

INTERSPECIFIC HYBRIDIZATION IN NICOTIANA.
XII. THE AMPHIDIPOID *RUSTICA-PANICU-
LATA* HYBRID; ITS ORIGIN AND CYTO-
GENETIC BEHAVIOR¹

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Received March 3, 1930

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INTRODUCTION

Though a great deal has been discovered as to the behavior of partially fertile interspecific hybrids, in no case has there been a complete cytogenetic analysis and explanation of the results of selfing and backcrossing such hybrids and derivatives in successive generations. And yet such an analysis is basic for in no other way can we know the stable recombination possibilities of this class of species hybrids. A really complete study of even one hybrid would not only be of great value in predicting the behavior of others, but would undoubtedly throw much light on such questions as the relationship of the species concerned, possibility of crossing over between homologous chromosomes of different species, origin of true breeding hybrid derivatives, and cause of interspecific sterility. For this purpose one must first determine (1) the meiotic behavior of the chromosomes of the hybrid; (2) the chromosome constitution of the functional male and female gametes and (3) the chromosome constitution and behavior of the F₂

¹ The author is indebted to Doctor R. E. CLAUSEN for helpful advice and interest throughout the course of this investigation.

progeny. Obviously, in order to make much progress, the hybrid selected for study must have a rapid life cycle and ease of cytogenetic manipulation.

Accordingly, the *paniculata-rustica* hybrid has been selected for such an intensive study. It is one of many hybrids exhibiting the Drosera scheme and up to the present time is the only interspecific *Nicotiana* hybrid from species differing in chromosome number which although notably deficient in fertility may yet yield progeny on selfing. Although the classic example of interspecific hybridization, being among the first hybrids obtained by KOLREUTER (1759), yet relatively little progress was made in its analysis in pre-Mendelian times. As noted by FOCKE (1881) early hybridizers, especially GÄRTNER (1849), observed that in common with other hybrids its progeny exhibited reversion to the parental type and fertility. Finally EAST (1921) demonstrated that stable recombination products may be established from it. None of these studies took into account the true cytological situation.

A re-inquiry from the viewpoint of cytology was made by GOODSPEED, CLAUSEN and CHIPMAN (1926) who demonstrated that the chromosome numbers of the parents were *rustica* 24_{II} and *paniculata* 12_{II} , and that the F_1 exhibited conjugation regularly according to the Drosera scheme, $12_{II} + 12_I$. Preliminary studies of the backcross progenies were also made. More extended studies by the author (1929) revealed that the functional female gametes of the F_1 *rustica-paniculata* hybrid were in two classes, 68 percent having arisen through normal distribution of bivalents accompanied by approximately random distribution of univalents and the remaining 32 percent having approximately the somatic chromosome number of the F_1 hybrid. In the first type of distribution the average number of univalents in the viable gametes was 5.38 as compared with an expectation of 6 based on purely random distribution.

While the application of the above results to the genetic phenomena reported by EAST (1921) was not self evident, the prediction was made that the production of such a high proportion of diploid gametes must have a significant effect on the F_2 and play an important part in the production of constant *rustica* derivatives differing from the original type.

In order to complete the basic studies needed for an analysis of the subsequent generations a knowledge of the functional male gametes and viable zygotes resulting from union of such gametes with the two classes of functional female gametes above outlined is essential. The present paper reports the results of a study of (1) the progeny resulting from F_1 used as a male parent; (2) the F_2 generation; and (3) the relatively stable derivatives obtained by selfing of fertile F_2 plants.

MATERIAL AND METHODS

The variety *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 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 and like other *paniculata-rustica* hybrids is partially fertile. When crossed as female parent to the parental species capsules are produced containing an average of approximately 40 seeds each. One experiences much more difficulty in producing seeds by self-fertilization or by backcrossing as the male parent.

Chromosome numbers were determined by examination of aceto-carminic smears of P. M. C. Check determinations by root tip counts were made in some cases from material fixed in Navashin's modified solution and stained in 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 $\times 8$ aplanatic eyepieces were used in working with P. M. C., giving at table level figures magnified about 2700 diameters; with root tips, $\times 15$ aplanatic eyepieces were used, giving figures magnified about 5100 diameters.

THE FUNCTIONAL MALE GAMETES OF
 F_1 PANICULATA-RUSTICA

In the summer of 1928, 50 flowers of a *paniculata* plant growing in the greenhouse were emasculated and pollinated with F_1 pollen. One capsule resulted containing 56 seeds, many of which were shriveled. Only two seeds germinated and the seedlings differed from each other and from *paniculata*. One died before flowering. The other was very different from *paniculata* in both flower and leaf morphology and was both self and backcross sterile. It exhibited $12_{II} + 5_I$ at I-M. in the P. M. C.

The first series of backcrosses to *rustica* was made in the summer of 1928. One series of 70 pollinations resulted in 10 capsules with 29 seeds, 8 of which germinated. Six lived to flowering, at which time it was determined that two were identical with *rustica pumila*, completely fertile, and exhibited 24_{II} at I-M. The other four differed from each other and from *pumila*. Their chromosome numbers were $20_{II} + 4_I$ (2), $18_{II} + 6_I$ (1) and $21_{II} + 3_I$ (1).

