THE ISOLATION OF HAPLOID MYXAMOEBAL CLONES FROM A DIPLOID APOMICTIC ISOLATE OF THE MYXOMYCETE DIDYMIUM IRIDIS

by

Gary F. Jakupcin

Submitted in Partial Fulfillment of the Requirements

for the Degree of

Master of Science

in the

Biology

Program

Dr. John D. Jerina 8/4/78

Adviser Date

Dean Kent 8/8/78

Dean of the Graduate School Date

YOUNGSTOWN STATE UNIVERSITY

August, 1978

ABSTRACT

THE ISOLATION OF HAPLOID MYXAMOEBAL CLONES
FROM A DIPLOID APOMICTIC ISOLATE
OF THE MYXOMYCETE DIDYMIUM IRIDIS

Gary F. Jakupcin

Master of Science

Youngstown State University, 1978

Clones of myxamoebae which fail to produce plasmodia have been isolated from cultures of the Philippine-1 isolate of D. iridis. These clones have been maintained continuously in the laboratory for a period of more than three years without producing plasmodia. It has been demonstrated by Feulgen-DNA cytophotometry that the mean nuclear DNA content of such clones is equivalent to the haploid replicated (2C) value. Conversely, myxamoebal clones which produce plasmodia have an average nuclear DNA content which is equivalent to the diploid replicated (4C) value. These latter data are consistent with our earlier report that the Ph-1 isolate is apomictic (Therrien, C.D. and J.J. Yemma 1974. American Journal of Botany 61:400-404). The haploid Ph-1 clones have been demonstrated to be cross compatible with clones bearing the mating alleles A^1 and A^3 through A^{11}, and incompatible with clones bearing the mating allele A^2. Furthermore, plasmodia which result from the Ph-1_n x Pan 2-4 (A^7) cross have a mean nuclear DNA content which is equivalent to the diploid replicated value. Such data demonstrate that the Ph-1_n clones are sexually compatible with haploid amoebae of heterothallic isolates. The

significance of the isolation of these haploid clones with regard to a recent hypothesis related to the evolution of heterothallism in the Myxomycetes will be discussed.

TABLE OF CONTENTS

LIST OF FIGURES

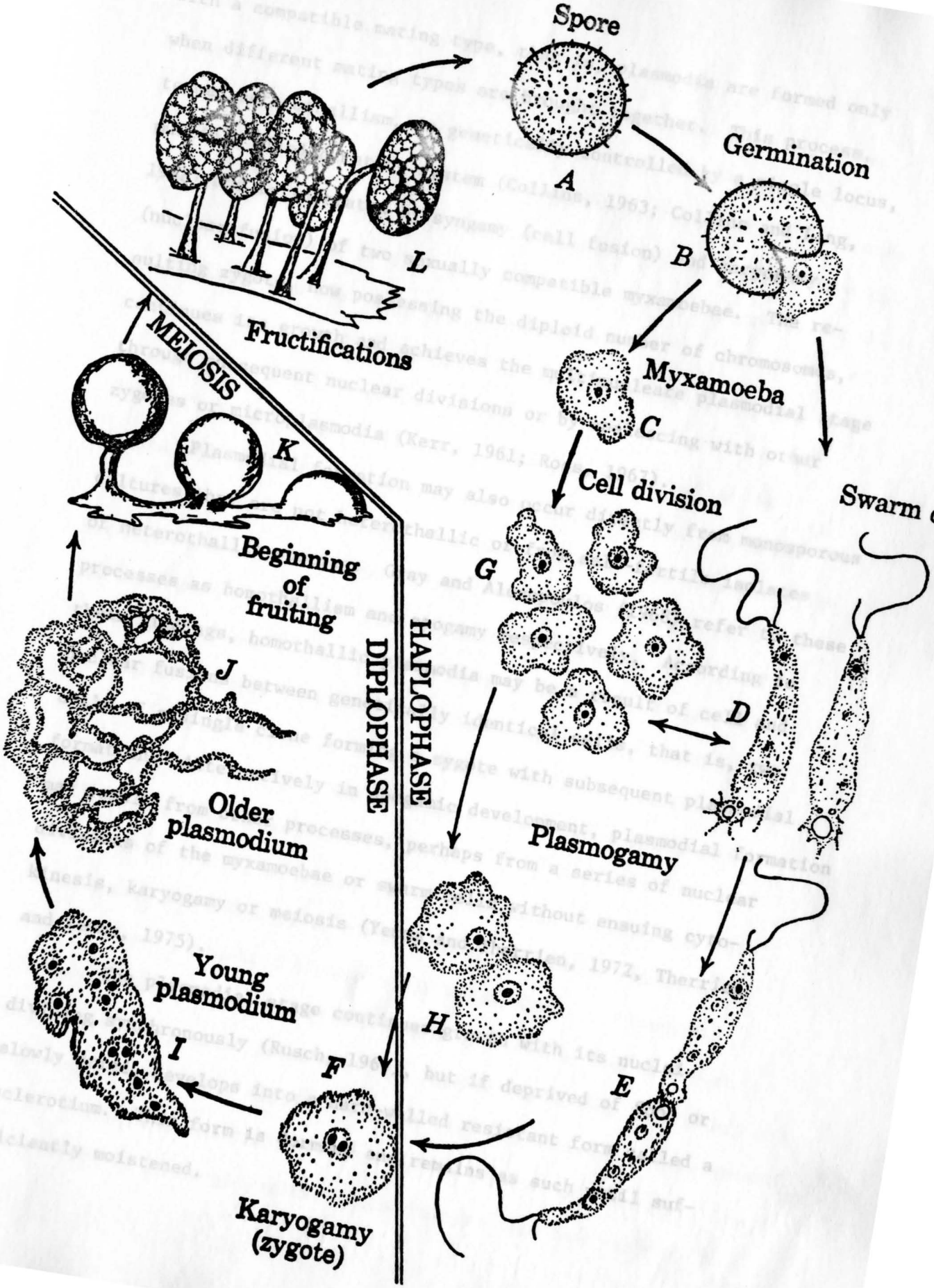
LIST OF TABLES

INTRODUCTION

The slime molds, or Myxomycetes, are eucharyotes which show the basic life cycle of higher eucharyotes in what may be its simplest form, the ability of a single cell to diverge from one cell type to another (Ashworth and Dee, 1975). The organism chosen for this study, Didymium iridis, is an acellular slime mold whose cellular divergence leads to a life cycle basically comprised of a unicellular myxamoeboid stage, a multinucleate plasmodial stage and a sporulation stage (Figure 1). Growth, isolation and study of these progressive changes provide an excellent investigative approach to the cell cycle, cyto-differentiation, cell movement and chemical changes characteristic of higher plants and animals.

In general, the myxamoeboid stage or haplophase of the life cycle, results from the germination of a spore. Upon germination, a single protoplast is released (Collins, 1961) and assumes the myxamoeboid form or, in an aqueous environment, develops two flagella and is referred to as a swarm cell. Both cell types are easily interconvertible. If the myxamoebae are provided bacteria as a food source, they will ingest the bacteria by means of pseudopodia and at intervals divide, resulting in a thriving population. As food is exhausted, the cells encyst and remain dormant until food is replenished.

The multinucleate plasmodial or assimilative stage may develop by several different processes. In some Myxomycete species, cultures grown from single spores will not give rise to plasmodia until mixed


Spore
A
Germination
B
L
Fructifications
MEIOSIS
K
Myxamoeba
C
Cell division
Swarm cel
Beginning
of
fruiting
G
D
J
DIPLOPHASE
HAPLOPHASE
Older
plasmodium
Plasmogamy
Young
plasmodium
H
I
F
E
Karyogamy
(zygote)

with a compatible mating type, that is, plasmodia are formed only when different mating types are brought together. This process, termed heterothallism, is genetically controlled by a single locus, multiple allelic mating system (Collins, 1963; Collins and Ling, 1968) and is initiated by syngamy (cell fusion) and karyogamy (nuclear fusion) of two sexually compatible myxamoebae. The resulting zygote, now possessing the diploid number of chromosomes, continues its growth and achieves the multinucleate plasmodial stage through subsequent nuclear divisions or by coalescing with other zygotes or microplasmodia (Kerr, 1961; Ross, 1967).

Plasmodial formation may also occur directly from monosporous cultures that are not heterothallic or from self-fertile isolates of heterothallic species. Gray and Alexopoulos (1968) refer to these processes as homothallism and apogamy respectively. According to their findings, homothallic plasmodia may be a result of cell and nuclear fusions between genetically identical cells, that is, two cells of a single clone forming a zygote with subsequent plasmodial formation. Alternatively in apogamic development, plasmodial formation may result from other processes, perhaps from a series of nuclear divisions of the myxamoebae or swarm cells without ensuing cytokinesis, karyogamy or meiosis (Yemma and Therrien, 1972, Therrien and Yemma, 1975).

The plasmodial stage continues growth with its nuclei dividing synchronously (Rusch, 1969), but if deprived of food or slowly dried, develops into a hard-walled resistant form called a sclerotium. This form is dormant and remains as such until sufficiently moistened.

Sporulation is an essential stage in the life cycle of a Myxomycete in which genetic reassortment and formation of gametes can occur. Spores allow an escape from conditions of starvation, cold or drought, surviving severe environmental conditions for months or even years (Alexopoulos, 1963). They can be dispersed to favorable situations by water or wind with resumption of the life cycle and the myxamoeboid form resulting from germination.

A comprehensive treatment of the Myxomycete life cycle is given by Gray and Alexopoulos (1968).

The sequential cell and nuclear events leading to the myxamoebal-plasmodial transition are key developmental processes in the Myxomycete life cycle. The mechanisms of this transformation, although elucidated to some extent by Kerr (1961), Koevenig (1964) and more recently by Ross (1967), Yemma and Therrien (1972) and Therrien and Yemma (1974; 1975), remain unresolved and open to speculation. Close examination of mature plasmodial cultures, which develop by either of the previously mentioned processes, always reveals viable myxamoebae surrounding the plasmodium which do not take part in this transitional process.

