MICROSPECTROPHOTOMETRIC AND GENETIC ANALYSIS OF HETEROPLOIDY IN THE MYXOMYCETE *DIDYMIUM IRIDIS*

by

John L. Semivan

Submitted in Partial Fulfillment of the Requirements
for the Degree of
Master of Science
in the
Biology
Program

Dr. John J. Gemmna 8-20-79
Adviser Date

Leo Land 8-24-79
Dean of the Graduate School Date

YOUNGSTOWN STATE UNIVERSITY

August, 1979

ABSTRACT

MICROSPECTROPHOTOMETRIC AND GENETIC ANALYSIS OF HETEROPLOIDY IN THE MYXOMYCETE DIDYMIUM IRIDIS

John L. Semivan

Master of Science

Youngstown State University, 1979

Microspectrophotometric and nuclear marker analyses were made of the chromosome distribution patterns in all life cycle stages for polyploid-haploid crosses in Didymium iridis over a period of four filial generations. Data was collected from swarmer, myxamoeba, plasmodia, and sporangial stages of the life cycle. Relative ploidy determinations of nuclear DNA contents were provided by cytophotometric measurements of Feulgen-DNA nucleal reaction nuclei. Mating type marker genetics also provided information on chromosome segregation in meiospores.

The results of the investigation indicates the production of plasmodia and meiospores with a variety of unexpected ploidy levels. Cytological examinations of progeny spores suggest that polyploid nuclei undergo meiotic stress and produce aneuploid myxamoeba. Genetic analysis of these myxamoeba indicates the production and transmission of progeny clones capable of expressing both parental mating types. The further observation of selfing myxamoeba suggests that in this system: chromosome elimination

after karyogamy of parental nuclei occurs and, a nuclear gene, gene product or, cytoplasmic factor has established a condition of enhanced cell fusion through the ability to institute membrane contact. It is yet to be determined if this behavior has a relationship to the production of aneuploid spores, however, cytoplasmic inheritance of the factor(s) involved is implicated.

ACKNOWLEDGEMENTS

The author would like to sincerely thank Dr. J.J. Yemma for his guidance and faith during the course of this study. Those deserving special mention are Mr. Len Perry and Miss Anna Marie Longo.

Finally, this thesis is dedicated to the author's parents and wife, for their continual support and encouragement.

TABLE OF CONTENTS

LIST OF SYMBOLS

SYMBOL	DEFINITION	UNITS OF REFERENCE
A	Area	μ
B	Area of the Measured Field	
c	Chromophore Concentration	
C	Correction factor for Unoccupied Space $C = (2-Q)^{-1} \ln (Q-1)^{-1}$	
E	Extinction	
I_o	Flux of Photons on chromophore	
I_s	Transmitted Flux	
k	Specific absorptivity constant of the chromophore at a a defined wavelength	
l	Path length through tissue	μ
L	Parameter equivalent to one minus the transmission (1-T) at a defined wavelength	
m	Chromophore mass in the measured field	
Q	Ratio of L_2/L_1	
T	Transmission of the Flux	
λ	Wavelength	nm

LIST OF FIGURES

FIGURE PAGE

LIST OF CHARTS

INTRODUCTION

Our understanding of biological processes depends upon the use of "model organism" systems easily and profitably adapted to analytical methodology. The true acellular slime molds, including Didymium iridis with its comparatively short life cycle, allow multi-faceted studies of cell cycle events during the processes of growth and differentiation while still maintaining the advantages of simpler microorganisms such as mass cell culturing of myxamoeba and plasmodia, natural synchrony of nuclear and morphological events, methodological access, and genetic analysis. Of particular interest are the characteristically plant and animal stages of the life cycle which offer varied opportunities for research in many areas of Biology. The complex and intriguing life cycle of the Myxomycete (See Plate 1) which generally involves an alternation of haploid and diploid trophic phases, has been well defined and discussed by Guttes, Guttes and Rusch (1961), Alexopoulos and Koevenig (1962), and Gray and Alexopoulos (1968).

The general life cycle of D. iridis is characterized by an alternation of two distinct vegetative states, a myxamoeba haplophase and a somatic plasmodia diplophase. This nuclear phase alternance cycle is under the control of a complex genetic system of differentiation and growth

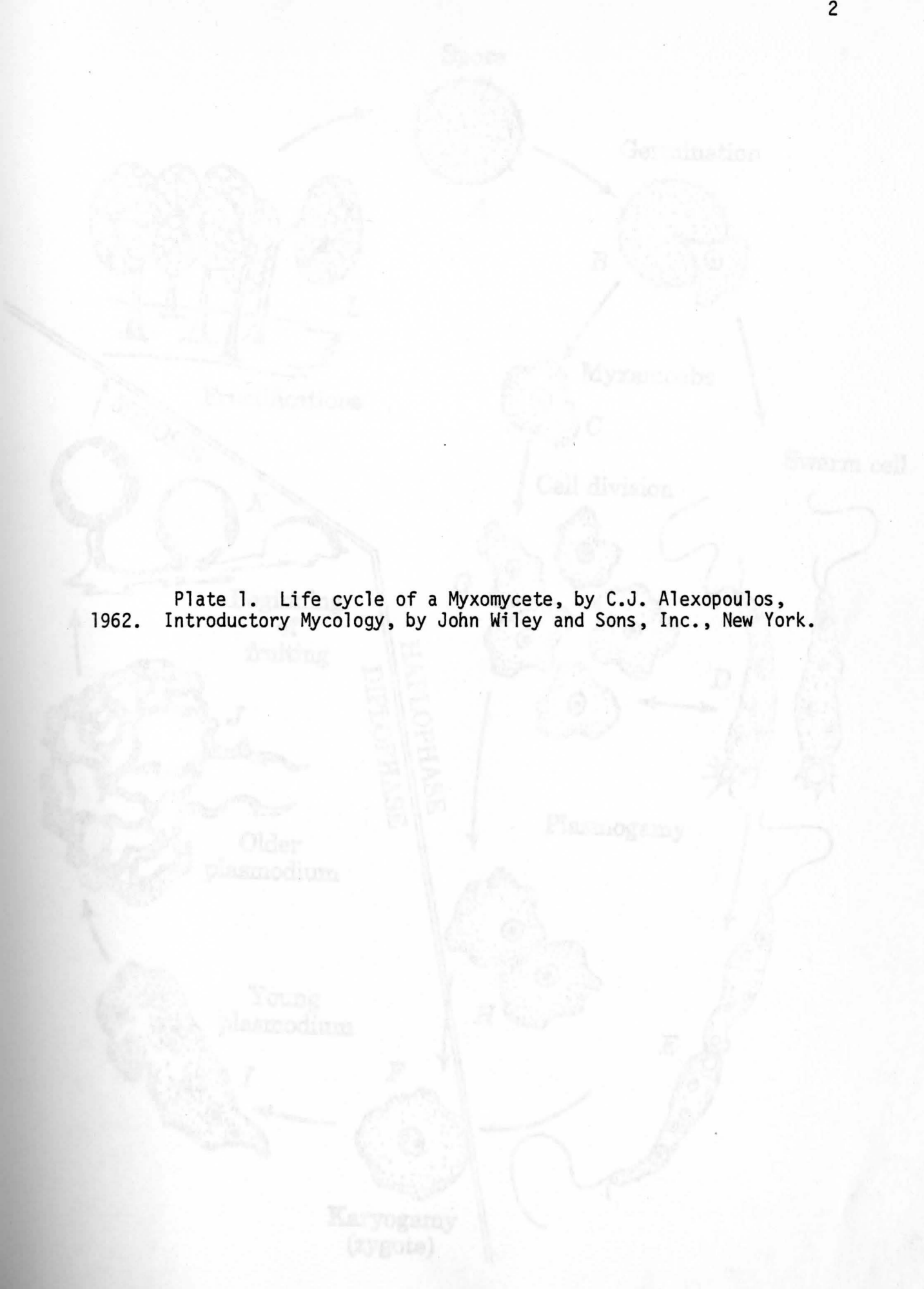
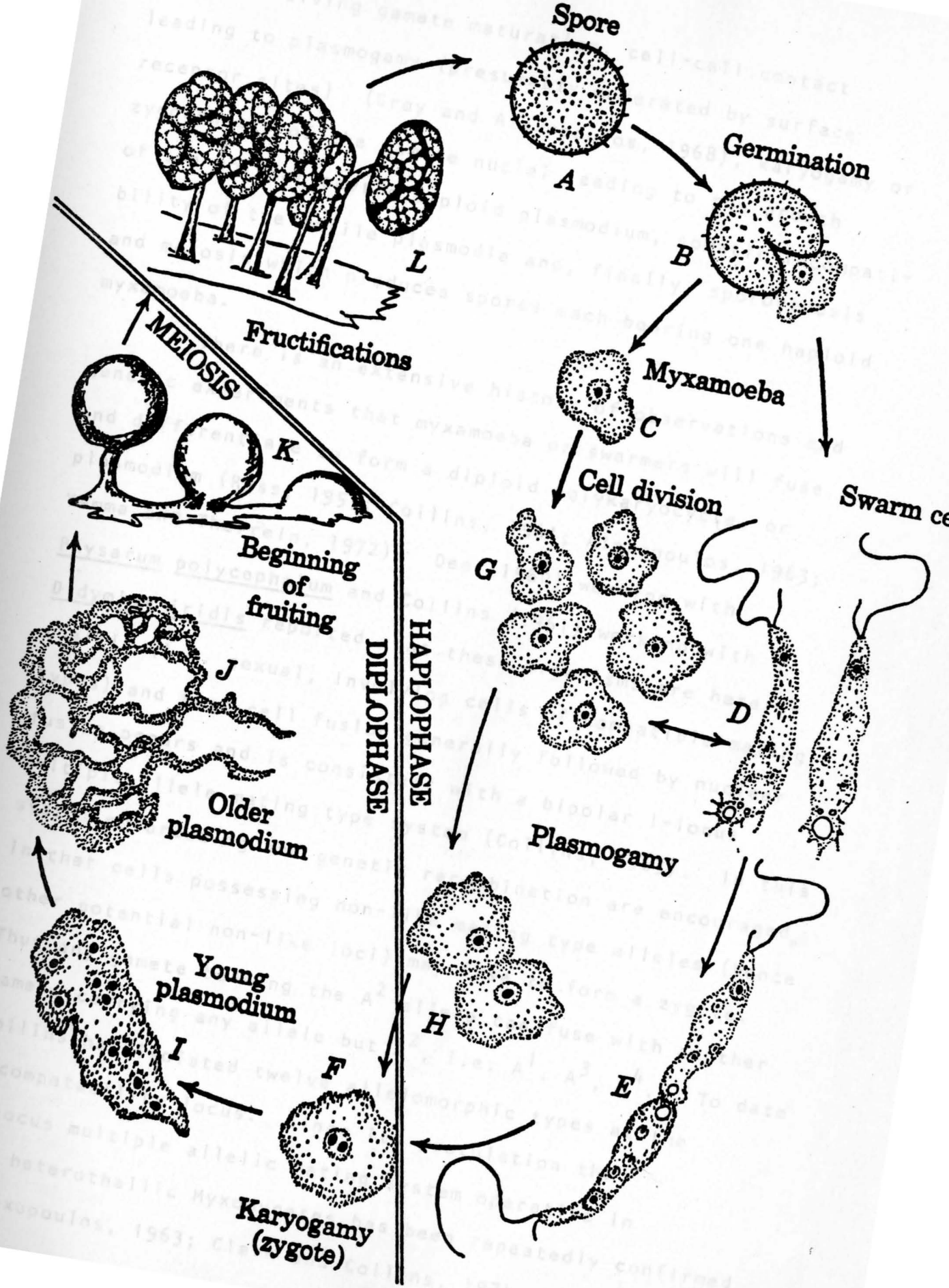


