NUCLEAR DNA CONTENT AND CHROMOSOME NUMBERS THROUGHOUT THE LIFE CYCLE OF THE COLONIA STRAIN OF THE MYXOMYCETE, PHYSARUM POLYCEPHALUM

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SUMMARY

Nuclear DNA content and ploidy have been determined at different stages of the life cycle of the Colonia strain of the myxomycete Physarum polycephalum. Analyses at the plasmodial stage showed that (a) Burton and Feulgen DNA analyses agreed within 15% with strains which ranged from 0.6 to 3.6 pg of DNA per nucleus; (b) S-phase in Colonia is during the early part of interphase as in the Wisconsin strain; (c) in heterothallic and heterothallic x Colonia crossed strains there are 10-12 pg of DNA and 70 chromosomes per nucleus and in Colonia 0.6 pg of DNA and 40 chromosomes.

Germinating spores of all strains contained one population of cells with about 0.5 pg of DNA and 40 chromosomes and another of larger cells with up to 2.5 pg of DNA and 200 chromosomes. The polyploid nuclei comprised 2-20% of the total in heterothallic strains, 2-65% in heterothallic x Colonia crosses and 25-75% in Colonia.

A method was devised for making chromosome spreads of amoebae grown on bacterial lawns. Cells were first exposed to dilute formaldehyde at 26 °C for 30 min, then spread on slides with hot lactic acid and stained. Such spreads of CLd (Colonia) and RSD4 (heterothallic) amoebae both contained about 40 chromosomes.

The data are consistent with the view that Colonia is haploid throughout its life cycle and that chromosome number is neither halved during sporulation nor doubled during plasmodial formation. However, the possibility exists that an alternance of ploidy occurs by way of the few diploid nuclei present in the plasmodium.

INTRODUCTION

Although the myxomycete Physarum polycephalum has been in use for more than a decade for biochemical studies of the cell cycle and of differentiation (see Jockusch, 1975, for the most recent review), very few of the investigations have employed genetic analysis. The main reason for this is that only a few genetically marked strains are available, owing to the technical difficulties involved in isolating recessive mutants from the usual heterothallic strains, where the mutant amoeba has to be mated with one of another mating type (mt) if a plasmodium is to be produced (Wheels, 1970; Haugli & Dove, 1972). A major breakthrough in solving this problem was the discovery of the Colonia strain (Wheels, 1970) because in this strain plasmodia develop within amoebal clones and mutants can be isolated directly.

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as already demonstrated for several nutritional mutants (Dee, Wheals & Holt, 1973; Cooke & Dee, 1974).

In spite of the obvious usefulness of Colonia strain, however, there are still some aspects of the life cycle which need to be clarified before the genetics of the organism can be completely understood, and among them is the question of whether there are ploidy changes during formation of the plasmodium or during sporulation. Wheals (1976) found recombinants among the progeny of a cross of Colonia and heterothallic (mt) amoebae and concluded that the strain was homothallic (mating type h) and that two mt amoebae fused, underwent karyogamy and developed into a plasmodium in the same way as heterothallic strains. However, when Cooke & Dee (1974a) did Feulgen analyses of nuclei of Colonia and heterothallic amoebae and of Colonia and Colonia x heterothallic crossed plasmodia, they found the relative DNA contents to be in the ratio of 1:1:1:2. They interpreted these data as meaning that karyogamy is involved in the development of a plasmodium from mt and mt amoebae but not in formation of a plasmodium from mt amoebae alone. They suggested that instead the plasmodium must arise either by apogamy, with a single amoebal cell developing into a plasmodium by repeated nuclear division, or by fusion or coalescence of 2 identical amoebae to give a dikaryotic cell, which then develops into a plasmodium. A recent cinematographic study by the same laboratory group (Anderson, Cooke & Dee, 1976) has almost completely ruled out the latter possibility and supported the idea of apogamy.

There are, however, other more trivial explanations for Cooke & Dee’s DNA data. First, there could actually be a change in ploidy during sporulation in Colonia but involving only the few diploid nuclei which are present in plasmodia. They might be able to undergo a normal meiosis to give haploid spores (Laffler & Dove, 1975). Two other possibilities were proposed by Yemma & Therrien (1972) to account for very similar DNA results with selfing and heterothallic strains of the myxomycete, Didymium iridis. (1) The selfed plasmodium was actually diploid but had its S-phase late instead of early in the mitotic cycle, as in the heterothallic strains of D. iridis and P. polycephalum (Nygaard, Guttes & Rusch, 1960), so that DNA measurements made before late interphase were of diploid G1- and/or S-phase, not of haploid G2.

