EVIDENCE FOR A MEMBRANE MATING RECEPTOR IN *DIDYMIUM IRIDIS*

by

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ABSTRACT

Evidence for a Membrane Mating
Receptor in Didymium Iridis

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Master of Science
Youngstown State University, 1983

Haploid myxamoebal mating types, Panamanian 2-7 A^8 and Honduran 1-2 A^1, of the acellular slime mold Didymium iridis, were isolated in pure culture on growth media. These isolates were then subjected to proper fixation, collection, purification, and staining utilizing the Feulgen reaction, and a microspectrophotometric analysis was performed to determine nuclear DNA content, and its relationship to the cell's ploidy level.

These two isolates were combined on growth media and the resulting plasmodia were similarly prepared and analyzed as the pure isolates. Both isolates were also placed on growth media modified by the addition of Concanavalin A. The resulting culture was also prepared and analyzed as above. All data collected was subsequently subjected to statistical analysis.

It was the purpose of this study, to determine if a membrane bound receptor is necessary for plasmodial formation between compatible mating types of D. iridis.

ACKNOWLEDGEMENTS

The author wishes to thank Dr. John J. Yemma for his invaluable assistance, encouragement, and guidance throughout the course of this study.

The author wishes to dedicate this thesis to his parents, Jay and Marlene Soltis; to his brother and sister, Kevin and Kelley; to his grandparents, Steve and Mary Yash; and to his girlfriend Karyn, whose support and encouragement made this study possible.

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CHAPTER 1

INTRODUCTION

The myxomycetes have provided a wealth of scientific information since they were first investigated by DeBarry in 1860. The eucharyotic Myxomycetes exhibits a unique life cycle, which has been utilized in extensive research including: studies of the cell cycle (Therrien and Yemma, 1974), mitotic synchronization (Rusch, 1969), and genetic studies (Collins and Ling, 1972).

The acellular slime mold Didymium iridis, the object of this study, has a life cycle (Figure 1) consisting of two basic vegetative stages: the first, a unicellular myxamoeboid stage, and the second, a multinucleate and sporulation stage.

The myxamoebal stage develops from the germination of the spore from which a single protoplast emerges (Collins, 1961). In the presence of an aqueous environment, the myxamoebae becomes biflagellate and is referred to as a swarmer. If taken from this aqueous environment, the myxamoebal form emerges once again. The myxamoebae thrive on certain bacterial strains such as Escherichia coli or Aerobacter aerogenes, ingesting them through means of pseudopodia and subsequently divide at certain intervals resulting in a quickly growing population. If the food source becomes exhausted or inade-

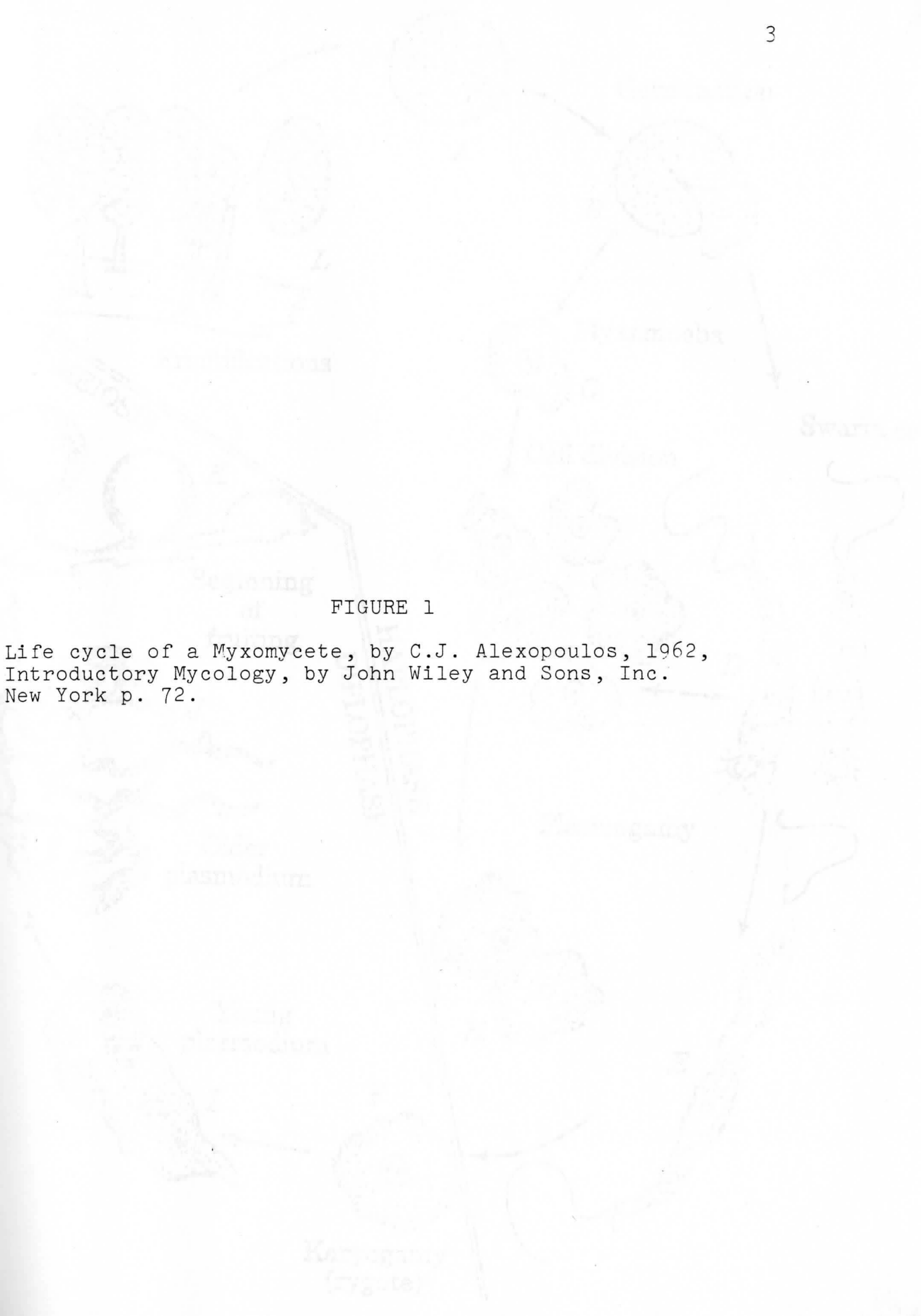
quate, the cells encyst until the environmental conditions become once again favorable (Alexopoulas, 1962; Gray and Alexopoulas, 1968).

Plasmodial development occurs in myxomycete species by several different processes. In some species, plasmodium will arise only after two mating types undergo syngamy and karyogamy respectively. This process is termed heterothallism and is genetically controlled by a single locus on the chromosome, containing multiple allelic possibilities (Collins, 1963; Collins and Ling 1968). After fusion of compatible mating types, a zygote results which has the diploid chromosome number. There is some question as to the method of zygote formation and subsequent plasmodial development. One of the purposes of this study is to investigate this process.