Ross (1967), in mating two heterothallic strains, was able to demonstrate an absence of syngamy among myxamoebae remaining in culture after plasmodial formation has taken place. He suggests that myxamoebae are not initially specialized cells, but have both vegetative and gametic potential. He further postulates that inducers secreted by myxamoebae initiate syngamy with the subsequent development of the diplophase eventually inhibiting any further cell fusion. The observation of extra-plasmodial myxamoebae has also been made by Dee

(1966) who implies some variation in the ability to form plasmodia among the cells of a clone. Whether these cells are in a state of genetic repression regarding the mating locus as previously reported by Ross (1967), or perhaps vary with respect to genetic content, biochemistry or ploidy from those cells which do produce plasmodia, is an interesting phenomenon, especially among homothallic strains, which up to now has never been adequately analyzed. The purpose of this investigation is to study this phenomenon more closely.

A previously reported homothallic strain of Didymium iridis, Philippine-1 (Therrien and Yemma, 1974; Collins, 1976), demonstrating the diploid content of DNA in both pre-plasmodial myxamoebae and plasmodial nuclei (Therrien and Yemma, 1974) was selected for examination. Cultures of post-plasmodial myxamoebae isolated from the vicinity of aged Philippine-1 (Ph-1) plasmodium have been maintained in this laboratory for several years and have never given rise to plasmodium. This post-plasmodial myxamoeboid clone will be designated as Ph-1_n.

Clark and Collins (1976), in studies of mating systems, reveal the existence of considerable variation in DNA content and chromosome numbers both between and within strains of Didymium iridis. They suggest that Myxomycetes are basically heterothallic, and that both homothallic and apogamic strains arise as satellite strains of the major heterothallic groups. They further theorize that selfing is the mechanism through which homothallic strains develop. This observation is based on the experiments performed by Yemma, Therrien and Ventura (1974) which demonstrate that heterothallic isolates possessing the selfing factor could be induced to form plasmodia 100% of the time through recloning. Clark and Collins also suggest that apogamy may

arise as a method of bypassing segregation problems due to the high occurrence of aneuploids and different DNA levels in mating clones.

The Philippine-1 isolate of Didymium iridis, being completely self-fertile (Therrien and Yemma, 1974), produces plasmodia 100% of the time in viable single spore isolations. Since some cells of a clone evidently do not participate in plasmodial formation (Dee, 1966; Ross, 1967), use of such an isolate provides a feasible way of analyzing possible variation among clonal cells regarding their potential or lack of potential to produce plasmodia without the mixing of cultures. Such an analysis could be accomplished through the comparison of DNA content of myxamoebae in culture with those isolated from the vicinity of the plasmodia, as well as DNA levels of other life cycle stages. Culturing techniques described by Collins (1963) allow for easy isolation of these various stages, and thus measurement of nuclear deoxyribonucleic acid by microspectrophotometry.

The Feulgen staining technique in conjunction with microspectrophotometry can be considered a valid approach to the investigation if the following conditions are met: (1) the Feulgen reaction is specific for DNA; (2) the amount of DNA can be used as an indication of ploidy. This criterion has been established in experimentation by numerous workers, including Mirsky and Ris (1949), Ris and Mirsky (1949) and Swift (1950). Further quantitative cytophotometric analysis has successfully been demonstrated in Didymium iridis by Therrien (1966), Yemma and Therrien (1972) and Therrien and Yemma (1974).

MATERIALS AND METHODS

Organisms and Culture Conditions

The isolates used in this study were originally supplied by Dr. O.R. Collins, Department of Botany, University of California. These isolates are designated as: Honduran 1-1 (Hon 1-1); Honduran 1-2 (Hon 1-2); Honduran 1-7 (Hon 1-7); Costa Rican 5-5 (CR 5-5); Panamanian 1-66-62.10 (Pan 1-66-62.10); Panamanian 1-66 (Pan 1-66); Costa Rican 2-25 (CR 2-25); Costa Rican 2-26 (CR 2-26); Panamanian 2-4 (Pan 2-4); Panamanian 2-6 (Pan 2-6); Panamanian 2-7 (Pan 2-7); Panamanian 3-1 (Pan 3-1); Panamanian 3-3 (Pan 3-3); Costa Rican 5-11 (CR 5-11); and Philippine-1 (Ph-1). The post-plasmodial myxamoebae clone of the Philippine-1 isolate ($Ph\text{-}1_n$) was previously isolated by Dr. John J. Yemma, Department of Biology, Youngstown State University.

All myxamoebal clones, including the post-plasmodial clone, were grown on solid media and innoculated with Escherichia coli according to the methods of Yemma and Therrien (1972). Initial cultures of the Ph-1 isolate were propagated from spores maintained in dry storage at $0^{o}C$ in their sporangium.

Populations of myxamoebae derived as direct descendents of single spores were required for certain aspects of this investigation. The isolation procedure involved: (1) streaking an entire sporangium on a sterile agar plate; (2) transferring a section of agar to a second plate, then streaking it to further dispense the spores; (3) isolating a section of agar containing a single spore with the aid of an insect dissecting needle; (4) transferring the agar section

to a culture medium plate previously innoculated with a suspension of E. coli; and (5) moistening the spore with the addition of a drop of sterile distilled water. Myxamoebae resulting from germinating Ph-1 spore isolates were subcultured every 24 hours to avoid the rapid plasmodial formation occurring within the isolate.

In order to obtain a representative myxamoebal population for the Ph-1 isolate, the spores of an entire sporangium were incubated in culture. Spores were initially separated by mechanically disintegrating a sporangium in 10 ml. of distilled water within a conical centrifuge tube. The suspension was then washed with several changes of distilled water before dispensing the mixture onto a culture plate previously innoculated with E. coli. After germination occurred, subculturing was performed prior to plasmodial formation.

Cultures of myxamoebae maintained on slants were transferred to culture plates previously innoculated with E. coli. Subcultures were made once the log phase of growth had been achieved. All cultures of myxamoebae were maintained in an incubator at 21°C (Yemma and Therrien, 1972; Yemma et al., 1974).

In order to obtain a plasmodium, subcultures of single spore isolates were sustained in culture until one spontaneously appeared, generally 24 to 36 hours after germination. The plasmodia, becoming sufficiently vigorous after three to five days, were placed under light-dark conditions to induce sporulation. Crosses between the various isolates analyzed were obtained by dispensing a 1 ml. cell suspension of each isolate onto culture media previously innoculated with E. coli. Control cultures were always simultaneously made. All necessary precautions were taken to avoid contamination.

Preparation of Material for Cytochemical Studies

Myxamoebae

(1) Cultures of myxamoebae were flooded with sterile distilled water to remove the cells from the culture media.

(2) Suspensions of myxamoebae were then transferred to conical centrifuge tubes and centrifuged at 5X gravity to remove any debris.

(3) These were in turn centrifuged at 70X gravity, the supernatent containing bacteria discarded, and the pellet flooded with 10% buffered formalin (pH 7.0). This was allowed to fix for a period of 12 to 18 hours.

(4) The fixed myxamoebae were then washed with two changes of 70% ethanol and allowed to post-fix in 70% ethanol for 12 hours.

(5) The suspensions were again centrifuged at 70X gravity, the supernatents decanted and the fixed myxamoebae retained. This step was repeated until a bacteria-free plug was obtained.

(6) Finally, the plug was resuspended in 70% alcohol and the cells affixed to previously albuminized slides.

Spores

(1) Sporangia were fixed at various hourly stages

in their development. Fixation was carried out in 10% buffered formalin (pH 7.0) for a period of 12 to 18 hours.

(2) The fixed sporangia were then washed twice in 70% ethanol and allowed to post-fix for 12 hours in the alcohol.

(3) After fixation, the tissue was dehydrated in a graded ethanol series, passed through two changes of xylene and imbedded in paraffin.

(4) Sections were cut at 5 microns and affixed to previously albuminized slides.

Plasmodia

Paraffin imbedded plasmodia used in this study were prepared and affixed to slides in a manner similiar to the preparation of spores.

Cytochemical Methods

The Feulgen nucleal reaction (Feulgen and Rossenback, 1924; as modified by Therrien, 1966; and Bryant and Howard, 1969) was employed for the specific localization of deoxyribonucleic acid. Prior to staining, paraffin sections of plasmodia and sporangia were cleared in xylene and then hydrated through a graded ethanol series to distilled water. Slides of myxamoebae were immersed in 70% ethanol, then rinsed in distilled water before proceeding through the following staining technique.

(1) Acid hydrolyze the tissue with 5.0 N hydrochloric acid

at room temperature for 43 minutes.

(2) Stain for one hour in Schiff's reagent. The basic fuchsin used to prepare the dye was obtained from Fisher Scientific Company (C.I. #42500).

(3) Rinse twice for 5 minutes in bisulfite rinse freshly prepared by adding 5 ml. of 1.0 N HCl and 5 ml. of 10% potassium meta-bisulfite to 100 ml. of distilled water.

(4) Rinse in distilled water, then dehydrate in a graded ethanol series.

(5) Clear in xylene, then mount in media of proper refractive index.

Cytophotometric Methods

All cytophotometric determinations were made on a Zeiss Type 01 microspectrophotometer equipped with a continuous interference monochrometer (Zeiss #47 43 10) for wavelength isolation. Prior to operation, the instrument was checked for proper alignment and photo-tube linearity. All measurements were made with a Planachromat oil immersion objective, N.A. 1.30 x 100.

The two-wavelength method of microspectrophotometry (Patau, 1952; and Ornstein, 1952) was utilized for the photometric determination of relative amounts of Feulgen stained DNA. This method eliminates the need for direct measurement of nuclear area (Mayall and Mendelsohn, 1970) and corrects for errors caused by heterogenous distributions of stained material. Experimental slides of each comparison test were stained simultaneously to obtain a uniformity in staining intensity and absorption curves.

An estimation of absorbing material depends upon the difference between transmissions at two wavelengths (1 and 2), where wavelength 2 corresponds to the maximum extinction (E_2) of a spectral absorption curve and wavelength 1 to the wavelength of half maximum extinction (E_1). A homogenous area of specimen is chosen for this measurement with the extinction equal to the log I_o/I_s; I_o is the intensity of the background light and I_s the intensity of light after passing through the specimen. Therefore, $E = I_o/I_s$ at wavelength 2, $E_1 = I_o/I_s$ at wavelength 1 and $2E_1 = E_2$. Wavelengths selected for this investigation conform to previous experimentation of Therrien and Yemma (1974) with the Ph-1 isolate.