(2) DNA content per chromosome doubled immediately before spore cleavage so that the spore nucleus could go through an essentially normal meiosis.

In order to answer the question of whether Cooke & Dee’s DNA analysis actually reflected ploidy, chromosome counts and DNA analyses were done at different stages of the life cycle of Colonia and of crosses of Colonia with heterothallic amoebae. Data presented in this paper show that Colonia strain had no delay in S-phase in the plasmodium, no ‘diploidization’ during spore formation and no net change in ploidy during the life cycle.
MATERIALS AND METHODS

Culture strains and methods

Plasmodial strains were chosen to include examples of plasmodia arising from mtb amoebae and from matings of mtb with heterothallic amoebae. The mtb strains used were C50 (Wheals, 1970), CL, CLd, LU348 and LU640; mtb x mtb crosses were LU624 x CLd, LU667 x LU5001d, LU626 x E65; mtb x mtb was CH188 x LU860 and mtb x mtb was CH207 x LU862. Three mtb x mtb crosses - LU648 x LU688, LU648 x LU1, and RSD9 x RSD8 - were also included, as well as M4cVIII, a strain grown from sclerotia which were originally collected in the field (Daniel & Baldwin, 1964). Except for RSD9 x RSD8 (Haugli, 1971), LU1 and M4cVIII, which are Wis1 derivatives (Dee, 1973), all strains had a Colonia background. Mating type and other characteristics of CH188 and CH207 were given by P. N. Adler, Massachusetts Institute for Technology, in a personal communication to J. Dee. Mating and plasmodial fusion types and nutritional markers (where present) of LU640, LU647 and LU860 have been reported by Cooke (1974) and of other CL and LU strains by Cooke & Dee (1974b).

Methods for maintaining inoculum stocks, growing plasmodia and for inducing sporulation were as described previously (Mohberg, Babcock, Haugli & Rusch, 1973). To avoid effects of ageing (McCullough et al. 1973) stock cultures were never carried as plasmodia on agar plates and all strains were either analysed within 3 or 4 weeks of mating or were stored as spherules. Genetic markers, such as the temperature sensitivity in E65 (Gingold, Grant, Wheals & Wren, submitted for publication) and the lysine requirement in LU348 should not have been expressed, since all cultures were grown at 26 °C in semi-defined medium (Daniel & Baldwin, 1964).

Amoebal strains used included two mtb strains with delayed plasmodial formation - CLd and C50 - and three mtb strains - LU648, a (Dee, 1966) and RSD4 (Haugli & Dove, 1972). RSD4 was grown axenically in protein-free medium (McCullough & Dee, 1976) and the others on Escherichia coli lawns on liver infusion agar (Dee & Poulter, 1970). Agar plate cultures to be used for DNA analysis were started with 5 x 10^6 cells per plate and were harvested at 2 to 3 x 10^6 per plate to ensure that all cells were actively growing.

Spore viability tests were done as follows: after plasmodia had fruited, dishes were kept at room temperature for 7-14 days. Sporangia were then harvested and broken in a Potter-Elvehjem homogenizer to release spores, which were washed 2-4 times with distilled water, counted in a haemacytometer and plated with E. coli. Plaques were counted after 4 or 5 days. Spores for DNA analysis and chromosome counts (see below) were washed in the same way and then germinated by incubation in water (1 to 2 x 10^8 spores in 5 to 8 ml in a 9-cm Pyrex Petri dish at 26 °C).

Isolation of nuclei

Plasmodial nuclei were isolated at metaphase for chromosome spreads and at M + 3-5 h for DNA analyses, according to methods already described (Mohberg & Rusch, 1971). Amoeba nuclei were isolated, using the same medium and blending time as for plasmodia, i.e. 0-1 % Triton X-100 and 15-30 s, instead of the 0-4 % and 1 min originally specified. Germinating spores were harvested after 4-5-5 h of incubation, by which time most were flagellated, and were handled in the same way as amoebae, except that the homogenate was centrifuged in 50-ml round-bottomed tubes for 5 min at 30 g to remove the bulk of the spore walls before it was passed through milk filter. Centrifugation through 1 M sucrose solution was necessary only with the plasmodial metaphase nuclei, which otherwise clumped so that chromosomes did not spread well.

