POLYPLOIDY ASSOCIATED WITH VARIETAL DIFFERENTIATION IN THE MEGASPERMA COMPLEX OF PHYTOPHTHORA

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(With Plates 73 and 74)

In a cytotaxonomic investigation of the large and small oogonial forms of Phytophthora megasperma Drechsler nuclear behaviour in the gametangia of both forms was consistent with a normal meiosis. Two divisions occurred, the first with extended prophase including pachytene, diplotene and diakinesis. Counts showed that four times as many nuclei were present in the gametangia after division, and the resulting nuclei were roughly half the size of the vegetative nuclei in nearby hyphae. Chromosome bridges were observed in both the first and second divisions. A large pale heterochromatic body attached to one pair of chromosomes was observed in the small oogonial form.

From counts at first metaphase, the chromosome number of the large oogonial form was c. 22–27, and of the small oogonial form c. 10–13. The two forms also differed in growth rate and general cultural characteristics. Varietal differentiation of these two forms (as P. megasperma var. megasperma and P. megasperma var. sojae Hildebrand respectively) therefore seems justified.

The discovery that meiosis including a metaphase stage with distinct chromosomes occurs in the sex organs of the Oomycetes (Sansome, 1963, 1965, 1966) provides an opportunity for studying the comparative cytology of this group. Within the Oomycetes the genus Phytophthora deserves special attention, as it is a large, economically important and taxonomically difficult genus, and contains a number of species being subjected to genetical investigation.

The genus contains several taxonomic complexes. One of these, the megasperma complex, consists of two main taxa, a form with large oogonia based on P. megasperma Drechsler (Drechsler, 1931) and a form with small oogonia based on P. sojae Kaufman & Gerdemann (Kaufman & Gerdemann, 1958). The latter was reduced to varietal status as P. megasperma var. sojae Hildebrand by Hildebrand (1959), a move supported by Waterhouse (1963). This situation has been the subject of much discussion, and the megasperma complex was consequently selected for a cytotaxonomic investigation.
Table 1. Growth rate, oogonial size and chromosome number of
P. megasperma var. megasperma and P. megasperma var. sojae

<table>
<thead>
<tr>
<th>Variety and isolate</th>
<th>Growth rate (mm/day)</th>
<th>Mean oogonial diameter (μm) and range</th>
<th>Estimated chromosome number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Var. sojae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P129</td>
<td>4.61</td>
<td>34.7 (26.4-41.1)</td>
<td>10-15</td>
</tr>
<tr>
<td>P130</td>
<td>3.72</td>
<td>34.9 (20.2-40.3)</td>
<td>10-15</td>
</tr>
<tr>
<td>Var. megasperma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P128</td>
<td>5.29</td>
<td>43.8 (37.2-48.1)</td>
<td>22-27 (-30)</td>
</tr>
<tr>
<td>P206</td>
<td>5.29</td>
<td>47.7 (37.2-54.3)</td>
<td>22-27 (-30)</td>
</tr>
<tr>
<td>P250</td>
<td>5.59</td>
<td>46.6 (35.7-55.0)</td>
<td>22-27 (-30)</td>
</tr>
</tbody>
</table>

MATERIALS AND METHODS

The following isolates were examined. P. megasperma var. megasperma: P128, isolated from Brassica sp. by J. Moore (IMI 56348); P206, isolated from roots of Aesculus hippocastanum by C. M. Brasier; and P250, isolated from soil around roots of Populus robusta by C. M. Brasier. P. megasperma var. sojae: P130 received from D. C. Erwin (his P405K); and P129, isolated from Glycine soja (IMI 131555 = ATCC18010).

Cultures were grown on carrot agar in darkness at 20 °C. Developing gametangia were examined cytologically as follows. The material was fixed in 3:1 absolute alcohol:glacial acetic acid after being immersed for 2 h in iced water to ensure turgidity and to slow down division. After fixation for 1-2 h the material was treated for 1-2 days in a 1:1 mixture of ether and absolute alcohol. The ether mixture was washed out with absolute alcohol and the material stained immediately or stored in 70% alcohol. The material was either stained overnight in aceto-carmine then mounted by the squash method, or left for 4 h in a 1-2% solution of orcein in 60% acetic acid and mounted in varying strengths of aceto-orcein solution.

