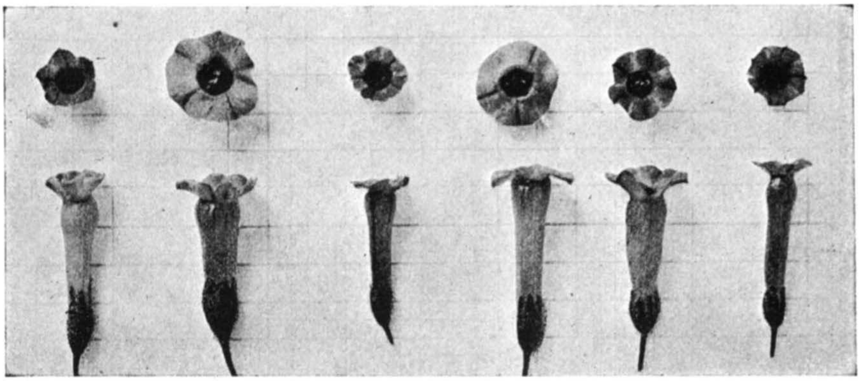


FIGURE 7.—Flowers of six different class 2 derivatives of F_1 *paniculata-rustica* ♀ × *paniculata* ♂.

F_1 *paniculata-rustica* × *rustica*

In the rustica backcross all of the seeds produced in five capsules were sown. These capsules contained a total of 183 seeds of which 94 germinated and 76 plants were raised to maturity. As in the previous population some seedlings died in the cotyledon stage and some of the plants failed to reach maturity principally because of insect and mosaic attack. Chromosome numbers of 67 plants were determined and are recorded in table 2 under the caption 1928. The previous results of GOODSPEED,

TABLE 2

Frequencies of chromosome numbers in progenies of F_1 rustica-paniculata ♀ × *rustica* ♂.

	CLASS 1, $h_{II}+k_{I}$ ($h+k=24$)													TOTAL	CLASS 2 CA 60 CHROMOSOMES
Number of bivalents + univalents	12_{II} + 12_{I}	13_{II} + 11_{I}	14_{II} + 10_{I}	15_{II} + 9_{I}	16_{II} + 8_{I}	17_{II} + 7_{I}	18_{II} + 6_{I}	19_{II} + 5_{I}	20_{II} + 4_{I}	21_{II} + 3_{I}	22_{II} + 2_{I}	23_{II} + 1_{I}	24_{II} + 0_{I}		
1926	—	—	—	—	—	—	7	—	4	—	—	—	—	11	
1928	—	—	—	3	8*	12	5	—	3	1	—	—	—	32	35
Total	—	—	—	3	8	12	12	—	7	1	—	—	—	43	35

* Including one plant with $16_{II}+9_{I}$ chromosomes.

CLAUSEN, and CHIPMAN (1926) are also included in the table under the caption 1926.

An examination of the data contained in table 2 again reveals the existence of two main classes as regards chromosome number (1) 43 plants belonging to the series $h_{II}+k_{I}$ with values of h ranging from 15 to 21 and k from 9 to 3, and (2) 35 plants having approximately $24_{II}+12_{I}$ or 60 chromosomes. The average value of k in class 1 was 6.46 or to make the situation directly comparable with the data from the *paniculata* backcross, an average of 5.54 univalents are distributed to the *rustica* backcross progeny by the F_1 hybrid parent. Included among these plants is an exceptional individual which uniformly exhibited $16_{II}+9_{I}$ in I-M (figure 8). The bivalents were all clearly such and could not be confused with univalents. This plant is comparable with the $13_{II}+8_{I}$ individual in the *paniculata* derivatives and probably also arose by equational non-disjunction in one of the univalents in the formation of the female gamete.

