GENETIC AND CYTOLOGICAL EVIDENCE FOR A DIPLOID
LIFE CYCLE IN PYTHIUM APHANIDERMATUM

by
Charles Winsor Dennett

A Thesis Submitted to the Faculty of the
DEPARTMENT OF PLANT PATHOLOGY
In Partial Fulfillment of the Requirements
For the Degree of
MASTER OF SCIENCE
In the Graduate College
THE UNIVERSITY OF ARIZONA

1 9 7 6

STATEMENT BY AUTHOR

This thesis has been submitted in partial fulfillment of requirements for an advanced degree at The University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the Library.

Brief quotations from this thesis are allowable without special permission, provided that accurate acknowledgment of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the Graduate College when in his judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

SIGNED: Charles Demuth

APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

M. E. Stanghellini
M. E. STANGHELLINI
Professor of Plant Pathology

July 28, 1975
Date

TABLE OF CONTENTS

	Page
LIST OF ILLUSTRATIONS	iv
ABSTRACT	v
 CHAPTER	
1. INTRODUCTION	1
2. A MEMBRANE-CHROMATIN ATTACHMENT MODEL OF MITOTIC NUCLEAR DIVISIONS	3
Introduction	3
Materials and Methods	4
Results	4
Discussion	10
3. GAMETANGIAL NUCLEAR DIVISIONS	13
Introduction	13
Methods	14
Results	14
Discussion	15
4. SEGREGATION OF CHLORAMPHENICOL TOLERANCE	18
Introduction	18
Materials and Methods	18
Results	20
Discussion	20
LITERATURE CITED	23

LIST OF ILLUSTRATIONS

Figure	Page
1. Hyphal early division figures	5
2. Hyphal late division figures	7
3. Schematic of membrane-chromatin attachment model	9
4. Gametangial diplotene-diakinesis figures	16

ABSTRACT

A cytological and genetic examination of Pythium aphanidermatum was undertaken to resolve the continuing controversy concerning the location of meiosis in the life cycle of the Oomycetes. The hyphal nuclei were examined in order to contrast these with meiotic figures seen in the gametangia. A new model is suggested to explain the non-classical fungal mitosis using the assumption of chromatin-nuclear-membrane attachment. Diplotene-diakinesis figures were photographed in both the antheridia and oogonia. A chloramphenicol tolerant mutant was induced with ethyl methansulfonate and the oospore progeny segregated 1 drug sensitive to 5.83 drug tolerant. Zoospores from the same mutant failed to segregate, indicating heterokaryosis was not responsible for the oospore segregation. These results are most simply the result of heterozygous diploid somatic nuclei and gametangial meiosis. Both the cytological and genetic data suggest a life cycle with diploid nuclei in the mycelium. These results give further support to the claims that Oomycetes are unlike most other fungi, because they have diploid somatic nuclei.

CHAPTER 1

INTRODUCTION

The somatic nuclei in most fungi are haploid. Fusion of these nuclei subsequent to plasmogamy is followed by meiosis which restores the haploid condition. Certain members of the Oomycetes, however, are believed to possess a diploid type of life cycle. These fungi have diploid somatic nuclei which presumably undergo meiosis in the gametangia. Plasmogamy and karyogamy then restore the diploid somatic nuclear condition.

Gametangial meiosis was first suggested for the Oomycetes in the late nineteenth century (Trow, 1895). Support for a haploid type of life cycle dominated the literature in the next 60 years (Dick and Win-Tin, 1973) until Sansome (1961, 1963, 1965) presented evidence for a diploid scheme. The very small size of the nuclei is one of the major causes of this controversy concerning the actual type of life cycle found in the Oomycetes.

Alternate approaches have been attempted to avoid relying on cytology. Microspectrophotometric evidence supports gametangial meiosis for Apodachyla (Howard and Bryant, 1971) and Saprolegnia (Bryant and Howard, 1969).

Genetic evidence comes mainly from Phytophthora species and ranges from inconclusive to supporting the diploid type of life cycle (gametangial meiosis) (Khaki and Shaw, 1974).