Measurements of absorbing material were made on nuclei centered at the optical axis and enclosed within a photometric field adjusted to allow a minimum amount of unoccupied space at its circumference. The amount of dye (M) within this field (A) was determined by the equation $M = KAL_1C$. K, the absorptivity constant required for determination of absolute values, was eliminated from the equation in this study of relative DNA determinations. L_1 and L_2 were calculated from transmissions readings ($T = I_s/I_o$) taken at wavelength 1 and wavelength 2 for each nucleus; $L_1 = 1-T_1$ and $L_2 = 1-T_2$. C, the correction factor for the distributional error (Swift and Rasch, 1956; Leuchtenberger, 1958) was determined from the ratio (Q) between L_1 and L_2. ($Q = L_2/L_1$). The Q value permits an elimination of the influence of unoccupied portions of measured area since the extinction ratio at the two wavelengths is 2:1. A series of C values and corresponding Q ratios can be found in a table formulated by Patau (1952).

Calculations of relative values of DNA were performed on an IBM 370 Model 145 computer.

RESULTS

The results of Feulgen-DNA measurements are presented graphically as histograms. The histograms represent nuclear DNA frequency distributions of cellular populations and provide a means for detection of any shifts in DNA content. Measurements are reported in units as relative amounts of DNA. Results of the genetic analysis conducted during this investigation are illustrated in both chart and tabular form.

Microspectrophotometric Analysis of Nuclear DNA in Didymium iridis

Selection of Wavelengths

The two-wavelength method of microspectrophotometry was utilized in this study. The wavelengths (560 nm and 505 nm) were selected to correspond with maximum and half maximum absorption values of Feulgen-DNA spectral absorption curves previously established under similiar experimentation and instrumentation (Yemma and Therrien, 1972; Therrien and Yemma, 1974).

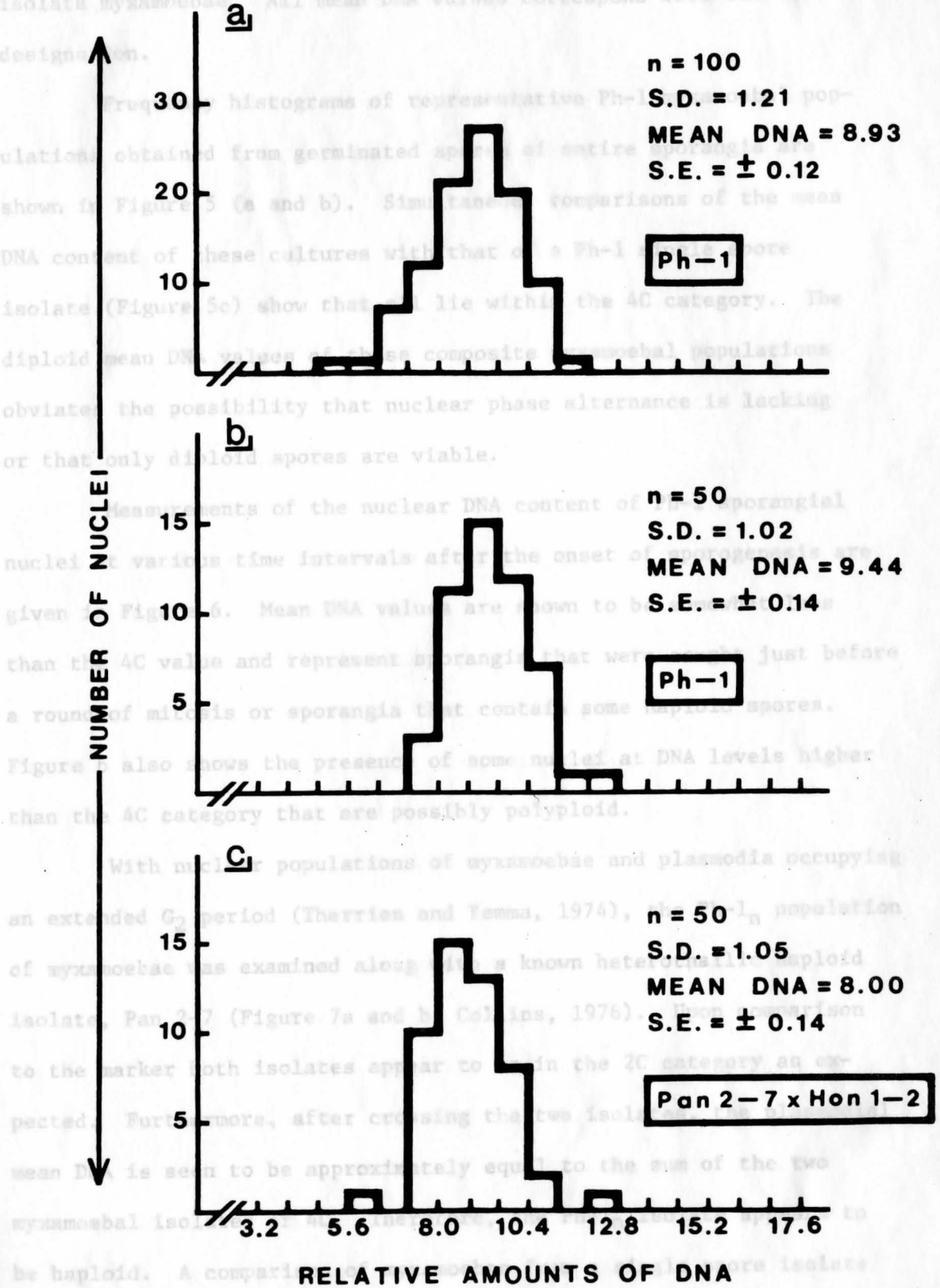
Deoxyribonucleic acid was measured in the myxamoebal, plasmodial and sporangial stages of the Ph-1 isolate, myxamoebal stage of the Ph-1_n and Pan 2-7 isolates, and plasmodial stage of the Ph-1_n x Pan 2-4, Ph-1_n x Pan 2-7, Ph-1_n x Hon 1-1 and Pan 2-7 x Hon 1-2 interisolate crosses. The resulting data is presented in the form of frequency histograms with the number of nuclei on the abscissa and the dye concentrations (in arbitrary units) on the ordinate. Visualization and comparison of such DNA profiles facilitates the

detection of mitotic activity, DNA synthetic activity and ploidy within or among cell populations. A representative random sample of 50 to 100 nuclei in each stage of the life cycle studies was measured and analyzed. Therefore, the histograms were not influenced by the chance occurrence and measurement of a small population of nuclei of a different ploidy level. The results are shown in Figures 2-9.

Analysis of Feulgen-DNA

Swift's (1950) designations of C, 2C and 4C are used to describe haploid, diploid and tetraploid DNA contents, respectively. It has been previously demonstrated that plasmodia formed from heterothallic isolates is diploid (Therrien, 1966) and that plasmodial nuclei are predominately in the replicated diploid state 99% of the time (Yemma and Therrien, 1972). The plasmodium resulting from the cross between the Pan 2-7 and Hon 1-2 isolates (Figure 2c) has the 4C amount of DNA and serves as an appropriate ploidy marker. The occurrence of nuclear phase alternance as well as ploidy can be determined in each life cycle stage studied by making comparisons to the marker.

Histograms representing Ph-1 myxamoebae and the Ph-1 plasmodium shown in Figure 2 (a and b) suggest that if the Pan 2-7 x Hon 1-2 plasmodium is in category 4C, then the Ph-1 myxamoebae and the Ph-1 plasmodium must also be in category 4C. This is consistent with data presented by Therrien and Yemma (1974) stating that myxamoebae of the Ph-1 isolate are at the same ploidy level as the Ph-1 plasmodium and supports their observation that the plasmodium of this isolate develops by apogamy.

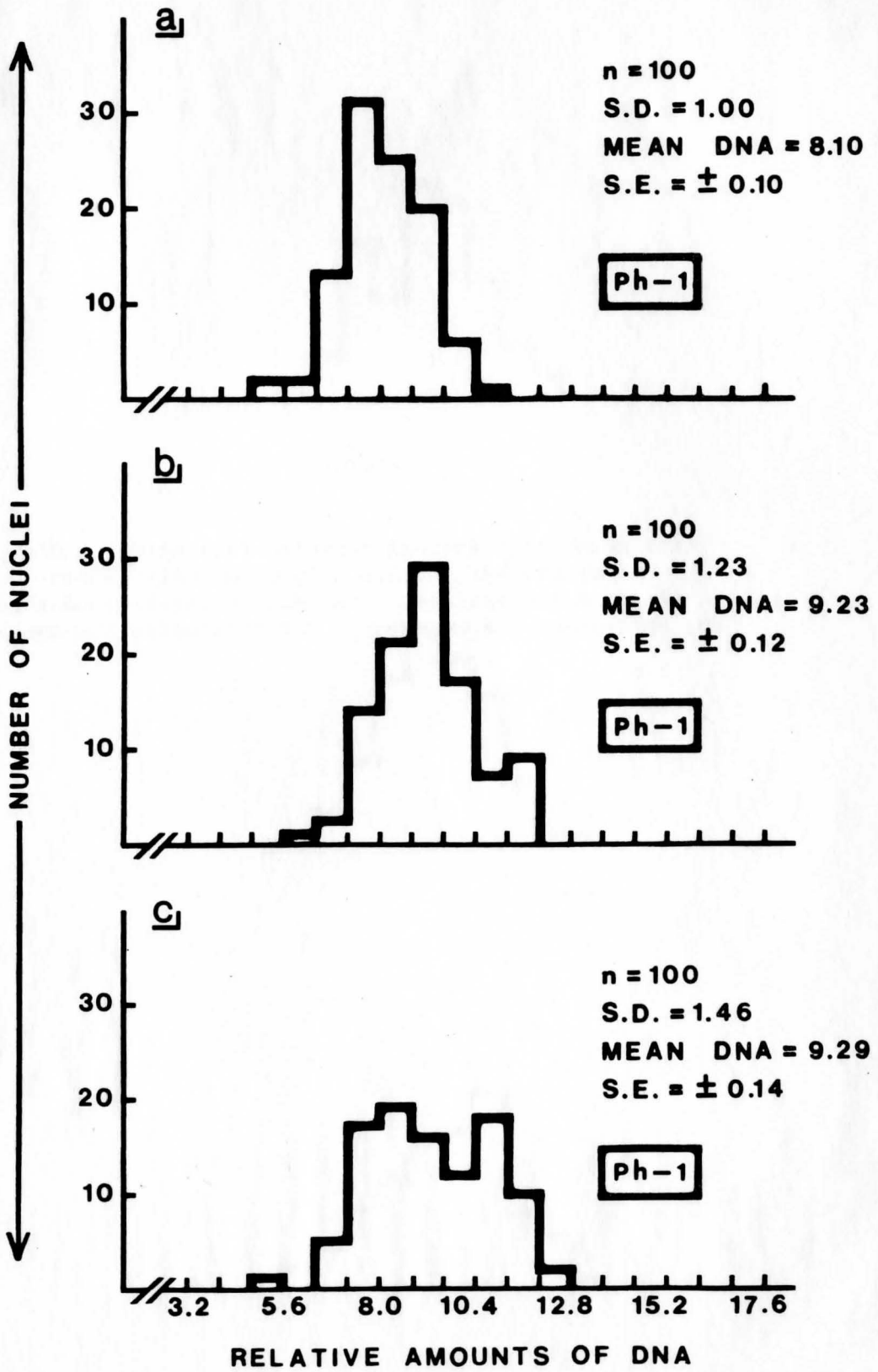

a
n = 100
S.D. = 1.21
MEAN DNA = 8.93
S.E. = ± 0.12
Ph—1
b
n = 50
S.D. = 1.02
MEAN DNA = 9.44
S.E. = ± 0.14
Ph—1
c
n = 50
S.D. = 1.05
MEAN DNA = 8.00
S.E. = ± 0.14
Pan 2—7 x Hon 1—2
NUMBER OF NUCLEI
30
20
10
15
10
5
15
10
5
3.2
5.6
8.0
10.4
12.8
15.2
17.6
RELATIVE AMOUNTS OF DNA

