Plasmodium formation without change in nuclear DNA content in *Physarum polycephalum*

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SUMMARY

The Colonia isolate of *Physarum polycephalum* produces plasmodia within amoebal clones. Wheals demonstrated genetically that amoebae of the C50 strain of this isolate, when crossed with heterothallic amoebae, yielded recombinant progeny. He concluded that nuclear fusion and meiosis occurred in these crosses and suggested that nuclear fusion was also involved in plasmodium formation in clones. He thus designated the strain 'homothallic'.

In the present work genetic evidence is presented which indicates that the Colonia strain CL, when crossed with heterothallic strains, also yields recombinant progeny and thus undergoes nuclear fusion and meiosis. Microdensitometric measurements of nuclear DNA content are reported which indicate that CL amoebae are haploid like heterothallic amoebae, and crossed plasmodia are diploid. However, clonally formed CL plasmodia were found to have the same G2 nuclear DNA content as CL amoebae. This observation excludes the possibility of nuclear fusion when plasmodia form within clones of CL amoebae and therefore the strain cannot be homothallic. Two alternatives, apogamy and coalescence, are proposed as the most likely mechanisms for clonal plasmodium formation in strain CL.

1. INTRODUCTION

The Colonia isolate of *Physarum polycephalum* differs from heterothallic isolates in being able to complete the life-cycle within single clones. Uninucleate amoebae give rise to a macroscopic multinucleate plasmodium which produces spores under appropriate conditions. The spores hatch to release uninucleate amoebae capable of repeating the life-cycle.

The Colonia isolate was originally described by Von Stosch, Van Zul-Pischinger & Dersch (1964), who suggested that the strain was 'homothallic' but did not give detailed evidence. Wheals (1970) genetically analysed the progeny of crosses between a Colonia derivative strain (C50) and heterothallic strains and showed that the ability of C50 amoebae to form plasmodia within clones was determined by a single allele at the mating-type locus (mt) which he designated mtb. His genetic results indicated that both nuclear fusion and meiosis occurred in such crosses. Wheals therefore suggested that within clones of Colonia amoebae, plasmodial development also involved nuclear fusion, i.e. that the strain was homothallic. If this is so, nuclei of Colonia plasmodia would be expected to have
twice the ploidy of the amoebae. The aim of the present work was to test this prediction by comparative measurements of nuclear DNA content of amoebae and plasmodia derived from the Colonia strain CL and representative heterothallic strains.

The average nuclear DNA content at different stages in the life-cycle of heterothallic strains of *P. polycephalum* has been measured by Mohberg & Rusch (1971), who concluded that in these strains, amoebae were haploid and plasmodia diploid. Therrien (1966), on the basis of microdensitometric measurements of nuclear DNA content in *Didymium nigripes*, concluded that his strain was homothallic, haploid amoebae fusing to produce diploid zygotes and plasmodia. Kerr (1968) discussed Therrien's results and questioned his conclusions. She reported chromosome counts on strains of *D. nigripes* and discussed time-lapse cinematographic studies by N. Kerr (1967). S. Kerr (1968) concluded that there was no ploidy level difference between amoebae and plasmodia and that nuclear and cytoplasmic fusion were not necessary for plasmodia formation.

2. MATERIALS AND METHODS

(i) *Strains.* The heterothallic amoebal strains a and i have been previously described (Dee, 1966). Strains CL (Colonia Leicester) and CLd (CL delayed plasmodia formation) were derived by successive cloning from C50 (Wheals, 1970) and their isolation will be described elsewhere (Cooke, manuscript in preparation). Under appropriate conditions CL amoebae form plasmodia in 100% of amoebal plaques and when suitably subcultured such plasmodia are able to complete the life-cycle. CLd amoebae also produce plasmodia within individual clones but do so only after a characteristic delay (7-10 days). LU523 is a heterothallic (*mt*) strain derived by backcrossing (to CLd) a *mt* progeny clone from the cross *a* x CLd.

(ii) *Loci.* mt: mating type. Alleles *mt* x *mt*: heterothallism (Dee, 1966); *mt*: plasmodia formation within amoebal clones ('homothallism'; Wheals, 1970).

F and n: plasmodial fusion type (Poulter & Dee, 1968; Poulter, 1969). Identity at both F and n loci is a prerequisite for plasmodial fusion.

sax: sensitivity to axenic medium (Poulter, 1969; Wheals, 1973). Plasmodia homozygous for sax fail to grow when subcultured to axenic medium.