Plate 1. Life cycle of a Myxomycete, by C.J. Alexopoulos, 1962. Introductory Mycology, by John Wiley and Sons, Inc., New York.


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

events involving gamete maturation, cell-cell contact leading to plasmogamy (presumably moderated by surface receptor sites) (Gray and Alexopoulos, 1968), karyogamy or zygogenesis of the gamete nuclei leading to the growth of the polykaryocytic diploid plasmodium, somatic incompatibility of the motile plasmodia and, finally, sporogenesis and meiosis which produces spores each bearing one haploid myxamoeba.

There is an extensive history of observations and genetic experiments that myxamoeba or swarmers will fuse and differentiate to form a diploid polykaryocycte, or plasmodium (Ross, 1957; Collins, 1961; Alexopoulos, 1963; Yemma and Therrein, 1972). Dee (1960) working with Physarum polycephalum and Collins (1961) working with Didymium iridis reported that these organisms are heterothallic (i.e. sexual, involving cells of compatible mating types) and that cell fusion generally followed by nuclear fusion occurs and is consistent with a bipolar 1-locus multiple allele mating type system (Collins, 1963). In this system outbreeding and genetic recombination are encouraged in that cells possessing non-like mating type alleles (hence other potential non-like loci) may fuse to form a zygote. Thus any gamete bearing the A^2 allele can fuse with another gamete carrying any allele but A^2, i.e. A^1, A^3, A^4... To date Collins has isolated twelve allelomorphic types at the incompatability locus. Since its postulation the 1-locus multiple allelic mating system operative in the heterothallic Myxomycetes has been repeatedly confirmed (Alexopoulos, 1963; Clark and Collins, 1974; Collins, 1961,

1963, 1965, 1974, 1975, 1976; Collins and Ling, 1964; and Dee, 1960, 1962, 1966).

Most of the work on sexual cell fusions in the myxomycetes has been either descriptive (Ross, 1957, 1967a; Koevenig, 1964; Gray and Alexopoulos 1968) or related to the genetics of the multiple allelic basis of incompatibility (Dee, 1966; Collins, 1976). Following Cayley's (1929) initial suggestion, Ross, Shipley and Cummings (1973) have proposed a model system concerning the functional aspects of the mating type alleles at the biochemical level of the plasma membrane. Their study included a fusion kinetics assay which suggested a chemical communication system wherein each myxamoebal mating type in a heterothallic cross continually secretes an "inducer" substance which will physiologically condition their sexually incompetent membranes. Mutual chemical induction involving expression of the mating type gene would lead to re-programming of the plasma membrane with specific proteins functional in recognition. Another possible explanation of the mode of action of the inducer (or the mating type cistron) may be indirect, involving exposure of pre-existing cryptic membrane sites as has been shown to occur in transformed Golden hamster cells (Inbar and Sachs, 1969; Ross, 1967). In a later article Ross and Shipley (1973) have obtained experimental evidence, which further suggests a requirement of protein synthesis for the process of sexual cell fusions. Their results are in agreement with studies done on other eukaryotes (Poste, 1970). Whatever the mechanism; the

myxamoeba are able to function as sexually competent gametes distinguishing between self and non-self. Collins and Ling (1968) then Yemma (1971) working with self-fertile isolates of D. iridis postulated that plasmodial formation in heterothallic crosses may be the result of mating type gene de-repression, whereas in a common mating type situation the mating type gene would remain repressed; thus the mating type gene may be a regulator gene and its expression would lead to plasmodial formation. Such a system could conceivably explain heterothallism at the level of molecular genetics. As Wheals (1970) pointed out, "... the mode of action of the mating type locus is not known; it could be affecting amoebal fusion, or nuclear fusion, or both." However, presenting recent evidence consistent with Yemma (1971) and Ross et. al., (1973) Yemma and Therrein (1975) were able to demonstrate microspectrophotometrically that upon mixing myxamoeba of compatible mating types, stable zygote formation occurred rapidly in a non-random process, implying specific membrane activity (i.e. cell fusion). While much of the actual sequence of events is still largely unknown, both investigators support a common mechanism for the function of the mating type gene, updating Wheals' earlier caution.

Poste (1970, 1971 in other studies) discussed virus-induced cell fusions considering cell fusions during differentiation events and proposed a Membrane Fusion Reaction Theory. Their theory includes consideration of the myxomycete plasmodium (Poste, 1970) and is representative

of a common mechanism for fusion between biological membranes. Basically, their scheme for fusion is accomplished in four stages beginning with the essential prerequisite of membrane contact. In most cells the outer coats of each limit the cells to a 100-200A proximity, while for fusion, intermembrane distances must be less than 10A. Therefore, lysosomal degredation of the outer cell coat (glycocalyx) resulting in microvilli formation is necessitated. Ross (1967a) using phase-contrast optics noted that before syngamy occurred a period of cell-cell proximity was necessary; possibly involving degredation of outer membrane layers. Contact then allows induction involving hyper-polarization of the apposed membranes which would lead to an altered electrostatic potential within the membrane. This event is then responsible for the displacement of Ca^{++} and depressed ATP activity of the membrane. The third step, actual fusion, occurs only after stages 1 and 2 and results in the establishment of stable intermembrane molecular bonding. Finally, the fused membranes will return to a "stable" state in a process essentially the reverse of induction. (For review see Poste and Allison, 1971). Studying fusion cells of five species of myxomycetes, Abe (1934) reported a difference in oxidation-reduction potential and electrical charge of their membranes suggestive of the induction stage. Ross and Shipley (1973) using D. iridis have obtained limited results of inhibited cell fusions with the lysosomal stabilizer, diphenhydramine, giving support to the Membrane

Fusion Theory.

The capacity for plasmodial formation leading to completion of the life cycle has been the subject of intense research in recent years. It is becoming increasingly apparent that myxamoeba have adapted several pathways of differentiation into the plasmodial stage involving many fascinating, though largely unknown mechanisms. Present evidence suggests that the Myxomycetes may be classified into two subgroups, dependent upon their utilization of a mating type system or non-sexual modes. The sexual compatibility system (heterothallism) is widespread, occuring throughout a diverse range of taxa, and is considered as being the primal condition of the Myxomycetes. In this system each strain of myxamoeba form clones which are self-sterile and cross-fertile.

