The Nicotiana rustica-paniculata Amphidiploid Derivatives

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Amphidiploids arising from species hybrids in which chromosome conjugation is altogether lacking are relatively constant, highly fertile, and show regular bivalent pairing. However, most of the ones recently reported exhibit some tendency to quadrivalent formation having originated from F₁ hybrids in which some at least of the chromosomes are sufficiently homologous to pair. Examples are Primula kewensis (Newton and Pellew, 1929), Nicotiana tabacum-sylvestris (Ruben, 1929), Nicotiana rustica-paniculata (Lammerts, 1931), and Crepis rubra-foetida (Poole, 1931). Assuming random dissociation of the quadrivalents and survival of the resulting gametes and zygotes one would obtain by continuous selfing numerous derivative lines. Any tendency to pair preferentially would delay attainment of uniformity. Complete substitution of homologs in a given quadrivalent group might make the resulting plants weakly viable or sterile, thus also making it impossible to get uniform lines. This is apparently the case in the experimentally formed new species Digitalis mertonensis reported by Buxton and Darlington (1931) where about a quarter of the plants in each generation are partially sterile.

The results of continuous selfing of the amphidiploid N. rustica-paniculata hybrid are accordingly of interest in view of the above possibilities. As reported by the author (1931) it arose from the union of diploid gametes produced by the F₁ hybrid between N. rustica (2₄II) and N. paniculata (1₂II). This hybrid shows the Drosera type of pairing during the first metaphase of the reduction division there being 1₂II+1₂I. Accordingly quadrivalents are formed during meiosis in the

1) Hybrids in which the chromosome number is diploid for both parental species.
amphidiploid, the number varying from $12_{IV} + 12_{II}$ to $36_{II}$ at I-M. F$_3$ populations from various F$_2$ plants were comparable in the degree of variability exhibited. Giant and dwarf types as well as a few completely sterile plants were present in each line. These sterile plants were numerically complete having 72 chromosomes and were therefore the result of chromosome substitution. Five of the most striking F$_3$ variants were selfed. In three lines complete uniformity was obtained by the F$_5$ generation. The other two were as yet variable but with the exception of three light green variants to be described later, F$_7$ generations were all uniform. These derivative lines resemble the original F$_3$ parents in most characters and some idea of their distinctive features may be obtained by reference to photographs of the original plants (Lammerts 1931). The flowers and leaves of four lines are shown in figures 1 and 2.
Within a given line the plants were comparable in fertility as judged by the number of seeds per capsule and the amount of good pollen. The various lines differed in these respects, some being much more fertile than others. The results obtained from averaging examinations of 10 plants in each population are as follows:

<table>
<thead>
<tr>
<th>Derivative</th>
<th>Good pollen per 1000 grains</th>
<th>Number of seeds per capsule</th>
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<tbody>
<tr>
<td>G 31030</td>
<td>75–80%</td>
<td>150–175</td>
</tr>
<tr>
<td>G 31031</td>
<td>60–65%</td>
<td>125–150</td>
</tr>
<tr>
<td>G 31032</td>
<td>85–90%</td>
<td>150–200</td>
</tr>
<tr>
<td>G 31033</td>
<td>75–80%</td>
<td>150–200</td>
</tr>
<tr>
<td>G 31034</td>
<td>45–50%</td>
<td>50–75</td>
</tr>
</tbody>
</table>

Though the differences exhibited by lines G 31030–33 may not be significant there is no doubt that line G 31034 is markedly less fertile than for example G 31032.

Chromosome numbers of 70 plants were determined by II–M studies, 15 from each of the first four lines and 10 from line G 31034. All had 72 chromosomes with the exception of five plants which had 71 (2), 73 (1) 74 (1), and 78 (1) respectively. The three variants in line G 31034 referred to above proved most unusual. They showed 12II + 12I, 11II + 13I and 10II + 1IV + 12I at I–M (Figs. 3 and 4) and therefore developed from unfertilized female gametes. Jørgensen (1928) obtained haploids from Solanum nigrum (36II) which formed 12II + 12I at I–M. He accordingly concludes that the haploid set in these and other similar