(iii) *Genotypes of amoebal strains.* a: mt1; f1; n1; sax-. i: mt2; f2; n2; sax+. CL (and CLd): mt*; f2; n1; sax+. LU523: mt1; f1; n1; sax+.

(iv) *Cultural conditions.* Amoebae were maintained in two-membered culture with *Escherichia coli* at 26 °C on liver infusion agar (LIA) containing 1 g Oxoid liver infusion agar powder per litre of 2% agar. CL amoebae may be maintained on LIA plates without plasmodia formation in plaques by (a) regular subculturing every 3-4 days or (b) transferring to 4 °C after 4 days incubation. All plasmodia were routinely cultured at 26 °C on a semidefined agar medium (SDM) (Dee & Poulter, 1970). Production of spores, spore plating and isolation of progeny clones were carried out by methods previously described (Wheals, 1970).

(v) *Plasmodium formation.* Plasmodium formation by amoebae derived from a
single clone was achieved by inoculating a dilute (6%) SDMagar plate with approximately 10³ amoebae together with a drop of bacterial suspension, allowing the drop to absorb and incubating for 3–5 days. The same procedure was adopted for crossing; the drop of bacterial suspension being inoculated with amoebae of different mating types. The resulting plasmodia were subcultured to SDM agar containing 250 μg/ml streptomycin (SDMS) to kill any remaining bacteria.

(vi) **Plasmodial fusion tests.** Methods have been fully described by Poulter & Dee (1968).

(vii) **Nuclear isolation.** A modified version of methods previously described by Mohberg & Rusch (1971) was used for isolation of plasmodial nuclei. Plasmodia were harvested in G₂ phase, the time in the mitotic cycle being determined by phase-contrast microscopic observation of glycerol/ethanol fixed smears (Mittermayer, Braun & Rusch, 1965). Five plasmodia 6–7 cm in diameter were carefully scraped from SDM agar into 200 ml of ice-cold medium A: 250 mM sucrose, 20 mM Tris (pH 7.2 with HCl), 20 mM-MgCl₂, 10 mM Mercaptoethanol, 0.1% (w/v) Triton X-100. The suspension was homogenized in an MSE Ato-Mix blender coupled to a Berco variable resistance transformer. Crossed plasmodia were homogenized at a nominal 50% of mains voltage (240 V) half speed for 15 sec and set at full speed for 30 sec. To obtain clean preparations from clonally formed plasmodia however, it was found necessary to homogenize at full speed for 1 min. After standing in ice for 5 min to allow froth to settle the homogenate was filtered through a cotton-wool milk filter pad sandwiched between two 7.5 in. fabric milk filters (Grant & Poulter, 1973). The filtered homogenate was decanted into four 50 ml conical glass tubes and centrifuged for 10 min (0 °C) at 2000 rev/min in a Sorval HS-4 rotor. The pellet was resuspended in 100 ml of medium B (as medium A but omitting Triton X-100), and centrifuged as described above. The final pellet was resuspended on 0.3 ml medium B, frozen in liquid N₂ and stored at −20 °C.

To ensure that the amoebae used for nuclear isolation were in logarithmic phase (therefore mostly in G₂) and not encysted (G₁) (Mohberg & Rusch, 1971), LIA plates were inoculated with approximately 50 amoebae per plate and 0.1 ml bacterial suspension. When large plaques had developed (after 4–5 days incubation) a further 0.2 ml of bacterial suspension was added, the plates respread and incubated for 2 days. Harvesting these plates by flooding with 5 ml ice-cold water and gentle scraping with a glass spreader yielded approximately 5 × 10⁶ amoebae/plate with no visible cysts.

Amoebal suspensions, essentially freed of bacteria by repeated washing in ice-cold water (Mohberg & Rusch, 1971), were then subjected to the nuclear isolation procedure outlined above. Homogenization was at full speed for 1 min and centrifugation at 2200 rev/min. Phase-contrast microscopic observation of the final suspension showed a preparation which was not distinguishable from isolated plasodial nuclei. However, after Feulgen staining (see below) it became apparent that the amoebal ‘nuclear’ preparations consisted of whole amoebae with only the nucleus being stained. Stain intensity in the cytoplasm was determined and found to be negligible. The cause of the apparently identical appearance of whole
amoebae and isolated plasmodial nuclei was found to be the presence of Triton in medium A. Amoebal cells simply suspended in medium A (or 0.1% Triton alone) were found to round off immediately and assumed the characteristic appearance of isolated plasmodial nuclei and this was not reversed when the cells were transferred to medium B.