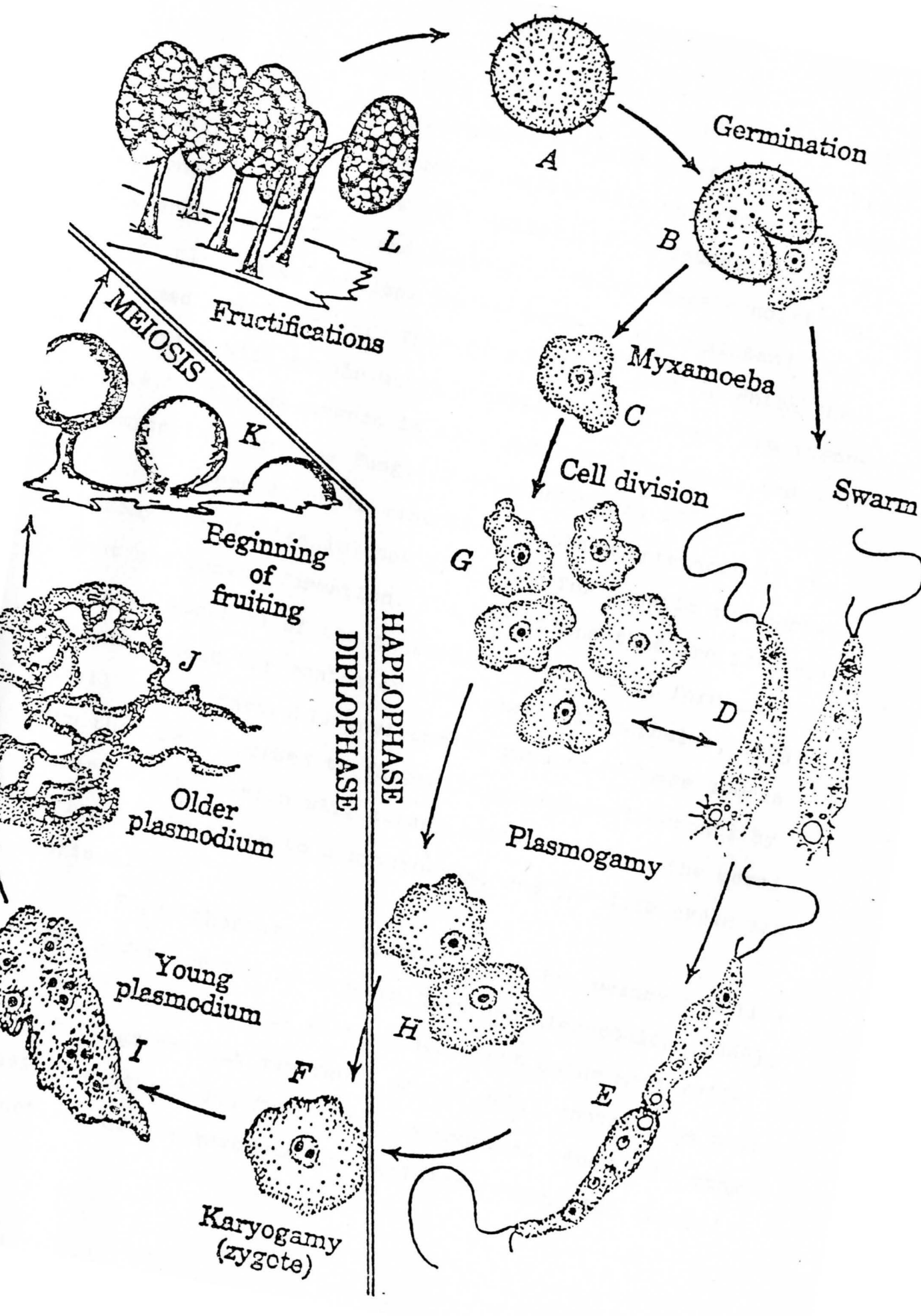
It has been suggested by some investigators that plasmodial development from zygotes occurs through the fusion or coalescence of many zygotes, subsequent nuclear division and differentiation of a single zygote, or fusion of microplasmodia (Kerr, 1961; Ross, 1967).

Other ways plasmodia have been observed to develop is through homothallism, a fusion of genetically identical cells (Collins, 1976), or apogamic development, which possibly occurs through a series of nuclear divisions of myxamoebae or swarm cells without cell division, nuclear fusion, or meiosis (Yemma and Therrien, 1972; Therrien and Yemma, 1974).

FIGURE 1



Life cycle of a Myxomycete, by C.J. Alexopoulos, 1962, Introductory Mycology, by John Wiley and Sons, Inc. New York p. 72.


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

After plasmodial formation, the organism will continue to grow with synchronous nuclear divisions (Rusch, 1969), unless it encounters unfavorable environmental conditions, such as a lack of a suitable food source, or intolerable temperature and humidity. Under these conditions, the plasmodium develops into a hard-walled resistant form termed a sclerotium. This is a dormant form in which the organism will remain until conditions once again are favorable. This phenomenon is also observed throughout the Eumycotina, or true fungi (Moore-Landecker, 1982).

Sporulation is essential to the maintenance of the myxomycete species in that it allows for genetic reassortment and gamete formation. In conditions that are intolerable to survival of the plasmodia, spores will form and remain viable for months or even years (Alexopoulos, 1963) until a more favorable environment returns. These spores can then be dispersed to favorable growth environments by water or wind, which will subsequently result in the germination of the spore to a myxamoebae, and the life cycle may continue.

For a thorough representation of the myxomycete life cycle, reference may be made to Gray and Alexopoulos (1968).

Today, there is general agreement among most cellular biologists that membranes of all cells contain the same basic structural and functional components. Among the many functions of the membrane are cellular recognition (Ashwell

and Morell, 1977) and receptors for hormones and other outside mediators of cell physiology (O'Keefe and Cuatrecasas, 1975).

Membranes are composed of two basic species of compounds: proteins and lipids. The lipid found in the membrane functions to give it its overall structural properties, and is arranged in a bilayer configuration where the two layers are approximately 40 angstroms apart. These lipids are amphipathic, with the polar heads of the molecules facing outward from the bilayer and the hydrophobic tails running perpendicular to it.

The protein component of the membrane accounts for 10 to 20% of its total composition and functions as enzymes and receptors, providing the distinct functional properties that membranes exhibit (Fox, 1972).

Membrane proteins can be divided into two classes depending on their location in or on the membrane (Capaldi, 1974). One class contains those proteins that are located only on the membrane surface, and are termed extrinsic proteins. The second class is composed of those proteins that actually penetrate the surface of the membrane bilayer, and those that actually extend all the way through and thus are termed intrinsic.

Membrane proteins have been shown to exhibit asymmetry (Rothman and Lenard 1977). Asymmetry can be observed in the membrane, where proteins of the extracytoplasmic side have been glycosylated, and the proteins of the cyto-

plasmic side have not. It is these glycoproteins of the extracytoplasmic side of the membrane that are of interest to this study and their involvement in the formation of membrane receptors (Crumpton, 1975). Membrane proteins are shown to move laterally through the semi-fluid lipid bilayer, and that the lateral mobility may be important to the action of cell surface receptors (Edidin et al., 1973), and to their binding with the lectin, concanavalin A used in this study (Gillette et al., 1973).

In the past very little has appeared in the literature concerning membrane receptor structure and function in the myxomycetes. Robert Molday, Rory Jaffe and Daniel McMahon, from the division of biology, California Institute of Technology, Pasadena (1976), did electron microscopy studies utilizing "microspheres" coupled to either concanavalin A or wheat germ agglutinin to visualize topographic distribution of cell surface lectin receptors in the cellular slime mold, Dictyostelium discoideum. Martha Ulbrick Gillette, R.E. Dengler and M.F. Filosa of the Departments of Zoology and Botany, Scarborough College, University of Toronto (1974), utilized con A to study its binding surface receptors and its subsequent induction of premature phosphodiesterase activity in D. discoideum.

Lectins are plant proteins found throughout the plant kingdom. These proteins exhibit a strong binding to glycoproteins. This binding between the lectin and glycoproteins involve specific binding sites on the lectin

molecule that recognize specific carbohydrate groupings on the glycoprotein (Sharon and Lis, 1972; Lis and Sharon, 1973).

