

INHERITANCE OF MONOSOMICS IN *NICOTIANA RUSTICA*

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Comparatively few investigations have been concerned with a study of monosomics ($2n-1$ types) and their mode of inheritance. The only extensive work is that of KIHARA (1924) and NISHIYAMA (1928) in *Triticum* and the analysis of the monosomics fluted and corrugated in *Nicotiana Tabacum* by CLAUSEN and GOODSPEED (1926). In these cases $n-1$ pollen grains rarely functioned and selfing gave substantially the same results as backcrossing. Accordingly WATKINS (1925) and CLAUSEN (1926) both argued that the rapid return to parental chromosome number of the derivatives obtained by selfing hybrids showing the *Drosera* type of reduction division was largely due to this phenomenon. Gametic elimination, selective functioning of ovule classes and zygotic elimination were not necessarily involved or at least played a minor role. Similarly selfing of plants obtained by backcrossing the F_1 *rustica-paniculata* hybrid to *N. rustica* (24_{II}) resulted in a return to the chromosome number of that species (unpublished data). A study of as large a series of monosomics as possible was undertaken with the idea of determining whether the results of the above investigators in other genera and species were applicable here. An unexpected amount of variation in the behavior of individual monosomics was disclosed by these studies which are presented in the present paper.

METHODS

A report on the progenies obtained by backcrossing the F_1 *rustica-paniculata* hybrid to *N. rustica* has been made (LAMMERTS 1929). The fertile plants therein described were both selfed and backcrossed. The plants of the backcross were again examined using the usual aceto-carminic technique and those having the highest number of bivalents were selected and backcrossed. By continuing in this way after several backcrosses eleven different lines were obtained from as many parent plants showing $23_{II}+1_I$ at metaphase-I. These cultures were grown in the greenhouse during the winter of 1931 and the chromosome number of about 20 plants in each line was determined. It was found that in general the monosomics (those showing $23_{II}+1_I$ at I-M) were of distinctive morphology, though in some

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populations other factors causing variability made the correlation less perfect. In each population a plant most nearly resembling *N. rustica* but having only $23_{II} + 1_I$ was selected so as to eliminate other possible sources of variation as much as possible. Each was selfed by limited pollination; that is, about 75 to 100 pollen grains were placed on each stigma. As the total number of seeds in a capsule is approximately 500, by pollinating in this way possible competition between n and $n-1$ grains is eliminated. The same plants were also crossed as female parents to *N. paniculata* (12_{II}), and as male parent to *N. rustica* using the same limited pollination technique as in selfing. Besides two types were crossed as female parents to *N. rustica* in order to see if the proportion of functional n and $n-1$ female gametes would be comparable to that obtained in the cross to *N. paniculata*. The cultures from these pollinations were grown in the summer of 1931. Classification by morphological characters was not difficult and any doubtful cases were verified by examination of the pollen mother cells.

TABLE 1
Transmission data pertaining to monosomics of N. rustica.

MONOSOMIC TYPE (DESCRIPTION OF APPEARANCE IN <i>N. rustica</i> .)	CROSSED TO <i>N. paniculata</i>		USED AS FEMALE PARENT		USED AS MALE PARENT		SELFED	
	$12_{II}12_I$ HYBRIDS	$12_{II}11_I$ HYBRIDS	NORMAL	MONO- SOMIC	NORMAL	MONO- SOMIC	NORMAL	MONO- SOMIC
A. Wide enlarged leaf; thickened stem	59	62	23	8*	42	70
B. Indistinguishable from normal type	22	69	104
C. Slightly asymmetrical flower with acute corolla lobes	54 56	27 19	112 89	1 11	70	47‡
D. Enlarged flower; partially sterile	33	69	38	65	94	..	34	62
E. Acute light green leaf; narrow corolla lobe	16	86	112 67	32 24	41 42
F. Small irregular leaf; flower ruffled	80 75 72	29 32 29	85 65 93	39 15 1	60 56 64	33 27 33
G. Rounded dark green cordate leaf; corolla lobes flat	63	53	31	77	104	1	47	52

* Based on observation of morphological differences only.

‡ Also segregating for large leaf type.