This study was undertaken to determine whether Pythium aphanidermatum (Edson) Fitzp. has a diploid type of life cycle. The understanding of somatic nuclear divisions is a prerequisite to distinguishing these from meiotic divisions. This study is separated into three parts. Chapter 2 is devoted to the description of somatic nuclear division and a new model for such divisions is presented. Chapter 3 deals with the gametangial divisions and Chapter 4 deals with a genetic instead of a cytological approach to determine the type of life cycle found in Pythium aphanidermatum.

CHAPTER 2

A MEMBRANE-CHROMATIN ATTACHMENT MODEL OF MITOTIC NUCLEAR DIVISIONS

Introduction

In order to cytologically distinguish meiotic divisions, an understanding of somatic divisions is required.

Stained fungal chromatin generally exhibits the following mitotic figures which differ from mitosis of higher plants and animals (Dowding and Weijer, 1962; Robinow, 1957a, 1957b; Robinow and Caten, 1969; Duncan and MacDonald, 1965; Day, 1971):

1. Apparent lack of a metaphase plate.
2. Nuclear membrane continuity throughout division.
3. Apparent absence of a well developed spindle.
4. Apparent beaded chains of chromatin in the form of filaments, rings, and crescents.
5. The track or double filament stage.

Currently there are two contrasting models of somatic nuclear divisions in fungi. One involves chromosomes linked in chains (Dowding and Weijer, 1960, 1962; Laane, 1967; Namboodiri and Lowry, 1967; Duncan and MacDonald, 1965). The other is a more classical scheme of independent

chromosome segregation driven by a simple spindle apparatus (Aist and Williams, 1972). A new model, termed the membrane-chromatin attachment model is proposed here which takes into account both of these models.

Materials and Methods

Pythium aphanidermatum isolates were grown in 1-10% centrifuged V-8 juice for 2-5 days and fixed in ethyl alcohol-acetic acid (3:1) for one hour. Orcein (2 g in 100 ml 45% acetic acid, heated and filtered) and 5N HCl were mixed (1:1) in a Coors spot dish. One cm³ of the growing tips of the mycelial mat was then stained for 30 minutes followed by a 5 min rinse in 45% acetic acid and cleared for 10 min in the clearing agent (1 chloral hydrate: 1 clove oil:1 lactic acid:1 xylene:2 phenol). After clearing, the mycelium was rinsed in 45% acetic acid and mounted in the same or in water to improve contrast.

The Feulgen technique (Bryant and Howard, 1969) and the carmine stain (Stephenson, Erwin, and Leary, 1974) gave equivalent results to the orcein stain.

Results

The interphase nucleus (Figure 1A) is roughly spherical in shape, containing faint threads of chromatin and a large Feulgen negative nucleolus. The chromosomes are postulated to remain attached to the nuclear membrane along its equator. This assumption is not proven here (see

Figure 1. Hyphal early division figures -- A: Interphase nucleus with nucleolus X2800; B: track X1800; C: ring X1500; D: rings separating X1800.

Figure 1. Hyphal early division figures.

Discussion) but leads to the following explanation of the mitotic cycle.

Nuclear division begins with a thickening of the chromosomes until the nucleus contains tangled double parallel strands--the "track" stage (Figure 1B).

Prophase contraction continues until metaphase is reached. The chromosomes now appear as tiny ($0.1\ \mu\text{m}$) beads attached to the membrane along $2/3$ of the equator. The appearance of the chromatin is probably determined by the space limitations as well as deformations caused by cytoplasmic streaming forces or by independent nuclear motility forces (Wilson and Aist, 1967). The latter forces are believed to originate in the microtubules passing from the cytoplasm to the nucleus. A ring [the "saturn's ring" of Robinow (1963)] was observed from a polar view of the metaphase nucleus in hyphae of wide diameter or antheridia and oogonia (Figure 1C,D).

In the narrow confines of the hyphae, the metaphase nucleus can take on the appearance of an oblong filament (Figure 2A). This stretching is caused perhaps externally by tensional forces from the nuclear motility microtubules (Heath, 1974). The membrane attachment model could explain this stretching by condensation forces of the chromosomes themselves. If the chromosomes are anchored to the membrane at more than one point, these points might be pulled together by chromosomal prophase contraction.