Concanavalin A, makes up two to three percent of the protein of the Jackbean, and has been shown to preferentially agglutinate cancer cells (Inbar and Sachs, 1969). This lectin along with others has been shown to have mitogenic qualities as well (Greaves and Janossy, 1972). As previously mentioned, lectins bind with a specificity for certain sugars. Con A is specific for alpha-D-mannopyranose and alpha-D-glucopyranose with unmodified hydroxyl groups at C-3, C-4 and C-6, and is a tetramer with a subunit molecular weight of 27,500. It will dissociate to a dimer at pH 5.8 or lower (Kornfied and Ferris, 1975).

Con A is unusual in that it has no alpha helical structure. Fifty-seven percent of the 237 residues in the monomer exist in three sheets of Beta structure. There are three binding sites on each monomeric unit, a manganese, a calcium, and a carbohydrate binding site respectively (Figure 2). The carbohydrate site does not bind substrate until the two metal binding sites are filled. It is thus suggested that a conformational change in the protein accompanying the ion binding is essential to the creation of the sugar binding site (Metzler, 1977; Brown et al., 1977; Sherry et al., 1978).

Concanavalin A has been utilized by several researchers to preferentially bind to cell surface glycopro-

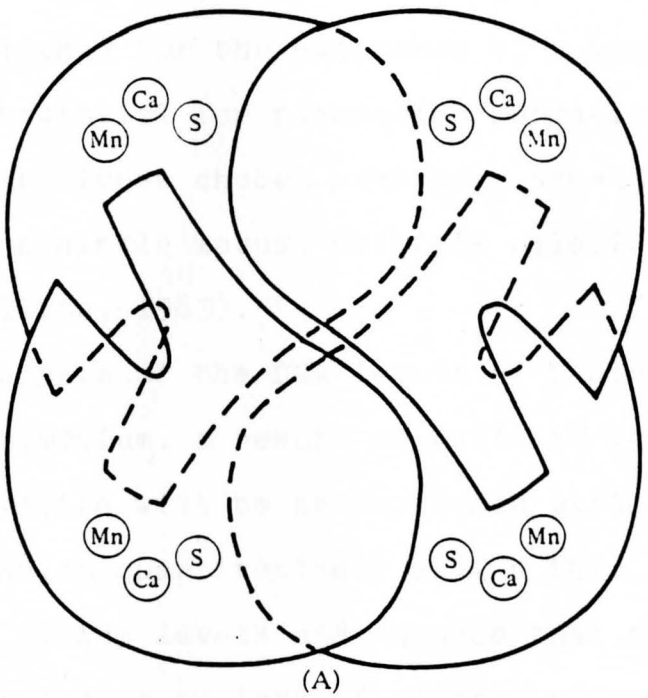


FIGURE 2

A schematic representation of the tetrameric structure of Concanavalin A, in Biochemistry: the Chemical Reactions of Living Cells. David E. Metzler. 1977. Academic Press, New York, New York. p. 282.

teins: namely, Maher and Molday (1981) with neuroblastoma cells, Okada (1981) with human erythrocytes, and Molday et al. (1976) and Gillette et al. (1973), both using Dictyostelium discoideum.

This study proposes to utilize the glycoprotein binding properties of Concanavalin A, in conjunction with the acellular slime mold, Didymium iridis, in order to demonstrate a specific membrane receptor; more specifically, to present evidence for the existance of a membrane bound receptor, essential for plasmodial formation from myxamoebal clones. The clones chosen have been shown to be heterothallic, where a single locus, multiple allelic mating system occurs (Collins, 1963).

Analysis of the DNA levels of haploid myxamoeba and diploid plasmodium, a result of cellular fusion and thus zygote formation will be accomplished utilizing the Feulgen reaction, which quantitatively stains DNA. By observation of nuclear ploidy levels and changes that may occur because of cellular fusion or lack of changes under experimental conditions, a hypothesis can be made as to the absence or presence of the theorized mating receptor in the membrane of D. iridis.

Quantification of the Feulgen-stained DNA was accomplished by microspectrophotometry, utilizing the two wavelength method as described by Patau (1957), and Ornstein (1952). Microspectrophotometry used in conjunction with the Feulgen staining technique is a reliable method of obtaining

ploidy levels, as proven by Mirsky and Ris (1949), Ris and Mirsky (1949), and Swift (1950). Furthermore, quantitative spectrophotometric analysis has been successfully accomplished in D. iridis by Therrien (1966), Yemma and Therrian (1972) and Therrien and Yemma (1974).

CHAPTER II

MATERIALS AND METHODS

The isolates used in this study were originally supplied by Dr. O. R. Collins, Department of Botany, University of California. Isolates are designated Honduran 1-2 (Hon 1-2) and Panamanian 2-7 (Pan 2-7).

All myxamoebal clones as well as plasmodia were grown on solid media, previously innoculated with Escherichia coli, according to the methods of Yemma and Therrien (1972).

Control cultures of myxamoebae were obtained by innoculating half strength cornmeal agar with E. coli and incubated for 24 hours at 25°C. The media was then innoculated with the respective myxamoebal clones, which were taken from stock slant cultures and incubated at 21°C. Subcultures were then made once log phase of growth had been achieved, and as previously described, were maintained in an incubator at 21°C (Yemma and Therrien, 1972; Yemma et al., 1974).

In order to obtain plasmodial controls, subcultures of Hon 1-2 A^1 and Pan 2-7 A^8 were placed on growth media (table 1), and prepared and incubated as above for 5 to 7 days. After significant plasmodial growth was obtained, plasmodia were further subcultured onto plasmodial growth media (table 1) and incubated an additional 5 to 7 days before harvest.

Experimental cultures were produced by crossing Hon 1-2 A^1 and Pan 2-7 A^8 on Concanavalin A media (table 1).

TABLE 1

Media

Growth Media (Half-Strength Cornmeal Agar)

8.0 grams Difco Agar
8.5 grams Difco Cornmeal Agar
1.0 Liter Distilled Water

Plasmodium Media

5.0 grams Difco Agar
1.0 gram Difco Peptone Agar
1.0 gram Lactose
1.0 Liter Distilled Water

Concanavalin A Media

8.0 grams Difco Agar
8.5 grams Difco Cornmeal Agar
0.2 grams Concanavalin A (Sigma)
1.0 Liter Distilled Water

All other preparations and incubation conditions were similar to those previously described for myxamoebae. The cultures were incubated for nine days and subsequently harvested.

Two of the experimental culture plates exhibited unexpected plasmodial growth. These plasmodia were isolated and transferred to plasmodia media and further incubated for harvest and subsequent analysis.

Experimental controls were simultaneously run with the experimental cultures and carried out under exactly the same conditions except that half-strength cornmeal agar was utilized instead of Concanavalin A media. After plasmodia development, the isolates were transferred to plasmodia media, and allowed to develop for 5 to 7 days before harvest.

Culture harvest and preparation for cytochemical studies

Myxamoebae

1. Cultures of myxamoebae were flooded with 10% buffered formalin (pH 7.0) and allowed to fix for 12 to 13 hours.
2. Myxamoebae were then carefully removed from the surface of the media, transferred to conical centrifuge tubes and centrifuged 5 X gravity to remove any debris.
3. These were centrifuges 15 minutes at 70 X gravity and the supernatant containing mostly bacterial contamination was discarded. The pellet was subsequently flooded with 70% ethanol and allowed to postfix for 12 hours.

4. After the postfix period, the suspensions were again centrifuged for 15 minutes at 70X gravity and resuspended in 70% ethanol. Samples of the suspension were checked microscopically to determine the extent of bacterial contamination. This process was repeated until a bacteria-free plug was obtained.
5. The plug was then finally resuspended in a small volume of 70% ethanol and the cells affixed to previously albuminized slides

Plasmodia

1. Plasmodia were flooded with 10% phosphate buffered formalin (pH 7.0) and allowed to fix 12 hours. Phosphate buffered formalin was then added in a gentle stream at the interface between the media and the plasmodial isolate to float it free.
2. The fixed plasmodia were then washed twice 70% ethanol and allowed to post-fix in 70% ethanol for 12 hours.
3. After fixation, the plasmodia were dehydrated in a series of graded ethanol, passed through two changes of xylene, and imbedded in paraffin.
4. Sections were cut at 4 microns and affixed to previously albuminized slides.