Preparation of chromosome spreads

Plasmodial chromosome spreads were made by heating metaphase nuclei in lactic acid and staining with aceto-orcein (LaCour, 1941) as described by Mohberg et al. (1973). The procedure for making spreads of spore chromosomes was as follows. Washed spores were
incubated in water for 75 min before sampling was begun. Then, at 15- or 20-min intervals for the next 2 h an 0·8-ml aliquot was transferred to a 50-ml conical tube, where it was made to 10 ml with water and mixed with 0·5 ml of 37% commercial formalin. After 30 min at room temperature, the suspension was diluted with 40 ml of ice-cold nuclear isolation medium (NIM) and set aside in cracked ice until the entire 6 or 8 samples had been harvested. Tubes were then centrifuged at 1000 g for 10 min and washed twice with 40 ml of NIM (to prevent formation of a brown background residue with aceto-orcein.) Pellets were finally suspended in about 0·2 ml of NIM by vortexing for 1 min. Two drops were mixed on a slide with 2 drops of 66% (v/v) lactic acid and heated over a small flame to spread chromosomes. Chromosomes were stained with aceto-orcein or were fixed in 3:1 ethanol-acetic acid and stained with Giemsa's (10 min in a 1:10 dilution of stock Giemsa's in 6·7 mM phosphate buffer at pH 6·8). Although metaphase was usually found in the 90- to 120-min samples, in different experiments with the same dish of spores it might occur as early as 75 and as late as 180 min after plating. Amoebae (early to mid log phase) were washed twice with ice water, then were treated with formalin, spread and stained in the same way as the germinating spores.

Analytical methods

DNA analyses were done on isolated nuclei either by the Burton (Burton, 1956) or by the Feulgen method, using the procedure of Darlington & LaCour (1962), as modified by Cooke & Dee (1974). Chicken erythrocyte nuclei were used as an internal standard (Rasch, Barr & Rasch, 1971). The nuclei were isolated by lysing oxalated chicken blood in 0·1% saponin in 1% saline and centrifuging at 1000 g for 10 min to pellet nuclei. The pellet was washed once or twice with NIM, suspended in NIM, parcelled out in amounts sufficient for 20-30 slides and stored frozen until needed. DNA content of *Physarum* nuclei was calculated on the assumption that erythrocyte nuclei contained 2·45 pg of DNA, a rough mean of the values given by Rasch et al. (1971).

RESULTS

Relation of DNA content to chromosome number in plasmodia

Since data on DNA content of *Physarum* nuclei have been expressed both in arbitrary Feulgen units (Bovey & Ruch, 1972; Cooke & Dee, 1974; Adler & Holt, 1974; Laane & Haugli, 1976) and in pg DNA by Burton analysis (Mohberg et al. 1973), a series of plasmodial strains, ranging from 0·6 to 3·6 pg DNA per nucleus, were analysed by both methods to establish a basis of comparison. The 2 DNA assays (Table 1) were in reasonable agreement throughout the range, although the Feulgen values usually were about 15% lower. Chromosome counts were also in rough agreement, for most strains with 0·6 pg of DNA per nucleus had 35-40 chromosomes and strains with 1·0-1·2 pg had about 70 chromosomes, giving an average DNA:chromosome ratio of 0·015. Ratios for C50 and RSD5 x RSD2 were considerably higher, presumably because both strains contained a large proportion of polyploids and they tend to give low chromosome counts because they are hard to spread completely.

As expected from the findings of Cooke & Dee (1974a), the *mt1 x mt h* strain LU624 x CLd, and the *mt1 x mt k* strain with a Colonia background, LU648 x LU688, both had about the same nuclear DNA content as the Wis 1 heterothallics, M2cVIII and RSD5 x RSD8, and almost twice the DNA content of the *mt h* strains—CL, LU640 and B11.