Figure 2. Hyphal late division figures -- A: Filament metaphase X1800; B: early anaphase X3300; C and D: late anaphase X1300.

Figure 2. Hyphal late division figures.

The filament is seen as a projection of the equatorial belt of chromatin. The stretched nucleus is approximately 7 μ m in length and resembles the beaded filament of Weiher (Dowding and Weiher, 1962). The third type of metaphase nucleus is spherical and packed with heavily staining chromatin beads.

Anaphase separation (Figure 2B,C,D) is probably of very short duration since it was rarely observed in stained material. This rapidity is in agreement with in vivo observations of Fusarium mitotic divisions (Aist and Williams, 1972). An anaphase duration of 13 seconds out of a total mitotic duration of 5.5 minutes was reported. The separation begins in a direction perpendicular to the hyphal axis (Figure 2B). Rotation of the nucleus (Aist and Wilson, 1968) results in late anaphase separation directed parallel to the hyphal axis.

Unilateral separation of chromatin does not always occur, occasionally "bridge like" connections occur between daughter groups of chromatin (Figure 2B,C). These are apparent in the division of filaments and correspond to the filament ends seen broken in Figure 2D, which are still united in Figure 2C. The rings divide as complete double rings or crescents, without "lagging" chromatin material (Figure 1D) (Weiher and Weisberg, 1966). Figure 3 represents a schematic diagram of the membrane-chromatin attachment model.

Figure 3. Schematic of membrane-chromatin attachment model -- A: Ring metaphase; B: ring anaphase; C: filament metaphase; D: filament anaphase; E: late anaphase; F: "V" anaphase; G: apparent "bridges."

Discussion

Cytological evidence presented in this paper has led to a model of somatic nuclear divisions to explain the unusual mitosis seen in Pythium aphanidermatum. The model relies on the membrane remaining intact during division and can explain chromosome segregation in the absence of a spindle apparatus. Other fungi with similar somatic chromatin figures may also conform to this model (Aist and Wilson, 1968).

A spindle apparatus has been demonstrated in certain fungi with recent application of electron microscopy. These microtubules seem to range from a single parallel band in Saccharomyces (Robinow and Marak, 1966), Shizosaccharomyces (McCully and Robinow, 1971) to increasingly complex arrangements with chromosomal microtubules (Saprolegnia [Heath and Greenwood, 1968]; Fusarium [Aist and Williams, 1972]; Phytophthora [Hemmes and Hohl, 1973]; Thraustotheca [Heath, 1974]; Catenaria [Ichida and Fuller, 1968]). Until more EM data are available, the presence of a mitotic spindle cannot be assumed for all genera of fungi.

The mechanism of genome separation in the fungi without spindles could be due to differential membrane growth between the attachment points as suggested for bacteria and yeast (McCully and Robinow, 1971), or a floating of attachment points on the membrane, as envisioned

by the fluid mosaic membrane model (Singer and Nicolson, 1972).

An intermediate possibility of genome separation mechanism could be similar to that of Saccharomyces. In this case the formation and elongation of a single central band of microtubules causes separation of the nuclear poles. This could also produce the anaphase-telophase figures seen here (Figure 2C,D) as well as the separating filaments (Figure 3).

Two current models of chromosome segregation can both be satisfied in terms of the membrane attachment model. The classical mitotic scheme requires chromosomal spindles to separate the individual chromosomes. The other model involves chromosomes separating as beaded filaments. The classical scheme is supported mainly by EM evidence. In Fusarium (Aist and Williams, 1972) and Thraustotheca (Heath, 1974) thin sections show the microtubule-chromosome attachment points (kinetochores) not connected to the membrane. It is possible that the bulk of the chromosome may remain membrane bound and when stained produce the filaments, crescents and other light microscope figures like those seen in Pythium aphanidermatum as well.

In higher organisms there are many reports of chromatin-membrane attachment, and the concept is becoming increasingly popular in the literature. Permanent chromosome attachment to the nuclear membrane is well established in

bacteria. The points of attachment function as DNA replication sites and probably in genome separation (Clay, Katterman, and Bartels, 1975). The attachment seems permanent in Gossypium and it has been suggested there is also a structural function as well as an involvement in DNA synthesis (Clay et al., 1975). Electron micrographs clearly show the attachment to the membrane of the permanently condensed chromosomes of the alga Gyrodinium cohnii (Kubabi and Ris, 1969).