Plasmodia from experimental cultures

These plasmodia were found to be very fragile, developed infrequently (approximately 1.8% of total crosses), and were non-viable. They were harvested, prepared and affixed to slides in a manner similar to the preparation of myxamoebae.

Cytochemical Methods

The Fuelgen nuclear reaction (Feulgen and Rossenback, 1924; as modified by Therrien, 1966; and Bryant and Howard, 1969) was employed for the stoichiometric staining of deoxyribonucleic acid. Slide preparations of myxamoeba and the plasmodium sample that was prepared in the manner of the myxamoebae, were immersed in 70% ethanol, then rinsed in distilled water before proceeding through the staining sequence. Prior to staining, paraffin sections of plasmodia were cleared in xylene, and then hydrated through a graded ethanol series to distilled water before they were subjected to the following staining sequence.

1. The tissue was hydrolyzed in 5N HCL for 45 minutes at room temperature.
2. Rinse in distilled water.
3. Stained for one hour in freshly mixed Schiff's reagent (4 parts), fortified with 10% potassium-metabisulfite (1 part). The basic fuschin used, was manufactured by Fisher Scientific Company (C.I. #42500).

4. Staining was followed by three five minute rinses in freshly prepared bisulfite rinses (Yemma, 1972).
5. Rinse in distilled water and dehydration in a graded ethanol series was then performed.
6. Finally the tissue was cleared in xylene, and cover slipped and mounted with permount.

Cytophotometric Methods

The cytochemical methods employed include Fuelgen staining and a combination of the techniques of microscopy and spectrophotometry. It is possible then, to offer absorption spectroscopy to individual cells, thereby allowing the amount of DNA per nucleus to be measured and computed (Swift, 1950) within a heterogenious cell population (Ris and Mirsky, 1949).

All quantitative DNA measurements were made using a Zeis type 01 Microspectrophotometer with a quartz iodide light source. The objective used was a Zeis oil immersion objective X100 N.A. 130, at an optovar setting of 1.25X, with Zeis immersion oil, 518C Din 5884. A continuous interference filter mono chronomator was used for selection of the desired wavelength. Before all readings, instrument alignment and phototube linearity response was checked. An absorption curve was used to determine the maximum and minimum wave-

lengths (Fig. 3, Results Section). All readings were conducted at the two respective wavelengths determined by the curve: 560 and 505.

The two wavelength method of microspectrophotometry (Patau, 1952: and Ornstein, 1952) was utilized for a photometric determination of relative amounts of DNA stained by the Feulgen reaction. This method was chosen because it negates a direct measurement of nuclear DNA (Mayall and Mendelsohn, 1970) and also corrects for errors caused by heterogenious distributions of stained nuclear material. All myxamoebae and plasmodia populations of slides were simultaneously hydrolyzed and stained to assure uniformity in stain intensity and thus absorption, relative to the respective cells DNA content.

An estimation of the absorbing material depends upon the difference in transmission at the two selected wavelengths, previously determined on the absorption curve. These wavelengths will be termed 1 and 2 respectively. Wavelength 2 will correspond to the maximum extinction of the absorption curve (E_2) and wavelength one to the wavelength, half the maximum extinction (E_1).

In this experiment, wavelength 2 was determined to be 560 nm., and wavelength 1 was 505 nm. Four microspectrophotometric readings are necessary to determine relative nuclear DNA content. Nuclei were picked at random, centered in the field aperature which was just larger in diameter than the nucleus itself (4 micron aperature), and readings were made: I_{01}, I_{S1}, I_{02}, I_{S2} respectively. The readings were then

utilized in determining the amount of chromophore (M), by the equation, M = KAL,C. The absorptivity constant K, was omitted since relative not absolute values were needed. L, was calculated by the equation $L_1 = (1-T)$, and L_2 = by the equa $L_2 = (1-T_2)$. T_1 and T_2 were determined by the wavelength readings, and the equations $T_1 = {}^{I_{S1}}/_{I_{01}}$ and $T_2 = {}^{I_{S2}}/_{I_{02}}$. C, the correction factor for the distributional error (Swift and Rasch, 1956; Leuchtenberger, 1958) was determined from the ratio (Q) between L, and L_2 ($Q = {}^{L_2}/_{L_1}$). The Q value is used to eliminate the influence of unoccupied space in the measured area around the nucleus, this is permitted 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 the main computer at Youngstown State University through a program written by Dr. John Yemma.

CHAPTER III

RESULTS

The results of this study are presented in both tabular form and as histograms. The latter method of presentation facilitates the observation of even minor shifts in DNA that may occur in template activity, synthetic activity or changes in ploidy level regarding different cell or nuclear populations. Only interphase nuclei were observed during this study; hence, none of the measurements include division figures.

The number of nuclei measured were plotted on the ordinate and the relative dye concentration of Feulgen stained DNA was correspondingly plotted on the abscissa in arbitrary units.

Use of the two wavelength method of microspectrophotometry necessitates the establishment of an absorption curve for the particular dye-molecular complex being investigated. Stained myxamoebal nuclei were used to establish the spectral absorptive curve according to the method of Yemma and Therrien (1972). The maximum absorption for the Feulgen-DNA complex was at a wavelength of 560nm, with the half maximum value at a wavelength of 505nm (Figure 3).