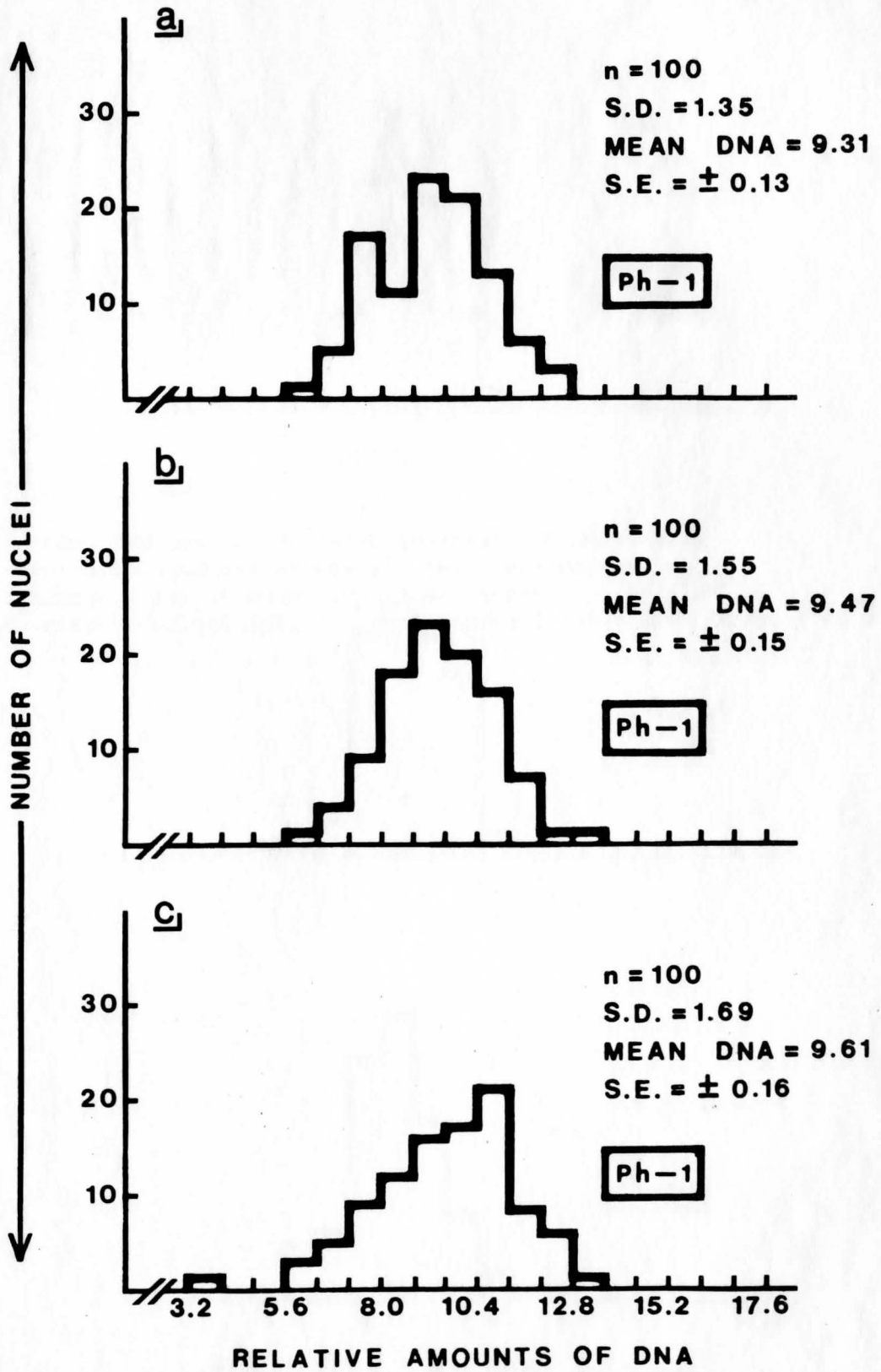
Figures 3 and 4 show comparisons among Ph-1 single spore isolate myxamoebae. All mean DNA values correspond with the 4C designation.

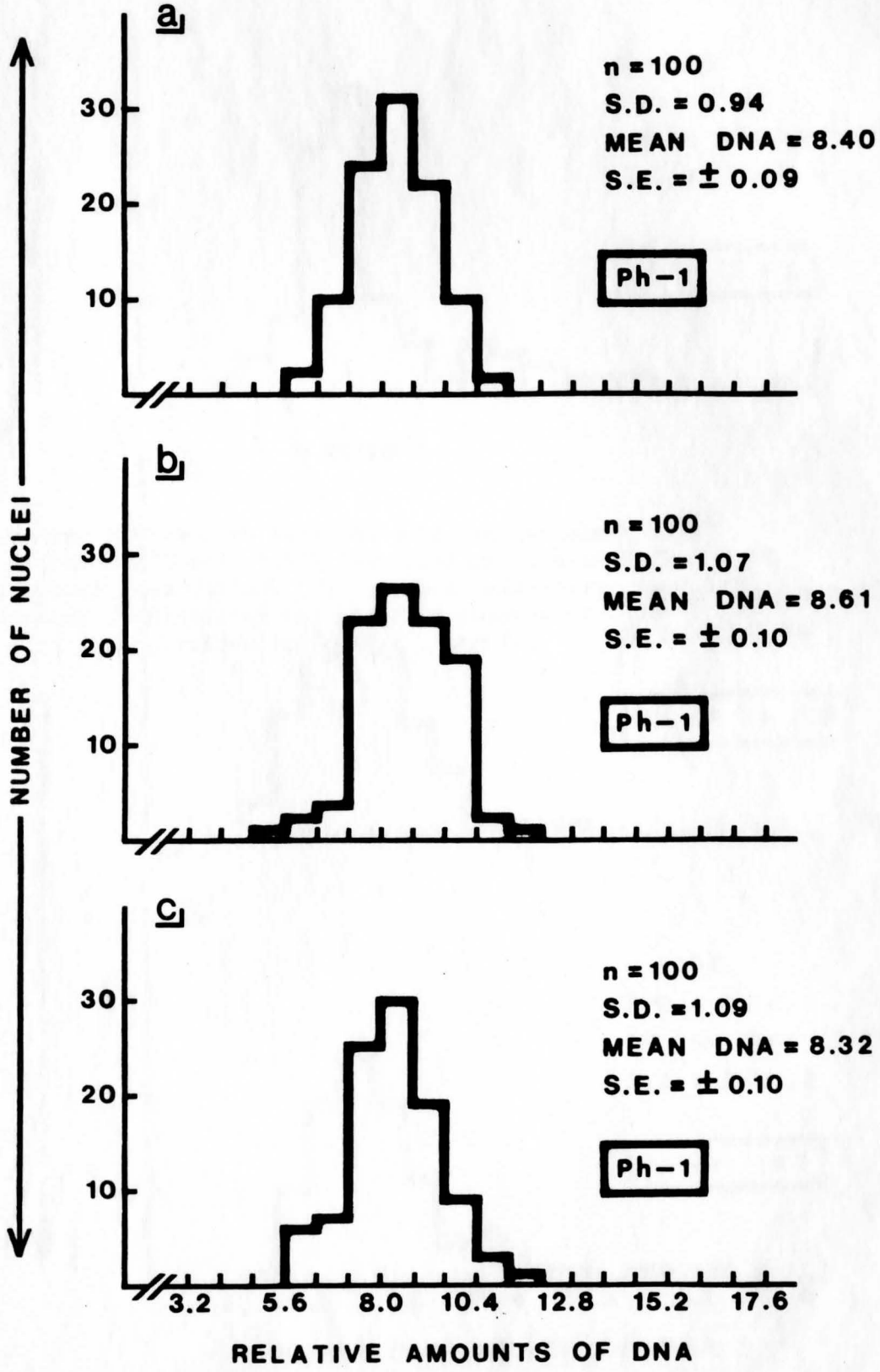
Frequency histograms of representative Ph-1 myxamoebal populations obtained from germinated spores of entire sporangia are shown in Figure 5 (a and b). Simultaneous comparisons of the mean DNA content of these cultures with that of a Ph-1 single spore isolate (Figure 5c) show that all lie within the 4C category. The diploid mean DNA values of these composite myxamoebal populations obviates the possibility that nuclear phase alternance is lacking or that only diploid spores are viable.