Nonrandom segregation of labeled DNA in bacteria (Lark, 1966) and Aspergillus (Rosenberger and Kessel, 1968) provide further evidence of permanent association of parental DNA. An organelle which segregates along with the DNA--a kinetochore or possibly the nuclear membrane--can carry the parental DNA to one pole while the replicated (non labeled) DNA goes to the other.

CHAPTER 3

GAMETANGIAL NUCLEAR DIVISIONS

Introduction

Cytological evidence of the location of meiosis in the majority of the Oomycetes studied has relied on the following evidence:

1. Multiple association of bivalents.
2. Reduction in nuclear diameter as meiotic divisions proceed.
3. Bridge and fragment at anaphase.
4. Metaphase plate unique to meiosis.

Some caution must be used when extrapolating from higher plants to fungi. The arguments of Sansome have been criticized because of the fact that some of the figures she described occurring in the gametangia are also seen in the hyphae (Stephenson et al., 1974).

The mitotic track figure in Pythium aphanidermatum can easily be mistaken for pachytene bivalents, because of the paired double stranded appearance common to both.

Nuclear size should not be used as evidence of meiosis. Meiosis reduces the amount of DNA in each G1 nucleus by one-half. A sphere whose volume is halved.

will show a radius decrease of only 21%. Such a decrease would be difficult to measure on the very small nuclei of Pythium aphanidermatum.

Bridges and fragments are fairly common in mitotic anaphase of Pythium aphanidermatum (Figure 2C,D) and in Fusarium (Aist and Williams, 1972) and should not be used as evidence of meiosis in fungi.

What is lacking is evidence of the diplotene-diakinesis stages characteristic of meiosis. These stages have been published by Win-Tin according to Dick and Win-Tin (1973, p. 146). If these figures are found and shown not to resemble any nuclear figures seen in the hyphae of the organism under study, the divisions may be considered meiotic.

Methods

The fungus was grown and stained as previously described.

Results

The general pattern of nuclear behavior in the gametangia of Pythium aphanidermatum agrees with that reported for related organisms (Dick, 1972). Many nuclei enter the developing oogonium until a septum is formed. Attachment of the antheridium occurs when there are approximately six nuclei in the oogonium and 1-4 nuclei in the antheridium. One to several meiotic events in each

gametangium produce the gamete nuclei. A fertilization tube passes a gamete nucleus (or nuclei) into the oogonium. Karyogamy has not been observed because of stain clearing difficulties in the developing oospore. Several nuclei do remain within the antheridia and from 6-8 nuclei are seen degenerating external to the developing oospore wall.

Diplotene-diakinesis figures have been observed for the first time in both antheridia (Figure 4A,B) and oogonia (Figure 4C,D). The synapsed chromosomes are found in groups of three within the gametangia. This observation supports a diploid life cycle with a chromosome number of six.

Discussion

The diplotene-diakinesis stages observed in the gametangia are distinct from the somatic figures. The meiotic chromosomes are large; their length being of the same order as the diameter of the entire somatic nucleus. In Neurospora a similar increase occurs. The tiny bead-like somatic chromosomes are about 0.1-0.3 μm while each of the seven diakinesis bivalents are approximately 3.3 μm long (Singleton, 1953).

Somatic nuclei can be found with looping filaments and some with these loops apparently crossing each other. The bivalents at diakinesis have two arms which loop and intersect at chiasma points and usually have two unpaired

Figure 4. Gametangial diplotene-diakinesis figures -- A
and B: Antheridia X1600; C: oogonium X2000;
D: squashed oogonium X4800.

Figure 4. Gametangial diplotene-diakinesis figures.

arms extending beyond this chiasma. The chance of finding groups of three somatic nuclei, each with loops, intersections and pairs of extending single arms is very remote. The association of the meiotic chromosomes in groups of three within the gametangia is consistent with a diploid lifecycle with three pairs of chromosomes in the somatic nuclei.