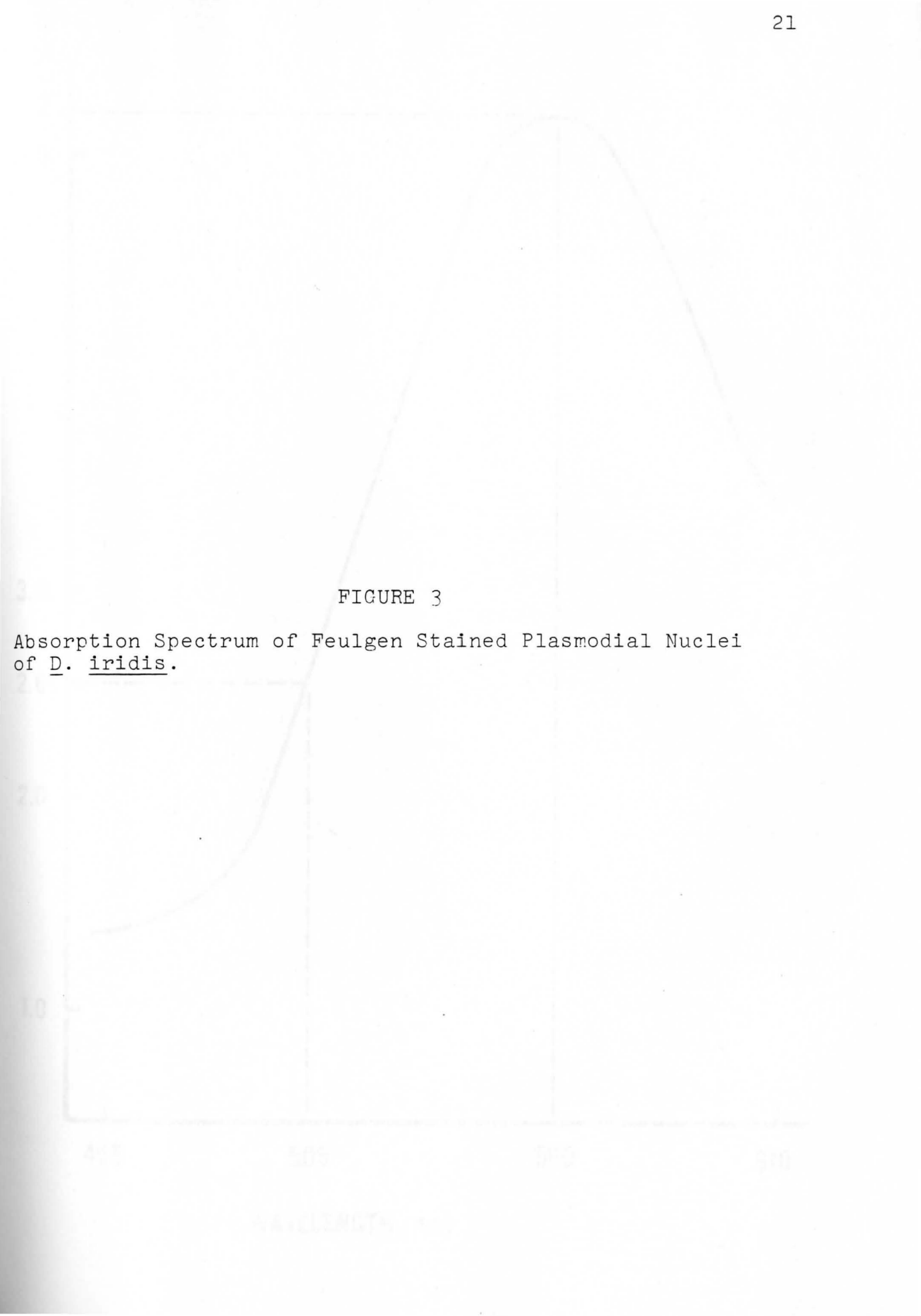
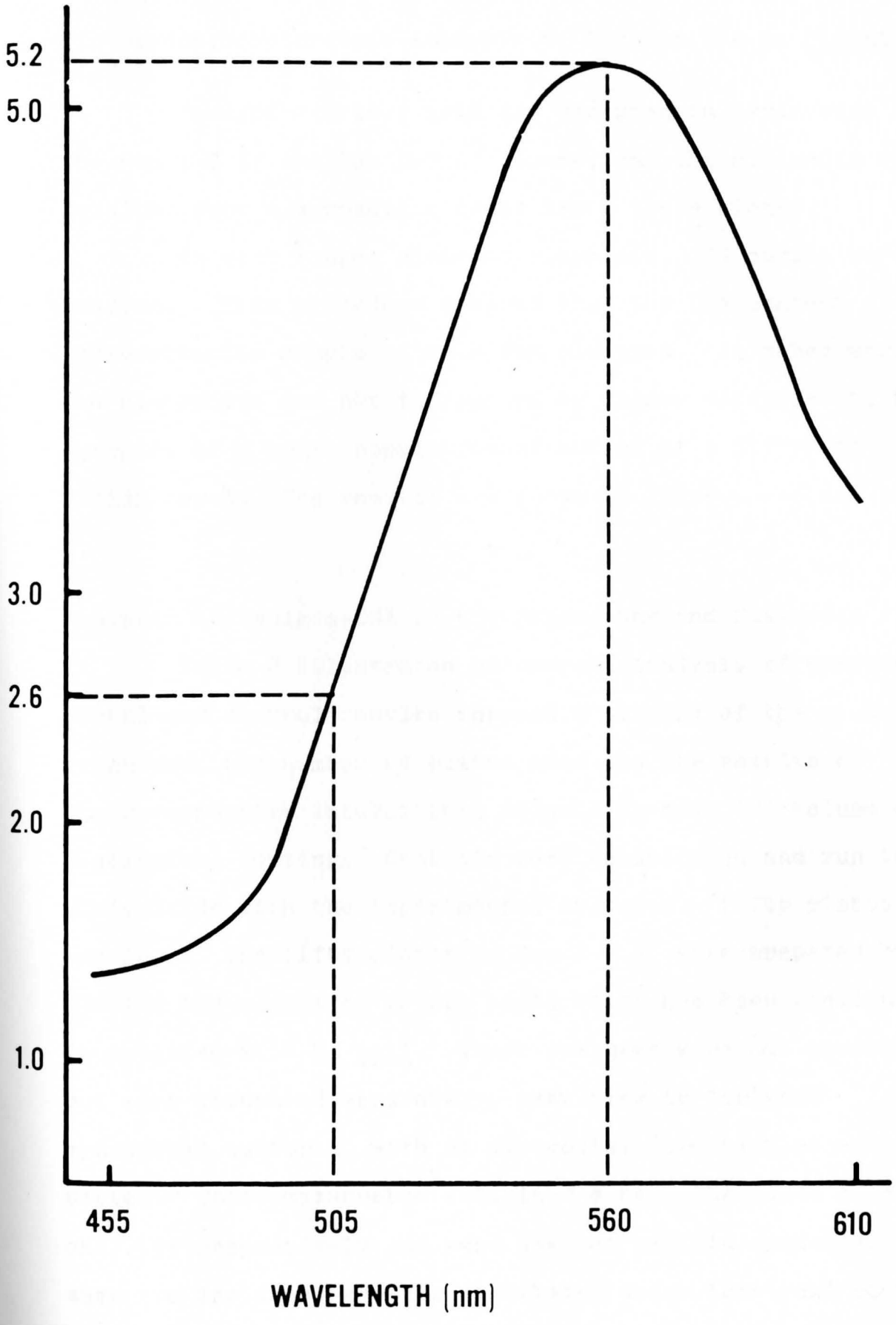


FIGURE 3

Absorption Spectrum of Feulgen Stained Plasmodial Nuclei of D. iridis.


5.2
5.0
3.0
2.6
2.0
1.0
455
505
560
610
WAVELENGTH (nm)

Microspectrophotometric Analysis of Nuclear DNA in Didymium iridis

Deoxyribonucleic acid was measured in myxamoebae of the Hon 1-2 A^1 and Pan 2-7 A^8 clones, and the plasmodia which resulted from a compatible cross among these clones.

In each stage, clone or plasmodia, 100 nuclei were analyzed. This procedure insured that the DNA content of a representative sample of each was measured. In other words the histograms are not influenced by chance measurement, for example, of a small population of nuclei of a different ploidy level. The results are shown in figures 4-6 and table 2.

Analysis of Feulgen-DNA in the Myxamoebae and Plasmodia

Table 2 illustrates an overall analysis of experimental and control results through a listing of the various organisms, the number of plates used and the results of their respective incubations, as well as mean DNA values and statistical testing. Controls were established and run in conjunction with the experimental cultures. Fifty plates of Hon 1-2 A^1 and fifty plates of Pan 2-7 A^8 were prepared by placing the clones on growth media which had been previously innoculated with E. coli. These cultures were not crossed but were incubated separately, resulting in isolated myxamoebal cultures, with no plasmodial formation as expected. These myxamoebae exhibited a mean DNA value of 3.16 and 2.93 respectively and were used as haploid controls. The same two isolates were also incubated under identical condi-

tions but in a mixed culture so that a cross would occur. After proper incubation, it was observed that 40 out of 50 plates produced plasmodia, or 80 per cent. The mean DNA content was determined to be 7.09 (figure 4) by microspectrophotometry and was utilized as the diploid control. The experimental cultures were prepared identically to the plasmodial controls, again by placing the two isolates in mixed culture, except, 200 ug/ml concanavalin A was added to the growth media. Of a total of 110 plates prepared, two plates curiously exhibited plasmodial growth, yet both the myxamoebae and the plasmodia isolated from this experimental population had haploid mean DNA values upon comparison to control values.