*S*-phase was located in the mitotic cycle of the CL plasmodium by Feulgen analysis.
DNA content and ploidy in Colonia

At early prophase, approximately 15 min before metaphase, there was 0.63 pg of DNA per nucleus; at reconstruction, just after separation of the daughter nuclei, 0.29 pg; at M + 3 h, 0.57 pg and at M + 5 h, 0.65 pg. A Polkinhorne (unpublished data) did a similar experiment with C50 and found that there also DNA doubling was 90% completed during the first 4 h after mitosis.

Table 1. DNA content and chromosome numbers in Colonia, Colonia x heterothallic crosses and heterothallic strains of plasmodia

<table>
<thead>
<tr>
<th>Strain</th>
<th>DNA per nucleus, pg</th>
<th>Mean chromosome number†</th>
<th>DNA per chromosome, pg††</th>
</tr>
</thead>
<tbody>
<tr>
<td>M3cVIII</td>
<td>1.23 ± 0.08 (4)</td>
<td>1.01</td>
<td>0.0148</td>
</tr>
<tr>
<td>RSD9 x RSD8</td>
<td>1.04 ± 0.07 (6)</td>
<td>1.14</td>
<td>0.0158</td>
</tr>
<tr>
<td>C50</td>
<td>0.88 ± 0.03 (5)</td>
<td>0.72</td>
<td>0.0206</td>
</tr>
<tr>
<td>RSD5 x RSD2</td>
<td>3.64 ± 0.10 (6)</td>
<td>3.30</td>
<td>0.0208</td>
</tr>
<tr>
<td>CL</td>
<td>0.71 ± 0.02 (4)</td>
<td>0.62</td>
<td>0.0159</td>
</tr>
<tr>
<td>LU640</td>
<td>0.68 ± 0.01 (2)</td>
<td>0.63</td>
<td>0.0173</td>
</tr>
<tr>
<td>BR</td>
<td>0.73 ± 0.02 (5)</td>
<td>not done</td>
<td>—</td>
</tr>
<tr>
<td>LU624 x CLd</td>
<td>1.19 ± 0.03 (6)</td>
<td>1.02</td>
<td>0.0140</td>
</tr>
<tr>
<td>LU648 x LU1</td>
<td>1.15 ± 0.05 (4)</td>
<td>1.01</td>
<td>0.0144</td>
</tr>
<tr>
<td>LU648 x LU688</td>
<td>not done</td>
<td>1.09</td>
<td>0.0159</td>
</tr>
</tbody>
</table>

DNA analyses and chromosome counts were made as described in Materials and methods. Burton analyses of the first 4 strains are from Mohberg et al. (1973).

• DNA content in pg per nucleus ± standard error of the mean; no. of samples analysed in parentheses.

•• Fifty Physarum nuclei and 10 or 20 chicken erythrocyte nuclei were measured for each sample. Data are given as mean DNA content, including polyploids.

† Mean chromosome number, including polyploids.

DNA content and chromosome numbers in growing amoebae

Since the results of the preceding experiment indicated that the Feulgen analysis could be used with nuclei having as little as 0.3 pg of DNA, and since this method requires much less starting material than the Burton procedure and is less sensitive to interference from bacteria, whole spores and agar (Mohberg & Rusch, 1971), it was used for all analyses of amoebae and germinating spores done in this study. Mid log phase CLd and C50 amoebae contained 0.6 pg of DNA; LU648, 0.46 pg; strain a, 0.41 pg, and RSD4, 0.34 pg. CLd and C50, like bacterially grown RSD4 and RSD5 (Mohberg & Rusch, 1971), appear to be predominantly in haploid G2-phase; whereas the other strains, particularly the axenically grown RSD4, are chiefly in S-phase.

Countable chromosome spreads were rarely found among exponentially growing amoebae, not only because metaphase, as in the plasmodium, occupies only about 5 min of the 8-12 h generation time; but also because the few spreads present were usually obscured by mitochondria and partially digested bacteria (Fig. 10, f). When amoebae were pretreated with formalin (see Materials and methods), the problem with ingested bacteria was reduced, apparently by causing cells to eject most of their
DNA content and ploidy in Colonia

cytoplasmic inclusions and spreads like those in Fig. 1E, G could be found. Counts of 8 such spreads of CLd gave a mean chromosome number of 38, with a standard deviation of ± 5.4 and a range of 27–48. RSD4 had a mean of 42 chromosomes with a standard deviation of ± 4.0 and a range of 35–49 in 12 spreads.