Because of the failure to secure a really representative progeny from which to draw conclusions as to pollen transmission a final attempt was made in the summer of 1929. Two *rustica pumila* plants growing in the field were caged and great care was used in emasculation to avoid the possibility of selfing. In spite of precautions, however, a check made by leaving 20 emasculated flowers unpollinated resulted in 3 capsules, two with 3 or 4 seeds per capsule and one with a well filled capsule. Hence the control was not perfect, and there is a possibility that all capsules of the backcross which set seed may have some selfed seed. This possibility must be considered in an interpretation of backcross results. One hundred backcross pollinations, using the pollen from 5 F_1 anthers on each pistil, resulted in 49 capsules with 322 seeds, many of which were badly shrunken. Ninety-three seeds germinated and about 78 plants survived to time of flowering.

A group of 12 plants were morphologically identical with *rustica pumila*. These were examined and found to exhibit 24_{II} at 1-M. Some of these plants may actually be the result of backcrossing and carry *paniculata* chromosomes incapable of causing noticeable morphological effect when in heterozygous condition. Selfed seed was saved from some of them in order to test this last possibility. For the present, however, it is believed best to consider these plants as the result of accidental self-pollination due to faulty control.

A number of plants were severely attacked by *Botrytis* sp. and failed to flower sufficiently for P. M. C. studies to be made. Five of these were strikingly different from *rustica pumila* and undoubtedly would have exhibited less than 24_{II} . The results of the chromosome determinations of

TABLE 1
Chromosome numbers of the progeny resulting from *rustica pumila* ♀ × F_1 ♂.

	24_{II}	$23_{II}+1_I$	$22_{II}+2_I$	$21_{II}+3_I$	$20_{II}+4_I$	$19_{II}+5_I$	$18_{II}+6_I$
1928	2	=	=	1	2	=	1
1929	28*	3	=	1	=	3	3
Total	30	3	=	2	2	3	4

* Including one plant with $24_{II}+1_I$, which was distinct from *rustica*.

the 38 remaining plants investigated are given in table 1 under the caption 1929, together with previous determinations under the caption 1928.

At least four of the plants with 24_{II} were distinct from one another and from *rustica pumila* as shown in figure 1. The other 24_{II} plants flowered

much later than the above group of twelve plants definitely classed as *rustica pumila* but were otherwise very similar to them. They were all highly self fertile. The four distinctive 24_{II} plants and the three plants having $23_{II}+1_I$ at 1-M in a population of only 75 plants are very good evidence for great selectivity in favor of the 24 and 23 chromosome gametes. The distinctive plants with 24_{II} also indicate that the pollen is capable of transmitting the *paniculata* homologues as readily as the egg.

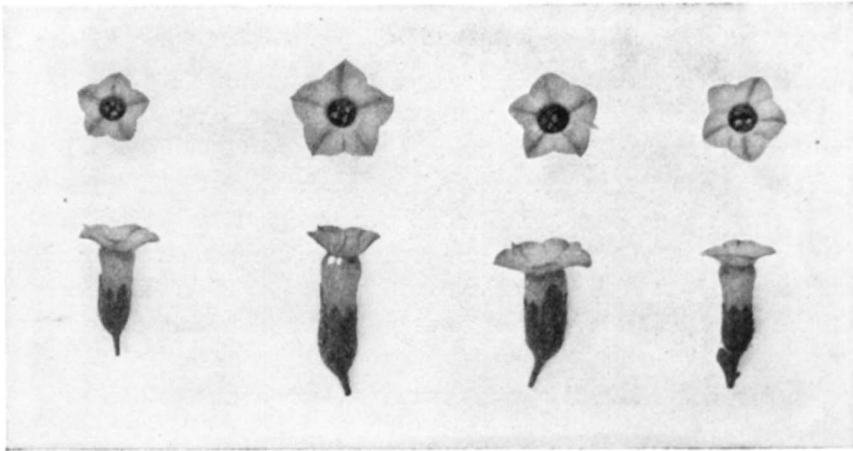


FIGURE 1.—Flowers of four 24_{II} plants of the backcross progeny of *N. rustica pumila* ♀ × *F*₁ *rustica-paniculata* ♂. The variation shows that *F*₁ pollen transmits *paniculata* homologs which express themselves when in heterozygous condition.

The other plants were highly variable and comparable to the reciprocal backcross in this respect in that no two were identical. One plant with $21_{II}+3_I$ and several with $18_{II}+6_I$ and $19_{II}+5_I$ were fully as sterile and probably more so than the *F*₁. There seemed to be no correlation between chromosome number and fertility for the plant with $21_{II}+3_I$ was as sterile as the one with $18_{II}+6_I$, and, furthermore, one plant with $19_{II}+5_I$ exhibited quite a high degree of fertility.