(viii) Estimation of nuclear DNA content. Preparations were rapidly thawed and a drop of each air-dried on a slide before being fixed in acetic alcohol for 1 h. Specimens were stained by the Feulgen method (Darlington & La Cour, 1962) with the modification that hydrolysis was by 5 M-HCl at room temperature for 45 min (Itakawa & Ogura, 1954). Stain intensity was measured with a Vickers M 85 Scanning Microdensitometer (Vickers Instruments Ltd). Fifty nuclei of each preparation were each scanned once using the following settings: slit width 20, wavelength 55 and spot size 2.

3. RESULTS

(i) Identification of crossed plasmodia from heterothallic × mtₜ matings

In a cross involving heterothallic and mtₜ amoebae two types of plasmodia are possible, namely those arising directly from mtₜ amoebae and those resulting from crossing between the heterothallic and mtₜ amoebae. To identify the two classes of plasmodia formed in this type of cross the plasmodial fusion system was used (Poulter & Dee, 1968). Thus a plasmodium which resulted from a cross between amoebae of the genotypes mt₁f₁n₁ (a) and mtₚfₚnₚ (CLd) would have the genotype f₁f₂n₁n₁ (fusion group III) whereas plasmodia formed in clones of CLd (or CL) amoebae fuse with tester plasmodia of fusion group V (f₂f₃n₂n₁). Therefore of the plasmodia forming on a cross plate, those arising directly from mtₜ amoebae might be expected to behave as group V plasmodia and those arising from a cross as group III plasmodia.

To set up a cross such as a × CLd, a drop of bacterial suspension on DSDM agar was simultaneously inoculated with a and CLd amoebae. As soon as individual plasmodia became visible (3–5 days) several were separately subcultured to SDMS agar and allowed to grow into large vigorous plasmodia which were then tested for their fusion behaviour against appropriate testers. It was found that each plasmodium could be unambiguously assigned to either fusion group V or fusion group III. The plasmodia could therefore be classified as having arisen either directly from CLd amoeba or by mating between a and CLd amoebae (i.e. crossed).

(ii) Comparison of nuclear DNA content of amoebal strains a, CL, CLd and plasmodia a × CLd and a × CLd

One crossed plasmodium (a × CLd)₁ identified as described above was selected for estimation of nuclear DNA content. Genetic analysis of the progeny of (a × CLd)₁ (see below) confirmed that this plasmodium had resulted from the sexual fusion of a and CLd amoebae.
Fig. 1 shows measurements of nuclear DNA content of Feulgen-stained log.
phase amoebae of strains \( a, CL, CLd \) (see Materials and Methods) and isolated
nuclei of plasmodia \( CL, CLd, a \times i \) and \( (a \times CLd)1 \). The \( CL \) and \( CLd \) plasmodia
used were produced in single clones.

![Graph showing DNA content of amoebae and plasmodia](image)

**Fig. 1.** Microdensitometric estimation of amoebal and plasmodial nuclear DNA
content. All samples were Feulgen stained as a single batch. Mean values and
standard errors are shown in parentheses.

It can be seen that, as expected, \( a \times i \) plasmodial nuclei have twice the DNA
content of \( a \) amoebal nuclei, agreeing with the conclusion of Mohberg & Rusch
(1971) that heterothallic amoebae are haploid and plasmodia diploid.

The crossed plasmodium \( (a \times CLd)1 \) also has a nuclear DNA content twice that
of \( a \) (and \( CLd \)) amoebae. However, the \( CL \) and \( CLd \) plasmodia have a nuclear
DNA content approximately the same as that of \( CL, CLd \) and \( a \) amoebae. These
data strongly suggest that:
(a) CLd amoebae can undergo cell and nuclear fusion with heterothallic amoebae to produce diploid plasmodia.

(b) CLd (and CL) plasmodia are haploid and plasmodia formation within clones of this strain of *P. polycephalum* is accomplished without nuclear fusion.