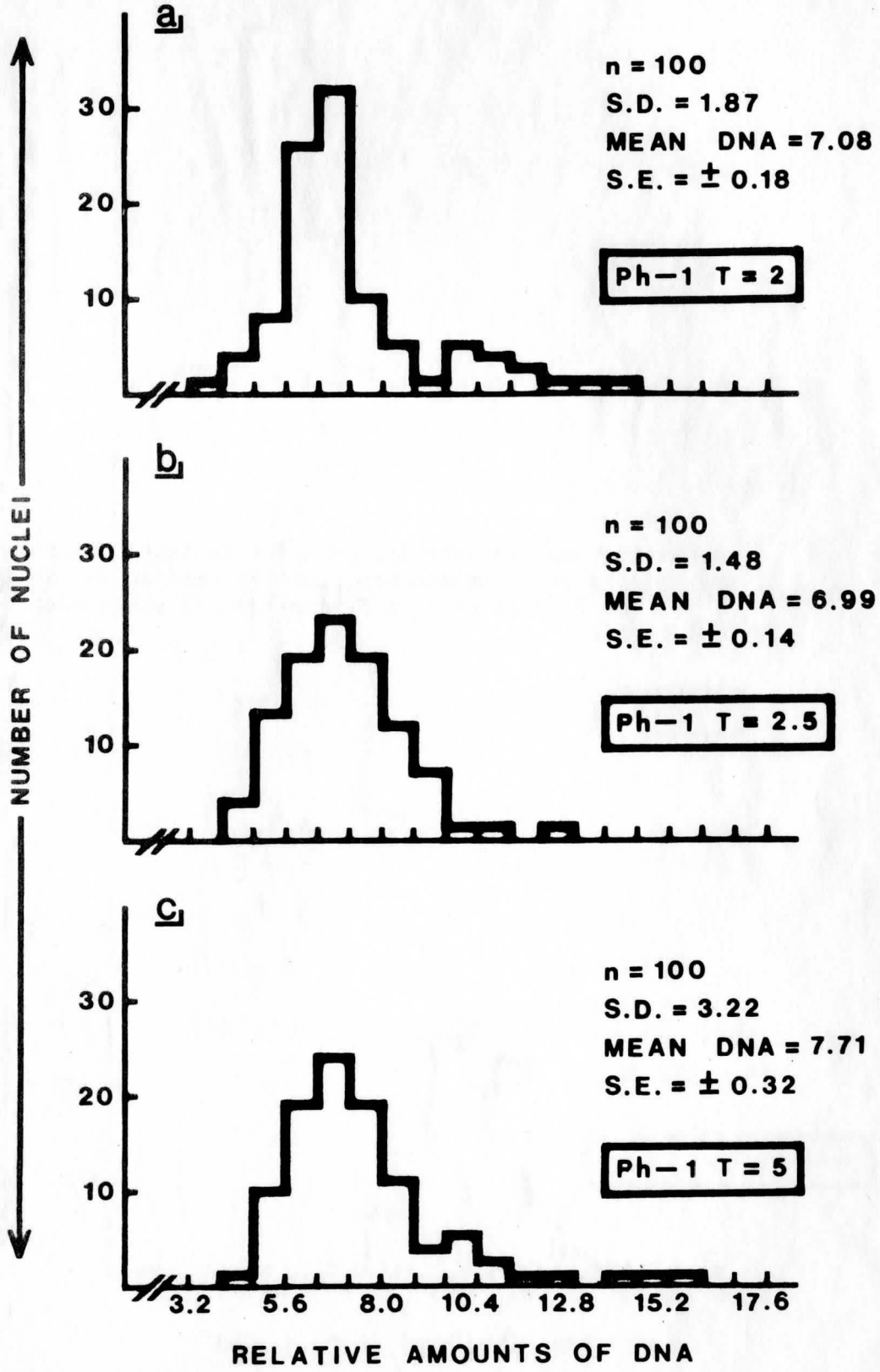
Measurements of the nuclear DNA content of Ph-1 sporangial nuclei at various time intervals after the onset of sporogenesis are given in Figure 6. Mean DNA values are shown to be somewhat less than the 4C value and represent sporangia that were caught just before a round of mitosis or sporangia that contain some haploid spores. Figure 6 also shows the presence of some nuclei at DNA levels higher than the 4C category that are possibly polyploid.

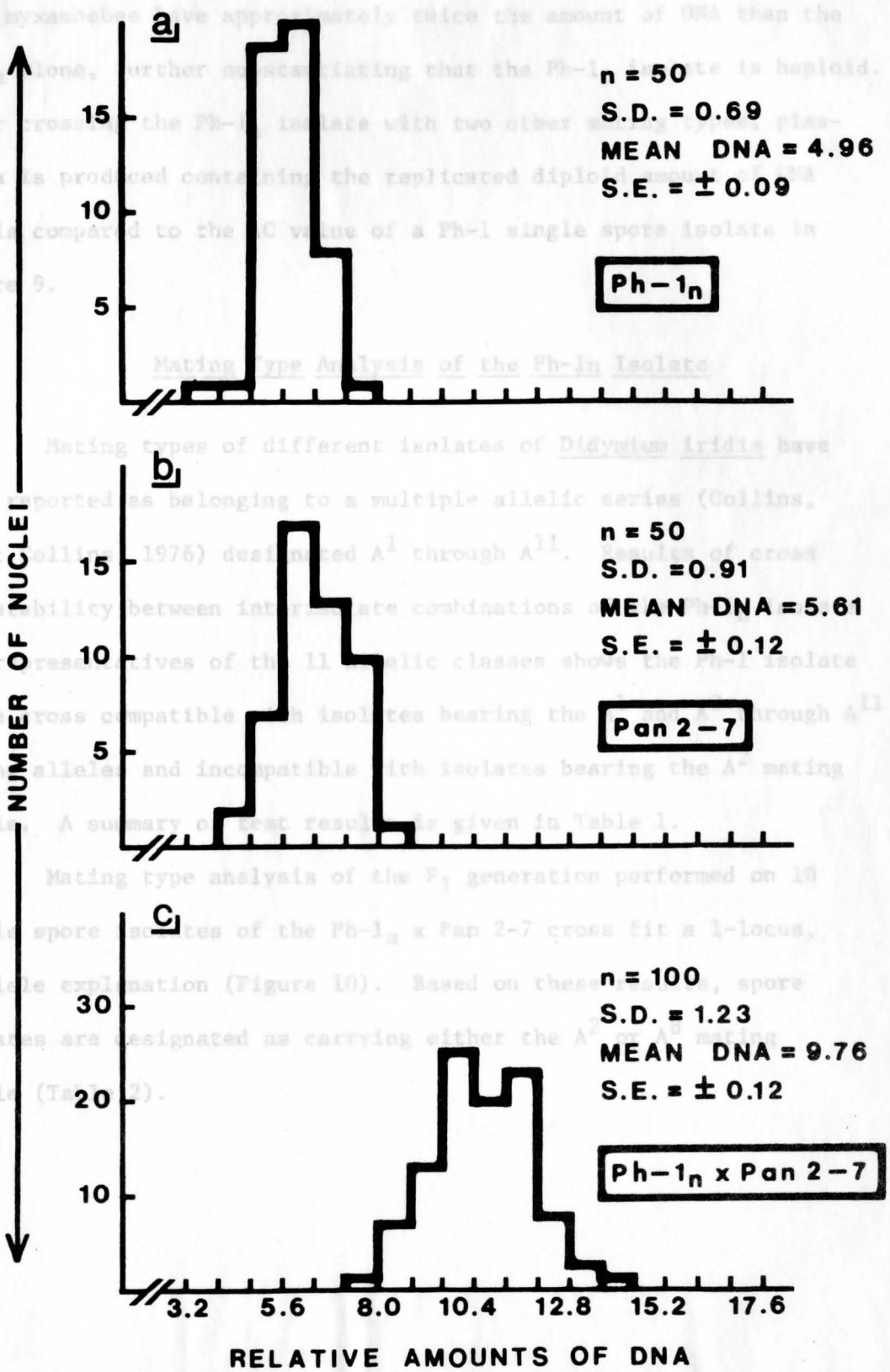
With nuclear populations of myxamoebae and plasmodia occupying an extended G_2 period (Therrien and Yemma, 1974), the Ph-1_n population of myxamoebae was examined along with a known heterothallic haploid isolate, Pan 2-7 (Figure 7a and b; Collins, 1976). Upon comparison to the marker both isolates appear to be in the 2C category as expected. Furthermore, after crossing the two isolates, the plasmodial mean DNA is seen to be approximately equal to the sum of the two myxamoebal isolates or 4C. Therefore, the Ph-1_n isolate appears to be haploid. A comparison of myxamoebae from a single spore isolate


a)
n = 100
S.D. = 1.00
MEAN DNA = 8.10
S.E. = ± 0.10
Ph-1
b)
n = 100
S.D. = 1.23
MEAN DNA = 9.23
S.E. = ± 0.12
Ph-1
c)
n = 100
S.D. = 1.46
MEAN DNA = 9.29
S.E. = ± 0.14
Ph-1
NUMBER OF NUCLEI
30
20
10
3.2
5.6
8.0
10.4
12.8
15.2
17.6
RELATIVE AMOUNTS OF DNA


a
n = 100
S.D. = 1.35
MEAN DNA = 9.31
S.E. = ± 0.13
Ph−1
b
n = 100
S.D. = 1.55
MEAN DNA = 9.47
S.E. = ± 0.15
Ph−1
c
n = 100
S.D. = 1.69
MEAN DNA = 9.61
S.E. = ± 0.16
Ph−1
30
20
10
NUMBER OF NUCLEI
3.2
5.6
8.0
10.4
12.8
15.2
17.6
RELATIVE AMOUNTS OF DNA


a
n = 100
S.D. = 0.94
MEAN DNA = 8.40
S.E. = ± 0.09
Ph−1
b
n = 100
S.D. = 1.07
MEAN DNA = 8.61
S.E. = ± 0.10
Ph−1
c
n = 100
S.D. = 1.09
MEAN DNA = 8.32
S.E. = ± 0.10
Ph−1
30
20
10
NUMBER OF NUCLEI
3.2
5.6
8.0
10.4
12.8
15.2
17.6
RELATIVE AMOUNTS OF DNA


a
n = 100
S.D. = 1.87
MEAN DNA = 7.08
S.E. = ± 0.18
Ph−1 T = 2
b
n = 100
S.D. = 1.48
MEAN DNA = 6.99
S.E. = ± 0.14
Ph−1 T = 2.5
c
n = 100
S.D. = 3.22
MEAN DNA = 7.71
S.E. = ± 0.32
Ph−1 T = 5
30
20
10
NUMBER OF NUCLEI
3.2
5.6
8.0
10.4
12.8
15.2
17.6
RELATIVE AMOUNTS OF DNA