Other methods of plasmodial differentiation show variations of this basic pattern, presumably through the action of gene regulatory agents (Collins and Ling, 1968); however, all suggest an evolutionary trend towards adaptation of the life cycle to particular geographic environments (Clark and Collins, 1976). Many non-heterothallic strains are known to complete their life cycle by intercellular matings within the same clone in a homothallic mating process. Considered a sexual process, cells of like mating type, sex and genotype fuse forming a homozygotic plasmodium which yield monotypic progeny. Homothallism is probably a secondary mechanism where the selection pressure for production of viable spores is greater than that for genetic recombination,

i.e. it is a method to regenerate a population of haploid myxamoeba under restrictive environmental conditions (Carlile, 1973). Homothallism in the Myxomycetes, including D. iridis, has been reviewed by Gray and Alexopoulos (1968) and studied by Therrein and Yemma (1974) and Collins (1976).

Several species, previously classified as homothallic based upon their life cycle behavior, have now been shown to differentiate by another method. This takes place by a non-sexual process (apogamy) where single myxamoeba initiate mitosis without cytokinesis producing a multinucleate plasmodium. Agpogamic development is widespread among the Plant Kingdom (Bold, 1960) and is characterized in the fungi by a lack of nuclear phase alternance; karyogamy and meiosis never take place. Historically, von Stosch (1935, 1937, 1964) has offered much of the evidence for the importance of apogamy as a means of completing the life cycle in the myxomycetes. There is still much debate as to whether many "homothallic" strains are in fact apogamic. Kerr (1967), 1968) has produced convincing evidence that their strain of D. nigripes, previously classified as homothallic, differentiates apogamically. He used both time-lapse microcinemaphotography and comparative metaphase chromosome counts to show that neither plasmogamy nor karyogamy occurs, and that there is no ploidy difference between myxamoeba and plasmodia. The face that the myxamoeba (haploid state) and plasmodia (diploid state) show different styles of mitosis has been used by some investigators as a means to show the

advent of the diplophase in the organisms life cycle (Kerr, 1967; Koevenig and Jackson, 1966). However, there is mounting evidence to show that the establishment of the diploid state (an obligatory n-2n condition) is not a necessary prerequisite to plasmodial formation (Ross, 1966). Therrein and Yemma (1974), using quantitative DNA cytophotometry, developed a method to determine the mechanism of plasmodium development based upon comparative ploidy measurements of nuclei in various life cycle stages. They were able to show that the Ph-1 isolate of D. iridis, thought to be homothallic, lacks nuclear phase alternance and is therefore apogamic. In their comparative study they offer evidence supportive to von Stosch's (1964) suggestion that self-fertile isolates of normally heterothallic strains develop apogamically. It is of interest that the Ph-1 myxamoeba are 4C ploidy as are the plasmodia; at this point it is unknown if an 8C nuclei is produced in the sporangium.

Wheals (1970) conducting genetic investigations with the Colonia isolate of P. polycephalum concluded that the strain was homothallic. His analysis indicated that the ability to clonally form plasmodia (self) was due to a specific allele (mt_h) at the mating type locus. However, Cooke and Dee (1974) then Anderson, Cook and Dee (1976) have presented similar microdensitometer and time-lapse cinematographic data which clearly show that the Colonia isolate is apogamically formed, as previously determined by von Stosch (1964). Adler and Holt (1974a) in a genetic-re-investigation of Colonia were able to get diploid

plasmodia by crossing with mt_3 and mt_4 myxamoeba at terperatures of 30 C. The increased temperature seems to select against clonal (apogamic) differentiation favoring greater numbers of crossed plasmodia. The cellular mechanism for this selective behavior is unknown. Analysis of F_1 progeny however, revealed myxamoeba which developed apogamically and myxamoeba of the heterothallic mating type in a 1:1 ratio. This segregation ratio still supports Wheals' contention that a specific allele of the mating type locus (mt_h) controls clonal plasmodial formation, however certain heterothallic strains are partially isogenic to Colonia and form plasmodia by preferential selection of alternative developmental pathways. Recently, Yemma (unpublished data) has obtained cross plasmodia with the Ph-1 and a heterothallic isolate of D. iridis. The isogenic nature of Colonia and Ph-1 may illustrate the heterothallic to true apogamic transition process. The fact that Colonia nuclei are hemizygous at all loci and express recessive mutations has made this strain invaluable for genetic analysis. The nuclear cycle events are, however, incomplete, since there is no data available which would indicate whether meiosis (preceded by chromosomal doubling) occurs in Colonia.

Probably the most perplexing of all developmental pathways is clonal plasmodium formation, or selfing. Here, myxamoeba of otherwise self-sterile heterothallic isolates differentiate into plasmodia without the necessity of crossing with compatible myxamoeba. The problem of selfing has been most extensively studied in D. iridis. Selfing,

earlier was thought to occur due to the cleavage of binucleate spores (Dee, 1966; Ross, 1967) or to a mutation at the mating type locus (Collins and Clark, 1966). However, Collins and Ling (1968) using genetic-marker F_1 analysis concluded that selfing is an apogamic process. Their data proposed three possible explanations for selfing in heterothallic isolates of known mating type. Since a given clonal line can give rise to subclones with varying capacities for selfing, a cytoplasmic mode of inheritance is feasible. Another mechanism might be the production of aneuploid amoeba during log phase growth. These "hyper-haploid" (aneuploid) amoeba might then mimic the diploid condition and be a possible source of the observed fact that the n-2n situation need not be strictly fulfilled. The third hypothesis was re-emphasized by Yemma (1971) and involves the derepression of modifier or suppressor loci, perhaps at the mating type locus, postulated to function as a regulator gene. The majority of the work involving the selfing phenomenon has concentrated on the cytoplasmic inheritance and aneuploid amoeba hypotheses.

Considering that a log phase cell population contains approximately $7X10^6$ myxamoeba per 15X16 mm Petri plate (Collins and Ling, 1968) occasional abnormal mitotic divisions or replication errors are statistically probable. Kraemer, et al., (1972) presents an excellent discussion on the origin and determination of heteroploid (i.e. haploid, aneuploid, polyploid) mammalian cell populations using Feuglen cytophotometry and flow microflourometry in conjunction

with karyotype analysis. These investigators have studied the dispersion in number (about the mode) and structure of chromosomes or parts of chromosomes as a result of such mechanisms as abnormal non-dysjunctions, endomitotic reduplications, multipolar mitoses, translocations and centric fusions and fissions. They state, "All these mechanisms should be correlated with shifts in cellular DNA content, and indeed this has been demonstrated when cellular DNA content and chromosome number mode could both be monitored in a series of related stemlines." Kraemer et al., (1972), however, offer evidence that in certain situations heteroploidy may not necessarily reflect increases in cellular DNA contents and they present a discussion, based upon their data, to account for this opposition to "accepted dogma".

Working with the cellular slime mold, Dyctiostelium discoideum, Sussman and Sussman (1962) demonstrated a heteroploid cell population existing in stable haploid, stable diploid and metastable classes. Although precise quantitation of nuclear DNA is available, the problem of exact chromosome counts in many myxomycetes (including D. iridis) is still controversial due to the small size and apparently large number of chromosomes. Recent methodological advances though, are rectifying this impasse (Therrein and Collins, 1976). Ross (1966) using direct chromosome counts, demonstrated the existence of heteroploid

(haploid to polyploid) myxamoeba and plasmodia in four strains of Myxomycetes. He points out the variability in chromosome number both between strains and sublines from the same strain. His data also indicates that polyploid nuclei are functional in completion of the life cycle. A further study by Koevenig and Jackson (1966) encountered a small percentage of polyploids (4%) in P. polycephalum, viewing the plasmodium as a productive source for autopolyploid nuclei. The authors speculate that this may be due to abnormal karyogamy or to abnormal plasmodial mitosis leading to an autopolyploid condition. These studies, and others by Kerr (1967, 1968) have related chromosome number to cultural conditions (e.g. axenic vs. bacterial culture) and to age of culture. McCullough et al., (1973) and Adler and Holt (1974b) have correlated changes in nuclear DNA content and myxamoeba properties in P. polycephalum as a result of extended culture and senescence. Mohberg and Rusch (1971) then Mohberg et al., (1973) working with P. polycephalum and Colonia extended these findings by demonstrating variation in DNA values, correlated to chromosome counts, in several sublines of one isolate. Their findings showed myxamoeba, plasmodia and spores of haploid, diploid and polyploid values. It is becoming increasingly apparent that the Myxomycetes, as a group, exist in a heteroploid range of variable stability, and that this condition is natural in both the assimilative as well as the myxamoebal stages. The aneuploid spores are related to poor germination rates, a frequent observation made in the literature

(Therrein and Collins, 1976). It must be noted, however, that the ploidy associated with poor germination is near diploid or greater amounts of DNA. Work is progressing in several laboratories in a comprehensive effort to relate DNA measurements to chromosome counts and the effects of these parameters on spore viability and mating behavior.