(iii) \((a \times CLd)1\) progeny analysis

The plasmodium \((a \times CLd)1\) was allowed to spore and 140 progeny amoebal clones isolated. Analysis of these progeny clones yielded the results shown in Table 1. The ratio \(mt_b : mt_t\) showed significant deviation from 1:1 at the 1% level and the reason for this is not clear. However, the allele ratio \(sax^+ : sax^-\) was 1:1 and the ratio of recombinants:parentals for \(mt\) and \(sax\) was also 1:1. This is conclusive evidence that the plasmodium \((a \times CLd)1\) resulted from a cross between \(a\) and CLd amoebae and that all progeny analysed arose from meiosis in diploid heterozygous nuclei.

|          | \(sax^+\) | \(sax^-\) | Total |
|----------|-----------|-----------|-------|
| \(mt_t\) | 29        | 25        | 54    |
| \(mt_b\) | 40        | 46        | 86    |
| **Total**| **69**    | **71**    | **140**|

Table 1. Progeny analysis of the plasmodium \((a \times CLd)1\)

\(mt_t\) amoebae: failed to form plasmodia within clones. Plasmodia produced when crossed to \(mt_b\), \(sax^-\) tester amoebae.

\(mt_b\) amoebae: produced plasmodia within clones. \(sax^-\) plasmodia: plasmodia (produced in tests of mating type) which failed to grow when subcultured to axenic medium (SDMS) were classified as \(sax^-\).

(iv) Comparison of nuclear DNA content in successive generations of \(mt_h\) and \(mt_t\) amoebae and plasmodia

To test the conclusions given in (ii) above, measurements of nuclear DNA content were made on a further eight strains of amoebae and plasmodia which were derived as shown in Table 2. Amoebae of strain CLd were mixed with amoebae of the heterothallic \(mt_t\) strain LU523. Plasmodia isolated from the cross plate were found to be of two fusion types – those which fused with a tester plasmodium of fusion group V, as expected if they were derived directly from CLd amoebae, and those which fused with a tester plasmodium of group III, as expected if they were formed by mating between CLd and LU523 amoebae. One plasmodium of each of these types was chosen as a representative plasmodium from which progeny amoebae and plasmodia were isolated.

The nuclear DNA content of all the representative strains named in Table 2 was estimated and the results are shown in Figs. 2 and 3. Again all material was
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Feulgen stained as a single batch to enable valid comparisons to be made. Also included in the staining series as control samples were nuclei of the CL plasmodium and the a x i plasmodium from (ii) above.

Table 2. Derivation of successive generations of amoebae and plasmodia for estimation of nuclear DNA content

| Amoebal mating: | LU523 x CLd |
|----------------|-------------|
| Plasmodia isolated from cross plate | |
| Fusion group: | V III |
| Number isolated: | 8 9 |
| Representative plasmodia: | (LU523 x CLd)3 (LU523 x CLd)15 |
| Progeny amoebae isolated | |
| Mating type: | mt_h mt_h mt_1 |
| Number isolated: | 10 4 6 |
| Representative amoebal clones: | LU670 LU577 LU574 |
| Plasmodia formed | |
| Representative plasmodia | LU670 LU577 LU574 x mt_2 |

Measurements of nuclear DNA content were made on the named representatives of each class (see Figs. 2 and 3). As indicated, these representatives of each generation were also used to produce the next generation. Amoebae which gave rise to plasmodia within clones were classified as mt_h. Amoebae which only gave plasmodia when test mated with mt_a amoebae were classified as mt_1.

The histograms Fig. 2 (a)–(c), show the measurements for the representative group V plasmodium, a progeny amoebal clone and the plasmodium which this clone produced. Both plasmodia have nuclear DNA contents close to that of the amoebal strain and similar to that of the control CL plasmodium (Fig. 2d), supporting the previous conclusion that plasmodia formed in clones of mt_h amoebae are haploid.

Fig. 3 (a) shows the nuclear DNA content of the plasmodium (LU523 x CLd)15 which was classified as crossed on the basis of its fusion behaviour. As expected the plasmodium appears to be diploid, its DNA content being similar to that of the control (a x i) (Fig. 2e).

