

Cytofluorimetric evidence for meiosis in gametangial nuclei of *Phytophthora drechsleri*

BY A. MARTIN MORTIMER AND DAVID S. SHAW

School of Plant Biology, University College of North Wales, Bangor, Gwynedd, U.K.

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SUMMARY

Relative DNA contents of samples of nuclei from hyphal tips and from gametangia at different stages of development have been obtained by cytofluorimetry. The occurrence of nuclei with 4C, 2C and 1C DNA complements in gametangia and mainly 2C complements in hyphae indicates that meiosis takes place in the gametangia of *Phytophthora drechsleri*.

1. INTRODUCTION

Although both genetical and cytological studies with several *Phytophthora* species have provided evidence that these fungi are diploid (Sansome, 1965; Shaw & Khaki, 1971; Boccas, 1972; Elliott & MacIntyre, 1973), some doubt still remains about the nature of the divisions in the gametangia (Stephenson, Erwin & Leary, 1974*a, b*). We have used a cytofluorimetric technique (Ruch, 1970) to measure the relative DNA contents of nuclei at stages in the life-cycle of *Phytophthora drechsleri* to ascertain whether nuclear divisions in the developing gametangia are mitotic or meiotic.

2. MATERIALS AND METHODS

Compatible isolates 6500 and 6503 of *Phytophthora drechsleri* were mated (Khaki & Shaw, 1974) and material containing hyphal tips and oogonia and antheridia of different ages was selected for examination. Specimens were fixed in either Newcomer's fixative (Newcomer, 1953) or acetic-alcohol (1:3). After hydrolysis for 10 min in cold 6 N-HCl, the material was stained with the fluorochrome BAO (2,5-bis-3-4[4'-aminophenyl-(1')]-1,3,4-oxadiazole). Squash preparations were examined in transmitted light phase-contrast and incident light fluorescence from UV illumination using a Zeiss Photomicroscope. In some instances the material was treated with helicase (L'Industrie Biologique Française, Gennevilliers) to facilitate squashing of the gametangia. Quantitative measurements of the relative DNA content of individual nuclei were obtained from the intensity of their fluorescence. Counts were also made of nuclei from developing gametangia.

3. RESULTS

Somatic nuclei from hyphal tips had a mean DNA content of 23.9 (± 0.78) units. The variation in the data was quite large and the distribution showed a tendency towards positive skewness (Fig. 1). In developing oogonia less than 28 h (stage 1) the mean number of nuclei was 12.5 and two nuclei were consistently observed in the associated antheridia. After 36 h a greater number of nuclei were found in both gametangia, in the range of 20–47 in oogonia and 2–8 in antheridia (stage 2).

The DNA contents of nuclei at these two developmental stages are presented in Figs. 2 and 3. The nuclei in gametangia at stage 1 separated into two distinct groups according to DNA content, having means of 23.7 (± 0.82) and 46.7 (± 0.70) units respectively (Fig. 2). After subsequent development (at stage 2) gametangial nuclei had a mean DNA content of 13.6 (± 0.55) units, this distribution being positively skewed (Fig. 3).

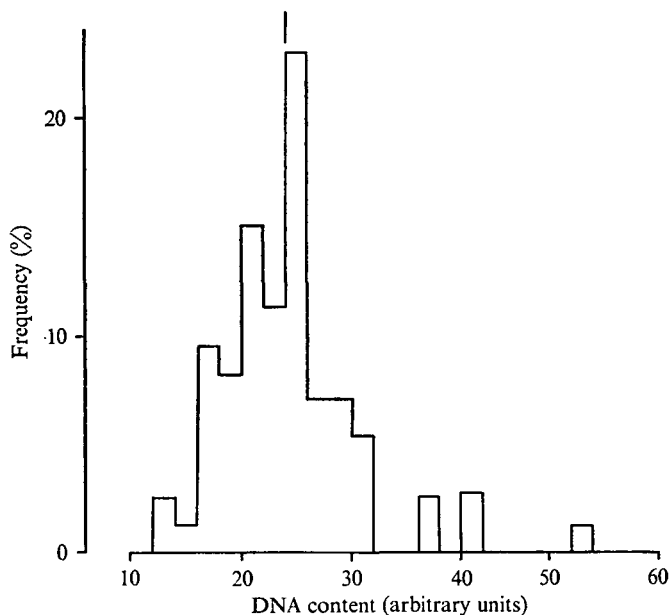


Fig. 1. The frequency distribution of DNA content in somatic nuclei from hyphal tips. Sample of 73 nuclei. The mean is indicated by a vertical line. Standard error = 0.78.