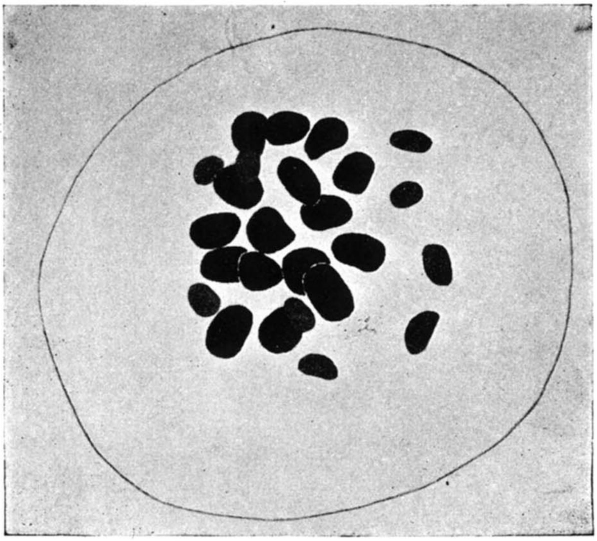
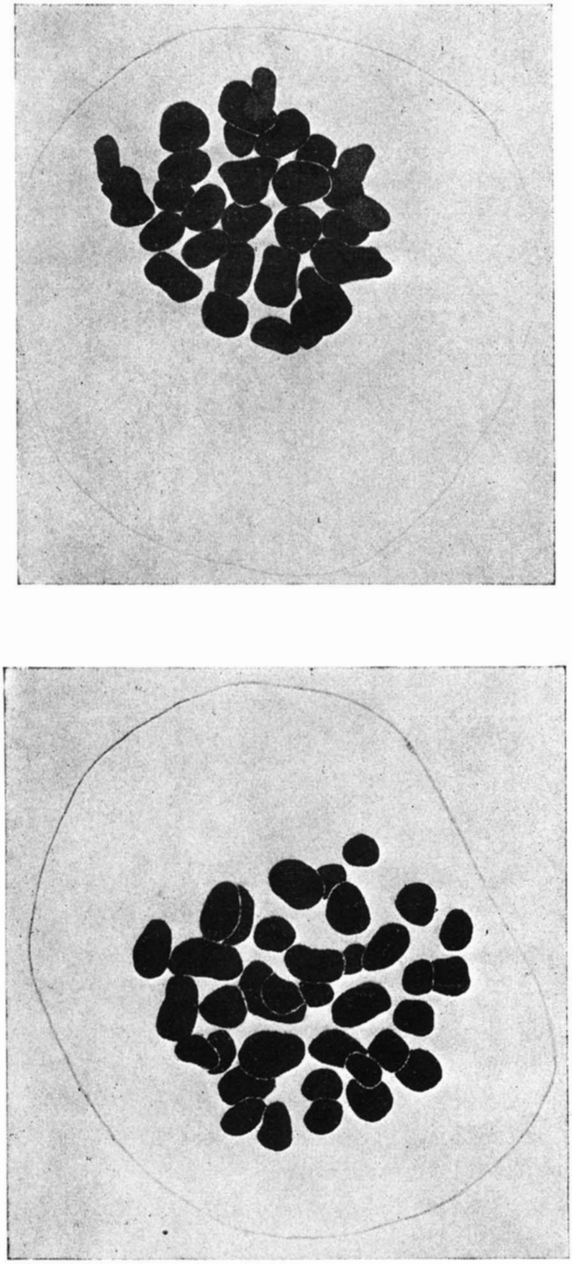


FIGURE 8.—$16_{II}+9_{I}$ derivative from F_1 *rustica-paniculata* ♀ × *rustica* ♂ I-M, polar view, univalent chromosome in gray. ×2700.

The 35 plants of class 2 are comparable with the class 2 plants of the *paniculata* backcross and arose in the same manner. Accurate chromosome counts of these plants from PMC are impossible because of their variable behavior, but as a class they are easily recognized. Within a



FIGURES 9 and 10.—Class 2 derivative from F$_1$ *rustica-paniculata* ♀ × *rustica* ♂. Figure 9: I-M, polar view showing apparently 28 chromatin elements. Figure 10: I-M, polar view, from same anther showing other extreme condition, that is, 35 chromatin elements $(24_{II}+12_{I}\pm)$. ×2700.