Both the mt_h (LU577) and mt_1 (LU574) progeny amoebae from this crossed plasmodium have the haploid DNA content (Fig. 3b, d). In agreement with the other results, the plasmodium formed in clone LU577 has a haploid DNA content (Fig. 3c) while the plasmodium LU574 x mt_2 (Fig. 3e) has a diploid value. All the results are therefore consistent with the conclusions summarized in (ii).
4. DISCUSSION

The data presented show that amoebae and plasmodia of the CL strain of *P. polycephalum* have the same nuclear DNA content and this content is half that of crossed plasmodia. Yemma & Therrien (1972) observed in ‘selfing clones’ of the usually heterothallic myxomycete *Didymium iridis* that amoebae and plasmodia had the same nuclear DNA content (2C), a value which was half that of crossed plasmodia (4C). They also observed that in all cases sporangial nuclei contained the 4C amount. They proposed that amoebae were haploid and in G_2 phase, that crossed plasmodia were diploid and in G_2 phase and that selfed plasmodia were also diploid but were in an extended G_1 phase. This does not seem to be the case for CL plasmodia because Sudbery (personal communication) has measured [³H]-thymidine incorporation in CL plasmodia and has found that DNA synthesis
commences immediately after mitosis in this strain and is complete within 3 h. These results indicate that as in heterothallic strains of *P. polycephalum* there is no G1 phase in CL plasmodia. Plasmodia in the present work were not used for nuclear isolation until at least 3 h after mitosis had been completed and were therefore in G2 phase. The present results are thus interpreted as indicating that there is no change in ploidy during plasmodium formation within clones of strain CL.

Mohberg et al. (1973) showed that plasmodia and spores of the Colonia strain C50 (see Materials and Methods) had the same average nuclear DNA content. In this and a previous study (Mohberg & Rusch, 1971) on *P. polycephalum*, heterothallic plasmodia in G2 had twice the DNA content of G2 amoebae and mature spores. Assuming that heterothallic amoebae and plasmodia have haploid and diploid nuclear DNA contents respectively, the present data strongly suggest that CL amoebae and plasmodia are both haploid. The average nuclear DNA contents estimated for C50 plasmodia and spores by Mohberg et al. (1973) were intermediate between those of heterothallic amoebae and plasmodia. The reason for this difference is not understood but is at present the subject of a joint investigation with Dr Mohberg in this laboratory. Chromosome counts (Mohberg et al. 1973) showed that plasmodia of CL (and other Colonia isolates) have 35–40 chromosomes whereas a × i plasmodia have predominantly nuclei with 45–50 or 75–80 chromosomes. Bradbury et al. (1973), however, reported a chromosome number of 22 for a × i plasmodia.

The fact that G2 nuclei from amoebae and plasmodia of strain CL have the same DNA content suggests that plasmodia formation in this strain occurs without nuclear fusion. This is in contrast with plasmodia formation in heterothallic strains and in crosses between mtb and heterothallic amoebae where nuclear fusion is indicated both by DNA measurements and by genetical results reported here and by Wheals (1970). Two alternative mechanisms for plasmodia formation within clones of CL amoebae seem possible:

(a) apogamy: a single amoebal cell developing into a plasmodium by repeated nuclear division without cell or nuclear fusion and without change in ploidy;

(b) coalescence: fusion of two or more genetically identical amoebae, without nuclear fusion, producing a dikaryotic cell which by repeated nuclear division gives rise to a plasmodium.

The present data do not allow one to distinguish between these alternatives although they do exclude the possibility in our strains of homothallic development since this requires nuclear fusion and consequent change in ploidy. Wheals (1970) found that a cross mtbf1 × mtbf2 gave rise to hybrid plasmodia (f1f2 fusion behaviour) and regarded this as additional evidence of nuclear fusion among mtb amoebae. This could also be explained by coalescence of amoebae giving rise to heterokaryotic plasmodia. In attempts to repeat these observations we have tested 50 plasmodia obtained after mixing mtbf1 and mtbf2 amoebae for their fusion phenotype. Of these only one exhibited hybrid fusion behaviour, the remainder being clearly either f1 or f2 in fusion behaviour. These results alone do not give conclusive evidence of
It is of great interest to understand the underlying processes involved in plasmodium formation since changes in morphology, nutrition and mode of nuclear division occur and it seems probable that differential gene activity is involved. It will also be of interest to understand the nuclear events associated with sporulation in Colonia isolates since meiosis occurs at this time in *P. polycephalum*. (Aldrich, 1967).