DISCUSSION

The absence of plants indicating diploid gamete formation in the above backcross progeny is very unexpected in view of the large number of them found in the progeny of the backcross, using *F*₁ as female, and the occurrence of a large number of amphidiploids in the *F*₂. However, as will be shown later, the pollen of the amphidiploid when used on *rustica* or *paniculata* fails to give viable seed, and the absence of expected class of plants

probably only indicates inability of F_1 pollen grains with somatic number of chromosomes to function on parental species rather than failure of their production. In other words, it is believed that the amphidiploids found in the F_2 are the result of union of diploid gametes, though as yet it has been impossible to demonstrate the presence of pollen grains with diploid number of chromosomes. Some evidence for this belief is found in the fact that only by hand pollination are any seeds produced by the F_1 , and secondly that examination of tetrad stage in F_1 meiosis shows a few dyads. It will be of interest to determine how readily F_1 pollen functions on the amphidiploid and the type of progeny resulting.

The selectivity is also peculiar in that no plants with $12_{II}+12_I$ corresponding to those with 24_{II} were found and differs strikingly from that type of selectivity reported by THOMPSON and CAMERON (1928) in backcross of *Triticum vulgare* ♀ × *vulgare-durum* F_1 ♂ where both the 21 and 14 chromosome grains were favored. Probably the cross of *N. paniculata* ♀ × F_1 *rustica-paniculata* ♂ would give a progeny with most plants having approximately 12_{II} , and the peculiar results so far obtained make it necessary to make this backcross in order to properly complete the analysis.

2. THE F_2 PROGENY OF *RUSTICA-PANICULATA*

In the spring of 1928, two F_1 plants were removed to the greenhouse where only *Crepis* species are grown. Inasmuch as they were thus isolated from parental pollen no caging or bagging was necessary. Several hundred hand pollinations, using pollen from five anthers on one stigma, resulted in 44 capsules containing a total of 114 seeds of which 63 germinated. Several plants died in the cotyledon stage and others were severely infected with *Botrytis* sp. Chromosome determinations of the 37 plants which flowered revealed three classes of plants as regards chromosome number.

The first class consisting of 4 plants evidently resulted from the union of haploid gametes. Their very regular I-M behavior made determination of chromosome number very easy. They exhibited $14_{II}+10_I$ (1) and $17_{II}+7_I$ (3). They differed from each other and though generally similar to *rustica pumila* differed from it in size and shape of flowers and leaves as well as habit of growth. Though similar to the class 1 plants obtained by crossing F_1 ♀ × *rustica* and discussed in a previous paper (LAMMERTS 1929), repeated attempts to secure seed by selfing failed and though in one plant occasional seeds were formed the capsules dropped before maturity. The plants in this class are, therefore, more sterile than the F_1 and hence are practically completely sterile.

The second class consisting of 3 plants arose from the union of diploid gametes and haploid gametes. They were in general similar to *rustica* but differed from it and among themselves in detail of flower and leaf morphology. The chromosome behavior at I-M was highly irregular as contrasted with the class 1 plants and usually the number of large chromatin elements (bivalents and trivalents) was less than 24. The irregularity in these plants was greater even than that reported for the class 2 plants, obtained in the backcross of F₁ female to *rustica*. Because of this irregularity exact chromosome determinations were not possible. Two plants had 54-55 chromosomes and the other 57-58, as determined by II-M studies. They arose then from the union of diploid gametes containing approximately 36 chromosomes with haploid gametes having 18-21 chromosomes. Inasmuch as most of the functional F₁ haploid gametes have an average of 18 chromosomes, the number of chromosomes in these class 2 plants agrees with expectation. These plants were also completely sterile and thus differed from the class 2 plants referred to above.

The third class consisting of the remaining 30 plants arose from union of diploid gametes with approximately 36 chromosomes. Inasmuch as the amphidiploid races described in detail in part 3 of this paper were derived from these plants only general statements as to their appearance and cytology need be given here. They were similar in general characters such as possession of wide, rather heart shaped, thick leaves; flowers comparable to the F₁ in tube length and to *rustica pumila* in tube width; and high degree of fertility. They were distinct from one another, however, in growth habit and detail of leaf and flower shape. The chromosome behavior at I-M was highly irregular and determination of the chromosome number of most of them was only approximate, being made from II-M. All varied around 72 chromosomes.

DISCUSSION

In a preliminary paper SINGLETON (1928) also reports the occurrence of plants having 72 chromosomes in the F₂ progeny of *N. rustica-paniculata*. The number of plants is not given but they are described as being nearly completely fertile when selfed. In a letter to the author he furthermore states that plants closely resembling *paniculata* were not recovered in his rather large F₂ population, and that the plants resulting from union of incomplete haploid gametes were very sterile. Our results are then in general quite comparable.