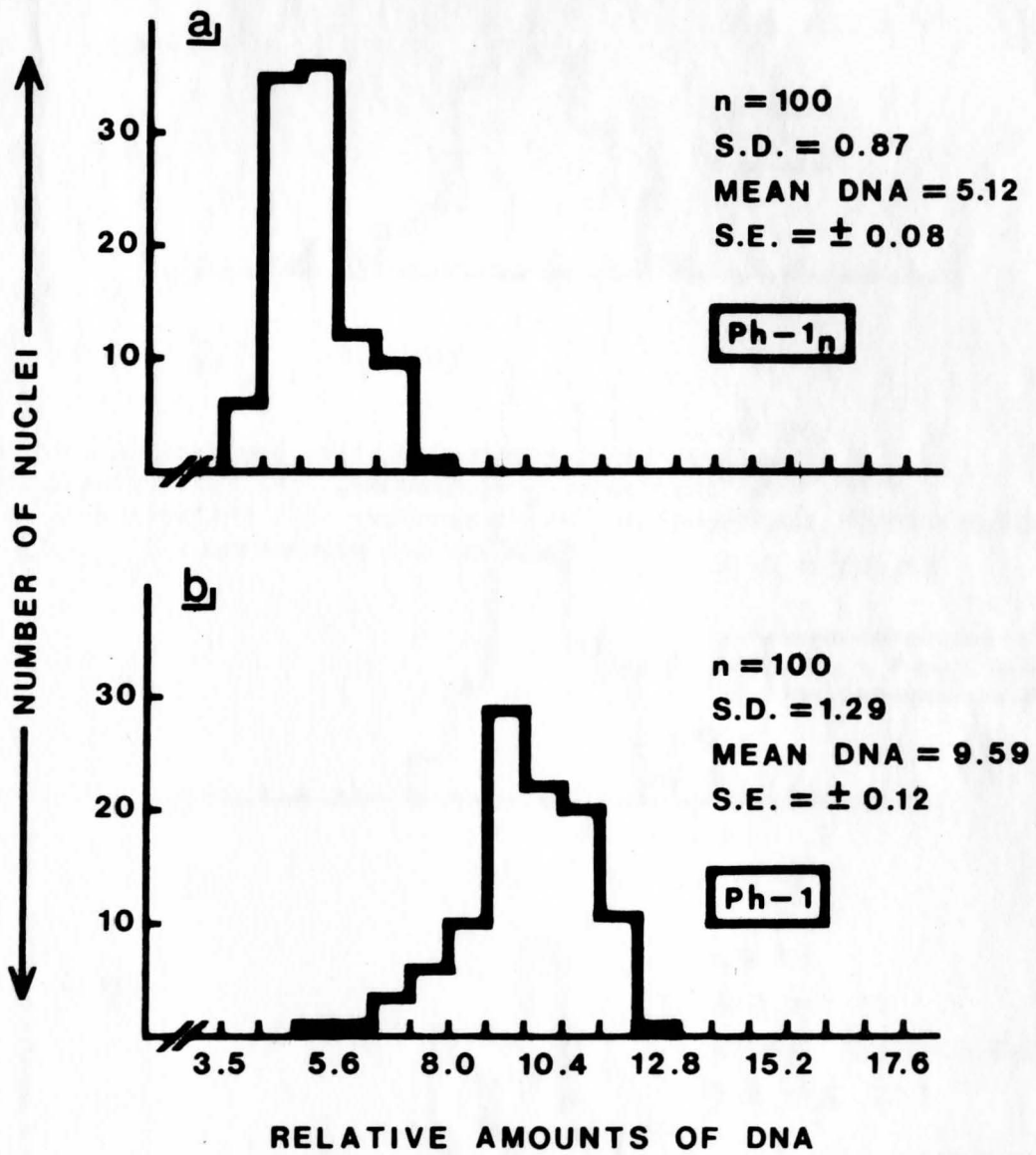

a
n = 50
S.D. = 0.69
MEAN DNA = 4.96
S.E. = ± 0.09
Ph-1n
b
n = 50
S.D. = 0.91
MEAN DNA = 5.61
S.E. = ± 0.12
Pan 2-7
c
n = 100
S.D. = 1.23
MEAN DNA = 9.76
S.E. = ± 0.12
Ph-1n x Pan 2-7
NUMBER OF NUCLEI
5
10
15
20
30
3.2
5.6
8.0
10.4
12.8
15.2
17.6
RELATIVE AMOUNTS OF DNA

off the Ph-1 clone with Ph-1_n myxamoebae (Figure 8) shows that the Ph-1 myxamoebae have approximately twice the amount of DNA than the Ph-1_n clone, further substantiating that the Ph-1_n isolate is haploid. After crossing the Ph-1_n isolate with two other mating types, plasmodia is produced containing the replicated diploid amount of DNA and is compared to the 4C value of a Ph-1 single spore isolate in Figure 9.

Mating Type Analysis of the Ph-1n Isolate

Mating types of different isolates of Didymium iridis have been reported as belonging to a multiple allelic series (Collins, 1963; Collins, 1976) designated A^1 through A^{11}. Results of cross compatability between interisolate combinations of the Ph-1_n isolate and representatives of the 11 allelic classes shows the Ph-1 isolate to be cross compatible with isolates bearing the A^1 and A^3 through A^{11} mating alleles and incompatible with isolates bearing the A^2 mating allele. A summary of test results is given in Table 1.

Mating type analysis of the F_1 generation performed on 10 single spore isolates of the Ph-1_n x Pan 2-7 cross fit a 1-locus, 2 allele explanation (Figure 10). Based on these results, spore isolates are designated as carrying either the A^2 or A^8 mating allele (Table 2).


a
30
20
10
n = 100
S.D. = 0.87
MEAN DNA = 5.12
S.E. = ± 0.08
Ph−1n
b
30
20
10
n = 100
S.D. = 1.29
MEAN DNA = 9.59
S.E. = ± 0.12
Ph−1
NUMBER OF NUCLEI
3.5
5.6
8.0
10.4
12.8
15.2
17.6
RELATIVE AMOUNTS OF DNA

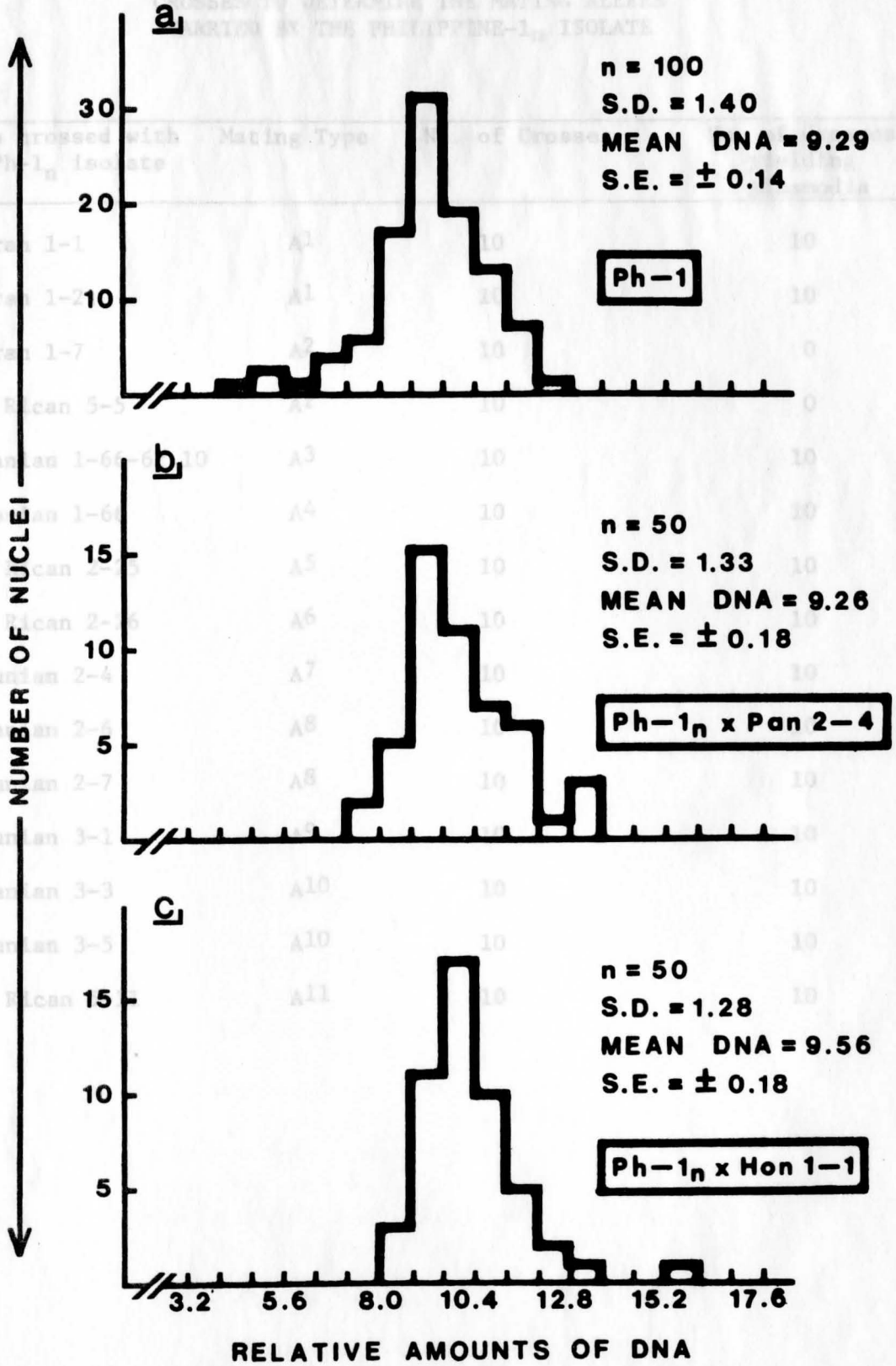

a
n = 100
S.D. = 1.40
MEAN DNA = 9.29
S.E. = ± 0.14
Ph−1
b
n = 50
S.D. = 1.33
MEAN DNA = 9.26
S.E. = ± 0.18
Ph−1n x Pan 2−4
c
n = 50
S.D. = 1.28
MEAN DNA = 9.56
S.E. = ± 0.18
Ph−1n x Hon 1−1
NUMBER OF NUCLEI
30
20
10
15
10
5
15
10
5
3.2
5.6
8.0
10.4
12.8
15.2
17.6
RELATIVE AMOUNTS OF DNA