Swift (1950) designated a system for the description of ploidy levels: C, corresponding to unreplicated haploid; 2C, corresponding to replicated haploid; and 4C, corresponding to replicated diploid. It has been previously demonstrated that plasmodia formed from heterothallic isolates, such as those utilized in this study, are diploid (Therrien, 1966), and the nuclei are in the replicated diploid state 99% of the time (Yemma and Therrien, 1972), thus corresponding to the 4C, replicated diploid, amount of DNA, and will serve as a diploid DNA control marker. In this same study, isolates that were not crossed and subsequently analyzed were noted to be 2C, or replicated haploid, since they were approximately one half the value of the plasmodial nuclei resulting from a cross of the two respective isolates.

It should be noted that in this study the two isolates, Pan 2-7 A^8 and Hon 1-2 A^1 showed a unimodal distribution of DNA, which is in agreement with previous studies done by Yemma and Therrien, 1972; and Collins, 1976. Since their relative DNA content was 2C, then it can be said that they were in G_2 arrest, in other words, the G_1 stage, unreplicated haploid, was absent. Thus, since control values determined in this experiment show the same unimodal distribution in the histograms, as well as in ploidy level as the previous studies mentioned; and taking the diploid (replicated) plasmodium to be 4C, the controls prepared in this study are valid and may be used in comparison to experimental isolate DNA values for subsequent analysis and interpretation.

Analysis of Feulgen-DNA in Pan 2-7, Hon 1-2, and Pan 2-7 X Hon 1-2

The nuclear measurement of Pan 2-7 and Hon 1-2 was performed and compared with the value for nuclear DNA in the plasmodium resultant from a cross between the two isolates. The resultant histograms may be viewed in figure 4. It can easily be noted by inspection of the histograms, that the plasmodium resulting from the cross of the two isolates, Pan 2-7 X Hon 1-2, has a nuclear DNA value near the sum of the two respective separate isolates.

Analysis of Feulgen-DNA in Hon 1-2 X Pan 2-7 Experimental Cross

Figures 5 and 6 represent histograms for the experimental cross between Hon 1-2 A^1 and Pan 2-7 A^8 on media con-

TABLE 2

Comparison of Experimental Yield

	Hon 1-2 Control	Pan 2-7 Control	Hon 1-2 X Pan 2-7 Control	Hon 1-2* X Pan 2-7 Exp. Cross
No. of Plates	50	50	50	110
No. Yielding Plasmodia	0	0	40	2**
$\bar{X}$ DNA Myxamoebae	3.16 $\pm$0.71	2.93 $\pm$1.02	-	3.28 $\pm$0.84
$\bar{X}$ DNA Plasmodia	-	-	7.09 $\pm$1.08	3.88 $\pm$1.20

*Grown on media containing 200 ug/ml Con A.
**Plasmodia determined to have developed apomicticly.

taining 200 ug/ml of the plant lectin, concanavalin A. Upon macroscopic examination of these cultures, only two plates or 1.8 per cent of the total (110) plates exhibited plasmodial growth.

Figure 5 represents a microspectrophotometric analysis of Feulgen-stained DNA in the experimental population that exhibited no plasmodial growth. The analysis revealed mean DNA values similar to those of either clone isolate and are thus designated 2C, which correspondingly is half the mean DNA value of the 4C plasmodial control.

Figure 6 represents relative Feulgen-DNA values for nuclei isolated from 2 plasmodium that were formed during the experimental cross. These plasmodia were small, fragil, and of low viability. Their DNA values were similar to the experimental crosses that produced no plasmodia, and similarly will be assigned a ploidy value of 2C, which again is approximately one half that of the diploid control plasmodial value of 4C. Yemma and Therrien, 1974, reported that self sterile clones gave rise to haploid plasmodia on occasion without benefit of cross; 13.2 per cent in Hon 1-2, and 10.0 per cent in Pan 2-7. These were considered to have developed apomictically and were not the result of fusion between cells to form zygotes and subsequently plasmodia. It should be noted that the two plasmodia forming plates in this study, comprises approximately 1.8 per cent of the total number of plates crossed utilizing growth media containing concanavalin A.

FIGURE 4

Histograms representing Feulgen-DNA control values for *Didymium iridis*. (a) Hon 1-2 A^1 myxamoebae; (b) Pan 2-7 A^8 myxamoebae; (c) Hon 1-2 X Pan 2-7 plasmodium.

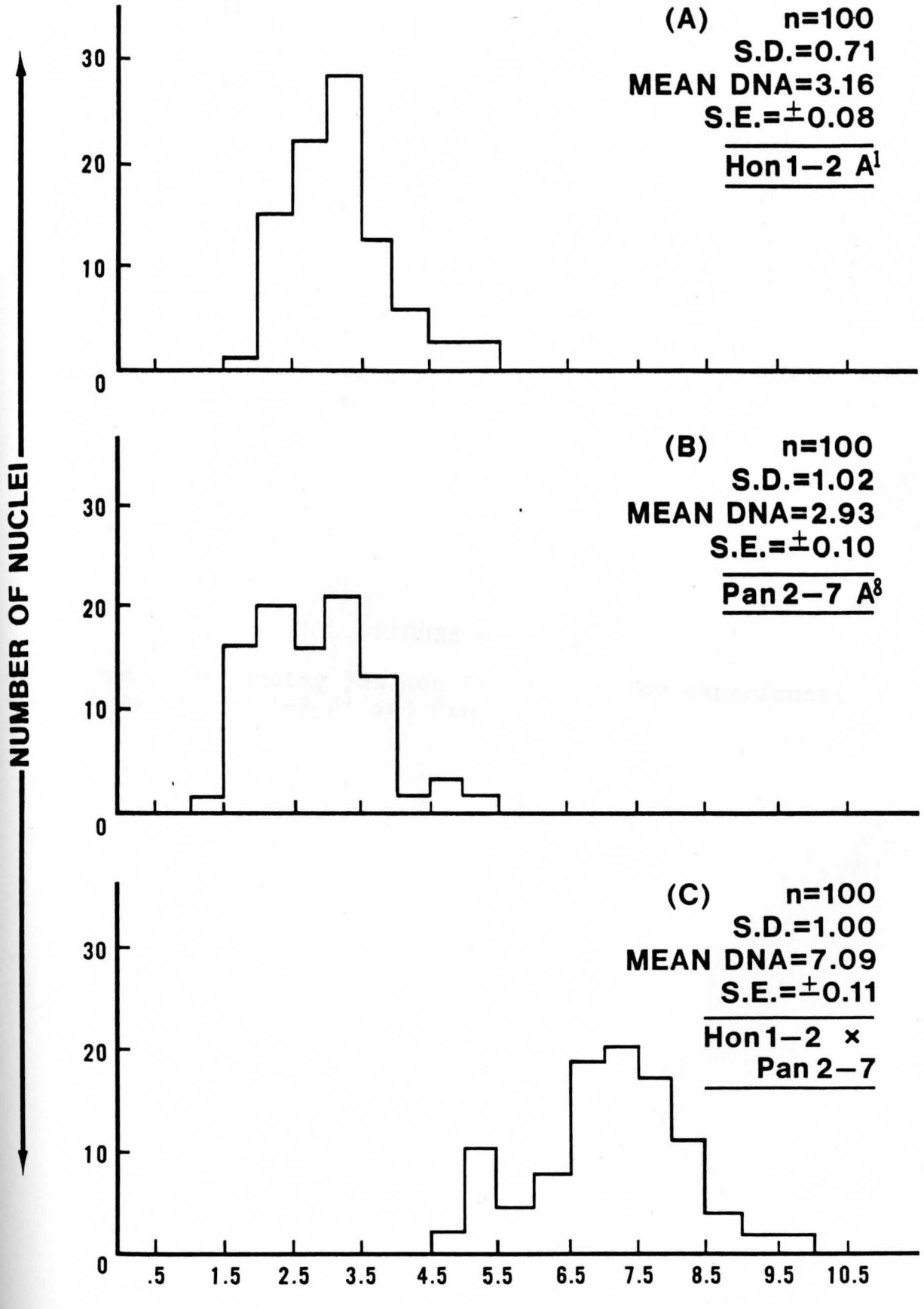

(A) n=100
S.D.=0.71
MEAN DNA=3.16
S.E.=±0.08
Hon 1–2 A1
(B) n=100
S.D.=1.02
MEAN DNA=2.93
S.E.=±0.10
Pan 2–7 A8
(C) n=100
S.D.=1.00
MEAN DNA=7.09
S.E.=±0.11
Hon 1–2 ×
Pan 2–7
NUMBER OF NUCLEI
0
10
20
30
.5
1.5
2.5
3.5
4.5
5.5
6.5
7.5
8.5
9.5
10.5
RELATIVE AMOUNTS OF DNA

FIGURE 5

Histogram representing Feulgen-DNA values for experimental cross between Hon 1-2 A^1 and Pan 2-7 A^8.