The absence of any plants resembling *paniculata* or of fertile *rustica* derivatives in our class 1 plants is according to expectation. In view of the

random survival of the female gametes and the large number of functional male gametes with less than 24 chromosomes the possibility of recovering 12_{II} or 24_{II} plants in the F_2 is very slight indeed. Though the data are here limited, studies of progenies resulting from selfing of a $17_{II} + 7_I$ backcross plant show that the *rustica-paniculata* hybrid and its derivatives behave according to KIHARA'S scheme, namely, the surviving zygotes are those with a total number of bivalents and univalents equal to 24. Accordingly most zygotes arising from the union of F_1 haploid gametes do not survive. The proportion of class 2 and 3 plants should, therefore, be relatively higher than the calculated expectation based on the proportion of diploid female gametes. This is indeed the case as regards the class 3 plants, for actually about 80 percent of the F_2 are amphidiploid. Only 8 percent of the F_2 are class 2 plants, however. Most of the zygotes resulting from the union of incomplete haploid gametes and diploid gametes are therefore inviable. Though some of the F_1 sterility is due to failure of F_2 zygotes in accordance with KIHARA'S scheme, this failure obviously is not the complete explanation, inasmuch as we have already observed that the F_1 used as male parents exhibits a sterility comparable to that exhibited on selfing.

3. THE RELATIVELY STABLE DERIVATIVES OBTAINED BY SELFING FERTILE F_2 PLANTS

(a) *Origin and behavior of amphidiploid lines*

The class 3 plants of the F_2 progeny were rather uniformly fertile. They have been described as rather similar in general characters. Inasmuch as they exhibited much variability in chromosome number and in detail of leaf and flower morphology, it was decided to continue the study in F_3 by selecting the five F_2 plants most distinct from each other in morphology and chromosome number. A brief description of these plants and their behavior will give some idea of the variability in class 3.

Plant 28643/15 was 78 cm high; the leaves were linear and not heart shaped as the majority of F_2 class 3 plants, curled at the edges and glossy. Irregular development of leaf tissue caused areas of light and dark color. Twenty-six percent of the pollen was good as determined by aceto-carmin staining. The plant exhibited 70 chromosomes at II-M. Five capsules containing 81 seeds were sown of which 60 germinated, giving rise to F_3 line, 29001.

Plant 28643/20 was 120 cm high; the leaves were heart shaped, broader, and shorter than those of *rustica pumila*. Seventy-seven percent of the pollen was good. The plant had 72 chromosomes at II-M. One capsule

containing 160 seeds, most of which germinated, gave rise to F_3 line, 29002. This plant is perhaps typical of the F_2 class 3 plants.

Plant 28643/47 was 102 cm high; the leaves were quite long, rather narrow, and thickened due to irregular development of the leaf tissue which also gave them a mottled green color. The plant exhibited 71 chromosomes plus a fragment at II-M. One capsule containing 125 seeds, most of which germinated, gave rise to F_3 line, 29003.

Plant 28643/48 was 113 cm high and had leaves similar to those of 643/20. It had 74 chromosomes at II-M and one capsule containing 150 seeds gave rise to F_3 line, 29004.

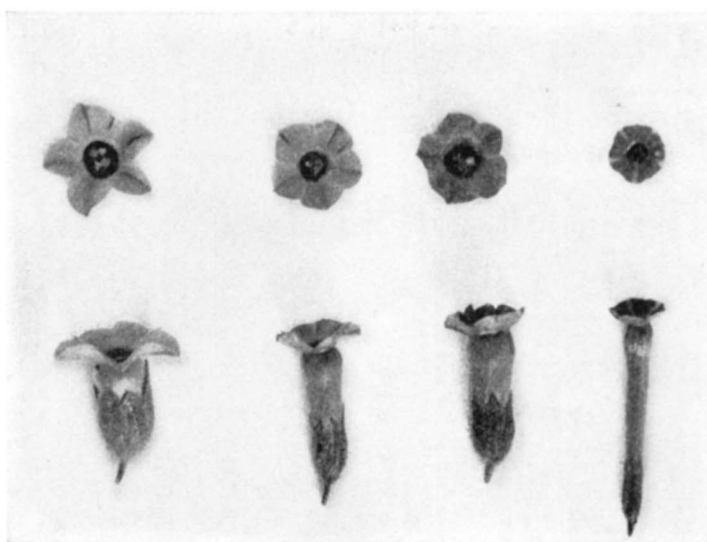


FIGURE 2.—Comparison of (1) *N. rustica pumila*, (2) F_1 hybrid, (3) amphidiploid hybrid and (4) *N. paniculata* flowers.

Plant 28643/50 was 117 cm high. The leaves were similar in shape to those of 643/20 but more emarginate. It had 77–78 chromosomes at II-M and one capsule containing 143 seeds gave rise to F_3 line, 29005.

All these plants as well as the remaining class 3 plants were very similar in flower size and shape, though, of course, exhibiting some variability, especially in leaf morphology and chromosome number; and the selected plants were considered representative. Only one plant in the entire class, namely, 28643/15 was at all infertile. The others, though much less fertile than *rustica*, which averages about 550 seeds per capsule, were comparable with one another, averaging about 150 normal-appearing seeds per capsule. Besides these there are many shriveled seeds which fail to germinate.