In order to make it possible to count a more meaningful number of chromosome spreads, several agents were tested for their ability to block mitosis in amoebae growing on bacterial lawns on agar. However, none were successful. Griseofulvin, which blocks mitosis in plasmodia (Gull & Trinci, 1974) and the herbicide, isopropyl 3-chlorophenyl carbamate (CIPC), at levels of 50–100 μg per ml were inhibitory but did not arrest cells in metaphase. Colchicine (0.16%), actinomycin C (15 μg per ml) and vinblastine sulphate (50 μg per ml) did not affect growth.

DNA content and chromosome numbers in germinating spores

Since a metaphase blocker for amoebae could not be found, germinating spores, which undergo a synchronous nuclear division shortly after hatching (Howard, 1931), were tested as a substitute for a synchronous amoeba culture. This was successful with mt × mt and mt × mt_h crosses, where spore germination was between 10% (RSD9 × RSD8) and 70% (LU647 × LU501d, CH207 × LU640 and CH188 × LU640). However, in mt_h strains, where hatching was between 0.2% in CL (even after 3 passages through the life cycle) and 2% in C50 and LU348, it was not possible to find enough chromosome spreads for reliable histograms and the proportion of nuclei of different ploidy levels had to be estimated from Feulgen DNA data.

Typical chromosome spreads of germinating spores are shown in Fig. 2. Chromosomes tended to be shorter and thicker than in the plasmodium and were more frequently split so that individual chromatids were visible (Fig. 2F, G), but this may have been caused by the formalin treatment, rather than a real difference in chromosome structure. Spreads in Fig. 2C through F contain from 42 to 50 chromosomes and are apparently in the ‘normal’ range for both mt and mt_h strains. In addition, highly polyploid nuclei (Fig. 2G) were found among spores of all strains, with the proportion being lower in the heterothallic than in the mt_h strains, as below.

The mt × mt_h cross, LU648 × LU688, contained 1.1 pg of DNA and about 70 chromosomes in the plasmodial nucleus (Table 1) and 0.5 pg of DNA (same as in the growing LU648 amoeba) and between 40 and 45 chromosomes in the bulk of the germinating spore nuclei (Fig. 3). The remainder of the spores seemed to be

Fig. 1. A, B, chromosome spreads of typical Colonia and Colonia × heterothallic crossed plasmodia. A is from CL, B from LU647 × 501d. Spreads were photographed through a phase-contrast microscope at 1500×. c–g, spreads of amoebae after treatment with dilute formalin and hot lactic acid. c, CLd swarm cells, showing flagella, flagellar apparatus and nuclei; in the cytoplasm are mitochondria and granules which are presumably remnants of food vacuoles. d, ‘spread’ of partially digested bacteria from a myxamoeba; structure at lower left is an unspread nucleus. e, RSD4 amoeba chromosomes. f, telophase in CL amoeba, showing chromosomes and mitochondria (at arrows). g, CLd amoeba chromosomes. c–e were stained with Giemsa, all others on the plate with aceto-orcein. Photography and magnification are given for A and B.
Fig. 2. Chromosome spreads in germinating spores. A, partially spread metaphase in a C50 spore; mitochondrion at arrow. B, telophase in a C50 spore. C–F, chromosome spreads of spores of C50 (C), RSD9 x RSD8 (D), and LU648 x LU688 (E, F). G, polyploid spread of LU648 x LU688 spore with approximately 150 chromosomes. Staining of D was with Giemsa and of others with aceto-orcein. Photography as in Fig. 1. Magnification of A–E same as for G.
DNA content and ploidy in Colonia

Diploid or triploid. Mean DNA content of the smaller nuclei was 0.51 pg at 1.25 h, 0.56 pg at 2.25 h (just prior to nuclear division in this experiment), and 0.53 pg at 4.75 h after plating of the spores in water. This indicates that hatching amoebae are predominantly in haploid G2-phase, and that there is an S-phase shortly after nuclear division so that cells are again in G2-phase within a few hours after dividing. Germinating spores of RSD9 x RSD8 and M3cVIII gave DNA and chromosome histograms (not shown) like those of LU648 x LU688, except that in the former there were about 20% polyploids and in the latter only 2%.