In consideration of aneuploid myxamoeba with respect to selfing, evidence is mounting which tends to relate these events. Adler and Holt (1975) recently demonstrated that some selfing clones of Physarum exhibit near diploid amounts of DNA. Genetic analysis showed the selfer (CPF) plasmodia to be heterozygous for the mating locus, thus implicating disomy of the chromosome bearing the mating type locus. To date this condition has not been validated for D. iridis, but it is not being ruled out. Collins and Therrein (1976) reviewing the evidence state, "Moreover, it is possible that further studies will turn up selfers in D. iridis which are disomic for one or more chromosomes, or are otherwise aneuploid." Yemma and Therrein (1972) examining selfed plasmodia concluded that they were diploid unreplicated (2C), since the plasmodial nuclei showed values one-half those of replicated diploid (4C) nuclei located in sporangia. More recently Therrein and Yemma (1975) reviewing this problem, demonstrated unequivocably that the selfed plasmodia is replicated haploid and that diploidization occurs prior to or during sporogenesis. Thus the mode of plasmodial formation in their selfer clone appears to be apogamic. They state, however, "...these results do not

rule out the possibility that swarm cell fusion (plasmogamy) occurs prior to plasmodial formation, only that nuclear fusion (karyogamy) does not occur at that time." Of interest is the fact that homokaryogamy did occur, in seeming support of Skupienski's (1927) report that karyogamy may occur later in the life cycle, and that "...it certainly appears to be part of the sequence of events during selfing in this species." (Therrein and Yemma, 1975). Thus while some of the selfers mechanism shows some of the initial life cycle events of apogamic strains (Therrein and Yemma, 1974), there are nuclear cycle differences in that karyogamy and possibly meiosis do occur. The information has led Clark and Collins (1976) to postulate selfing as an intermediate state in the transition from a heterothallic to a homothallic strain; whereas apogamic strains are a response to polyploid mitotic sterility problems and remain totally non-sexual by passing karyogamy and meiosis. Yemma et al., (1974) also reinforced an earlier observation that selfing may involve cell fusion possibly mediated by a (viral) cytoplasmic factor.

That selfing in D. iridis has a cytoplasmic component was convincingly demonstrated by Yemma, Therrein and Ventura, (1974). Using mating type as a nuclear marker, a non-selfing isolate was crossed in separate experiments to two selfing isolates of known selfing frequency. From these crosses the resulting meiotic segregants were analyzed for selfing frequency. The capacity to self was inherited by all F_1 segregants, regardless of nuclear mating type, with the

non-selfing isolate forming plasmodia at a higher frequency than either of the two original selfing parents. Upon recloning, the capacity to self reached 100% in the F_3 generation for the original non-selfing nuclear cell, and its increased frequency upon recloning, led to the hypothesis that a cytoplasmic factor is operant. Since all plasmodial nuclei exist in a common cytoplasm, spore delimitation would allow for the acquisition of the factor by any progeny. Also, the non-selfing isolate is more sensitive to the action of this factor; however, the nature and action of this factor are as yet unknown. The presence of a virus or virus-like particle is a distinct possibility since Olive (1975) has demonstrated their occurance in the fungi. Thus the evidence to date seems to link selfing and other alternate modes of plasmodial formation to events which are nuclear, or cytoplasmic, or both.

The present work was stimulated by findings reported by Therrein and Collins (1976) which show that a polyploid clone (CR 2-26, from the Costa Rican 2 isolate) when crossed to a compatible haploid clone (CR 5-5, from the Costa Rican 5 isolate) results in the apogamic induction of the haploid myxamoeba to form haploid plasmodia. The induction phenomena occurs 100% of the time and is thought to be due to a factor or event related to the polyploid myxamoeba. An F_1 analysis of this plasmodia shows that the progeny are of one mating type allele only, the CR 5-5 haploid isolate.

Haugli, et al., (1971) analyzing a haploid-polyploid interisolate cross between heterothallic

myxamoeba of P. polycephalum isolated progeny which were able to clonally produce plasmodia (selfing). Alder and Holt (1975) extended this study by examining spores derived from the selfer plasmodia. As mentioned previously, the F_2 segregants included selfer myxamoeba as well as myxamoeba bearing each of the original parental heterothallic mating types (i.e. haploid and polyploid mating type cells) which gave rise to the F_1 selfer plasmodia. They concluded from this that the F_1 selfer myxamoeba are heterozygous for the mating type locus. The heterozygous selfer myxamoeba were also shown to contain near-diploid amounts of DNA. Their examination of selfer plasmodia revealed a lack of nuclear phase alternance, a condition similar to the Colonia (mt_h) plasmodia.

Clark and Collins (1976) and Collins and Therrein (1976) have discussed the occurance of aneuploid and polyploid spores in heterothallic mating types of D. iridis. The latter authors present a justification of nuclear DNA content and ploidy designations, specifically for the polyploid isolate (CR 2-26) used in this study.

It is the purpose of this investigation, in view of the previous information, to record the differentiation patterns of interisolate crosses between a polyploid clone (CR 2-26) of the Costa Rican -2 isolate and a haploid clone (CR 5-5) of the Costa Rican -5 isolate. It is also the intent of this study to determine the meiotic segregation patterns of these interisolate crosses. The objectives of this inquiry, then are: 1) to determine nuclear ploidy

levels in both plasmodial and subsequent spore isolate nuclei of D. iridis; and 2) to delineate the meiotic segregation patterns of these crosses using the mating type allele as a nuclear marker. A central query of this thesis is to answer the question, "Are there differential ploidy levels in resultant meiotic segregants, and if so, can they be correlated to events such as selfing or other differentiation modes?" And, "Is there correlation to other previous observations using similar designs?"

In order to accomplish the former, nuclear DNA activity was determined by using the Feulgen staining technique in combination with absorption spectrophotometry. The two-wavelength method of chromophore measurement developed by Patau (1952) and Ornstein (1952) was used exclusively.

The development of microspectrophotometry as a valid analytical tool began with the ultraviolet (UV) absorption measurements of chromatin at 260 nm (reviewed by Caspersson, 1936; Swift, 1966). Concurrent histochemical work was being carried out that identified the UV absorber as nucleic acids (Pearse, 1968). Also, biochemical research has provided evidence of a "DNA constancy" hypothesis, in that all cells of a particular organism possess a uniform amount of DNA (Boivin, Venderly and Venderly, 1948, 1949; Ris and Mirsky, 1949).

Caspersson (1936), in a critical appraisal of the methodology, found that protein interference at 280 nm and non-specific light losses gave inconsistent results for quantitative UV studies. Drawing upon an earlier

observation made by Wyckoff (1932) that the UV absorption patter at 257 nm was chemically similar to the morphological pattern of Feulgen stained nuclei Caspersson (1936), then Pollister, Swift, Mosses and Leuchtenberger (1947, 1949) were able to apply the Feulgen stain to absorption in the visible light region.

Feulgen-DNA quantitations can be considered a valid approximation to the problem when the following conditions are satisfied: 1) microspectrophotometric determinations are accurate; 2) the Feulgen reaction is specific for DNA only; and 3) the DNA quantitation can be used as a reliable indicator of ploid.

Davies and Walker (1953), Patau (1952) and Ornstein (1952) present a detailed discussion concerning the accuracy of the two-wavelength method in compensating for distributional error, or the nonhomogeneous spatial distribution of the chromophore-substrate molecules.

By measuring the amounts of nuclear DNA in several animal species by biochemical extraction and spectrophotometry Ris and Mirsky (1940) established the accuracy of microspectrophotometry in quantitative analysis. Their values were within 10% of each other.

Lessler (1953) and Kasten (1959) have confirmed the specificity of the Feulgen reaction, and the direct correlation between color intensity and nuclear DNA concentrations.

Evidence for a direct relationship between DNA and ploidy was presented by Ris and Mirsky (1949) working with

several animal species. Their data confirmed the observations from chromosomal behavior during meiosis that mature spermatazoa contained approximately half the DNA of somatic cells. Swift (1950) comparing nuclear DNA measurements in murine and frog specimens also showed corroborative results in a 2:1 ratio for spermatozoa and somatic cells. Ris and MIrsky (1949) also presented data suggesting the existence of a polyploid series existing in certain tissues. Moore (1952) further established this hypothesis by confirming the 2:1 ratio of somatic to spermatozoa cells and showing a 1:2:4:8 polyploid series in liver and other tissues. She concluded that this geometric series was not due to experimental error but to the natural biological properties of the organism.

Rusch (1973) has suggested a revision of the terminology of the G-1 phase of the cell cycle involving differentiation events in both normal and carcinogenic states, based upon his work with P. polycephalum. The advantages the Myxomycetes offer make this organism a useful model of basic research. Genetic investigations concerning the role of the mating type gene and modifying factors in plasmodial formation, a basic model of specific gene activity leading to differentiation, depend upon a comprehensive approach to the life cycle events. Such studies will surely lead to a better understanding of nucleo-cytoplasmic relations in differentiation events.

MATERIALS AND METHODS

Tissue Preparation

All isolates of *Didymium iridis* used during this investigation were obtained from Dr. O. R. Collins, Department of Botany, University of California, Berkeley. Clones of the heterothallic isolates were designated CR 2-26, CR 5-5, Hon a-2A^2, Hon a-1A^1, Hon a-7A^2, A-2-, and BIP-4. They are of compatible mating types expressing the A^6, A^2, A^2, A^1, A^2, A^2, and A^7 mating alleles, respectively.

Cultivation of Material

The original single spore isolates of Didymium iridis were allowed to germinate into clones of myxamoeba. These were sustained on slants of half-strength 2% corn meal agar (CM/2) which were previously inoculated with *Escherichia coli*. In this two-membered culture system, the *E. coli* metabolize the corn meal while the cells utilize the bacteria as a food source. Culture plates of sterilized media were streaked with 2 ml of a dilute *E. coli* suspension which developed into an even bacterial lawn within a 24 hour period. These plates were used for subculturing of the stock clones.