THE MONOSOMIC TYPES

Seven of the twelve monosomics that should be obtained theoretically from this hybrid were recovered. These are designated A–G respectively. Data on their inheritance together with a brief description of each is given in table 1. Some idea of the changes in the morphological appearance of the *rustica-paniculata* hybrids brought about by the removal of a single chromosome may be had by reference to figures 1 and 2. These are ex-

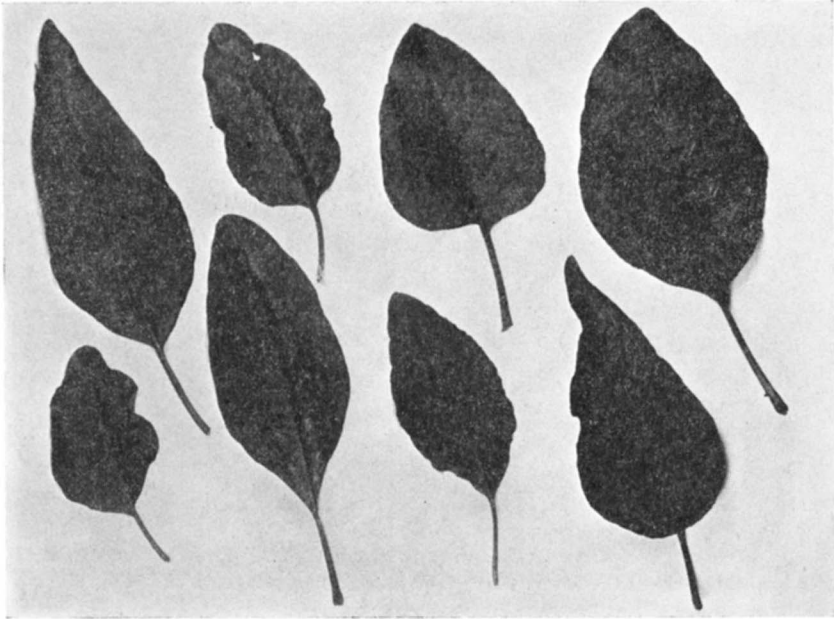


FIGURE 1.—Leaves of the $12_{II}+11_I$ *N. rustica-paniculata* hybrids. At bottom left to right are types A–D; top, left to right, E–G, and normal $12_{II}+12_I$ hybrid.

amples of the nullosomic condition and the effect is of course less in the monosomics of the corresponding selfed progenies where only one member of the pair is removed. Besides the type recorded in table 1 there were found in each selfed and backcross population from 2 to 7 variants which in certain cases were dwarfs or differed in details of flower morphology. Three unusually luxuriant plants were also found. These variants were selfed and in most cases have given only normal progeny. They were apparently due merely to environmental influences. Some, however, have given progenies exhibiting further variation and these are now being investigated. Although practically all the variants were examined, in no case

were any trisomics found. Only two variants were found in the hybrid populations. One of these appeared in the cross of type A to *N. paniculata*. It was unusually luxuriant and had thick wide leaves with irregular margins. The plant resembled *N. solanifolia* in growth habit but was a true hybrid, the pollen mother cells showing $12_{II}+12_I$ at metaphase-I. It was unfortunately as highly sterile as the normal hybrids so that further study could not be made. The lack of variants in the hybrid populations is not clear but may be connected with a slighter sensitivity to environmental influences on the part of the hybrids which were very vigorous.

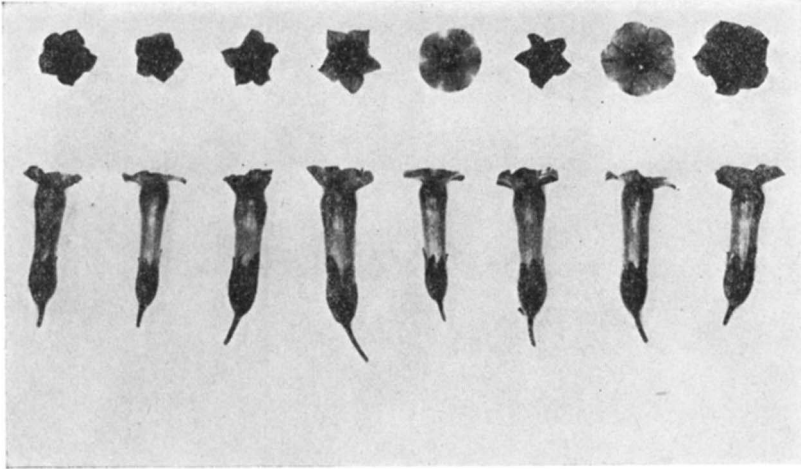


FIGURE 2.—Flowers of $12_{II}+11_I$ *N. rustica paniculata* hybrids. From left to right are types A-G and at extreme right the normal $12_{II}+12_I$ hybrid. About two-thirds natural size.

