Additional Information on the Mechanism of Chromosome Substitution in Nicotiana

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Substitution of foreign chromosomes is a necessary first step in certain plant breeding programs which utilize wild species. One such program has been concerned with the substitution of the chromosome carrying the genetic base for mosaic resistance from *Nicotiana glutinosa* for one of *N. tabacum* which was accomplished for the first time by Holmes (1938). Only a thorough understanding, qualitatively and quantitatively, of the mechanism of substitution will provide a rationale for planning future programs of this type.

Holmes started by crossing an amphidiploid *N. tabacum* x *glutinosa* for several generations to *N. tabacum*. The substitution occurred in an early generation. On the basis of a subsequent analysis Gerstel (1946) suggested that either multivalent formation or non-conjunction may have led to the formation of gametes which contained the substituted chromosome from the wild species. Clausen and Cameron (1957) then described in greater detail a model by which a trivalent could disjoin in such a fashion that an intact foreign chromosome would be substituted in a spore.

Earlier evidence indicated that substitution is achieved with relative ease early in the backcrossing program. In Holmes' (1938) work substitution heterozygosity was achieved in the first backcross from the amphidiploid in two out of three trials. Chaplin and Mann (in press) also obtained occasional substitution of chromosomes from *N. paniculata*, *N. plumbaginifolia* and *N. rustica* in the gametes of the first segregating generation, of the type *tabacum-tabacum-paniculata*, and Apple (unpublished) also achieved substitution of a *N. plumbaginifolia* chromosome carrying resistance to blackshank in an early generation. Therefore, we studied the 5x generation, derived from a cross of amphidiploid *N.*

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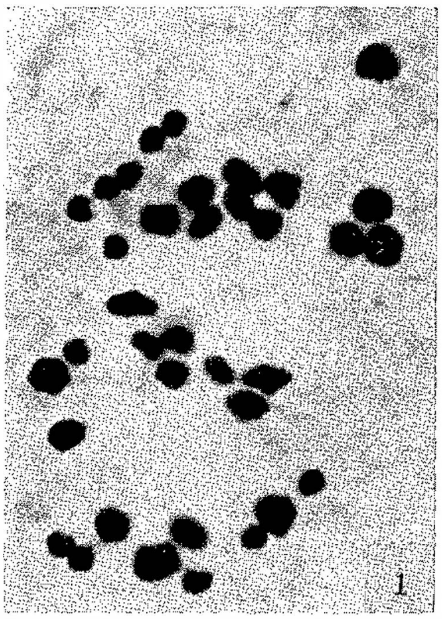

Figure 1. Meiotic metaphase with 24 pairs of *N. tabacum* chromosomes and 12 *N. glutinosa* univalents.

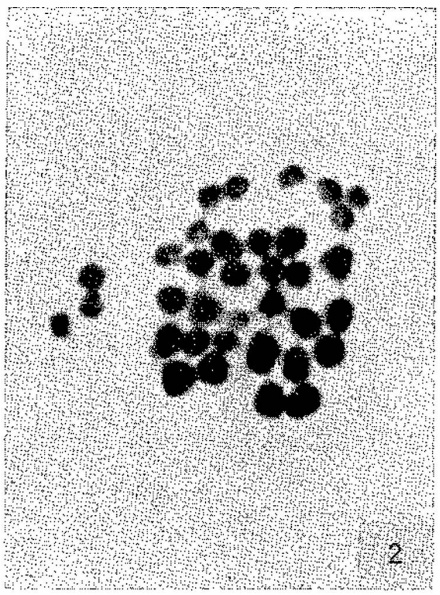

Figure 2. Meiotic metaphase with 24 pairs of *N. tabacum* chromosomes and 11 *N. glutinosa* univalents.

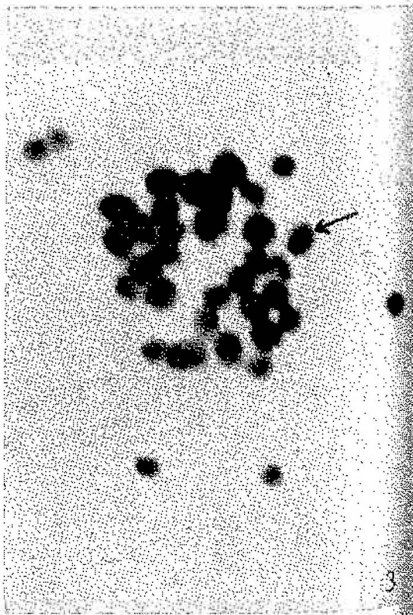

Figure 3. Meiotic metaphase with 23 *tabacum* pairs, 1 *N. tabacum* univalent (large arrow) and 12 *N. glutinosa* univalents (small

tabacum x *glutinosa* (6x) x *N. tabacum* (4x) in order to explore further the mechanism of substitution.