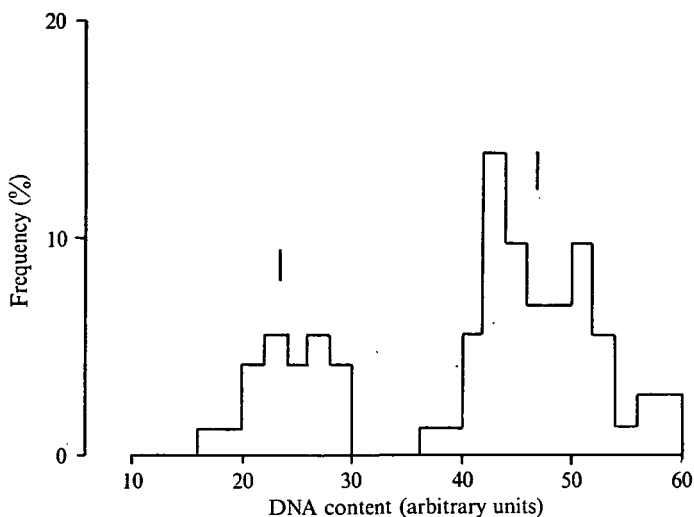


Fig. 2. The frequency distribution of DNA content in gametangial nuclei (Stage 1). Sample of 72 nuclei. The means of the two groups are indicated by vertical lines, standard errors are 0.82 and 0.70, left and right respectively.

4. DISCUSSION

If nuclear divisions in the gametangia are mitotic, the distributions of DNA content in both hyphae and gametangia may be expected to be broadly similar. Some nuclear DNA may be expected to be replicating. The positive skewness in the distribution of DNA content of somatic nuclei and the presence of some nuclei in the young gametangium (stage 1) with DNA contents in the range of 30–60 units are evidence for replication. The population of nuclei in the gametangia 10 h later with a mean DNA content of 13.6 units when compared with the mean somatic content (23.7 units) indicate, however, that a reduction in nuclear DNA has occurred. The reduction in DNA at this time and the large numbers of nuclei with this reduced DNA content in the gametangia and their absence from hyphae indicates that the gametangium is the site of meiosis and that these nuclei

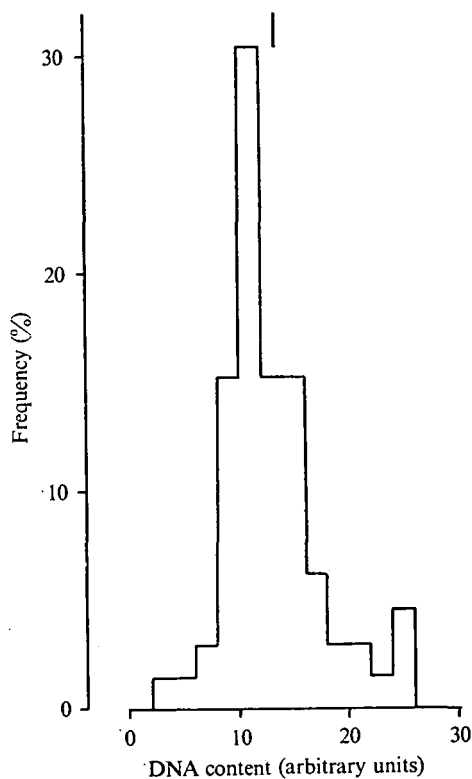


Fig. 3. The frequency distribution of DNA content in gametangial nuclei (stage 2). Sample of 66 nuclei. The mean is indicated by a vertical line. Standard error = 0.55.

are products of meiosis. In consequence hyphal nuclei will be diploid. This evidence is fully consistent with cytological data (Galindo & Zentmyer, 1967) and with genetical data (Khaki & Shaw, 1974) from matings of these same isolates of *P. drechsleri*; and with cytological evidence for other members of the Peronosporales including several *Phytophthora* and *Pythium* species, *Sclerospora graminicola*, *Bremia lactuca*, *Peronospora parasitica* and *Albugo candida* (Sansome, 1961, 1963, 1965, 1966; Sansome & Harris, 1962; Huguenin & Boccas, 1970; Tommerup, Ingram & Sargent, 1974; Sansome & Brasier, 1973; Sansome & Sansome, 1974).

Since the cytological evidence for gametangial meiosis in several members of the Saprolegniales (Barksdale, 1968; Dick & Win-Tin, 1973) is reinforced by microspectrophotometric data (Bryant & Howard, 1969; Howard & Bryant, 1971) and in one study by genetical findings (Lasure & Griffin, 1974) it is reasonable to suggest that the life-cycle of the Oomycetes is basically a diplontic one. The hyphal nuclei of these fungi may then carry recessive alleles which will be exposed to selection after sexual or parasexual recombination. The opportunities for the production of segregants during vegetative growth of clones must contribute to the success of *Phytophthora* spp. and particularly to those like the late blight fungus of potato, *P. infestans*, which frequently occur in the field as a single mating or compatibility type (Savage *et al.* 1968).

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