Examination of table 1 will show that crosses to *N. paniculata*, backcrosses and selfed progenies were in general comparable as regards the proportion of monosomic plants found. Only in type G do we find differences of possible significance. Here the percentage of nullo-G hybrids is 45 as compared with 71 percent in the female backcross and 52 percent in the selfed lines. This variation probably reflects a great variability in the frequency of lagging of the univalent during the reduction division resulting in varying percentages of micronuclei and $n-1$ gametes. Also in type F where $n-1$ pollen functions occasionally we find a higher proportion of monosomics in selfed lines than in the backcross. Nevertheless for comparative purposes we may class the three populations of a given type together. This has been done in table 2.

It may readily be seen that the monosomics fall into two sharply de-

finned groups consisting of (1) types A, B, D, E, and G in which 56 to 75 percent of the functional gametes are $n-1$ and (2) types C and F where only 31 percent are $n-1$. The variation in group 1 may well be only a random one although the range is so great as to make it seem likely that the B and E chromosomes lag and accordingly are eliminated much more frequently than A and G with D intermediate in behavior. Direct comparative cytologic study on percentages of lagging as revealed by the number of micronuclei would possibly enable one to decide this question. There can be no doubt however that chromosomes C and F are in a class by themselves. The low percentage of functional $n-1$ gametes from these types does not seem to be the result of poor viability. Germination was high (about 95 percent) and the monosomic plants, especially those of

TABLE 2
Functional female gametes from N. rustica monosomics.

MONOSOMIC TYPES	24-CHROMOSOME GAMETES	23-CHROMOSOME GAMETES	TOTAL	PERCENT $n-1$
A	101	132	233	56
B	22	69	91	75
C	180	83	263	31
D	105	196	301	65
E	90	250	340	73
F	590	183	773	31
G	141	182	323	56

type C, were quite as vigorous as normal plants. Furthermore it is these very types which show an appreciable amount of transmission through the pollen, further evidence that the small number obtained through the female parent probably is not due to a lower viability of these types. It would appear therefore that some other phenomenon is operating to cut down the percentage of functional $n-1$ female gametes. Possibilities under investigation are (1) selective development at the four celled stage of megasporogenesis, (2) frequent failure of $2n-1$ zygotes to complete normal seed development, and (3) double division of the univalent involved.

DISCUSSION

One of the most obvious features of the various cultures was that in every case the differences between the monosomics and the normals in *N. rustica* were less pronounced than the corresponding differences in the cultures obtained by crossing the same parent to *N. paniculata*. This was most noticeable in the case of type B which was so similar to the normal

that classification in the selfed progeny was impossible. However, in the hybrid population the nullosomics were readily recognized. The same phenomenon was described by CLAUSEN (1926) in his studies of the fluted and corrugated monosomics of *N. Tabacum*, another *Nicotiana* species with $n=24$. Here the regular F_1 *sylvestris-Tabacum* hybrid has 12 *sylvestris* chromosomes paired with 12 *Tabacum* homologs leaving 12 unpaired *Tabacum* chromosomes. The fluted and corrugated hybrids have 12 bivalents and only 11 univalents. In other words these hybrids were nullosomic as regards the fluted and corrugated chromosomes, there being no homologs of them in *N. sylvestris*. Likewise examination of the *rustica-paniculata* hybrids resulting from the union of 23 chromosome gametes and *N. paniculata* pollen showed $12_{II}+11_I$ at I-M in each of the seven types. These types were then comparable to those reported by CLAUSEN in *N. Tabacum* and the greater contrast observed in hybrids is because we are here dealing with a nullosomic instead of a monosomic effect. The corresponding condition in *N. rustica* would necessitate the production of plants with both members of a given pair eliminated instead of only one. This has so far proved impossible although every effort has been made including limited self pollination on the shortened style of a type F plant. In no case were any plants exhibiting 23 bivalents at first metaphase-I or extreme development of the monosomic complex found although most of the variants were examined with this idea in mind. Undoubtedly this lack of nullosomics is mainly due to the rarity with which $n-1$ pollen grains function. It is unnecessary to assume that the nullosomic condition would have a lethal effect although in types F and C such may well be for here we have an appreciable number of $n-1$ pollen grains functioning. In the case of type F 28 percent of the functional female gametes and 18 percent of the functional male gametes are $n-1$. Accordingly about 5 percent of nullosomics would be expected. Since none was formed even in progenies resulting from limited pollination on shortened style, one may conclude that the nullosomic has very low viability.