Materials and Methods

The amphidiploid parent of the experimental material was synthesized from a line of *N. tabacum* (var. 402) which carried the recessive yellow green (*yg*) and *N. glutinosa*. The other parent of the 5x generation studied was yellow green tobacco. Two families were used: E700, in which the rare yellow green segregants were saved at the cotyledonary stage from a very large population and the green ones discarded and a much smaller family E701 from which all plants were raised to maturity. Only a limited number of plants from both groups were analyzed cytologically.

Young flower buds were fixed in Carnoy's (6:3:1) for 24 hours and the material was then transferred to a mixture of 3 parts of 95% alcohol and 1 part of propionic acid saturated with ferric acetate. Anther smears were made according to the propiono-carmine technique (Swaminathan *et al.*, 1954).

Results and Discussion

The observations made on 37 plants of the 5x generation are summarized in **Table 1**. Twenty-four of these had 60 chromosomes, that is the 24 *N. tabacum* pairs and 12 *N. glutinosa* singles (**Figure 1**) expected in this generation with normal disjunction in the amphidiploid parent. Twelve plants had only 59 chromosomes. Ten of these lacked a *N. glutinosa* chromosome, as was evident from the modal conjugation of 24 pairs (*N. tabacum*) and 11 univalents (*N. glutinosa*) in the plants of this group (**Figure 2**). The other two plants with 59 chromosomes lacked a *N. tabacum* chromosome, since they formed usually 23 pairs of 13 univalents. In one of these two plants one could observe directly that one of the univalents came from tobacco, since it was larger than any chromosome of the *N. glutinosa* genome (**Figure 3**). The remaining plant had only 57 chromosomes (**Figure 4**).

Table 1. Chromosome conjugation in 37 offspring from the cross amphidiploid x *N. tabacum*

Chromosome No.	60	59 (lacking glut. chr.)	59 (lacking tab. chr.)	57‡ (lacking 3 chr.)
No. of plants*	24**	10†	2	1
$24_{II} + 12_{I}$	*424*	2 + 5 (?)	—	—
$23_{II} + 13_{I}$	1	2 + 4 (?)	*53*	1 + 1 (?)
$24_{II} + 11_{I}$	—	*182*	1	—
$23_{II} + 11_{I}$	—	2	—	—
$23_{II} + 14_{I}$	*5*	—	—	—
$22_{II} + 11_{I}$	—	1	—	—
$22_{II} + 13_{I}$	—	—	—	*69*
$22_{II} + 15_{I}$	*1*	—	—	—
$25_{II} + 9_{I}$	—	2	—	—
$25_{II} + 11_{I}$	1	4	—	—
$1_{III} + 23_{II} + 11_{I}$ (?)	3	—	—	—
$1_{III} + 23_{II} + 10_{I}$ (?)	—	1	—	—
$1_{III} + 22_{II} + 12_{I}$ (?)	1	—	—	—
$1_{III} + 21_{II} + 13_{I}$	—	1	—	—
Total	436	206	54	71

*At least 10 metaphases were scored in each plant.
**One yellow-green E700 plant in this group.
†Three yellow-green E700 plants in this group.
‡This plant lacked either 2 tab. and 1 glut. chromosomes or one tab. pair and one tab. single.

Table 1 reveals a surprising regularity of conjugation. In a large majority of cells (underscored) the maximum number of *N. tabacum* pairs was formed and only very few cells (total of 6, broken underscorings) had non-conjugated *N. tabacum* pairs. Six additional cells may have contained trivalents; but this is a maximum figure, since any doubtful case was scored as a trivalent and probably most of these were overlaps rather than true conjugants. Seven cells contained 25 bivalents, which may have been indicative of autosyndesis between *N. glutinosa* chromosomes; there may also have been some misinterpretation of overlaps, the more so since Webber (1933) did not find any metaphase pairing in haploid *N. glutinosa*. A few cells deviated from the normal total counts of 60, 59 and 57, respectively. There may have been some errors in counting caused by overlaps, etc., or the abnormal cells may have been caused by occasional premeiotic aberrations in distribution.

The data failed to indicate any appreciable amount of either nonconjunction or trivalent formation in the 5x generation, the events which had been postulated to result in chromosome substitution. If one divides the frequency of cells which gave any indication of the two phenomena by twelve, one arrives at a very small chance indeed for the substitution of any particular *N. glutinosa* chromosome. It should be mentioned, however, that Chu (1951) has reported a somewhat higher frequency of the pertinent events. Among 56 cells of a *N. tabacum-tabacum-glutinosa* pentaploid he found three with a trivalent and nine cells in which fewer than 24 pairs were formed.