The 2% CM/2 medium was prepared by mixing 8.5 grams of Difco Corn Meal agar with 8.0 grams Difco plain agar in 1.0 liter of distilled water and then autoclaved. The media was then pipetted as 20 ml aliquots into 100 X 20 mm Petri plates (Collins, 1963; Alexopoulos and Gray, 1968; Yemma et al., 1974). Once the myxamoeba reached the log phase of growth each clone was subcultured to fresh culture plates by transferring small pieces of agar containing adhering myxamoebae. Transfers of the amoeba were made every seven days. All stock cultures were periodically examined under the microscope for fungal and bacterial contaminants; any infected plates were discarded and replaced.

To obtain a cross plasmodium, one mixes amoebal suspensions of two compatible mating types (CR 2-26 and CR 5-5) on a fresh culture plate previously innoculated with E. coli (Collins, 1963; Therrein, 1966). Once the plasmodia have grown into a relatively large size, usually 1/3 the area of the plate, they were transferred to fresh culture plates pre-sprinkled with pieces of sterilized oats. This method allowed the plasmodia to grow into large workable organisms and required about 4-7 days. Several "selfed" plasmodia (collins and Ling, 1968; Yemma and Therrein, 1972; Yemma et al., 1974) were also analyzed in this investigation. These plasmodia were maintained as the cross plasmodia. Both myxamoebal and plasmodial cultures were always maintained and incubated at 23 degrees C.

In order to obtain sporangia, viable stock plasmodia were transferred to culture plates. These were allowed to remain at room temperature under normal light-dark conditions until sporulation occurred, usually within 2-6 days. In some instances, plasmodia differentiated into the sclerotial (spherule) phase of their life cycle and the following procedures were required for their sporulation: the sclerotia were transferred to fresh culture plates with an excess of water added and then incubated. Within 3-7 days plasmodia appeared and the process was repeated as above (Therrein, 1966; Alexopoulos and Gray, 1968; Yemma, 1971). In order to produce clones from a single spore, the following method was employed: sporangia developed from cross plasmodia were isolated with fine-tipped forceps and streaked in 2 ml of water with a sterile spatula across a dry plate of sterile plain agar. Individual spores were then isolated using a sterile fine needle under an A.O. stereomicroscope at 20X and transferred to Petri plates containing sterile 2% CM/2. These plates were checked using a compound light microscope at 150X to ensure that only one spore was transferred. 1 ml of a dilute E. coli suspension was added to the single spore isolate, and the plates were allowed to incubate (Collins, 1963; Yemma, 1971). When germination occurred it did so within one to two weeks. Myxamoeba resulting from single spore germination were transferred to fresh 2% CM/2 culture plates and grown as previously described. All plates, however,

were incubated and periodically examined for 45 days: beyond which point they were assumed non-viable (Collins, 1963, 1965).

Where genetical analysis of single spore isolates derived from F_1, F_2 and F_3 clones were necessary, these were backcrossed to the Parental types, as well as to several tester clones of known mating type, and their mating types determined. Mating type designations are indicated by A and a numerical superscript, e.g. A^2, A^6 (Collins, 1963). All crosses were made and maintained as previously described. The plates were examined for a three-week period for the presence of plasmodia. If at the end of that time plasmodia were absent, the myxamoeba originally used in the cross were assumed as being of the same mating type.

Preparation and Treatment of Material for Cytochemical Studies

Swarmer Cells

(1) For each cell type 15 culture plates of log phase myxamoeba were flooded with isotonic .25M Phosphate Sucrose Buffer (.25M PSB) pH 6.5 and allowed to set for 1 hour. This ensured a high yield of swarmers.

(2) The swarmer suspensions were pipette-transferred to 40 ml conical centrifuge tubes and centrifuged at 70X gravity for 20 minutes in a swinging bucket centrifuge. The bacterial laden super-

natant was discarded by vacuum suction (since swarmers are motile) and the swarmer pellet was resuspended in buffer and the process repeated twice more.

(3) The relatively bacterial-free swarmer plug was then re-suspended in 10% neutral buffered formalin, vortexed, and centrifuged at 70X for 15 minutes. The supernatant was suctioned off and the swarmer plug re-suspended in fresh 10% neutral buffered formalin and allowed to fix for a period of 12-18 hours according to the method of (Kasten, 1959; Swift, 1950).

10% neutral buffered formalin is prepared thus: 4.0 grams of NaH_2PO_4 abd 6.5 grams of anhydrous Na_2HPO_4 were made up to 900 ml with distilled water (w/v). 100 ml of commercial 40% formaldehyde is added and then put on a magnetic stirrer to ensure a solution (Therrein, 1966).

(4) The fixed swarmers were then centrifuged at 70X gravity, the supernatant discarded, and the pellet washed twice in 70% ethanol. The cells were then post-fixed for 12 hours in freshly prepared 70% ethanol.

(5) Swarmers were then centrifuged, the 70% ethanol discarded, and then re-suspended in sterile distilled water.

(6) This water suspension of swarmers was centrifuged at 5X gravity to remove any remaining debris.

(7) The suspensions were then centrifuged 70X gravity, and the pellet of fixed swarmers re-suspended in sterile distilled water.

(8) To ensure a completely bacterial free final preparation, the swarmers were centrifuged twice more at 70X gravity, and placed on labeled, previously albuminized slides. Multiple slides were made and allowed to air dry, then stored in closed slide boxes, since dust particles can interfere with the cytophotometry.

Plasmodia

(1) Growing plasmodia of uniform age were transferred from CM/2 plates onto 2% plain agar and allowed to migrate for 24 hours. This allowed for complete digestion of ingested bacteria.

(2) The surface of the cultures were then flooded with 10% neutral buffered formalin

(pH 7.0) and allowed to fix for 12-18 hours. The plasmodia usually floated to the surface, but when this did not occur gentle pressure from a pasteur pipette lifted them free. The plasmodia were washed twice for 1 hour each, in 70% ethanol then post-fixed for 12 hours in fresh 70% ethanol.

(3) Fixed plasmodia were then dehydrated along a graded ethanol series, passed through two changes of xylene and imbedded in paraffin wax M.P. 56 degrees C.

(4) Sections were cut at six microns, as determined from previous measurements of the polyploid nuclei to ensure no nuclei were damaged in processing, and affixed to previously labeled albuminized slides and allowed to air dry. Storage of slides was the same as for swarmers.

Sporangia

(1) Sporangia were fixed in 10% neutral buffered formalin during various hourly stages in their differentiation for a 12-18 hour period.

(2) These were also washed twice in 70% ethanol for 1 hour each, then allowed to post-fix in fresh 70% ethanol for 12 hours.

(3) The sporangia were dehydrated in a graded ethanol series, passed through two changes of xylene, then imbedded in paraffin wax, M.P. 56 degrees C.

(4) Sections were again microtomed at six microns in allowance for the theoretical probability of obtaining polyploid spores resulting from protoplast cleavage. These paraffin sections were then affixed to labeled albuminized slides and allowed to air dry. Storage of slides was the same as for the swarmers and plasmodia.

Cytochemical Methods

Feulgen - DNA Analysis

The Feulgen nucleal reaction was applied for the specific localization and quantitation of deoxyribonucleic acid (Feulgen and Rossenbeck, 1924; as modified by Therrein, 1966; and Bryant and Howard, 1969). The quantitative basis for the Feulgen reaction is due to the stable bond formed in a stoichiometric ratio between the Schiffs dye and the polyaldehyde nature of hydrolyzed DNA (apurinic acid). This bond in the new molecular dye-DNA complex has been shown to be of an Alkyl-sulfonic acid nature (Nauman, West, et al., 1960) and becomes a magenta color in a non-recolorization reaction; i.e. the original color of the

(1) All slides hydrolyzed in 5 N hydrochloric acid for approximately 50 minutes at room temperature.

(2) Stain for two hours at room temperature in Schiffs reagent, bis-N-aminosulfonic acid, (Lillie, 1951) which is fortified by addition of ten percent potassium metabisulfite (KMB) in approximately a 10 ml to 40 ml reagent (a 1 to 4 ratio). This insures against loss of SO_2 thus enhancing optimum reactivity. The basic fuchsin, a variable mixture of pararosanaline, rosanaline and Magenta II, used in preparation of the dye was from Fisher Scientific Company (CI. #42500).

(3) Rinse three times for 5 minutes each in a bisulfite rinse freshly prepared by mixing 5 ml of 10% potassium metabisulfite and 5 ml 1 N hydrochloric acid in 100 ml of distilled water.

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

(5) Clear in two changes of xylene, then mount in Preservaslide.

Slides of swarm cells, plasmodia and sporangia were used as controls in order to insure that the Feulgen reaction was always specific and quantitative (Yemma, 1971).

dye is not restroed. This bond stability allows for the quantitation of relative DNA content in individual nuclei (Kaste, 1967; Ris and Mirsky, 1949). Schiffs reagent, a basic dye, possesses three NH_2 groups as reactive sites which, in the presence of sulfurous acid (HSO_3), will show a high specificity for polyaldehydes in a complex, non-first order reaction (Caspersson, 1936; DiStefano, 1948; Kaste, 1959, 1960, 1967; Ris and Mirsky, 1949; Taylor, 1959).