CHAPTER 4

SEGREGATION OF CHLORAMPHENICOL TOLERANCE

Introduction

Genetic data, microspectrophotometric and cytological studies have been presented as evidence in the unresolved controversy of whether the Oomycete vegetative stage is haploid or diploid (Hendrix and Papa, 1974; Gallegly, 1970). Genetic studies have been hampered by the difficulty in obtaining mutants and the low percentage germination of the sexual spores (oospores). Recently, Stanghellini and Russell (1973) reported that ingestion of culturally grown oospores of Pythium aphanidermatum by aquatic snails increased the percentage germination from 20 to 94%. Thus, Pythium aphanidermatum was chosen to test the ploidy of the homothallic mycelium by searching for segregation in the sexual progeny of a drug resistant mutant.

Materials and Methods

A chloramphenicol, CA (chloromycetin, Sigma Chemical Co., St. Louis, Mo.) resistant mutant of Pythium aphanidermatum was isolated by treating 1 ml of ingested oospores (approximately 10^7 spores) with 8 ml of 0.12 M ethyl methansulfonate (EMS) (Sigma Chemical Co., St.

Louis, Mo.) in sterile tap water for 2 hours at room temperature. The oospores were then washed by centrifugation. The supernatant was discarded and the oospores washed into a flask containing 200 ml of 10% V-8 juice and 600 µg/ml CA and incubated at room temperature on a shaker. After 5 days incubation, individual colonies (10-20 survived from 10^7 oospores) were transferred and tested for growth on CMA (corn meal agar 1.7 g/l) containing 600 µg/ml CA. One of these mutants was selected and used in this study. A final CA concentration of 1800 µg/ml was used in the testing media to distinguish clearly between the mutant and the sensitive isolate (growth in 100 hours--tolerant 40 mm, nontolerant 0-5 mm). When testing isolates for growth, transfers were made from colonies of the same age.

The CA resistant isolate was grown in liquid culture (deep petri plate with 30 ml water and 4 rolled oat flakes) for oospore production. Two to three week old mycelial mats were removed, rinsed and fed to water snails (Stanghellini and Russell, 1973). Washed excreted oospores were suspended in sterile distilled water and spread onto CMA plates and incubated at 35°C for 4-12 hours. Forty-one single germinating oospores were randomly selected from the mutant progeny, which showed a 94% oospore germination rate, and transferred to V-8 juice agar slants (10% Campbell's V-8 juice and 2% agar).

Zoospores were also randomly sampled from the same CA resistant parent. Zoospores were produced by transferring agar containing the margin of a colony to sterile water. After 4-8 hours at room temperature zoospores were released and then were spread on water agar and isolated.

Results

The single oospore progeny of the CA tolerant parent showed a segregation of 6 sensitive:35 tolerant in two trials (3:16 and 3:19). Zoospores were isolated to determine if this segregation resulted from segregation of a heterokaryon. No segregation was seen in 51 single zoospore isolates.

In order to expand these data, an attempt was made to test the second generation for segregation. This failed because of the almost complete oospore abortion found in the 41 single oospore colonies. This loss of second generation fertility was also reported by Khaki and Shaw (1974) and Castro and Zentmyer (1968).

Discussion

The oospore segregation from the CA tolerant parent can be explained with a diploid life cycle and a dominant mutation to CA tolerance. The wild type recessive genes were carried in the diploid hyphal nuclei and produced the CA sensitive oospores segregants when two recessive gametes united. In Aspergillus (Warr and Roper, 1965) and

Phytophthora (Khaki and Shaw, 1974) drug resistance is frequently dominant. The uninucleate zoospores produced colonies that failed to segregate, indicating each hyphal nucleus was at least heterozygous, if not homozygous, for CA tolerance.

The expected segregation ratio of the oospores is 1:3 not the observed 1:5.83. This ratio can be produced if some nuclei are homozygous for the mutation and some heterozygous. The mutant CA resistant parents of the heterothallic Phytophthora used by Khaki and Shaw (1974) were completely homozygous. They suggested a semi-dominant resistance allele which allows selection for the homozygous nuclei (of spontaneous or parasexual origin) because of their increased drug resistance over that of the heterozygous nuclei. The ratio observed here (1:5.83) would be produced by a parent with approximately one homozygous nucleus to three heterozygous ones.