The rather rapid return to the parental chromosome number on the part of the hybrid derivatives obtained by selfing fertile plants in the backcross of F_1 *rustica-paniculata* to *N. rustica* (24_{II}) is therefore mainly due to the infrequency with which pollen grains containing incomplete chromosome sets function.

A study of the comparative effects of removing whole chromosomes in diploid, theoretically amphidiploid and hexaploid species is interesting. BLAKESLEE and BELLING (1924) found that $n-1$ gametes from a $2n-1$ bud variant did not function in *Datura Stramonium*, a diploid species with

$n = 12$. In *Zea mays*, also a diploid species with $n = 10$, numerous translocations are being studied by ANDERSON, BURNHAM, and BRINK. As reported by BURNHAM (1930) approximately one-half of the gametes from plants heterozygous for a given translocation are deficient for a section of one chromosome and duplicated as regards the other. In some cases the deficiency is very small and yet such gametes do not function (see BURNHAM [1932]). Monosomics have as yet not been found. In *Oenothera Lamarckiana*, a plant which was $2n - 1/2$ gave only normal and $2n + 1/2$ plants when self pollinated and crossed to *Oe. Hookeri* (unpublished data of author). Evidently monosomics and deficiencies are not transmitted in diploid species.

N. Tabacum and *N. rustica* are both naturally occurring amphidiploid species having two sets of 12 chromosomes which are to some extent at least homologous though pairing is no longer possible. In both these species monosomics are transmitted readily through the egg but only rarely through the pollen and nullosomics have so far not been obtained.

Finally KIHARA (1924) and NISHIYAMA (1928) report that in *Triticum vulgare* and *spelta* ($n = 21$) male and female gametes both function and nullosomics having only 20_{II} may be obtained. By crossing monosomics involving different chromosomes plants having $19_{II} + 1_A + 1_B$ were obtained by NISHIYAMA (1928). These when selfed gave among others a few weak plants with only 19_{II} . Recently NISHIYAMA (1931) completed a study of fatuoid oats in *Avena sativa* ($n = 21$), another supposedly hexaploid species. He found the fatuoid character associated with a certain chromosome, *c*, which may occasionally be eliminated spontaneously. Such a monosomic is transmitted through both ovules and pollen and because of lagging of the univalent only about $1/6$ of the gametes are normal. Selfing therefore resulted in 256 homozygous fatuoids ($2n = 40$), 397 heterozygous fatuoids ($2n = 41$) and only 42 normals ($2n = 42$). Here then we have a frequent lagging and no selective elimination of gametes with the incomplete set of chromosomes.

We may therefore conclude from this comparative study that there is a direct relation between the degree of polyploidy and viability of $n - 1$ gametes and $2n - 2$ zygotes. The recovery of monosomics in a supposed diploid species would then be evidence of its polyploid nature. As pointed out by BRIDGES (1921) and CLAUSEN (1926) monosomics may be used advantageously for the determination of linkage groups and also linkage relations within a group. Their use in plants is however limited to amphidiploids and hexaploid species because of their inviability in diploids.

SUMMARY

Seven monosomics in *N. rustica* are described. The percentage of functional $n-1$ gametes varies in the individual cases but due to lagging is usually over 50 percent. In two cases, however, only 31 percent of the functional gametes were $n-1$. This was not the result of inviability because about 95 percent of the seed germinated and practically all the plants survived. Only three types were transmitted in appreciable frequency through the pollen.

Nullosonic plants ($2n-2$) were not obtained even after selfing with a limited amount of pollen on shortened styles.

Hybrid derivatives obtained by selfing fertile plants in the backcross of *N. rustica-paniculata* hybrid to *N. rustica* (24_{II}) return to the chromosome number of *N. rustica* mainly because pollen grains with incomplete chromosome sets rarely function. Zygotes lacking both members of a chromosome pair are probably eliminated even when produced.

Viability of $n-1$ gametes and $2n-2$ zygotes is evidence that a species is polyploid.

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