given aceto-carmine mount the number of chromatin elements may vary from cell to cell from a minimum of about 24 to a maximum of approximately 36. A plate with apparently $24_{II}+5_{I}$ is shown in figure 9. Rarely, plates may be found having apparently 24_{II} but in reality such figures are to be interpreted as $12_{III}+12_{II}$ the trivalent nature of some of the chromatin elements being more or less distinctly revealed on focusing with the fine adjustment. Other PMC of the same anther may exhibit approximately 36 chromatin elements ($=24_{II}+12_{I}$) as shown in figure 10. Intermediate conditions are more usual, indicating either variation in the original formation of trivalents or successive stages in the separation of loosely attached univalents from the more closely associated bivalents. Inasmuch as accurate counts of chromosome number in plants of class 2 cannot be made by examination of aceto-carmine smears of PMC attempts were made to make counts from root-tip preparations. A characteristic plate from one of these roots, exhibiting 59 chromosomes, is shown in figure 11. Determination of four other plants gave 58, 60, 58, and 56-58 (?) chromosomes.

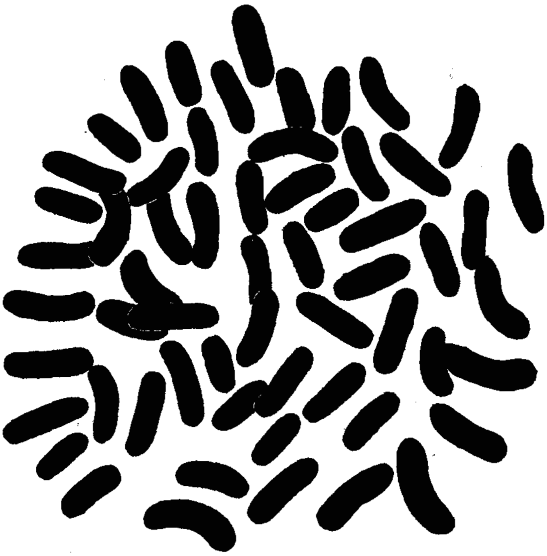


FIGURE 11.—Somatic plate of a class 2 derivative from F_1 *rustica* ♀ ×*rustica* ♂ showing 59 chromosomes. ×5100

As regards morphological features the plants of this backcross progeny were much less variable than the *paniculata* derivatives, although the diversity was so great that no two plants were identical. All were prevailingly *rustica* in type and many closely resembled the F_1 hybrid in general features. Variability is obviously to be expected among individuals belonging to class 1; but those belonging to class 2 were also variable, although not so strikingly so as the class 2 plants of the *paniculata* backcross. Characteristic flower types are illustrated in figures 12 and 13. The diversity among these class 2 plants evidently arises from the fact that the production of somatic gametes by the F_1 hybrid is usually accompanied by irregularities in chromosome distribution, particularly by the loss of a few chromosomes, so that the "somatic" gametes do not possess the entire somatic complement as is shown by the root-tip counts

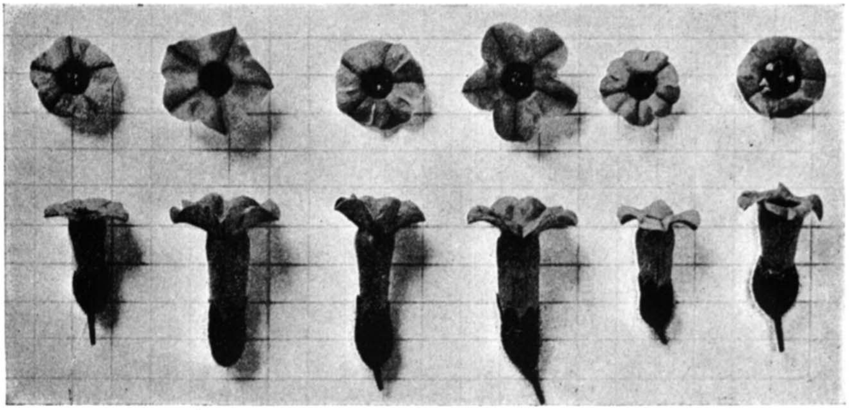


FIGURE 12.—Flowers of six different class 1 derivatives of F_1 *paniculata-rustica* ♀ × *rustica* ♂.