In searching for another cause of chromosome substitution the thirteen plants with fewer than 48 *N. tabacum* chromosomes are of interest, because they indicate occurrence of meiotic irregularities in the preceding generation, i.e., in the amphidiploid *N. tabacum* x *glutinosa*. This apparently led to loss of a *N. glutinosa* chromosome in ten of the 37 gametes which were sampled and to loss of a *N. tabacum* chromosome in two. The two plants with 23 tobacco pairs, 1 tobacco univalent and 12 *N. glutinosa* univalents were in fact substitution heterozygotes which, in turn, would produce a large proportion of gametes with a substituted

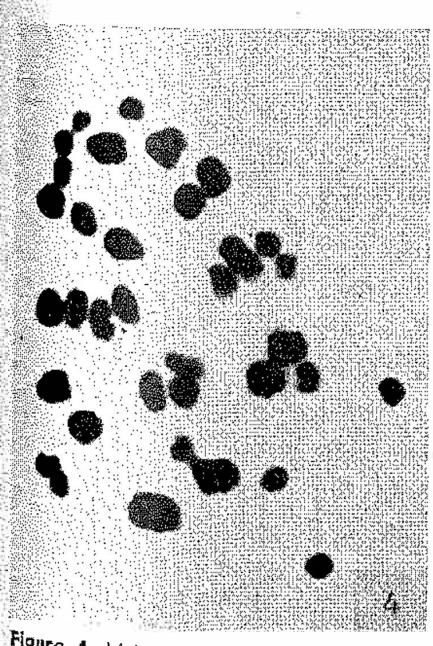

Figure 4. Meiotic metaphase with 22 pairs and 13 univalents.

N. glutinosa chromosome. This also applies to the plant with 57 chromosomes. Thus, it appears that the best opportunity to obtain substitution products in plant breeding operations occurs in the amphidiploid.

The amphidiploid itself was unfortunately unavailable for study at this time and only qualitative observations can be found in the literature. In their original report on "*N. digluta*" Clausen and Goodspeed (1925) reported minor irregularities in distribution as precocious splitting, lagging and microcyte formation. Similarly, Fardy and Hittier (1945) observed occasional multivalents and univalents at metaphase I and disturbances in regular chromosome distribution to the dyads. A consequence of such irregularities was illustrated by Müntzing (1935) who found that the plants of the F_6 generation from *N. digluta* had 68 or 69 chromosomes instead of the original 72.

The data presented in **Table 1** are interesting in another connection. Genetic segregation in *N. tabacum* x *glutinosa* amphidiploids had been observed by Gerstel and Phillips (1958). Amphidiploids constituted from yellow green tobacco and normal green *N. glutinosa*, like the parents of the present material, gave in testcrosses a total of 322 green to 4 yellow green offspring. The results presented (see footnotes in **Table 1**) suggests that only a part of the yellow green plants from such a cross are caused by mendelian segregation; another, and probably the larger part are the result of the loss of a *N. glutinosa* chromosome. The twelve *N. glutinosa* chromosomes could not be distinguished from each other at meiosis, but the fact that three out of four of the yellow green plants studied had 59 instead of 60 chromosomes is suggestive. Therefore, differential affinity of tobacco for tobacco and *N. glutinosa* for *N. glutinosa* chromosomes is even stronger than genetical study alone would suggest. The multivalents recorded by Fardy and Hittier (1945) for amphidiploid *N. tabacum* x *glutinosa* thus appear doubtful and the material should be re-examined from that viewpoint. Since there is such strong affinity of tobacco chromosomes it appears improbable that trivalent formation in later generations can be an important factor in achieving chromosome substitution. No explanation can be offered at present for the greater tendency for non-conjunction in the amphidiploid than in pentaploid generation. Since the amphidiploids have more chromosomes than the pentaploids the suggestions offered in the literature for a reduction of pairing in polyploids, as compared with diploids may be applicable (see short review in Gerstel and Phillips, 1958).

Summary

Study of pentaploids *N. tabacum-tabacum-glutinosa* revealed the following:

a) The chromosomes behaved very regularly at meiosis, forming 24 *N. tabacum* pairs and 12 *N. glutinosa* univalents; non-conjunction of tobacco pairs was very rare and whether trivalents were formed at all could not be ascertained with certainty. The chances of achieving substitution in the pentaploid generation are therefore very poor.

b) Of the 37 pentaploid plants sampled ten lacked a *N. glutinosa* chromosome (the sample was not random since selected plants of recessive phenotype had been included). Three plants lacked *N. tabacum* chromosomes and were substitution heterozygotes. Because of the appreciable chance for substitution the breeder should concentrate on amphidiploid generation.

c) Segregation in *N. tabacum* x *glutinosa* amphidiploids observed in the past was found to be due in part to loss of chromosomes carrying dominant marker genes; differential affinity of chromosomes at meiosis is thus very pronounced. The cause of the greater meiotic irregularity of amphidiploids as compared with pentaploids has not been determined.

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