The five F_3 lines resulting from these plants were comparable in every way. Furthermore, each line exhibited about the same type of variability, hardly two plants being identical. The variability was much greater than that of F_2 . No constant differences were noted which could serve to distinguish one line from another. Each line had dwarf and giant types, most plants, however, being intermediate between extremes, as represented by figure 7. Comparison of the relatively constant flower type with *rustica*

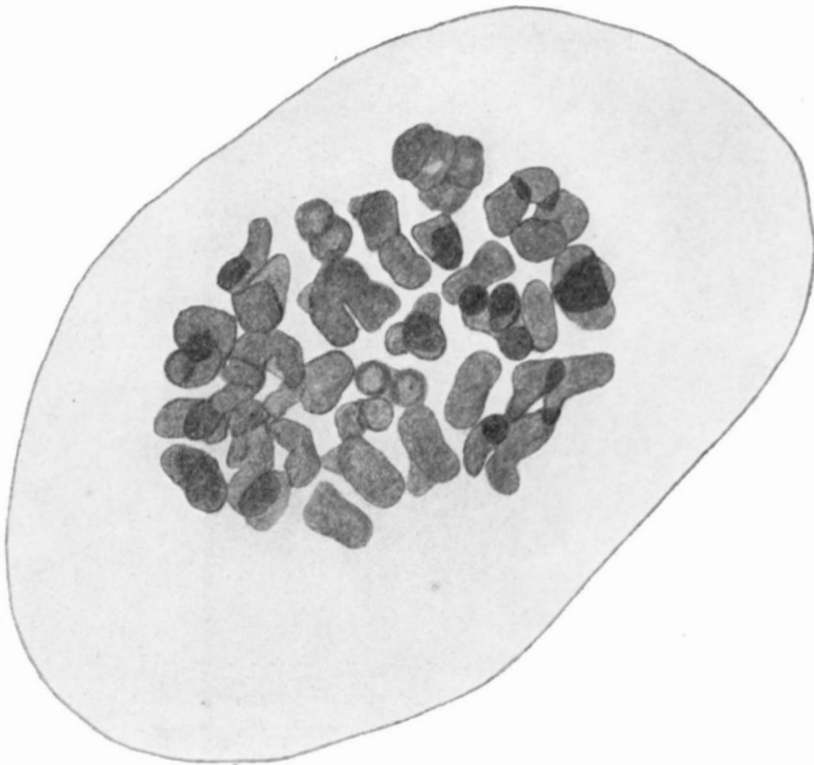


FIGURE 3.—I-M of amphidiploid plant showing $10_{IV}+16_{II}$.

pumila, the F_1 , and *paniculata* is made in figure 2. It is not to be understood from this figure that the amphidiploids showed no variability in detail of flower morphology, but that such as occurred was around this type as a mode. Finally the F_3 lines resembled *paniculata* in their long flowering period, this being especially noticeable under field conditions where *paniculata*, the F_1 hybrid and the amphidiploid, remained flowering long after *rustica pumila* and its derivatives.

Cytologically the plants were very similar in their chromosome behavior. Many plants were examined and all showed very irregular behavior at I-M. Within a given aceto-carmin mount the number of chromatin elements varied from approximately 24 to 36. The cell shown in figure 3 is characteristic. It was interpreted as having $10_{IV} + 16_{II}$. II-M counts checked this interpretation, inasmuch as 72 chromosomes were counted. Figure 4 shows

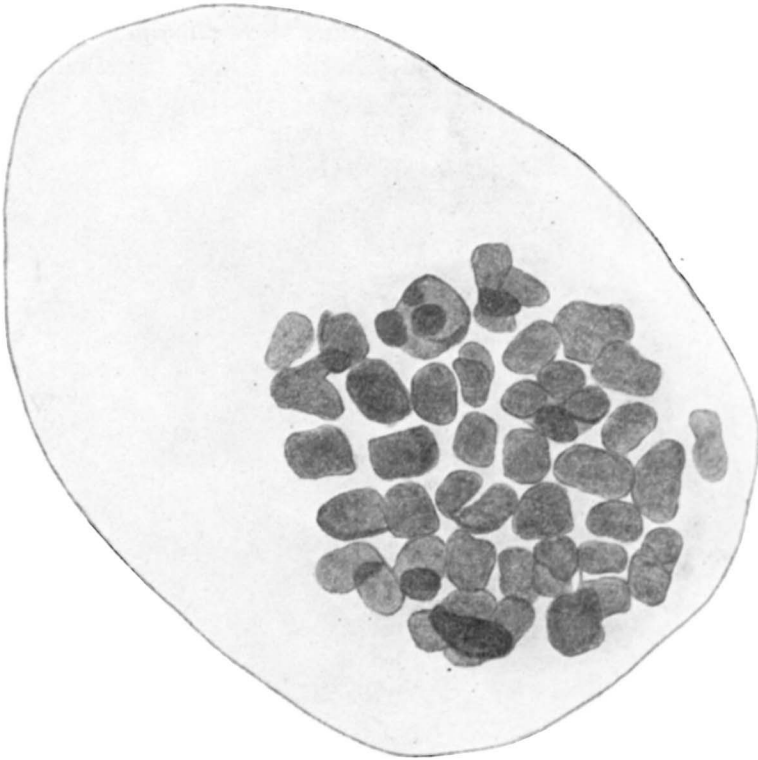


FIGURE 4.—I-M of amphidiploid plant showing $4_{IV} + 30_{II} + 2_{I}$.