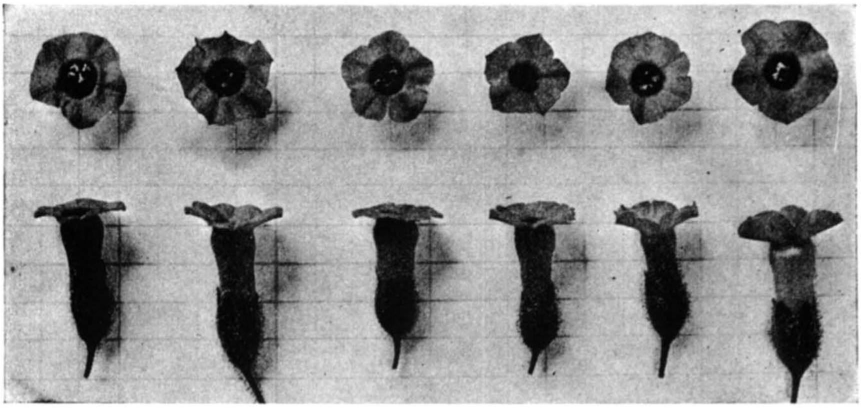


FIGURE 13.—Flowers of six different class 2 derivatives of F_1 *paniculata-rustica* ♀ × *rustica* ♂.

reported above. Apparently, however, the absence of one or two univalents in a $24_{II}+12_{I}$ plant does not cause such a distinct morphological change as a corresponding absence in a $12_{II}+24_{I}$ plant. Preliminary observations on fertility failed to disclose any obvious relation with chromosome number. Fertility was usually high in plants of class 1 as measured by seed production on selfing and backcrossing reciprocally to *rustica*, but at least one plant tested was completely sterile. Fertility in class 2 plants was even higher, only one plant failing to give selfed seed, and all were reciprocally backcross fertile. It was also possible to get seed from reciprocal backcrosses of class 2 plants to *paniculata*.

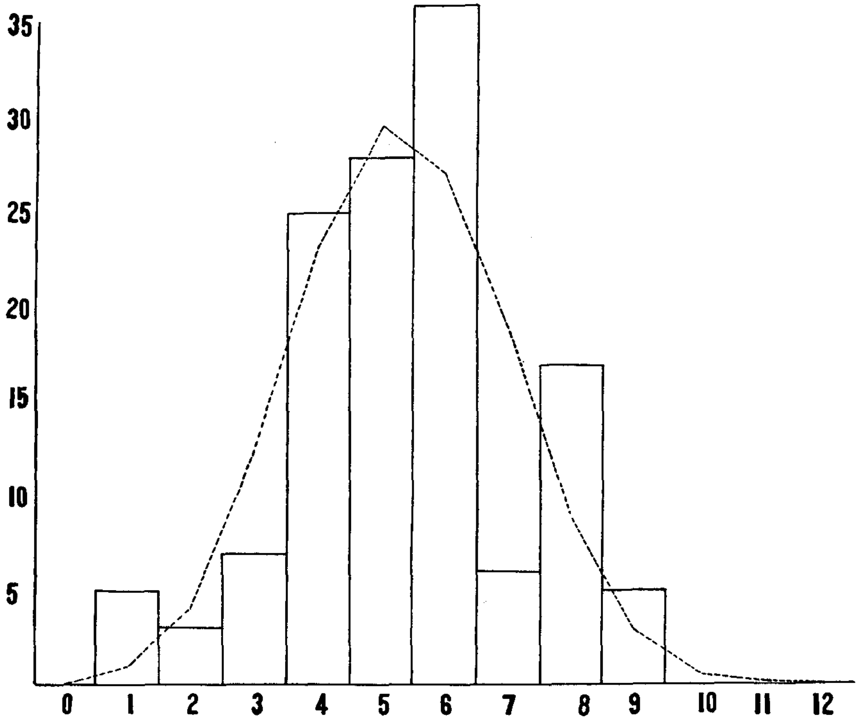


FIGURE 14.—Frequency polygon showing actual distribution of values of *i* from table 3 compared with calculated values obtained by expansion of binomial 132 (.552+.448).[12]

The above results demonstrate that the rustica backcross progeny arise from the same two classes of gametes as the *paniculata* backcross progeny; namely, (1) those produced by normal separation of bivalents and approximately random distribution of univalents and (2) somatic gametes from equational division of the entire chromosome complement accompanied by minor irregularities.