OBSERVATIONS

Cultural characteristics of the isolates

The radial growth rates of the mycelia of each isolate grown on carrot agar at 23° were measured over 5 days in a replicated experiment. These data are shown in Table 1, together with the mean oogonial diameters measured in samples taken from the same culture plates. The three P. megasperma var. megasperma isolates had a faster radial growth rate and fewer but larger oogonia than the two var. sojae isolates. The var. megasperma isolates also differed from the var. sojae isolates in gross morphology, the former having sparse aerial mycelium and the latter a more dense rather woolly aerial mycelium.

Nuclear behaviour in the gametangia

The aceto-carmine method gave better preparations with the three isolates of the variety megasperma, whereas aceto-orcein proved more successful for the two var. sojae isolates.
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Table 2. P. megasperma var. megasperma (P128) and P. megasperma var. sojae (P130): number of nuclei in prophase and metaphase stages (before division) compared with the number after division, in both antheridia and oogonia

<table>
<thead>
<tr>
<th></th>
<th>No. of oogonia or antheridia counted</th>
<th>No. of nuclei counted</th>
<th>Mean per gametangium</th>
</tr>
</thead>
<tbody>
<tr>
<td>P128 Oogonia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>64</td>
<td>1049</td>
<td>16.2</td>
</tr>
<tr>
<td>After</td>
<td>27</td>
<td>1699</td>
<td>62.8</td>
</tr>
<tr>
<td>P128 Antheridia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>83</td>
<td>170</td>
<td>2.0</td>
</tr>
<tr>
<td>After</td>
<td>35</td>
<td>251</td>
<td>7.2</td>
</tr>
<tr>
<td>P130 Oogonia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>30</td>
<td>434</td>
<td>14.5</td>
</tr>
<tr>
<td>After</td>
<td>21</td>
<td>1349</td>
<td>64.2</td>
</tr>
<tr>
<td>P130 Antheridia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>25</td>
<td>53</td>
<td>2.1</td>
</tr>
<tr>
<td>After</td>
<td>9</td>
<td>64</td>
<td>7.1</td>
</tr>
</tbody>
</table>

The two isolates of var. sojae produced numerous gametangia, a small proportion of which were distorted, with the oogonium extended into a beak and, in extreme cases, into a long hypha-like projection. The nuclei in these projections underwent the same type of divisions as those in the central portion. In several cases an extra oosphere was produced in the projection.

Nuclear behaviour in the gametangia was typical for Oomycetes. The initial number of nuclei in the oogonia and antheridia was reduced by the abortion of some of the nuclei, while the remainder increased in size and entered an extended prophase during which pachytene, diplotene and diakinesis stages could be observed (Pl. 74, figs. 11–15). Metaphase followed and at this stage or at diakinesis chromosome counts could be attempted. Spindle fibres were observed at metaphase in gametangia of P206 stained with aceto-carmine (Pl. 74, fig. 16). Anaphase was rarely observed, partly because it is a short stage and partly perhaps because of the cold treatment which may tend to hold the nuclei at metaphase.

The first division was soon followed by a second division without a prolonged prophase and without any increase in nuclear size. This division resembled the divisions in the vegetative nuclei in that metaphase chromosomes were rarely observed.

The nuclei produced as a result of these divisions were approximately half the size of those in the vegetative hyphae. This supports the view that the vegetative nuclei are diploid and that the divisions in the gametangia are meiotic, resulting in the production of haploid nuclei.

**Numerical evidence for two divisions in the gametangia**

Counts were made of nuclei at prophase and metaphase (before division) and of periplasm and antheridial nuclei (after division) in P128 and P130. The results are given in Table 2. The average number of nuclei in each oogonium and antheridium after division is approximately four times
Table 3. Number of chromosome configurations per nucleus in
P. megasperma var. sojae P130

<table>
<thead>
<tr>
<th>No. of configurations per nucleus</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of nuclei observed</td>
<td>6</td>
<td>23</td>
<td>16</td>
<td>27</td>
<td>18</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

that of the average before division, indicating that two divisions of the
nuclei have taken place.

The number of periplasm nuclei expected would be four times the
original number in the oogonium less one (representing the oosphere
nucleus) but with the addition of some or all of the antheridial nuclei
not involved in fertilization. Clusters of nuclei are sometimes observed in
the periplasm adjoining empty antheridia, so it seems that the surplus
antheridial nuclei move into the periplasm.