a more unusual condition, that is, $4_{IV} + 30_{II} + 2_{I}$. Rarely one finds a plate showing the maximum number of quadrivalents, that is, $12_{IV} + 12_{II}$. The II-M are very clear, and though it is difficult to find decisive plates, when located, they agree as to number. The determination of the five F_2 plants and F_3 plants selected for parents of the F_4 generation is usually based on counts of 5 II-M plates each. Figure 5 shows a II-M of a typical F_3 plant exhibiting 72 chromosomes.

Five F_3 plants were selected for the F_4 generation. As with the F_2 an endeavor was made to pick extreme types with the idea of establishing possible derivatives. Some idea of the appearance of these amphidiploids and

the variability exhibited by them may be obtained from reference to figures 6-10. The dwarf plant (figure 6) proved to be completely sterile. It exhibited 72_1 at II-M. There were very few sterile plants and it is interesting that in spite of a numerically complete double set of chromosomes, complete sterility is possible. Three of the other plants were determined as having approximately 72 chromosomes and one (figure 10) was definitely determined as having 73 chromosomes.

The four F_4 lines arising from these plants were distinct from one another. Each line exhibited variability but not to the extent found in F_3 ,

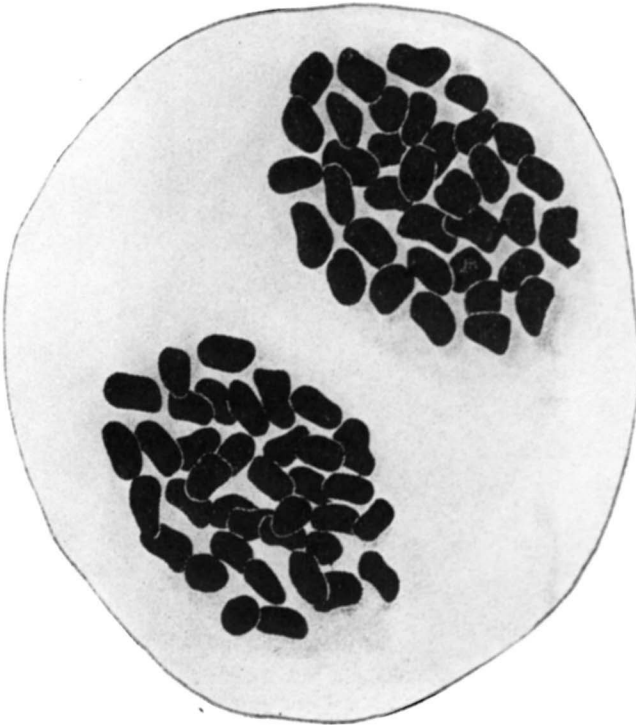


FIGURE 5.—II-M of amphidiploid plant showing 72 chromosomes.

and furthermore it was of a type distinctive for each line. In other words, four derivatives have been established from as many F_3 plants, and these show a greater degree of constancy than was found in the F_2 and F_3 . They are similar in chromosome behavior to the F_2 and F_3 , exhibiting a variable number of bivalents and quadrivalents. Investigations are being continued into the F_5 and further generations in order to determine subsequent behavior. The possibility of securing other more striking derivatives is also being investigated. It is planned to secure more F_3 material from F_2 seed