In quantitative cytophotometry color intensity depends on several variables: type of fixation, hydrolysis time, manufacturer of the stain, exposure time of tissue in Schiffs reagent and, cell thickness (Davies and Walker, 1953; Olkowski, 1976). Paraffin sections of plasmodia and sporangia were cleared in two changes of xylene, then rehydrated through a graded ethanol series to distilled water. The smears of swarm cells were immersed in distilled water for one minute. For each select tissue type to be read all nuclei were on one slide and all slides received identical treatment, thus allowing for legitimate comparisons. The prototype hydrolysis technique of 1 N hydrochloric acid at 60 C for 8-16 minutes (DeCosse and Aiello, 1966; Therrein, 1966) was replaced by hydrolysis in 5 N hydrochloric acid at room temperature for 50 minutes. This method was found to yield optimum results with both mammalian and the Myxomycete tissue used here (Fand et al., 1967; Yemma and Therrein, 1972). The Feulgen staining technique used is as follows:

Microspectrophotometric Methods

All cytophotometric quantifications were made using a Zeiss Type 01 microspectrophotometer with a 60 W Tungsten light source and all measurements were made with a Planochromat oil immersion objective, N.A. 1.30, X100 at an Optovar setting of 1.25X. A continuous interference-filter monochromator (Zeiss No. 47 43 10) was used for selection of the desired wavelengths and the diaphragm insert was set at a diameter of 0.8mm. Before each reading session an instrument alignment and phototube linearity check was made. Also, frequent checks for cleanliness of lens systems and the monochromator were made to aid accuracy.

The cytochemical methods employed include Feulgen staining and a combination of the techniques of microscopy and spectrophotometry. It is possible then, to apply absorption photometry to individual cells, thereby allowing the amount of DNA per nucleus to be measured and computed (Swift, 1966) within a heterogeneous cell population (Ris and Mirsky, 1949; Kasten, 1967).

The basis for chemical analysis by microscopic absorption spectrophotometry is achieved by application of the Beer-Lambert Law where, for any monochromatic wavelength:

$$E = \log Io/Is = -\log T = km/B = kcl \qquad (1)$$

where, E = extinction

I_o = flux of photons on chromophore

I_s = transmitted flux

T = transmission of the field

k = specific absorptivity constant of the chromophore at the defined wavelength

c = chromophore concentration

l = path length through tissue

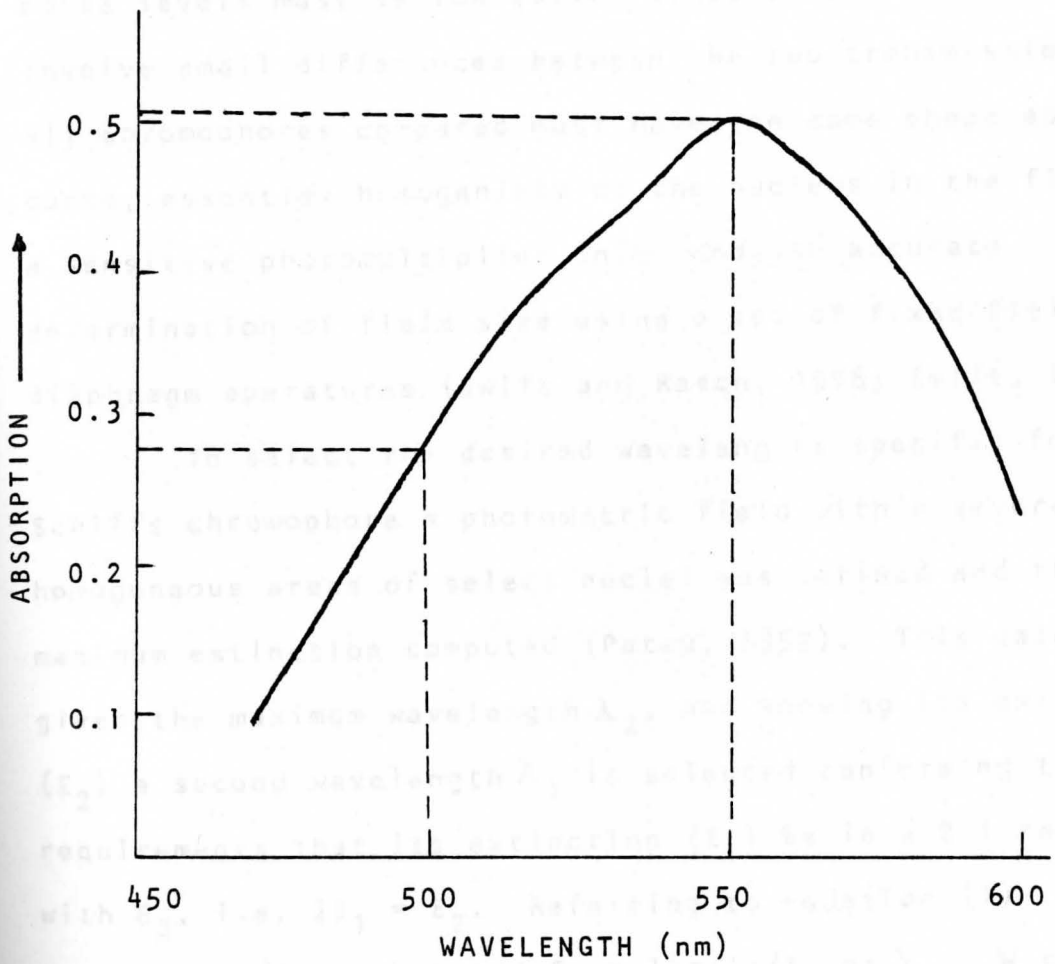
m = chromophore mass in the measured field

B = area of the measured field

Accurate absorption curves for the Schiffs chromophore, highly reproducible by photoelectric methods, are optimally obtained if the chromophore measured has a homogeneous distribution (Kasten, 1960; Swift, 1955). Kasten (1959b) has made numerous comparisons of in vitro and in vivo curves and found them consistent. For this investigation several absorption curves were made and a representative curve is shown in Figure 1.

In this study the Two-wavelength method of chromophore measurement, developed independently by Patau (1952) and Ornstein (1952) and Mendelsohn (1961) was used exclusively. Using theoretical considerations Patau (1952) and Ornstein (1952) determined that distortions in absorption, due to distributional error, are greater at higher rather than lower extinctions and through a rigorous mathematical process developed a model system wherein the source of error could be corrected for. In this system, the whole nucleus is circumscribed within the photometric field and the measurement of absorbing molecules is accomplished by transmissions at two reference wavelengths, chosen from the Feulgen-chromophore absorption curve, and the ratio between these

0.5
0.4
0.3
0.2
0.1
Absorption
550
600
WAVELENGTH (nm)


0.5
0.4
0.3
0.2
0.1
ABSORPTION
450
500
550
600
WAVELENGTH (nm)

absorptions could be set as a 2:1 ratio. Their method placed several critical requirements on the photometric system: since the method depends on the accuracy of the 2 wavelengths a monochromator giving the narrowest band width possible must be used instead of filters, an incident light source of high purity (the 60 W Zeiss Tungsten source is of such a nature) to irradiate the nucleus, instrument noise levels must be low (0.1%) since some measurements involve small differences between the two transmissions, all chromophores compared must have the same shape absorption curve, essential homogeniety of the nucleus in the field, a sensitive photomultiplier unit, and, an accurate determination of field size using a set of fixed field diaphragm aperatures (Swift and Rasch, 1956; Swift, 1966).

To select the desired wavelengths specific for the Schiffs chromophore a photometric field within several homogeneous areas of select nuclei was defined and the maximum extinction computed (Patau, 1952). This value gives the maximum wavelength λ_2, and knowing its extinction (E_2) a second wavelength λ_1 is selected conforming to the requirements that its extinction (E_1) be in a 2:1 ratio with E_2, i.e. $2E_1 = E_2$. Referring to equation (1) $E_1 = \log I_o/I_s$ at λ_1, and $E_2 = \log I_o/I_s$ at λ_2. With these equations inhomogeneous nuclear areas may be measured. When making measurements all nuclei were selected at random from the same slide. The nucleus was centered on the optical axis and circumscribed by an appropriate field

aperature in order that the least amount of unoccupied light enter the photometric field. At this point four readings were taken; I_{o1} I_{s1} I_{o2} I_{s2}

The amount of chromophore (absorbing material), (M), in the defined area (A), is then calculated from the following relationship:

$$M = kAL_1C \qquad (2)$$

The absorptivity constant, k, determined for the Myxomycetes by Therrein (1966), was ommitted in this study since only relative ploidy values were required. For each nucleus transmissions were made at λ_1 and λ_2 conforming to equation (1) where $T_1 = I_s/I_o$ and $T_2 = I_s/I_o$. From the transmissions, $L_1 = (1-T_1)$ and $L_2 = (1-T_2)$. By manipulation of the transmission into L_1 and L_2 parameters, a ratio, Q is generated so that $Q = L_2/L_1$; and this value corresponds to the 2:1 ratio deduced by Patau and Ornstein. If the measured area was homogeneous this ratio would be maintained; however, if distributional error was present (operant) the distortion at the higher extinction would alter the ratio. With the extinction ratio of the two wavelengths set as 2:1, Q may be used to calculate a correction factor (c), where $C = (2-Q)^{-1} \ln(Q-1)^{-1}$, thus allowing the elimination of the influence of any unoccupied portions of the measured area. Therefore, for any irregular, non-homogenous nucleus the total amounts of absorbing chromophores in a given area may be calculated. Patau (1952) has formulated a table giving Q to C values, where $1 \leq Q \leq 2$.