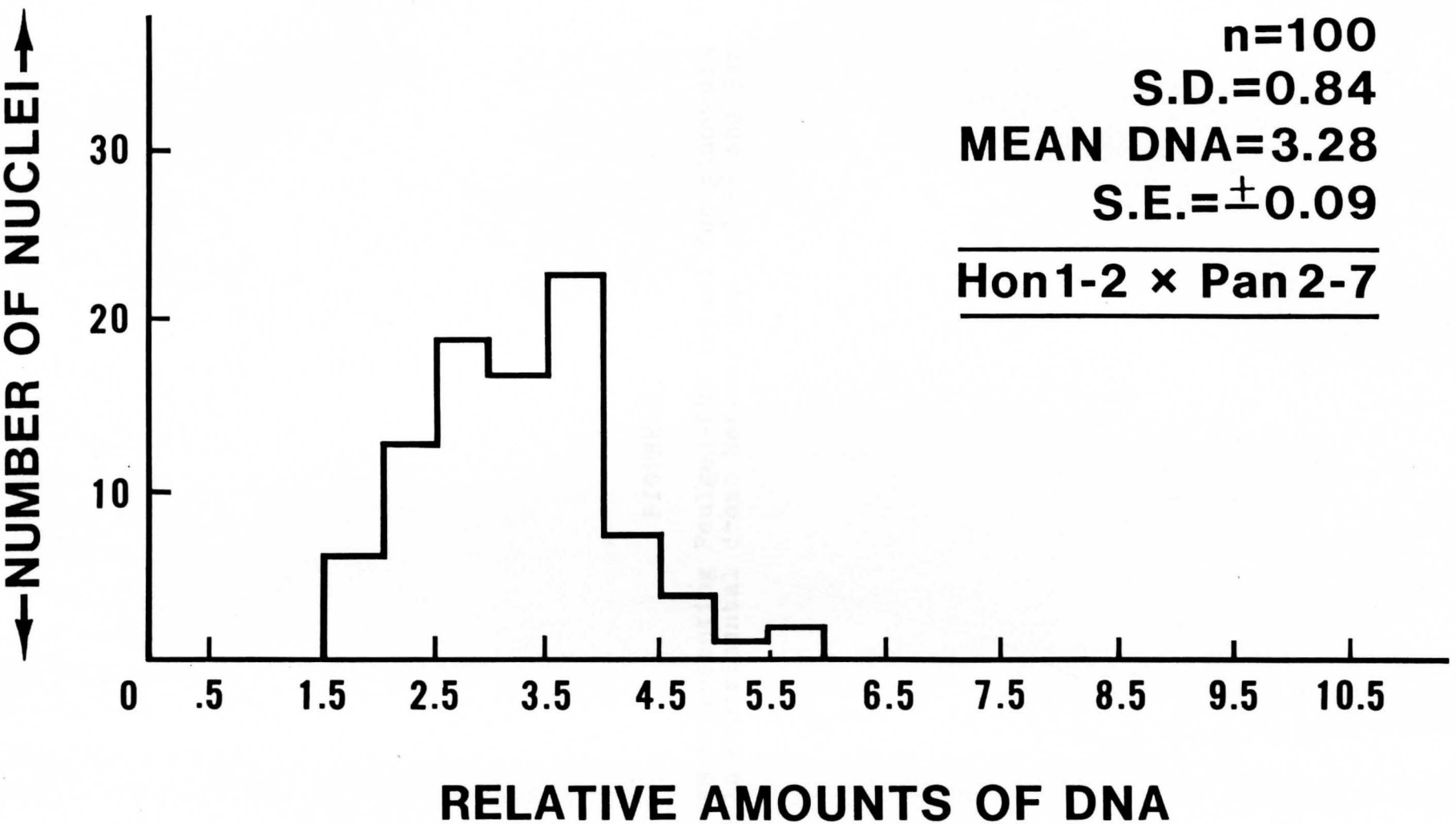
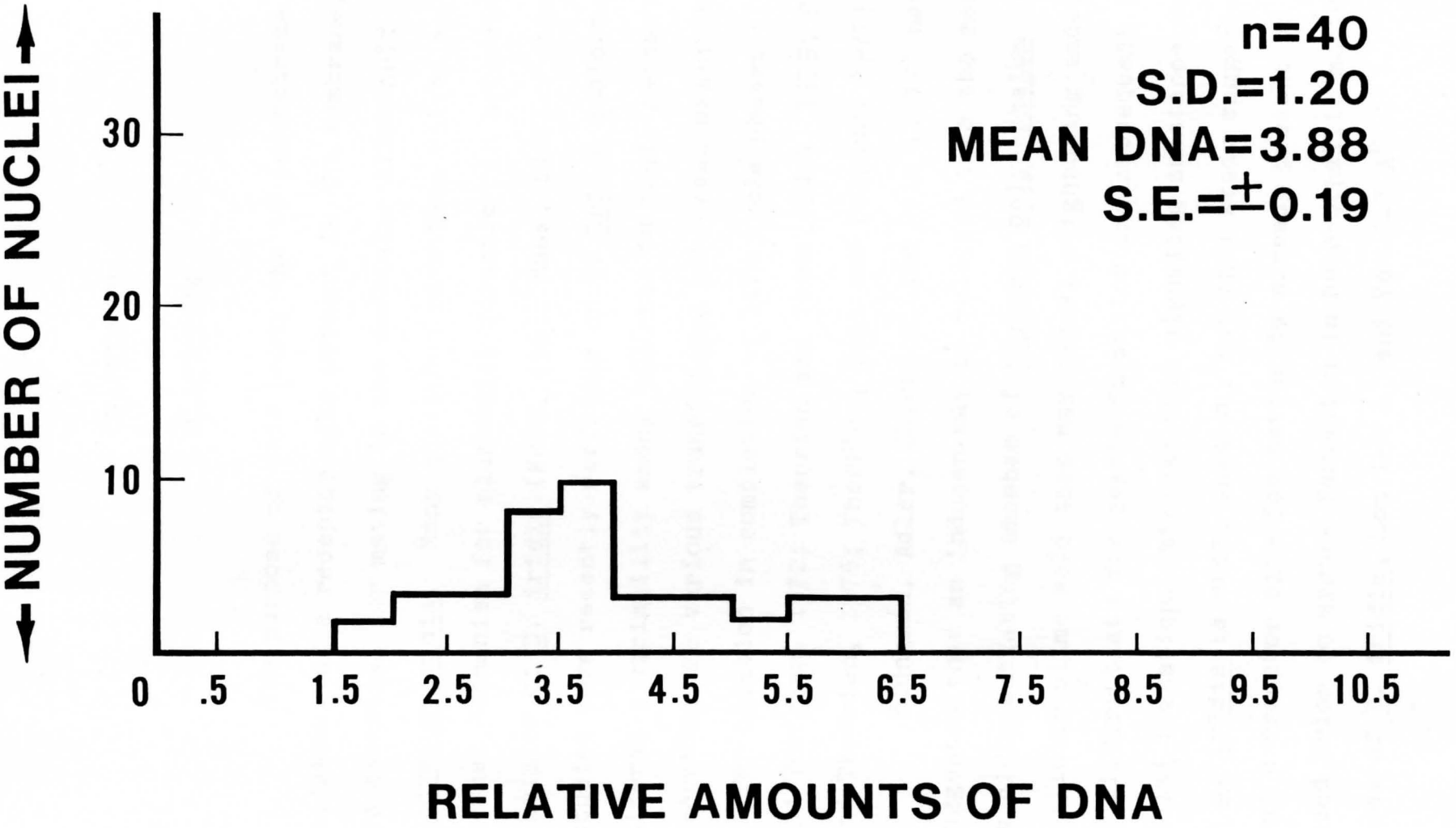