Fig. 3. Feulgen DNA analyses and chromosome counts in germinating spores (A, B) and plasmodium (C, D) of the mt1 x mt2 strain, LU648 x LU688. DNA readings were made on 50 nuclei of each sample and DNA content calculated by means of the chicken erythrocyte nuclei included as internal standards. (See Materials and methods.) Data were then grouped in intervals of 0.05 pg and plotted against frequency in percent. Chromosome histograms were made from counts of 22 spreads of germinating spores and 42 of plasmodial nuclei. In this and later histograms data for spores are in the upper panels and for plasmodia in the lower.

In both of the mt1 x mt2 crosses, LU647 x LU5001d and LU860 x E65, the plasmodial DNA profiles resembled those of the mt1 x mt2 strains and had a single main peak at about 1 pg of DNA (Fig. 4). Spores, however, had major peaks at 0.55 and 0.8 pg. Counts of 26 chromosome spreads of LU647 x LU5001d spores showed that 35% of the nuclei were in a peak with a mean of 48 chromosomes; 35% were in a second peak at 75 chromosomes, and the remaining spreads ranged from 90 to 250 chromosomes. Mating mt1 with either mt3 (CH188) or mt4 (CH207) gave plasmodial DNA profiles like LU648 x LU688 but in spores of CH188 x LU640.
In strains arising from \( mt_b \) amoebae only – LU640 (Fig. 4), C50 (Fig. 5), LU348 and CL (last two not shown) – 90% or more of the plasmodial nuclei had a DNA content of 0.6 pg and 2–10% had a content of 1.0–1.2 pg. However, in the germinating spores only 25–50% of the nuclei were in the 0.5 pg peak. The remainder contained 1.5–2 times as much DNA. Chromosome counts of C50 (Fig. 5) and 640 (not shown) showed that both diploid and polyploid nuclei were present.

**Fig. 4.** Feulgen DNA analyses of matings of \( mt_b \) with heterothallic amoebae. Strains used were the \( mt_b \) strain, LU640, and crosses of \( mt_b \) with \( mt_u \) LU647 x LUsooid, and with \( mt_t \), CH207 x LU640. A–C, spores: A, LU647 x LUsooid (\( mt_u \)); B, CH207 x LU640 (\( mt_t \)); C, LU640 (\( mt_t \)). D–F, the corresponding plasmodia. Data were obtained and plotted as for Fig. 3.

**DISCUSSION**

By combining the data of this and several earlier papers (Arescaldino, 1971; Mohberg & Rusch, 1971; Mohberg et al. 1973; Laane & Haugli, 1976) it is possible to deduce the DNA content and times of synthesis throughout almost the entire life cycle of the heterothallic, Wis 1-derived strains of *P. polycephalum*. The \( G_2 \)-phase DNA content is 1.0–1.2 pg per nucleus (4C) in the growing, starving and sporulating plasmodium, and it remains at this level until the first meiotic division, where it drops to half (2C), and the second meiotic division, where it is halved again, giving
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Fig. 5. Feulgen DNA analyses and chromosome counts in germinating spores (A, B) and plasmodium (C, D) of the mt${}_5$ strain, C50. Data were obtained and plotted as for Fig. 3. Chromosome histograms were based on 15 spreads of germinating spores and 50 of plasmodial nuclei.

1C. At some time between meiosis II and germination, DNA is replicated, since the hatching spore contains 0.5 pg of DNA, close to the 2C amount.

The change in ploidy indicated by the DNA data is supported by chromosome counts, which show that such mt${}_1 \times mt{}^2$ crosses as LU648 × LU688 and RSD9 × RSD8 have approximately 70 chromosomes per plasmoidal nucleus and 40 in the germinating spore. This corresponds to a haploid number of 40 instead of the 25 proposed earlier (Mohberg et al. 1973). The earlier counts were almost certainly too low because spores were not treated with formalin before spreading and the unstabilized chromosomes were destroyed during heating in lactic acid prior to staining.