TABLE 3

Distribution of values of i in the production of viable gametes of class 1 by F_1 paniculata-rustica ♀.

i=	0	1	2	3	4	5	6	7	8	9	10	11	12	TOTAL
paniculata backcross	—	5	3	4	17	16	24	6	10	4	—	—	—	89
rustica backcross	—	—	—	3	8	12	12	—	7	1	—	—	—	43
Total observed	—	5	3	7	25	28	36	6	17	5	—	—	—	132
Calculated 132 $(.555+.445)^{12}$	*.1*	*1.1*	*4.7*	*12.7*	*23.1*	*29.5*	*27.1*	*19.2*	*9.1*	*3.5*	*.85*	*.15*	*.007*	*131.1*

DISCUSSION

Inasmuch as the chromosome counts of the backcross progenies are in agreement as to the character of the viable female gametes produced by F_1 *paniculata-rustica*, they will be considered together in this brief discussion. As respects the regular gametes; that is, those resulting from normal disjunction of the bivalents and approximately random distribution of the univalents, the combined data of table 3 show that the average number of univalents included in these gametes is $5.38 \pm .11$. These distributions exhibit little evidence of selective viability of any chromosome number class, aside perhaps from the five gametes containing only one univalent in the *paniculata* backcross series. With an average of 5.38 univalents per gamete, the chance of inclusion of each univalent becomes $5.38 \div 12 = 0.448$, and a comparable calculated distribution may be obtained by expansion of the binomial expression, $132(.552 + .448)^{12}$ As shown by the frequencies in table 3 and graphic representation in figure 14, the calculated and observed distributions are in close agreement. Apparently, therefore, the slight deviation from purely random distribution exhibited in these results is to be ascribed to chance elimination of univalents. Direct studies of "tetrads" are in harmony with this conclusion, for they exhibit a small percentage of microcytes and micronuclei.

The production of "somatic" gametes is also attended by slight irregularities in chromosomal distribution, as is shown by the variations in morphological features of plants arising from them and also by direct determination of chromosomes numbers of such plants. Preliminary observations of F_1 PMC indicate that dyad production is very rare, but some of the instances which have been observed exhibit micronuclei and microcytes in addition to the two primary cells. Apparently this type of gamete production is more frequent in EMC than in PMC. The evidence of irregularity is in harmony with observations on dyad formation in other hybrids, as for example F_1 *sylvestris-tomentosa* (GOODSPEED and CLAUSEN, 1928), a high percentage of the dyads of which exhibit micronuclei and microcytes. In the *tabacum* haplonts also these authors report similar irregularities in dyad production. It is possible that in many instances such imperfect dyads, particularly those representing minus deviations in chromosome number, are non-functional, which may account for the observation of BLAKESLEE, MORRISON and AVERY (1927) that the offspring of haploid Daturas are mainly diploid, and that such chromosomal variations as occur are in the plus direction. KARPECHENKO'S (1927) determinations of chromosome numbers in the progeny of F_1 Brassica-Raphanus hybrids likewise indicate irregularities in the production of somatic gametes, but they also appeared uniformly to be in a plus direction. No doubt minus deviations are to be expected more frequently than plus ones, and in the material here reported only such irregularities were actually demonstrated, but this may be due merely to the small number of accurate determinations which were made.

It has usually been assumed that lack of conjugation promotes the production of somatic gametes. FEDERLEY (1928) and ROSENBERG (1928) in recent summaries bearing on this problem go so far as to state that in the cases of somatic gamete formation so far observed there is either complete or partial failure of pairing. Such a causal relationship between lack of pairing and somatic gamete formation may be present in most cases; but in the present instance observations of PMC indicate a highly regular conjugation according to the Drosera scheme, despite the fact that 32 percent of the viable female gametes are of the somatic category.