An alternate explanation could produce a 5:1 segregation ratio. This ratio would be produced by fusion of the heterozygous gametangial nuclei producing a tetraploid oospore (CACAcaca). Meiosis then occurs in the oospore and all but one of the nuclei degenerate. If this occurs randomly, a duplex segregation ratio of 5:1 will result. This explanation would change the location of meiosis, but would not change the diploid nature of the hyphae.

If this CA resistant mutant is controlled by a single nuclear gene, then the recovery of the wild type oospores from the mutant is incompatible with a haploid life cycle. This conflicts with most of the previous genetic data (Hendrix and Papa, 1974; Gallegly, 1970). The major criticism of this work has been centered on the very low germination of the oospores, usually from 0.1%-10%. The use of snail ingested oospores increases germination to 94% and also eliminates nonoospore propagules such as oogonia, antheridia, and hyphae. Two recent studies that do support a diploid life cycle both had a high per cent oospore germination (Khaki and Shaw, 1974; Elliott and MacIntyre, 1973).

LITERATURE CITED

- Aist, J. R., and P. H. Williams. 1972. Ultrastructure and time course of mitosis in the fungus Fusarium oxysporum. J. Cell. Biol. 55:368-389.
- Aist, J. R., and C. L. Wilson. 1968. Interpretation of nuclear division figures in vegetative hyphae of fungi. Phytopathology 58:876-877.
- Bryant, T. R., and K. L. Howard. 1969. Meiosis in the Oomycetes: I. A microspectrophotometric analysis of nuclear deoxyribonucleic acid in Saprolegnia terrestris. Amer. J. Bot. 56(9):1075-1083.
- Castro, J. F., and G. A. Zentmyer. 1968. Mortality of germinated F₂ oospores from crosses of F₁ single oospore cultures of Phytophthora infestans. Phytopathology 59:10. abstr.
- Clay, W. F., F. R. H. Katterman, and P. G. Bartels. 1975. Chromatin and DNA synthesis associated with nuclear membrane in germinating cotton. Proc. Nat. Acad. Sci. USA 72:3134-3138.
- Day, A. W. 1971. Genetic implications of current models of somatic nuclear division in fungi. Can. J. Bot. 50:1337-1347.
- Dick, M. W. 1972. Morphology and taxonomy of the Oomycetes, with special reference to Saprolegniaceae, Leptomitaceae and Pythiaceae. I. Sexual reproduction. New Phytol. 68:751-775.
- Dick, M. W., and Win-Tin. 1973. The development of cytological theory in the Oomycetes. Biol. Rev. 48:133-158.
- Dowding, E. S., and J. Weiher. 1960. Mitosis in Neurospora. Nature, Lond. 188:338-339.
- Dowding, E. S., and J. Weiher. 1962. Mitosis in Neurospora and Gleasinospora I. Genetica 32:339-351.

- Duncan, E. J., and J. A. MacDonald. 1965. Nuclear phenomena in Marasmius androsaceus (L. ex Fr.) and M. rotula (Scop. ex Fr.). Trans. R. Soc. Edinb. 66:129-141.
- Elliott, C. G., and D. MacIntyre. 1973. Genetic evidence on the life history of Phytophthora. Trans. Br. Mycol. Soc. 60(2):311-316.
- Gallegly, M. E. 1970. Genetics of Phytophthora. Phytopathology 60:1135-1141.
- Heath, I. B. 1974. Mitosis in the fungus Thraustotheca clavata. J. Cell Biol. 60:204-220.
- Heath, I. B., and A. D. Greenwood. 1968. Electron microscopic observation of dividing somatic nuclei in Saprolegnia. J. Gen. Microbiol. 53:287-289.
- Hemmes, D. E., and H. R. Hohl. 1973. Mitosis and nuclear degeneration: simultaneous events during secondary sporangia formation in Phytophthora palmivora. Can. J. Bot. 51:1673-1675.
- Hendrix, F. F., and K. E. Papa. 1974. Taxonomy and genetics of Pythium. Proc. Am. Phytopath. Soc. 1:200-207.
- Howard, K. L., and T. R. Bryant. 1971. Meiosis in the Oomycetes: II. A microspectrophotometric analysis of DNA in Apodachyla brachynema. Mycologia 63: 58-68.
- Ichida, A. A., and M. S. Fuller. 1968. Ultrastructure of mitosis in the aquatic fungus Catenaria anguillulae. Mycologia 60:141-155.
- Khaki, I. A., and D. S. Shaw. 1974. The inheritance of drug resistance and compatibility type in Phytophthora drechsleri. Genet. Res. Camb. 23: 75-86.
- Kubabi, D. F., and H. Ris. 1969. Division in the dinoflagellate Gynodinium cohnii. A new type of nuclear reproduction. J. Cell Biol. 40:508-528.
- Laane, M. M. 1967. The nuclear division in Penicillium expansum. Can. J. Genet. Cytol. 9:342-351.