In the antheridium, seven nuclei were often observed: presumably
the eighth nucleus had already fertilized the oosphere. The numerical
evidence therefore indicates that all or almost all of the nuclei in the
gametangia undergo two successive divisions.

**Chromosome numbers**

The chromosomes are extremely difficult to count because of their small
size and the difficulty of getting suitably flattened preparations. Two
factors increase this difficulty. First, there is a considerable difference in
size between the largest and smallest chromosomes, and the smallest
bivalents separate first. It is a problem to decide whether small bodies
are two small bivalents or two half-bivalents. Secondly, terminalization
is incomplete, so that certain configurations could be interpreted as two
overlapping bivalents or one bivalent with an interstitial chiasma. The
presence of interstitial chiasmata also makes it impossible to identify
multivalents except in very favourable positions.

However, the three isolates of the var. *megasperma* (Pl. 73, figs. 1–6)
had approximately twice as many chromosomes as the two var. *sojae*
isolates (Pl. 73, figs 7–10). The chromosome number for var. *megasperma*
was estimated to be between 22 and 30, the upper limit depending on
the assumption that some of the configurations were multivalents. It was
unexpectedly difficult to establish the chromosome number of var. *sojae*
although many metaphases were examined. It was therefore decided to
count the number of chromosome configurations without distinguishing
between bivalents, multivalents or univalents (Table 3). Efforts were made
to avoid counting early anaphases, but the counts showing 14 or 15
configurations could be those in which one or two bivalents had already
separated. The counts would seem to indicate a basic chromosome
number of between 10 and 15.

**Multivalents and chromosome bridges**

*P. cactorum* and *P. erythroseptica* (Sansome 1965, 1966), *P. infestans*
(Sansome & Brasier, 1973) and *P. cinnamomi* (Brasier, 1972) have been
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reported as having a haploid chromosome number of nine or ten. *P. megasperma* var. *sojae* may have a basic set of nine or ten chromosomes with one or two pairs of extra chromosomes partly homologous with the original set, a polysomic condition. The occasional observation of possible multivalents (Pl. 73, fig. 9 and Pl. 74, fig. 15) supports this view. If polysomy is present the different numbers of configurations at metaphase might not be due to difficulties in interpretation, but might indicate differences in pairing of the additional chromosomes.

In the strains P206 and P130 chromosome bridges were observed at first and second divisions which suggests the presence of inversions (Pl. 74, figs. 18, 20).

The ‘H’ body

In both isolates of var. *sojae* a large pale body, the ‘H’ (heterochromatic) body, was attached to one pair of chromosomes (Pl. 73, figs. 7, 8, 10). This could be the nucleolus which in other species has disappeared by this stage, or it could be heterochromatin. The ‘H’ body was observed in aceto-orcein preparations which were not overstained, and in suitable preparations was seen in all nuclei. ‘H’ bodies were not identified in var. *megasperma*, possibly because the preparations were usually stained with aceto-carmine.

DISCUSSION

Drechsler (1931) characterized *P. megasperma* as having oogonia with an average diameter of 47.4 µm and oospores of 41.4 µm and later Tompkins, Tucker & Gardner (1936) included in this species an isolate from cauliflower with smaller oospores, average 33 µm, redefining *P. megasperma* as having oospores greater than 30 µm. Hildebrand (1959), in a detailed investigation of a small-spored form from soybean described originally as *P. sojae* by Kaufman & Gerdemann (1958), concluded that this fungus was *P. megasperma*, but that its recognition as a variety (*P. megasperma* var. *sojae*) was justified. Waterhouse (1963) supported this view, citing the large- and small-spored forms as *P. megasperma* var. *megasperma* and *P. megasperma* var. *sojae* respectively. Nevertheless, the taxonomic status of the two forms has since been the subject of further discussion (Erwin, 1965; Savage et al. 1968; Waterhouse, 1970).

The present investigation shows that the two forms differ in chromosome number as well as in spore size and growth characteristics. This indicates that they should at least be accorded varietal status. Further work is needed to determine the evolutionary relationship between the two forms.