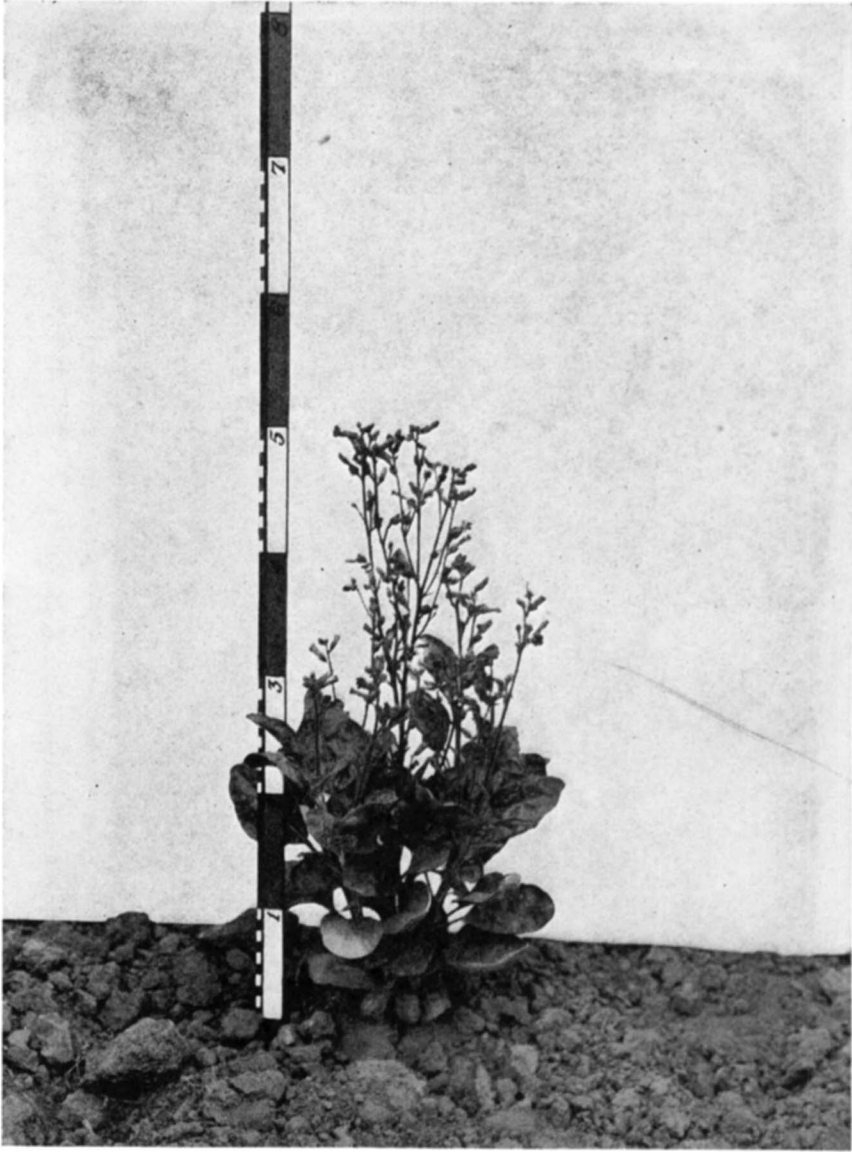


FIGURE 6.—A dwarf type plant found in an F_2 amphidiploid line. It exhibited 72 chromosomes at II-M and yet was completely sterile.

and to make selections of other plants for F_4 lines as was described above so as to get a clearer picture of the extent to which derivative lines may be built up.

(b) *Result of crossing amphidiploids with parental species*

(1) Reciprocal backcrosses to *rustica pumila*

Plant 29001 was used for making crosses with the parental species. It



FIGURE 7.—Typical F_2 amphidiploid plant. This plant exhibited 72 chromosomes at II-M.

is shown in figure 7. The chromosome number was definitely determined from a number of II-M plates to be 72.

When this plant was used as the male parent on *rustica pumila* well-filled capsules were obtained. The seed though somewhat shriveled ap-



FIGURE 8.—A striking variant found in an F_3 amphidiploid line. Note long flower tubes and narrow leaves. This plant exhibited approximately 72 chromosomes at II-M.

peared good. However, two tests resulted in only one germination from 538 seeds. The reciprocal backcross, using 29001/4 as female parent, produced well-filled capsules. From one capsule containing about 150



FIGURE 9.—A giant type F_3 amphidiploid plant exhibiting approximately 72 chromosomes at II-M.



FIGURE 10.—A giant type F_2 amphidiploid plant exhibiting 73 chromosomes at II-M.

seeds only 6 plants were obtained of which two survived to transplanting. A repeated germination gave similar results, only 8 in 168 seeds germinating.

(2) Reciprocal backcrosses to *paniculata*

When 29001/4 is used as the female parent the capsules, though at first normal in appearance, at maturity show only shriveled, chaffy seed which usually fails to germinate. In one test 8 capsules had only 4 possibly good seeds of which 2 germinated. It is as yet undetermined whether these are actually hybrid plants. Cross sections stained with haemotoxylin show that 9 days after pollination the ovules of the backcross have poorly developed endosperm and relatively smaller size than comparable ovules arising from self-pollination.

When 29001/4 is used as the male parent on *paniculata* the capsules contain only chaffy seed which failed to germinate.

DISCUSSION

The behavior of the amphidiploid on backcrossing to its parents is very unexpected in view of the known fact that zygotes arising from union of F_1 somatic gametes with parental pollen are viable and grow into the class 2 plants described in a previous paper (LAMMERTS 1929). The diploid gametes of the amphidiploid are presumably the same as those produced by the F_1 , and theoretically the amphidiploid should be fertile with its parents, instead of almost completely sterile. The Raphanus-Brassica hybrid investigated by KARPECHENKO (1927) behaves similarly with its parents. This phenomenon is as yet unexplained, though several suggestions as to its cause are being investigated. From the viewpoint of phylogeny the two cases are of especial interest. If these synthetic "species" existed under natural conditions cytogeneticists would be tempted to consider the sterility they exhibit with their parents as due to chromosome incompatibility and hence infer that the species investigated were only distantly related. In other words, the behavior of these two amphidiploids with their parents shows clearly that crossability is not a satisfactory index of species relationship.