The formula used for the standard deviation is: S.D. = $(X^2/N)^{\frac{1}{2}}$ where, X is the deviation from the mean and N is the number of nuclei scored. The standard error was calculated from the following relationship: S.E. = S.D./(N). For accuracy's sake, all relative DNA calculations as well as the statistical treatment were made using an IBM 370 Model 145 computer.

RESULTS

The results of the genetic analysis in this investigation are presented in chart form, a method facilitating observations correlsponding to the heterothallic mating system of *D. iridis*. Feulgen-DNA measurements are presented as frequency-distribution histograms to record any shifts in DNA contents. Only interphase nuclei were measured; except where otherwise noted. All DNA values are relative and hence are give in arbitrary units.

Feuglen-DNA Spectral Absorption Curve

The two-wavelength method of microspectrophotometry requires the generation of an accurate absorption curve for the chromophore-molecular complex being measured. Both myxamoebal and plasmodial nuclei were used to determine the spectral absorption curve for the Feulgen-DNA. From the curve it was established that the maximum absorption for the Feulgen-DNA complex is at a wavelength of 555 nm and the half-maximum value absorbed at a wavelength of 500 nm.

Microspectrophotometric Analysis of Nuclear DNA in *D. iridis*

Slides of swarmers, plasmodia and sporangia were stained with Schiffs reagent and relative quantitative measurements of deoxyribonucleic acid content were computed.

To account for any variability, a standard hydrolysis curve was carried out on both swarmer cell nuclei and plasmodial nuclei which produced the 50 minute hydrolysis figure. Optimum hydrolysis is essential in that the greatest stain intensity is obtained when all the purine bases have been removed, thereby exposing the reactive aldehyde groups (DiStefano, 1948). Control slides were run simultaneously with experimental slides in the staining procedure to ensure the strict specificity of the stain. Swarmer, plasmodia and sporangial slides treated with 5% TCA at 90° C then later Feulgen stained revealed a Feulgen negative pattern; indicating that DNA had been effectively removed (i.e. the Feulgen Reaction is specific for DNA).

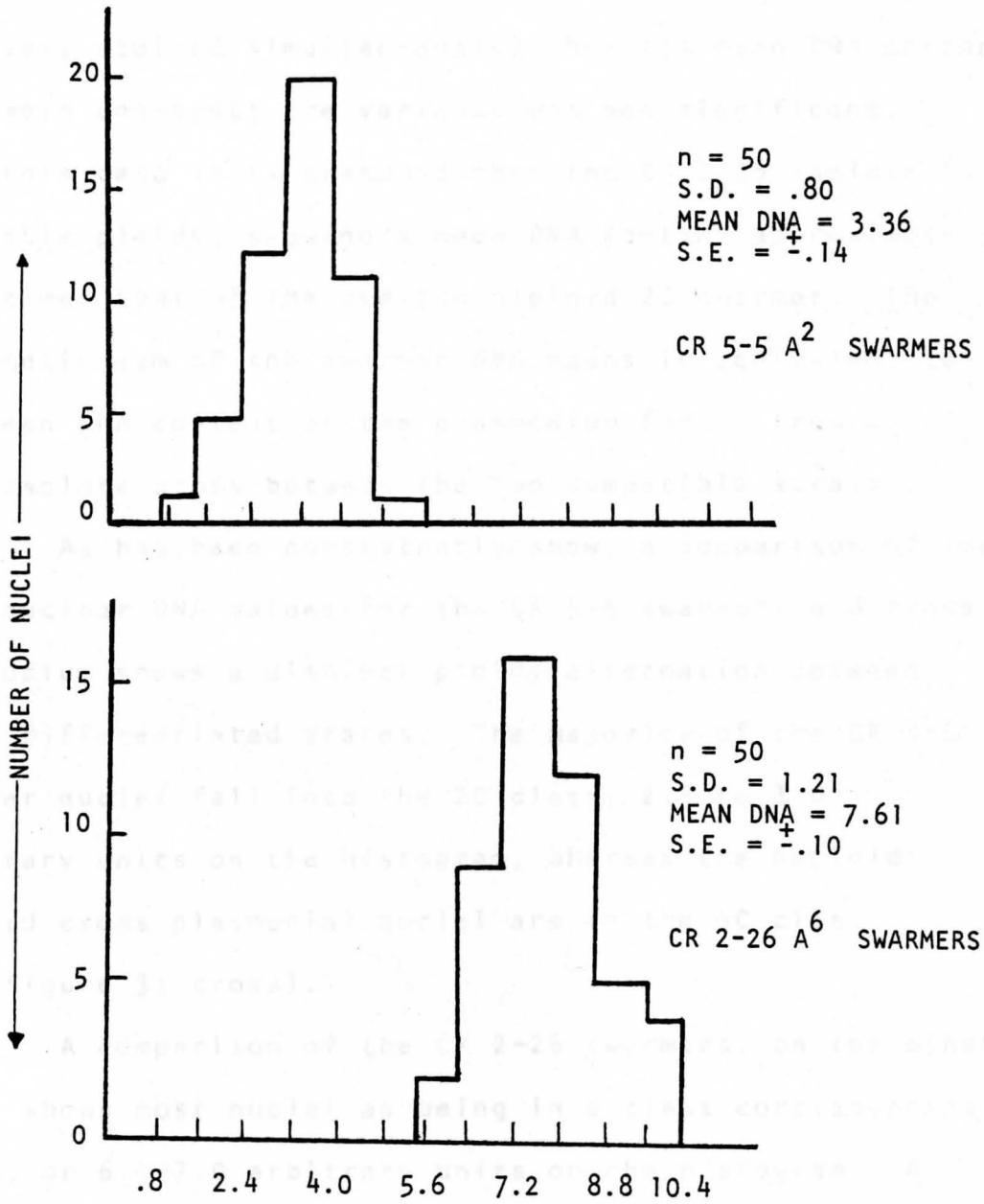
DNA was measured in the swarmer stage of the life cycle for the CR 5-5 and CR 2-26 parental clones, as well as all F_1, F_2 and F_3 generation spore isolates (except in several cases where myxamoeba were measured). DNA was also measured in the following stages of the life cycle: plasmodia and sporangia produced from interisolate crosses between compatible CR 5-5 and CR 2-26 clones and back-crosses between compatible parental and spore isolate clones. The data are presented as frequency-distribution histograms with the number of nuclei plotted on the ordinate and the relative dye concentration (in arbitrary units) on the abscissa. By visual inspection histograms facilitate detection of ploidy changes or mitotic activity in a given cell population. To determine accurate ploidy level

representatives, 100 nuclei were analyzed for each plasmodial and sporangia unit. Swarm cell ploidy levels were determined by a population size of 50 cells. This method insured that the DNA content of a representative sample of each stage was measured. Thus the frequency distributions are not influenced by the chance measurements of small populations of nuclei of a different ploidy level (e.g. some otherwise diploid plasmodia have been shown to contain approximately 4% polyploid nuclei; Koevenig and Jackson, 1966).

The terminology of Swift (1950) is used to describe the DNA content of nuclei, i.e., haploid, diploid, and tetraploid nuclei are designated, respectively, C, 2C and 4C. Higher ploidy levels are represented by corresponding multiplicity designation. The synthetic activity of the interphase nucleus is represented by the designations of Howard and Pelc (1953); G_1(pre-DNA synthetic gap), S(DNA synthesis), and G_2(post-DNA synthesis gap). The results are shown in figures 2-23.