Figs. 3 and 4. I–M polar view of the “haploids” G31034/6 and 7 showing 10II + 1IV + 12I and 11II + 13I. × 2700.
species is composed of two or more homologous sets of chromosomes. The "haploids" occurring in G 31034 are comparable to those found by Jørgensen and are indirect evidence in support of his general conclusion, having been obtained from plants known to have four sets of 12 homologous chromosomes. The return of these plants to the original F₁ chromosome constitution was accompanied by similar decrease in fertility, G 31034/6 showing 10₁Ⅱ + 1₁IV + 12₁ being even less fertile than the F₁ hybrid. Study of the progeny obtained by backcrossing this plant to N. rustica was made to determine the nature of the quadrivalent, i.e. whether the result of a reciprocal translocation or of all four members of a quadrivalent going to the same pole during the reduction division of the parent plant. Two plants of the backcross survived to time of flowering. First and second metaphase figures of the pollen mother cells were studied by means of aceto-carmine smears. One plant usually showed 17₁Ⅱ + 1₁III + 6₁ at I–M (Fig. 5) though often 18₁Ⅱ + 7₁ were observed. The total number of chromosomes in the two II–M plates was 43. The other plant was more irregular but usually 18₁Ⅱ + 1₁III + 5₁ were observed at I–M and a total of 44 chromosomes at II–M. A plant having an association of four chromosomes due to a reciprocal translocation would give on backcrossing plants having either normal bivalents or the apparently quadrivalent condition, and not plants exhibiting trivalents such as were found in the two plants examined. The presence of trivalents in these two plants is then evidence that the group of four associated chromosomes found in G 31034/6 resulted from the simultaneous non-disjunction of two quadrivalents each going as a unit to opposite poles, and is therefore a true quadrivalent and not the result of a reciprocal translocation. It is of course understood that the above assumption of simultaneous non-disjunction of two quadrivalents is rather unlikely from consideration of chance probability, but it nevertheless seems warranted by the evidence. The II–M studies of the amphidiploid revealed that distribution of chromosomes from

Fig. 5. I–M polar view of a plant obtained by backcrossing G3104/6 as female parent to N. rustica. 17₁Ⅱ + 1₁III + 6₁ are usually seen as shown. ×2700.
quadrivalent dissociation was occasionally uneven. The following II-M distributions were observed:

<table>
<thead>
<tr>
<th>36-36</th>
<th>35-37</th>
<th>34-38</th>
<th>33-39</th>
<th>32-40</th>
</tr>
</thead>
<tbody>
<tr>
<td>62</td>
<td>56</td>
<td>18</td>
<td>6</td>
<td>1</td>
</tr>
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Many of the resulting male gametes accordingly must have more or less than 36 chromosomes. The haploid plants in line G31034 give evidence for at least some uneven distribution of quadrivalents in the reduction division of the embryo mother cells also but the extent of such irregularity can only be estimated from a study of a large number of similar haploids or preferably by study of divisions leading to megaspore formation.

The relatively few plants having more or less than 72 chromosomes would seem to indicate (1) that pollen with more or less than 36 chromosomes usually fails to function in fertilization probably due to poor pollen tube growth and (2) that the reduction division in the embryo mother cells is more regular than in the pollen mother cells. Unfortunately seeds obtained by backcrossing the amphidiploid as either male or female parent to *N. rustica* germinate poorly. About one in 150 seeds tested germinates, however, so that backcross progenies may be obtained and the above assumptions concerning constitution of functional eggs and pollen may be tested.

As before stated the derivative lines are uniform in spite of the above described chromosome irregularities. The plants with more or less than 72 chromosomes were indistinguishable from the others. This is to be expected in view of the fact that removal of one chromosome from *N. rustica* (theoretically an amphidiploid) causes but slight morphological effect. (Lammerts 1932). The true breeding derivative lines indicate that (1) the dissociation of the quadrivalents in the original F₂ parents was not strictly preferential, and (2) complete substitution is possible in some quadrivalents at least. The rather rapid attainment of uniformity would also seem to indicate an approach to random dissociation, though some other interpretation such as balanced heterozygosis maintained by elimination of zygotes homozygous for certain chromosomes is more likely, especially in view of the fact that different lines vary in fertility, all being much below normal for the parental species.

The demonstration that distinctive true breeding amphidiploid lines varying in fertility and specific type may be obtained from hybrids exhibiting some pairing in the F₁ is very interesting from the
economic point of view in that it gives practical breeders a chance to select for types more adapted to specific needs at the same time retaining as a basis the same general intermediate expression of characters found in the diploid species hybrid.

Summary

(1) Amphidiploid plants combining the diploid chromosome numbers of *N. rustica* (24II) and *N. paniculata* (12II) were obtained in the F2 by the union of diploid gametes from the F1 hybrid (12II+12I). These plants showed a variable number of quadrivalents at the first metaphase and accordingly by continuous selfing gave rise to distinctive true breeding derivative lines most of which have 72 chromosomes.

(2) These uniform lines indicate that dissociation of quadrivalents in the original F2 plants was not strictly preferential and that complete substitution is possible in some quadrivalents at least.

(3) Three haploid plants were found in one line. These had 12II+12I, 11II+13I and 10II+1IV+12I and are comparable to the haploids obtained by Jørgensen from *Solanum nigrum* (36II).

Literature Cited