*P. megasperma* var. *sojae* appears to have more chromosomes than other species of *Phytophthora*, excluding var. *megasperma*. It was first thought that it might be triploid, judging by the variable number of configurations at metaphase and the extent of trivalent formation. However, most of the configurations appear to be normal bivalents, and later stages are more regular than one would expect in a triploid. Empty oogonia, due to failure of oosphere formation, are rare and Erwin & McCormick (1971) have reported up to 72% germination of oospores under favourable conditions. Also they found no variation in morphology or cultural growth among
twenty-three single oospore isolates. These observations suggest that it is unlikely that var. sojae is triploid.

The observation of meiosis in the gametangia of *P. megasperma* var. *sojae* and var. *megasperma*, as shown by the occurrence of two successive nuclear divisions leading to reduction in nuclear size, and by the occurrence of multiple associations and bridges, provides further cytological evidence that the Oomycetes are diploid (Sansome, 1963, 1965, 1966). Critical genetical evidence of diploidy has been provided by Shaw & Khaki (1971) in the heterothallic *P. drechsleri*, and by Elliot & MacIntyre (1973) in their elegant demonstration of mendelian inheritance in the homothallic *P. cactorum*.

The occurrence of diploidy and polyploidy rather than haploidy in the vegetative hyphae of *P. megasperma* has significance for the behaviour of the pathogen in the field, and for the interpretation of data for disease resistance and pathogenicity. The best-defined pathogenic races are to be found in *P. megasperma* var. *sojae* on soybean. Resistance of soybean varieties to races 1 and 2 of the pathogen appears to be controlled by single genes (Hartwig, Keeling & Edwards, 1968), and although little is known about the genetic control of pathogenicity in the fungus, a gene-for-gene system seems likely. If alleles for virulence in *P. megasperma* var. *sojae* are recessive to avirulence, as in rusts, then race expression will depend on the alleles being identical. This would enable the virulence alleles of the pathogen to be conserved in heterozygotes from which new races could arise by recombination and selection.

We are grateful to the Royal Society for financial support to E. Sansome. We also thank Dr D. C. Erwin for supplying a culture of *P. megasperma* var. *sojae*.

REFERENCES


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EXPLANATION OF PLATES 73 AND 74

All magnifications are approximate

PLATE 73

Metaphase I configurations in gametangia of P. megasperma var. megasperma. All × c. 6000

Fig. 1. P250, metaphase nucleus showing c. 25-30 bivalents (some out of focus).

Fig. 2. P206, antheridium with three metaphase nuclei.

Fig. 3. P128, metaphase nucleus in oogonium.

Fig. 4. P128, metaphase nucleus showing c. 25-30 bivalents (some out of focus).

Figs. 5, 6. P206, metaphase nuclei with multiple associations (arrowed).

Metaphase I configurations in gametangia of P. megasperma var. sojae

Fig. 7. P129, metaphase nucleus showing ‘H’ body (arrowed). x 6000.

Fig. 8. P129, metaphase nucleus showing ‘H’ body (arrowed) and c. 10-15 bivalents (some out of focus). x 6000.

Fig. 9. P130, metaphase nucleus with irregular configuration, possibly a trivalent (arrowed). x 6000.

Fig. 10. P130, oogonium and antheridium with metaphase nuclei showing ‘H’ bodies (arrowed). x 3500.

PLATE 74

Gametangial meiosis in P. megasperma

Fig. 11. P128, early prophase nucleus. Note nucleolus (arrowed). x 6000.

Fig. 12. P206, Pachytene nucleus. x 6000.

Fig. 13. P130, pachytene–diplotene nucleus with chiasma in bivalent (arrowed). x 6000.

Fig. 14. P128, Diplotene nucleus showing bivalent formation (arrowed). x 6000.

Fig. 15. P130, nucleus at diakinesis, with possible trivalent (arrowed). x 6000.

Fig. 16. P206, small oogonium with three metaphase nuclei in side view, showing spindles (arrowed). x 3500.

Fig. 17. P206, nucleus at anaphase I showing lagging chromosome (arrowed). x 6000.

Fig. 18. P130, telophase II nucleus with double bridge (arrowed), possibly due to irregular separation of a trivalent or of the ‘H’ body. x 6000.

Fig. 19. P130, prophase II nucleus showing nucleolus (arrowed). x 6000.

Fig. 20. P130, telophase II nuclei, one showing a bridge. x 6000.

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