Previous work on the isolation and genetic analysis of mutants in the Colonia strain of *P. polycephalum* (Dee, 1973; Dee, Wheals & Holt, 1973; Wheals, 1973) was based on the assumption that clones of *mth* amoebae formed diploid homozygous plasmodia. However, the results of these studies agree equally well with the assumption that the plasmodia are haploid. The strains *CL* and *CLd* are now being used in several laboratories to isolate and analyse nutritional and temperature sensitive plasmodial mutants after mutagenesis of amoebae. The present results on the nuclear DNA content of *CL* (*CLd*) do not throw doubt on the value of these strains for the genetic analysis of *P. polycephalum* as discussed in detail by Dee (1973).

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REFERENCES

Aldrich, H. C. (1967). The ultrastructure of meiosis in three species of *Physarum*. *Mycologia* 59, 127–148.

Bradbury, E. M., Matthews, H. R., McNaughton, J. & Molgaard, H. V. (1973). Subnuclear components of *Physarum polycephalum*. *Biochimica et Biophysica Acta* 335, 19–29.

Darlington, C. D. & La Cour, L. F. (1962). *The Handling of Chromosomes*, 4th ed. London: Allen and Unwin.

Dee, J. (1966). Multiple alleles and other factors affecting plasmodium formation in the true slime mould *Physarum polycephalum* Schw. *Journal of Protozoology* 13, 610–616.

Dee, J. (1973). Aims and techniques of genetic analysis in *Physarum polycephalum*. *Berichte der Deutschen Botanischen Gesellschaft* 86, 93–121.

Dee, J. & Poultier, R. T. M. (1970). A gene conferring actidione resistance and abnormal morphology on *Physarum polycephalum* plasmodia. *Genetical Research* 15, 33–41.

Dee, J., Wheals, A. E. & Holt, C. E. (1973). Inheritance of plasmodial valine requirement in *Physarum polycephalum*. *Genetical Research* 21, 87–101.

Grant, W. D. & Poultier, R. T. M. (1973). Rifampicin-sensitive RNA and protein synthesis by isolated mitochondria of *Physarum polycephalum*. *Journal of Molecular Biology* 73, 439–455.

Itakawa, O. & Ogura, Y. (1954). The Feulgen reaction after hydrolysis at room temperature. *Stain Technology* 29, 13–15.

Kerr, N. S. (1967). Plasmodium formation by a minute mutant of the true slime mould, *Didymium nigripes*. *Experimental Cell Research* 45, 646–655.

Kerr, S. (1968). Ploidy level in the true slime mould *Didymium nigripes*. *Journal of General Microbiology* 53, 9–15.

Mittermayer, C., Braun, R. & Rusch, H. P. (1965). The effect of actinomyein D on the timing of mitosis in *Physarum polycephalum*. *Experimental Cell Research* 38, 33–41.
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Möhring, J., Babcock, K. L., Haugli, F. B. & Rusch, H. P. (1973). Nuclear DNA content and chromosome numbers in the Myxomycete Physarum polycephalum. Developmental Biology 34, 228–245.

Möhring, J. & Rusch, H. P. (1971). Isolation and DNA content of nuclei of Physarum polycephalum. Experimental Cell Research 66, 305–316.

Poultier, R. T. M. (1969). Senescence in the Myxomycete Physarum polycephalum. Ph.D. Thesis, University of Leicester.

Poultier, R. T. M. & Dee, J. (1968). Segregation of factors controlling fusion between plasmodia of the true slime mould Physarum polycephalum. Genetical Research 12, 71–79.

Terry, C. D. (1966). Microspectrophotometric measurements of nuclear deoxyribonucleic acid content in two Myxomycetes. Canadian Journal of Botany 44, 1667–1675.

van Stosch, H. A., Van Zul-Pischinger, M. & Dersch, G. (1964). Nuclear phase alternance in the Myxomycete Physarum polycephalum, pp. 481–482. In Abstracts 10th International Botanical Congress, Edinburgh, 1964.

Wheals, A. E. (1970). A homothallic strain of the Myxomycete Physarum polycephalum. Genetics 66, 623–633.

Wheals, A. E. (1973). Developmental mutants in a homothallic strain of Physarum polycephalum. Genetical Research 21, 79–86.

Yemma, J. J. & Terry, C. D. (1972). Quantitative microspectrophotometry of nuclear DNA in selfing strains of the Myxomycete Didymium iridis. American Journal of Botany 59, 828–835.