TABLE 1

CROSSES TO DETERMINE THE MATING ALLELE CARRIED BY THE PHILIPPINE-1_n ISOLATE

Clones crossed with the Ph-1_n isolate	Mating Type	No. of Crosses	No. of Crosses yielding plasmodia
Honduran 1-1	A^1	10	10
Honduran 1-2	A^1	10	10
Honduran 1-7	A^2	10	0
Costa Rican 5-5	A^2	10	0
Panamanian 1-66-62.10	A^3	10	10
Panamanian 1-66	A^4	10	10
Costa Rican 2-25	A^5	10	10
Costa Rican 2-26	A^6	10	10
Panamanian 2-4	A^7	10	10
Panamanian 2-6	A^8	10	10
Panamanian 2-7	A^8	10	10
Panamanian 3-1	A^9	10	10
Panamanian 3-3	A^{10}	10	10
Panamanian 3-5	A^{10}	10	10
Costa Rican 5-11	A^{11}	10	10

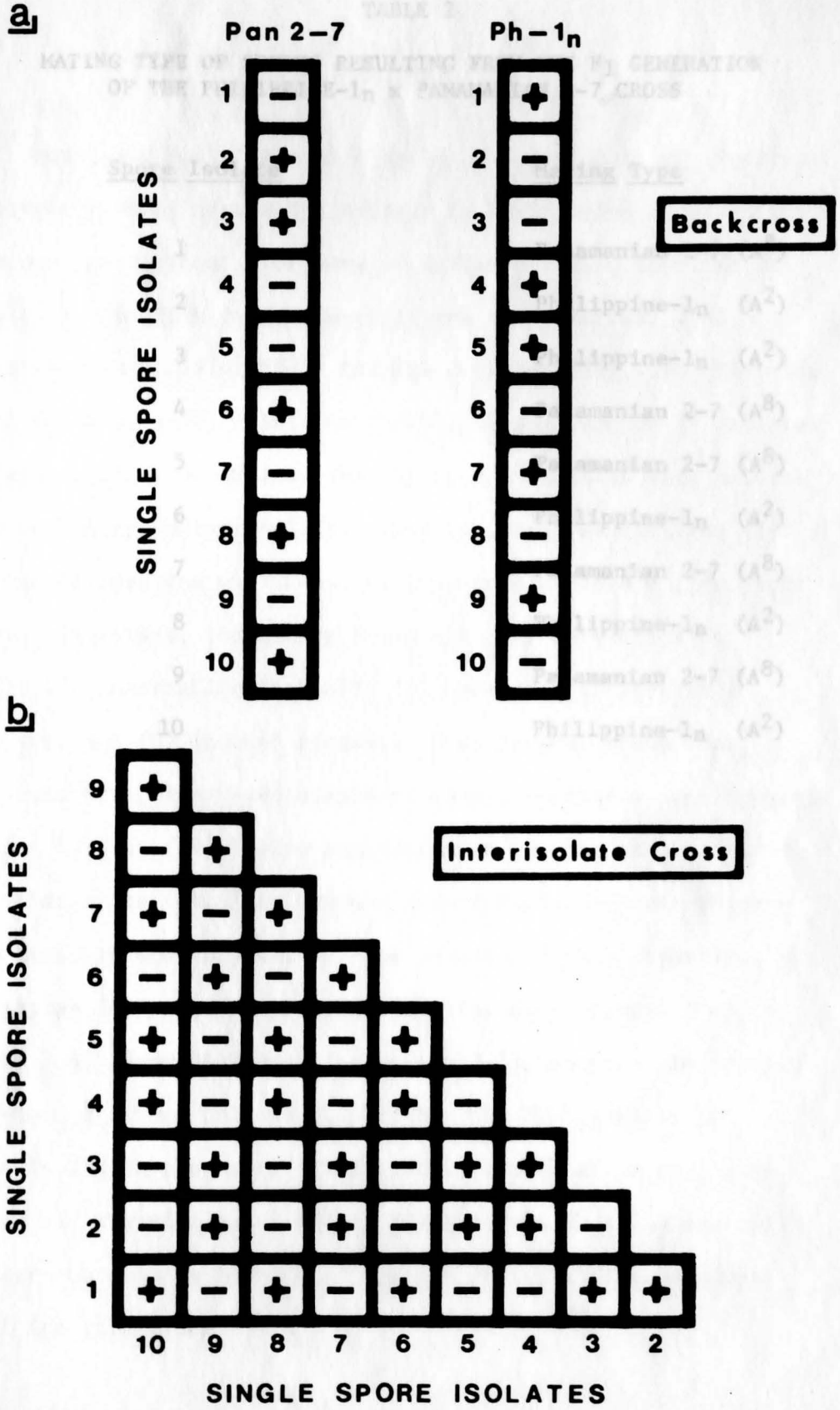

a
Pan 2–7
Ph–1n
SINGLE SPORE ISOLATES
1
2
3
4
5
6
7
8
9
10
Backcross
b
SINGLE SPORE ISOLATES
9
8
7
6
5
4
3
2
1
10 9 8 7 6 5 4 3 2
SINGLE SPORE ISOLATES
Interisolate Cross

TABLE 2

MATING TYPE OF SPORES RESULTING FROM THE F_1 GENERATION OF THE PHILIPPINE-1_n x PANAMANIAN 2-7 CROSS

Spore Isolate	Mating Type
1	Panamanian 2-7 (A^8)
2	Philippine-1_n (A^2)
3	Philippine-1_n (A^2)
4	Panamanian 2-7 (A^8)
5	Panamanian 2-7 (A^8)
6	Philippine-1_n (A^2)
7	Panamanian 2-7 (A^8)
8	Philippine-1_n (A^2)
9	Panamanian 2-7 (A^8)
10	Philippine-1_n (A^2)

DISCUSSION

Recent studies of the life cycle of Didymium iridis indicate considerable variability among isolates in their means of executing the various transitional processes of differentiation involved in completion of the life cycle phases (Yemma and Therrien, 1972; Therrien and Yemma, 1974, 1975; Collins and Therrien, 1976; Therrien, Bell and Collins, 1977). Collins (1976), on analysis of 27 isolates of Didymium iridis, determined that 11 of the isolates were heterothallic and 16 non-heterothallic. Therrien, Bell and Collins (1977) further subdivided the way these isolates complete their life cycle into five categories, indicating these as: (1) heterothallic (sexual); (2) homothallic (sexual); (3) apogamic (non-sexual); (4) selfing; and (5) induced apogamy. They further state that mating types have never been displayed among homothallic and apogamic isolates. Collins (1976) also suggests from results of preliminary testing that cross fertility between heterothallic and non-heterothallic isolates does not exist. The results of this investigation, demonstrating failure of nuclear phase alternance between Ph-1 myxamoebal and plasmodial nuclei (Figures 2-5), support the premise of Therrien, Bell and Collins (1977) that the Ph-1 isolate is apogamic in its development. This in turn supports the prior observations of Therrien and Yemma (1974) that the Ph-1 isolate fails to demonstrate a nuclear phase alternance during the transitional stage of its life cycle.

The results also demonstrate, through analysis of mean nuclear DNA content, a reduction in ploidy level from the diploid state of Ph-1 pre-plasmodial myxamoebae (Figures 2-5), plasmodia (Figure 2b) and sporangia (Figure 7), to a haploid level for myxamoebae isolated from the vicinity of aged plasmodia (Figures 7a and 8a) when appropriate comparisons are made to isolates demonstrating characteristic nuclear DNA values in various life cycle stages (Therrien and Yemma, 1974, 1975; Collins and Therrien, 1976). These haploid post-plasmodial myxamoebae, designated as Ph-1_n, further demonstrate the essential characteristics of a heterothallic isolate according to Therrien, Bell and Collins (1977) by: (1) never yielding plasmodia in isolated clonal cultures; (2) forming diploid plasmodia in crosses with established heterothallic haploid isolates (Figures 7c, 9b and 9c); (3) showing cross compatibility with isolates displaying the A^1 and A^3 through A^{11} mating types and non-compatibility with isolates bearing the A^2 mating type (Table 1); and (4) the recovery of mating types from the F_1 generation of the Ph-1 x Pan 2-7 cross (Figure 9 and Table 2). Therefore, the Ph-1 isolate, through the use of microspectrophotometry and genetic and cross-compatibility testing, shows the characteristics of an apogamic isolate in its ability to complete its life cycle, but also the characteristics of a heterothallic isolate through its ability to give rise to an active haploid counterpart, that is, when a culture of this isolate achieves the exponential phase of growth, a heterogeneous population of cells regarding nuclear ploidy level is evident, although the diploid value usually predominates (Figures 2-5).

The appearance of haploid myxamoebae in stable diploid strains and diploid myxamoebae in stable haploid strains has been reported by Sussman and Sussman (1962) in their work with the cellular slime mold Dictostelium discoideum. However, up to this time, no previous studies regarding this occurrence in the acellular slime mold have ever been reported. The occurrence of haploids among diploids or diploids among haploids in Sussman and Sussman's study was not encountered with analysis of myxamoebae in the exponential phase of growth, but only when myxamoebae were analyzed in early stationary phase. Even in this case, myxamoebae appeared only in the order of one in several hundred or less. The presence of a unimodal distribution regarding myxamoebal populations (Figures 2-5) has been explained by Yemma and Therrien (1972) and is due to the almost complete lack of a G_1 phase among growing cellular populations. Although the greatest number of cells in the Ph-1 population have the diploid amount of nuclear DNA, it is evident some nuclei in these populations have the haploid amount of DNA, although there is only a small number of haploid myxamoebae present prior to plasmodial formation. This can be explained by the fact that haploid myxamoebae are predominant in culture only after plasmodial formation has ceased. In this way, almost all of the diploid cells in culture would participate in plasmodial formation and thus allow any haploid myxamoebae to propagate to an appreciable number.

It has been observed in this study that plasmodial formation occurs very rapidly among Ph-1 isolates. Microplasmodia have been isolated as early as 24-36 hours after germination was observed, much earlier than the three to five day period required to reach log phase

and plasmodial formation among other Myxomycete isolates (Gray and Alexopoulos, 1968; Yemma and Therrien, 1972). The observed rapid plasmodial formation may be the result of the lack of gametic fusion and the permanent diploid condition of the myxamoebae or disomy at the mating locus. Adler and Holt (1975), working with Physarum polycephalum, were able to show clonal cultures that formed plasmodia with the absence of gametic fusion to be heterozygous at the mating locus and near diploid in DNA content. These reports indicate that the apparent disomic condition of the mating locus may be the only condition necessary for plasmodial formation. Thus, the diploid state at the mating locus, being a normal condition in crossed plasmodial cultures after gametic fusion, already exists among the diploid Ph-1 myxamoebae and may be a determining cause in quick apogamic plasmodial formation.