Sterility of interspecific hybrids is usually assumed to depend upon selective elimination of certain classes of gametes and in some instances there is objective evidence for this assumption. Thus it was shown by GOODSPEED and CLAUSEN (1927) that the predominating classes of viable gametes of the F_1 *sylvestris-tabacum* hybrid were those in which only 0-3 univalents were included. Here F_1 sterility may be explained in part as the result of inviability of most gametes having from 3-11 singles, and since according to chance distribution most gametes would be in this category, the sterility exhibited would be correspondingly high. However, in the case of F_1 *paniculata-rustica* this explanation does not apply inasmuch as the viable gametes apparently represent a random sample as respects chromosome number of the entire series. It is still possible that the sterility is selective, although the cytological data supply no positive evidence as to the manner in which the elimination of gametes is effected.

The bearing of these results upon the genetic findings hitherto reported, especially those of EAST (1921), is not entirely clear. It is impossible to bring cytological findings into proper relation with genetic results unless chromosomal determinations are made in connection with the genetic experiments. It would seem reasonable, however, to assume that the production of such a high proportion of somatic gametes must have a significant effect upon the character of the F_2 progeny, and it may also be possible that they play an important part in the production of constant *rustica* derivatives differing from the original parental type. These, however, are matters which we hope to take up in future studies in this series.

SUMMARY

1. Considering the two backcross progenies together, results of individual chromosome counts of 195 plants indicate that 68 percent of the viable female gametes of F_1 *paniculata-rustica* arise through normal distribution of bivalents accompanied by approximately random distribution of univalents.

2. The remaining 32 percent of viable gametes contain approximately the total somatic chromosome number of the F_1 hybrid.

3. In the first type of distribution, the average number of univalents included in the viable gametes was 5.38 as compared with an expectation of 6, based on purely random distribution. The evidence indicated that this slight deviation from expectation is to be ascribed to elimination of chromosomes during meiosis rather than to selective viability of different classes of gametes.

4. Root-tip counts of plants arising from somatic gametes demonstrate that these gametes rarely contain the complete somatic complement of chromosomes; but that in their production a few chromosomes are usually eliminated. The morphological diversity of the plants arising from these gametes is in agreement with this demonstration.

5. Occasional deviations from the above described methods of gamete formation were also found, specifically evidence of equational nondisjunction in the distribution of univalents and of doubling of the chromosome number in the production of somatic gametes.

6. Sterility of the F_1 is evidently not primarily due to inviability of gametes resulting from random distribution of univalents, inasmuch as viable gametes apparently represent a random sample, as respects the chromosome number, of the entire gametic series.

7. While the application of these results to the genetic phenomena is not self-evident, there can be no question as to the necessity of determining cytological conditions step-by-step in order to arrive at a proper analysis of the breeding data.

LITERATURE CITED

BLAKESLEE, A. F., MORRISON, G., and AVERY, A. G., 1927 Mutations in a haploid Datura Jour. Heredity 18: 193–199.

EAST, E. M., 1921 A study of partial sterility in certain hybrids. Genetics 6: 311–365.

FEDERLEY, H., 1928 Chromosomenverhältnisse bei Mischlingen. Verhandlungen des V. International Kongresses für Vererbungswissenschaft. Berlin, 1927. 1: 195–219.

GOODSPEED, T. H., CLAUSEN, R. E., and CHIPMAN, R. H., 1926 Interspecific hybridization in Nicotiana. IV. Some cytological features of the *paniculata-rustica* hybrid and its derivatives. Univ. California Publ. Bot. 11: 103–115.

GOODSPEED, T. H., and CLAUSEN, R. E., 1927 Interspecific Hybridization in Nicotiana. VI. Cytological features of *sylvestris-tabacum* hybrids. Univ. California Publ. Bot. **11**: 127–140.

1928 Interspecific Hybridization in Nicotiana. VIII. The *sylvestris-tomentosa-tabacum* hybrid triangle and its bearing on the origin of *tabacum*. Univ. California Publ. Bot **11**: 245–256.

ROSENBERG, O., 1928 Speciesbildung mit Vervielfältigung von Chromosomen. Verhandlungen des V. Internationalen Kongresses für Vererbungswissenschaft. Berlin, 1927. **1**: 332–341.

SETCHELL, W. A., 1912 Studies in Nicotiana. I. Univ. California Publ. Bot. **5**: 1–98.