It may seem rather unexpected that the segregation of the amphidiploid is apparent for the first time in the F_3 . Theoretically, however, the F_2 plants arise from somatic gametes genetically identical except in so far as non-disjunction has brought about loss or gain of chromosomes. The F_2 plants at meiosis have been shown to exhibit a variable number of quadrivalents and if these dissociated at random, gametes varying genetically would result. The F_3 plants, therefore, would vary both morphologically

and genetically and give rise on selfing to lines of distinctive variability. This has indeed actually proved to be the case and it is believed that the evidence presented proves that dissociation of the quadrivalents is not strictly preferential.

Although a number of amphidiploid hybrids have been recorded, in only a few cases have they been subjected to an extended study. The most complete studies are those of *Nicotiana digluta* (36_{II}) (CLAUSEN 1928); *Primula kewensis* (18_{II}) (NEWTON and PELLEW 1929); the Raphanus-Brassica hybrids (18_{II}) (KARPECHENKO 1927); and the Digitalis hybrids (56_{II}) (BUXTON and NEWTON 1928). These as well as the others recorded may be divided into two groups as regards origin. In one class are those which arose, not as a result of regular formation of diploid gametes by an F₁ hybrid, but rather by a chance doubling in the F₁ zygote (*Nicotiana digluta*, *Primula kewensis*) or by chance union of rarely formed diploid gametes by the F₁ as in the *Nicotiana sylvestris-Tabacum* amphidiploid (RYBIN 1928). The other class originate as a result of regular formation and functioning of diploid gametes by an F₁ hybrid and in this category may be placed the Raphanus-Brassica hybrid, and the Digitalis hybrid. Until recently it was assumed that regular formation of diploid gametes depended upon complete lack of pairing, inasmuch as cytologic studies by FEDERLEY in his Pygaera hybrids and the above hybrids were in this category (FEDERLEY 1928). Furthermore, in both the Digitalis and Brassica F₁ hybrids the only functional gametes were those having diploid or hyperdiploid chromosome numbers, while the great majority of gametes being random combinations of parental chromosomes were non-functional. In the case of the amphidiploid *rustica-paniculata* hybrid, however, the F₁ regularly exhibits the Drosera scheme, 12_{II}+12_I, and many of the haploid gametes, though incomplete, are functional. Besides there are formed regularly diploid gametes which give rise to amphidiploid F₂ plants. The supposition then that somatic gamete formation is necessarily dependent upon lack of pairing can no longer be entertained.

The question of the constancy of these hybrids is of interest from the standpoint of evolutionary significance. When an amphidiploid results from doubling up in a hybrid which exhibits complete lack of pairing, there is theoretically little chance for anything but constancy and such is indeed the observed behavior in the cases so far investigated. But when an amphidiploid originates from a hybrid exhibiting pairing, quadrivalent formation with consequent segregation into numerous strains is theoretically possible. The F₁ of *P. verticillata* × *floribunda* shows 9 loosely formed pairs at I-M. In spite of this, however, *P. kewensis* usually exhibits 16 bivalents

and only one quadrivalent. As a consequence numerous derivative lines have been established, the members of which vary around a type distinct from that of another line. Apparently recombination of *floribunda* and *verticillata* chromosomes is here occurring, leading eventually to the formation of numerous derivatives, all amphidiploid as regards chromosome number.

The amphidiploid *rustica-paniculata* hybrid also has the theoretical possibility of breaking up into numerous derivatives, inasmuch as quadrivalents are formed at meiosis. So far four such lines have been obtained. Whether these will attain the constancy of the parental diploid strains or continue to vary is as yet undetermined, but the high degree of constancy in the F_4 as contrasted with the F_3 lines would lead one to expect a higher degree of constancy in successive generations.

The demonstration that in spite of F_1 pairing according to the Drosera scheme diploid gamete formation occurs regularly, leading to production of amphidiploids, is important, for it shows that interspecific hybridization as a factor in polyploidy is not limited to hybrids in which no pairing occurs.

SUMMARY

(1) Study of the backcross progeny of *N. rustica pumila* ♀ × F_1 *rustica-paniculata* ♂ shows that there is great selectivity in favor of the 24 and 23 chromosome gametes. The pollen is also able to transmit *paniculata* homologs, inasmuch as the 24_{II} plants differed from one another morphologically.

(2) The results of selfing the F_1 are in general accord with expectation based on a study of functional male and female gametes. Three classes of F_2 plants are produced: (1) sterile plants arising from union of haploid gametes and having a total of 24 bivalents and univalents; (2) sterile plants resulting from union of diploid and haploid gametes and (3) from union of diploid gametes amphidiploid plants which, because of zygotic elimination of a great proportion of the diploid zygotes, make up approximately 80 percent of the F_2 progeny.

(3) These F_2 amphidiploids as well as the F_3 show a variable number of quadrivalents, bivalents and univalents at I-M. Chromosome determinations from II-M showed that the plants used for selfing had 72 or 73 chromosomes.

(4) Four derivative lines were obtained in the F_4 which, although not constant, varied about a characteristic type. This indicates that the dissociation of the quadrivalents is not strictly preferential.

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