- Lark, K. G. 1966. Regulation of chromosome replication and segregation in bacteria. *Bacteriol Rev.* 30:3-32.
- McCully, E. K., and C. F. Robinow. 1971. Mitosis in the fission yeast Shizosaccharomyces pombe: a comparative study with light and electron microscopy. *J. Cell Sci.* 9:475-507.
- Namboodiri, A. N., and R. J. Lowry. 1967. Vegetative nuclear division in Neurospora. *Am. J. Bot.* 54: 735-748.
- Robinow, C. F. 1957a. The structure and behavior of the nuclei in spores and growing hyphae of Mucorales. I. Mucor heimaies and Mucor fragilis. *Can. J. Microbiol.* 3:771-789.
- Robinow, C. F. 1957b. The structure and behavior of the nuclei in spores and growing hyphae of Mucorales. II. Phycomyces blakesleeanus. *Can. J. Microbiol.* 3:791-798.
- Robinow, C. F. 1963. Observation on cell growth, mitosis and division in the fungus Basidiobolus ranarum. *J. Cell. Biol.* 17:123-152.
- Robinow, C. F., and C. E. Caten. 1969. Mitosis in Aspergillus nidulans. *J. Cell. Sci.* 5:403-431.
- Robinow, C. F., and J. Marak. 1966. A fiber apparatus in the nucleus of the yeast cell. *J. Cell. Biol.* 29:129-151.
- Rosenberger, R. F., and M. Kessel. 1968. Non-random sister chromatid segregation and nuclear migration in hyphae of Aspergillus nidulans. *J. Bacteriol.* 96:1208-1213.
- Sansome, E. R. 1961. Meiosis in the antheridium and oogonium of Pythium debaryanum Hesse. *Nature (London)* 191:827-828.
- Sansome, E. R. 1963. Meiosis in Pythium debaryanum and its significance in the life history of the Biflagellatae. *Trans. Br. Mycol. Soc.* 46:63-72.
- Sansome, E. R. 1965. Meiosis in the diploid and polyploid sex organs of Phytophthora and Achyla. *Cytologia (Tokyo)* 30:103-117.

- Singer, S. J., and G. L. Nicolson. 1972. The fluid mosaic model of the structure of cell membranes. *Science* 175:720-731.
- Singleton, J. R. 1953. Chromosome morphology and the chromosome cycle in the ascus of Neurospora crassa. *Amer. J. Bot.* 40:124-144.
- Stanghellini, M. E., and J. D. Russell. 1973. Germination in vitro of Pythium aphanidermatum oospores. *Phytopathology* 63:133-137.
- Stephenson, L. W., D. C. Erwin, and J. V. Leary. 1974. Cytology of somatic and gametangial nuclei in Phytophthora capsici and Phytophthora megasperma var. sojae. *Can. J. Bot.* 52:2055-2060.
- Trow, A. H. 1895. The Karyology of Saprolegnia. *Ann. Bot.* 9:609-652.
- Warr, J. R., and J. A. Roper. 1965. Resistance to various inhibitors in Aspergillus nidulans. *J. Gen. Microbiol.* 40:273-281.
- Weijer, J., and S. H. Weisberg. 1966. Karyokinesis of the somatic nuclear of Aspergillus nidulans. I. The juvenile chromosome cycle (Feulgen Staining). *Can. J. Genetics and Cytology* 8:361-374.
- Wilson, C. L., and J. R. Aist. 1967. Motility of fungal nuclei. *Phytopath.* 57:769.