Several M$_{3c}$ derivatives (Mohberg et al. 1973) contained nuclei with 50–55 chromosomes, and what relation they have to the haploid or diploid number is unknown. However, on several occasions M$_{3c}$ cultures have been observed to drop to 40 chromosomes per nucleus after continuous submersed culture for long periods of time (a year or more). Either a population of microplasmodia with small nuclei slowly outgrows those with diploid nuclei, or diploid nuclei lose chromosomes until they are haploid and the nuclei with 50–55 chromosomes are an intermediate in the transition. We are trying to work out a method for doing at least a partial karyotype so that we can answer this question and also determine whether the range which we see in chromosome counts within individual strains is owing to aneuploidy or merely to poor spreading technique.
We have not established whether the polyploid cells among germinating spores can develop into amoebae colonies and finally into plasmodia. Conceivably these cells have hatched before meiosis is completed and later drop to a normal chromosome number, as can occur in another species of myxomycete, *Fuligo septica* (Cathcart & Aldrich, 1972). They may also not be viable, beyond being able to germinate, or they may grow so slowly that they are diluted out by normal amoebae and lost from the population. However, if they do survive, the ploidy of plasmodia from uncloned heterothallic amoebae probably is even more heterogenous than already thought, since a diploid (2n) amoeba might produce three different kinds of plasmoidal nuclei when mixed with a normal (1n) amoeba – 2n by mating, 2n by selfing and 1n by inducing the other amoeba to self (Therrien & Collins, 1976). On the other hand, it should be possible to take advantage of the polyploid amoebae to construct plasmodial strains with a wider range of ploidy than found among the various RSD crosses (Mohberg et al. 1973) and hopefully also to select strains which spherulate better than the RSD strains, many of which have been lost because of poor spherule viability.

Strains from *mt*₁, *mt*₃ and *mt₄* × *mt₅* matings all resembled heterothallic strains in that plasmoidal nuclei had approximately twice the DNA content and chromosome number as spores and amoebae, and differed principally in that polyploid amoebae were more numerous among germinating spores. In plasmodia arising solely from *mt*₁ amoebae, however, there were only 0.6 pg of DNA and about 40 chromosomes per nucleus and this did not change throughout starvation and sporulation up to the time of the precleavage mitosis (A. Polkinhorne, unpublished data; Mohberg et al. 1973). In germinating spores the average nuclear DNA content was actually higher than in the parent plasmodium, for in all 4 of the strains examined, at least 25% of the nuclei had the diploid DNA content and chromosome number. (Whether the germinating spores were also bi- and multinucleate, as reported for CL by Laane, Haugli & Mellem (manuscript submitted) is not known, since our DNA analyses were done on isolated nuclei and not whole cells.)

The data presented in this paper are in accord with Cooke & Dee’s (1974a, b) proposals: that there is no change in ploidy during the life cycle of Colonia; and that fusion of amoebae and karyogamy occur when a plasmodium arises from the mating of heterothallic amoebae with other heterothallics or with *mt₅* amoebae, but not when the plasmodium develops from *mt₅* amoebae alone. Since synaptonemal complexes have been found in CL spores by Laane et al. (submitted for publication) and in C₃₀ spores by I. Arescaldino-LaCorre (personal communication), it seems definite that meiosis at least begins. von Stosch, van Zul-Pischinger & Dersch, 1964, claimed that the meiosis was abortive, but the electron-microscope studies of Laane and co-workers suggest that the first division is completed and the second suppressed, so that recombination occurs without reduction in chromosome number.

As with the heterothallic strains, we know very little of either the origin or fate of the polyploid cells which hatch from Colonia spores, and here they are particularly worrisome, both because of their relative abundance and because of the complications they could cause in genetic experiments. It seems unlikely for two reasons that
diploid spores are the source, at least not the sole source, of the diploid nuclei found in Colonia plasmodia. First, we have found that uncloned CL and C50 spores with 50–75% polyploid nuclei gave plasmodia with 10% or less diploid and polyploid nuclei. Secondly, plasmodia of all Colonia strains examined thus far have contained at least 2% diploid nuclei, in spite of the fact that they were grown from cloned amoebae. It would seem rather that diploid plasmodial nuclei are produced from 1n nuclei by some event too rare to have been detected in the cinematographic study of Anderson et al. (1976), such as endomitosis or fusion of nuclei, either in the parental amoebal plaque or in the new plasmodium. Laffler & Dove (1975) have suggested that the 2n nuclei in the plasmodium go through a normal meiosis to give viable haploid spores. Although E/M observations (Laane et al. submitted for publication) indicate a pseudomeiosis with a single division, rather than a normal diploid meiosis with two divisions, it will probably not be possible to settle this question until the 2n nuclei can be traced through the entire life cycle.

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