Sporulation was difficult to induce in the Ph-1 isolate, with most plasmodial cultures directly forming sclerocia. In most cases, sporulation was accomplished only after replating the sclerocia on moist cornmeal agar containing a minimal amount of E. coli, followed by controlling the cultures under light-dark conditions.

Spore germination among all isolates analyzed was especially difficult to induce. The best results occurred when single spore isolations were made one week after sporulation was initiated. Spores isolated one and two days after the sporulation process resulted in a very low percentage germination.

The evolution of the Class Myxomycetes, as analyzed by Clark and Collins (1976), indicates that homothallism may be the most common form of propagation. They theorize that heterothallism arose

early in evolution, with homothallism being a most recent condition. Collins (1976) further suggests that Didymium iridis may be in a transitory state leading toward homothallism. The diverse ways that isolates of Didymium iridis now complete their life cycles are summarized by Therrien, Bell and Collins (1977). These, together with the seemingly interim condition of the Ph-1 isolate demonstrated in this report, tend to support this observation.

Permanent differences among various species of fungi are known to originate from instability (Lenhart, 1973). Methods of instability demonstrated among fungi are point mutations, chromosomal mutations, aneuploidy, non-disjunction, heterokaryosis, parasexual cycle and extranuclear factors (Moore-Landecker, 1972; Lenhart, 1973).

Myxamoebae and plasmodia of various ploidys have now been established among the Myxomycetes (Yemma and Therrien, 1972; Therrien and Yemma, 1974, 1975; Adler and Holt, 1974, 1975; Ross, 1966; Kerr, 1968; Mohberg et al., 1973), although the mechanisms of ploidy changes and instability have not been completely elucidated. The method by which the apparently diploid Ph-1 clone gives rise to haploid cells is at this time unknown, although the observations of a high rate of mitotic activity, a short or absent G_1 phase, and an extended G_2 phase among rapidly growing myxamoebae in culture (Yemma, 1971) indicate the possibility of the appearance of nuclei with a DNA content quite different from that which is normally observed during the cell cycle. Therrien and Collins (in press), for example, have observed the appearance of polyploid myxamoebae within normal haploid clones, apparently arising as the result of DNA synthesis without a following mitotic division. It is quite possible that the appearance of haploid

cells within a population of diploid cells, as observed in this study, is the result of cells dividing mitotically without the preceding DNA synthesis, thus leading to a reduction in ploidy.

Nuclear cycle variability has also been reported among strains and clonal lines of Physarum polycephalum (Mohberg et al., 1973; Adler and Holt, 1975; Collins, 1975). Nuclear cycle instability, as demonstrated in Physarum polycephalum, and as this study suggests for Didymium iridis, may lead to developmental and transitional changes among the isolates which may result in an evolutionary state of permanent variability.

Further experimentation with the Ph-1 isolate could be designed to analyze transfers of single spore isolations for ploidy at time periods beginning with early propagation, through exponential and log phase, followed by analysis of myxamoebae existing during early and late plasmodial growth. This type of study should be able to demonstrate a shift in mean DNA distribution from the diploid to the haploid state. Analysis for possible morphological differences between Ph-1 and Ph-1_n myxamoebae is presently taking place in this laboratory using plasma membrane isolation techniques developed by Yemma and Selanik (1977).

BIBLIOGRAPHY

Adler, P.N. and C.E. Holt. 1974. Change in properties of Physarum polycephalum amoebae during extended culture. Journal of Bacteriology 120:532-533.

Adler, P.N. and C.E. Holt. 1975. Mating type and the differentiated state in Physarum polycephalum. Developmental Biology 43: 240-253.

Ashworth, J.M. and J. Dee. 1975. The Biology of Slime Molds. Edward Arnold Limited, London. pp. 1-4.

Alexopoulos, C.J. 1963. The Myxomycetes II. The Botanical Review 29:1-78.

Brant, T.R. and K.L. Howard. 1969. Meiosis in the Oomycetes: I. A microspectrophotometric analysis of nuclear deoxyribonucleic acid in Saprolegnia terrestris. American Journal of Botany 56:1075-1083.

Clark, J. and O.R. Collins. 1976. Study on the mating systems of eleven species of myxomycetes. American Journal of Botany 63:783-789.

Collins, O.R. 1961. Heterothallism and homothallism in two myxomycetes. American Journal of Botany 48:674-683.

Collins, O.R. 1963. Multiple alleles at the incompatibility locus in the myxomycete Didymium iridis. American Journal of Botany 50:477-480.

Collins, O.R. 1975. Mating types of five isolates of Physarum polycephalum. Mycologia 67:98-107.

Collins, O.R. 1976. Heterothallism and homothallism: a study of 27 isolates of Didymium iridis, a true slime mold. American Journal of Botany 63:138-143.

Collins, O.R. and H. Ling. 1968. Clonally produced plasmodia in heterothallic isolates of Didymium iridis. Mycologia 60: 858-868.

Collins, O.R. and C.D. Therrien. 1976. Cytophotometric measurement of nuclear DNA in seven heterothallic isolates of Didymium iridis, a myxomycete. American Journal of Botany 63:457-462.

Dee, J. 1966. Multiple alleles and other factors affecting plasmodium formation in the true slime mold Physarum polycephalum Schw. Journal of Protozoology 13:610-6161.

Feulgen, R. and H. Rossenbeck. 1924. Mikroskopisch-Chemischer Nachweiss einer Nucleinsauve von Typus der thymonucleinsauve und die darauf berhende elektive Farburg von Zellkernen in mikroskopischen Praparaten. Z. Physiological Chemistry 135: 203-248.

Gray, W.D. and C.J. Alexopoulos. 1968. Biology of the Myxomycetes. Ronald Press Co., New York. 288 p.

Kerr, N.S. 1961. A study of plasmodium formation by the true slime mold, Didymium nigripes. Experimental Cell Research 23: 603-611.

Kerr, S. 1968. Ploidy level in the true slime mold Didymium nigripes. Journal of General Microbiology 53:9-15.

Koevenig, J.L. 1964. Studies on life cycle of Physarum gyrosum and other Myxomycetes. Mycologia 56:170-184.

Lenhart, K. 1973. Vegetative instability in fungi. I. The mechanisms of vegetative instability in Ascomycetes. Acta Universitatis Palackianae Olomucensis Facultatis Medicae 65:75-81.

Leuchtenberger, C. 1958. Quantitative determination of DNA in cells by Feulgen microspectrophotometry, pp. 219-278. In: J.F. Danielli (ed.), General cytochemical methods, Vol. 1, Academic Press, Inc., New York.

Mayall, B.H. and M.M. Mendelsohn. 1970. Errors in absorption cytophotometry: some theoretical and practical considerations, pp. 171-197. In: G.L. Weid and G.F. Bahr (eds.), Introduction to Quantitative Cytochemistry - II, Academic Press, New York.

Mirsky, A.E. and H. Ris. 1949. Variable and constant components of chromosomes. Nature 163:666-667.

Mohberg, J., K.L. Babcock, F.B. Haugli and H.P. Rusch. 1973. Nuclear DNA content and chromosome numbers in the myxomycete Physarum polycephalum. Developmental Biology 34:228-245.

Moore-Landecker, E. 1973. Fundamentals of the Fungi. Prentice-Hall, Inc., Englewood Cliffs, New Jersey. pp. 217-247.

Ornstein, L. 1952. The distributional error in microspectrophotometry. Laboratory Investigation 1:250.

Patau, K. 1952. Absorption microspectrophotometry of irregular shaped objects. Chromosoma 5:341.

Ris, H. and A.E. Mirsky. 1949. Quantitative cytochemical determination of deoxyribonucleic acid with the Feulgen nucleal reaction. Journal of General Physiology 33:125-146.

Ross, I.K. 1966. Chromosome numbers in pure and gross cultures of myxomycetes. American Journal of Botany 53:712-718.

Ross, I.K. 1967. Syngamy and plasmodium formation in the myxomycete Didymium iridis. Protoplasma 64:104-119.

Rusch, H.P. 1969. Some biochemical events in the growth cycle of Physarum polycephalum. Federation Proceedings 28:1761-1770.

Sussman, M. and R.R. Sussman. 1962. Ploidal inheritance in Dictostelium discoideum: stable haploid, stable diploid and metastable strains. Journal of General Microbiology 28:417-429.

Swift, H. 1950. Deoxyribose nucleic acid content of animal nuclei. Physiological Zoology 23:169-198.

Swift, H. and E. Rasch. 1956. Microphotometry with visible light, pp. 353-400. In: G. Oster and A.W. Pollister (eds.), Physical techniques in biological research, Vol. III, Academic Press, Inc., New York.

Therrien, C.D. 1966. Microspectrophotometric measurement of nuclear deoxyribonucleic acid content in two myxomycetes. Canadian Journal of Botany 44:1667-1675.

Therrien, C.D., W.R. Bell and O.R. Collins. 1977. Nuclear DNA content of myxamoebae and plasmodia in six non-heterothallic isolates of a myxomycete, Didymium iridis. American Journal of Botany 64:286-291.

Therrien, C.D. and O.R. Collins. In press. Apogamic induction of haploid plasmodia in a myxomycete, Didymium iridis. Developmental Biology.

Therrien, C.D. and J.J. Yemma. 1974. Comparative measurements of nuclear DNA in a heterothallic and a self-fertile isolate of the myxomycete Didymium iridis. American Journal of Botany 61:400-404.

Therrien, C.D. and J.J. Yemma. 1975. Nuclear DNA content and ploidy values in clonally-developed plasmodia of the myxomycete Didymium iridis. Caryologia 28:313-320.

Yemma, J.J. 1971. Quantitative analysis of DNA and nucleohistones in selfing strains of the myxomycete Didymium iridis. Ph.D. Dissertation, The Pennsylvania State University, University Park, Pennsylvania.

Yemma, J.J. and P.E. Selanik. 1977. A rapid method for isolation of the plasma membrane of the myxamoebae and swarm cells of the myxomycete Didymium iridis. Cytobios 18:183-193.

Yemma, J.J. and C.D. Therrien. 1972. Quantitative microspectrophotometry of nuclear DNA in selfing strains of the myxomycete Didymium iridis. American Journal of Botany 59:828-835.

Yemma, J.J., C.D. Therrien and S. Ventura. 1974. Cytoplasmic inheritance of the selfing factor in the myxomycete Didymium iridis. Heredity 32:231-239.