n=100
S.D.=0.84
MEAN DNA=3.28
S.E.=±0.09
Hon1-2 × Pan2-7
NUMBER OF NUCLEI
30
20
10
0
.5
1.5
2.5
3.5
4.5
5.5
6.5
7.5
8.5
9.5
10.5
RELATIVE AMOUNTS OF DNA

FIGURE 6

Histogram representing Feulgen-DNA values for plasmodia formed in experimental cross between Hon 1-2 A^1 and Pan 2-7 A^8.


n=40
S.D.=1.20
MEAN DNA=3.88
S.E.=±0.19
NUMBER OF NUCLEI
30
20
10
0
.5
1.5
2.5
3.5
4.5
5.5
6.5
7.5
8.5
9.5
10.5
RELATIVE AMOUNTS OF DNA

CHAPTER IV

DISCUSSION

The purpose of this study was to demonstrate the existence of a receptor site present in the membrane, that is essential for mating in the acellular slime mold Didymium iridis. Many questions have been raised as to the actual mechanism for plasmodial formation in heterothallic isolates of D. iridis (Kerr, 1961; Ross, 1967). Other studies more recently performed on D. iridis indicate considerable variability among isolates in their mechanism for executing the various transitional processes essential for differentiation in completion of life cycle phases (Yemma and Therrien, 1972; Therrien and Yemma, 1974, 1975; Collins and Therrien, 1976; Therrien, Bell and Collins, 1977).

Youngman, Adler, Shinnick, and Holt (1977) have suggested that an inducer may be released into the media by differentiating amoebae of Physarum polycephalum, an acellular slime mold that may signal neighboring amoebae to differentiate for zygote formation and subsequent plasmodial formation. Whether this signaling substance occurs in D. iridis is still unknown, but this study suggests that a receptor site for mating is already present or induced prior to zygote formation in heterothallic mating types of D. iridis, Hon 1-2 A^1 and Pan 2-7 A^8.

In this study, the demonstration of a membrane receptor for mating in D. iridis utilized the plant lectin concanavalin A. This lectin, has the ability to bind at the glycocalyx with specificity to the glycoproteins with alphaglucopyranose carbohydrate moieties attache (Lis and Sharon, 1972; Lis and Sharon, 1973). It has been further demonstrated by Gillette, Filosa and Dengler (1973) using the cellular slime mold, Dictyostelium discoideum that concanavalin A binds to membrane receptor sites and that it furthermore causes a four-fold increase in membrane-bound cyclic AMP phosphodiesterase activity. Molday, Jaffe and McMahon (1976) also using Dictyostelium discoideum labeled membrane receptors with concanavalin A-flourescent microsphere conjugates, to visualize membrane receptors by electron microscopy, demonstrated location and capping in membrane receptors. Molday and associates, in the same study, also utilized wheat germ agglutinin and demonstrated a difference in the binding pattern of this lectin in comparison to the concanavalin A, thus further supporting the specific binding properties of concanavalin A with membrane bound receptors in slime molds. It is then feasible that the application of the con A-receptor binding technique can demonstrate the presence of a membrane bound mating receptor in D. iridis.

In this study, the Pan 2-7 A^{8} and Hon 1-2 A^{1} were placed on growth media supplemented with the addition of

200 ug/ml of concanavalin A (Gillette at al., 1973). The control plates utilized the same organisms, culture, and growth conditions, with the exclusion of concanavalin A, exhibited 80% plasmodial formation. The experimental crosses (those with con A), exhibited almost complete exclusion of plasmodial formations.

Since these experimental plates produced virtually no plasmodial growth by macroscopic examination, it was hypothesized that cell fusion (mating) is necessary for diploid plasmodial formation between heterothallic isolates of D. iridis, and that this fusion was inhibited by concanavalin A binding to membrane glycoproteins comprising the mating receptor. Furthermore, this membrane mating receptor must contain alpha-mannopyranose and alpha-glucopyranose carbohydrate residues (Sharon and Lis, 1972; Lis and Sharon, 1973) since con A binds to these moieties. This hypothesis was confirmed by isolation of these organisms from experimental cultures and then subjecting them to proper fixation, staining, and microspectrophotometric analysis to determine the relative nuclear DNA content. The results of this analysis were then compared to controls by means of histograms, as well as by comparison of the statistical mean DNA values.

Upon comparison, the experimental cultures exhibited a relative mean DNA content of 3.28, which were similar to the values for the myxamoebal controls: 2.93 for Pan 2-7 A^8, and 3.16 for Hon 1-2 A^1. When comparing experimental culture values to diploid plasmodial controls, the concanavalin

A treated clones are observed to have approximately half the nuclear DNA content as the diploid controls, and are thus designated haploid. Since these cells were confirmed to be haploid, the possibility of cell fusion and subsequent zygote formation was ruled out.

It should be noted that two con A treated plates produced plasmodia in the experimental population. These plates comprised approximately 1.8% of the total plates used in the experimental cross. These plasmodia were very fragile, small and demonstrated low viability. The cultures were subsequently transferred to plasmodia media (see table 1; Materials and Methods) for further growth and development. They remained very small and fragile, and because of low viability are difficult to transfer. Following a seven day incubation period, the plasmodial isolates were properly fixed and stained for microspectrophotometric analysis.

Upon comparison of mean DNA values for the experimentally crosses plasmodia to the controls, it was evident that they were haploid. They had a mean DNA of 3.88, which was very near one half the relative DNA content of the diploid controls (7.09), and slightly higher than the haploid control values; 2.93 for Pan 2-7 A^8, and 3.16 for Hon 1-2 A^1. This slightly higher value can be explained by the fact that these plasmodial nuclei may be hyperhaploids (Collins and Ling, 1968) due to aneuploid development through apogamy, where the diploid heterozygous condition exists at the mating

locus, apparently satisfying requirements for plasmodial formation (Collins and Ling, 1968; Adler and Hold, 1975; Therrien and Yemma, 1974, 1975).

The results of this study correlate well and are in agreement with the studies of apomictic plasmodial development by heterothallic isolates of the acellular slime mold, previously reported by Adler and Holt (1975) in Physarum polycephalum, and Therrien and Yemma (1974, 1975) and Collins and Ling (1968) in Didymium iridis, which suggest two possible mechanisms for development. The two mechanisms they suggest for apomictic plasmodial development are the fusion of myxamoebae with plasmogany but no Karyogamy, or the nuclear division of a single cell without an accompanying cytokinesis.

This study provides rather conclusive evidence that fusion does not occur in apomictic development of plasmodia, since it can be assumed that concanavalin A had already bound to the mating receptors of the two myxamoebal isolates in the experimental culture plates before they came into intimate contact. This therefore suggests that apomictic plasmodial formation occurs through an abnormal mitotic division of a single cell nucleus, where mitosis is not followed by cytokinesis. This division involves a non-disjunction at the mating locus forming an aneuploid. This cell, through subsequent nuclear division and differentiation may then develop into a haploid plasmodium.

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