1. Analysis of F-DNA in Parental Swarmers

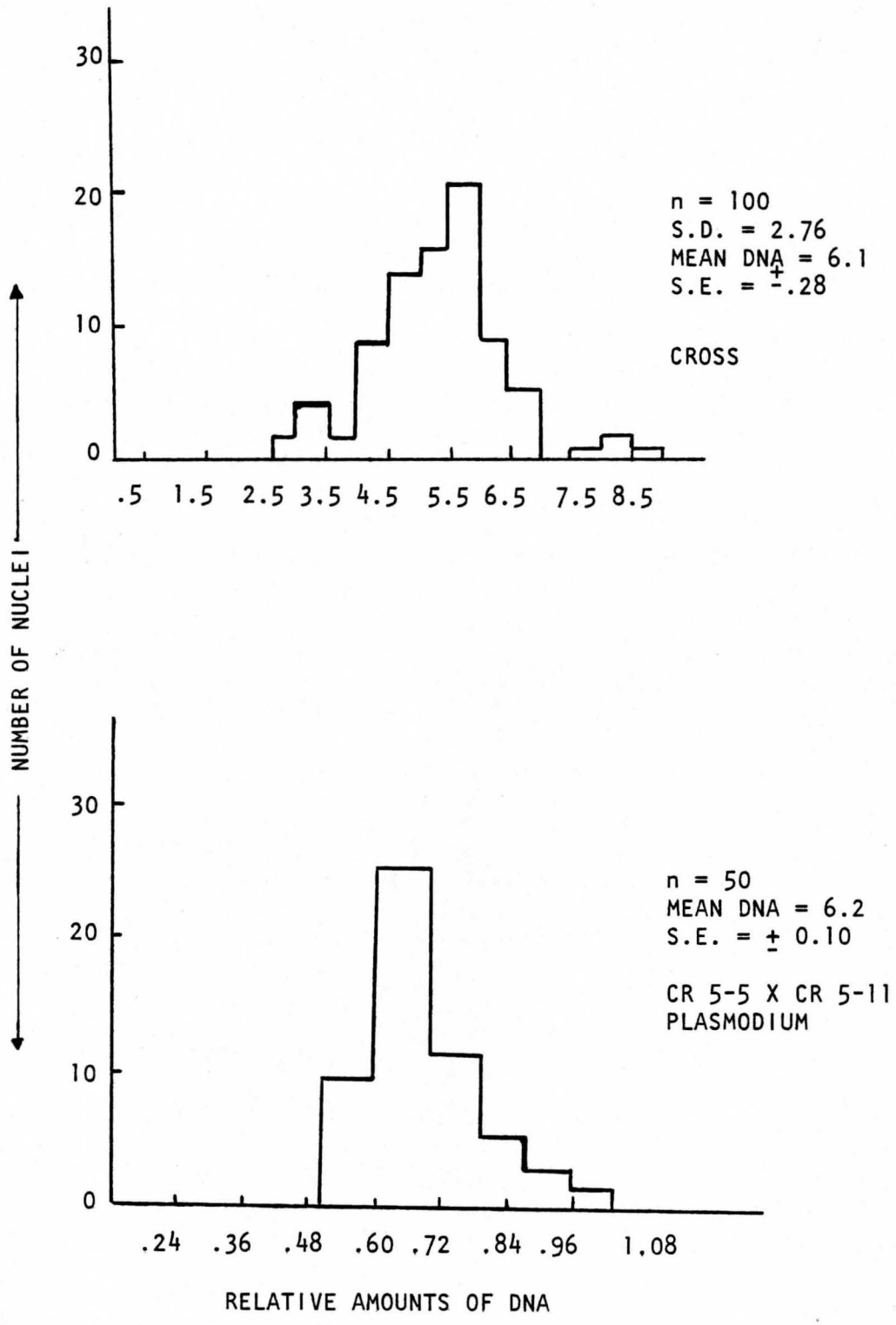
Measurements of nuclear DNA in the CR 5-5 and CR 2-26 swarmers are shown in Figure 2. The mean DNA content of the CR 5-5 swarmers is approximately one-half the diploid value (Figure 3). The mean DNA content of the CR 2-26 swarmers however, is about 14% greater than that of the cross diploid value. The data further indicates that the CR 5-5(A^2) mating type has approximately 44% the

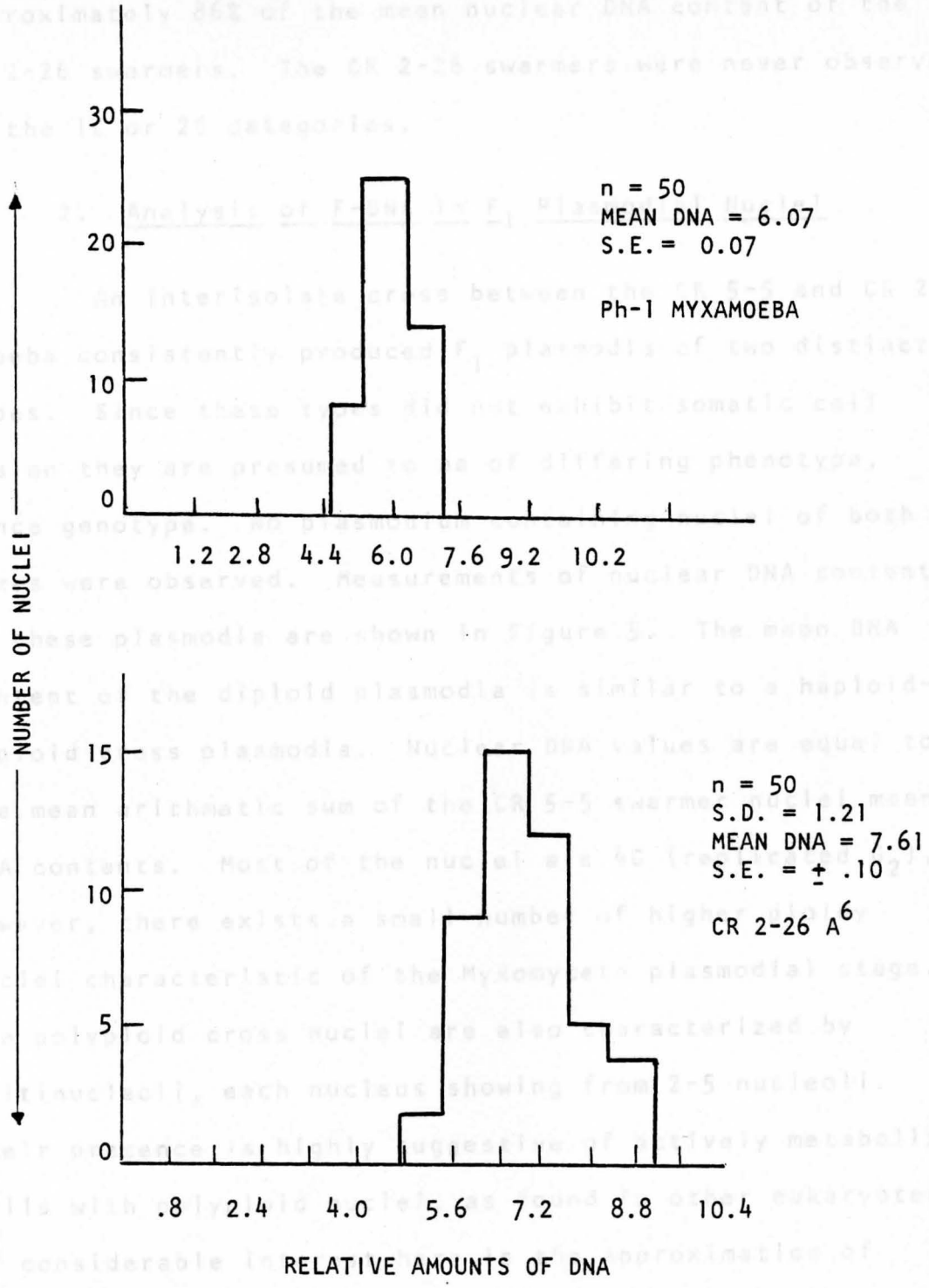

20
15
10
5
0
n = 50
S.D. = .80
MEAN DNA = 3.36
S.E. = ±.14
CR 5-5 A2 SWARMERS
15
10
5
0
n = 50
S.D. = 1.21
MEAN DNA = 7.61
S.E. = ±.10
CR 2-26 A6 SWARMERS
NUMBER OF NUCLEI
.8
2.4
4.0
5.6
7.2
8.8
10.4
RELATIVE AMOUNTS OF DNA

the nuclear DNA content of the CR 2-26 (A^6) allelomorph. Also, samples of the CR 2-26 isolate taken 8 months apart (however, stained simultaneously) show its mean DNA content to remain constant; the variance was not significant. From this data it is presumed that the CR 2-26 isolate is of stable ploidy, showing a mean DNA content approximately 2.15 times that of the average haploid 2C swarmer. The arithmetic sum of the swarmer DNA means is equivalent to the mean DNA content of the plasmodium formed from an interisolate cross between the two compatible strains.

As has been consistently show, a comparison of the mean nuclear DNA values for the CR 5-5 swarmers and cross plasmodium shows a distinct ploidy alternation between these differentiated states. The majority of the CR 5-5 swarmer nuclei fall into the 2C class, 2.5 to 3.0 arbitrary units on the histogram, whereas the haploid-haploid cross plasmodial nuclei are in the 4C class (see figure 3; cross).

A comparison of the CR 2-26 swarmers, on the other hand, shows most nuclei as being in a class corresponding to 4C, or 6.4-7.0 arbitrary units on the histogram. A similar situation of apparently stable 4C myxamoeba can be seem in the Ph-1 isolate of D. iridis (Figure 4). Therrein and Collins (1976) have recently reported on stable polyploid cell lines existing in D. iridis, including several sub-lines in the CR-2 isolate. Like the diploid cross plasmodia, the Ph-1 isolate contains


30
20
10
0
.5 1.5 2.5 3.5 4.5 5.5 6.5 7.5 8.5
n = 100
S.D. = 2.76
MEAN DNA = 6.1
S.E. = ±.28
CROSS
NUMBER OF NUCLEI
30
20
10
0
.24 .36 .48 .60 .72 .84 .96 1.08
n = 50
MEAN DNA = 6.2
S.E. = ± 0.10
CR 5-5 X CR 5-11
PLASMODIUM
RELATIVE AMOUNTS OF DNA

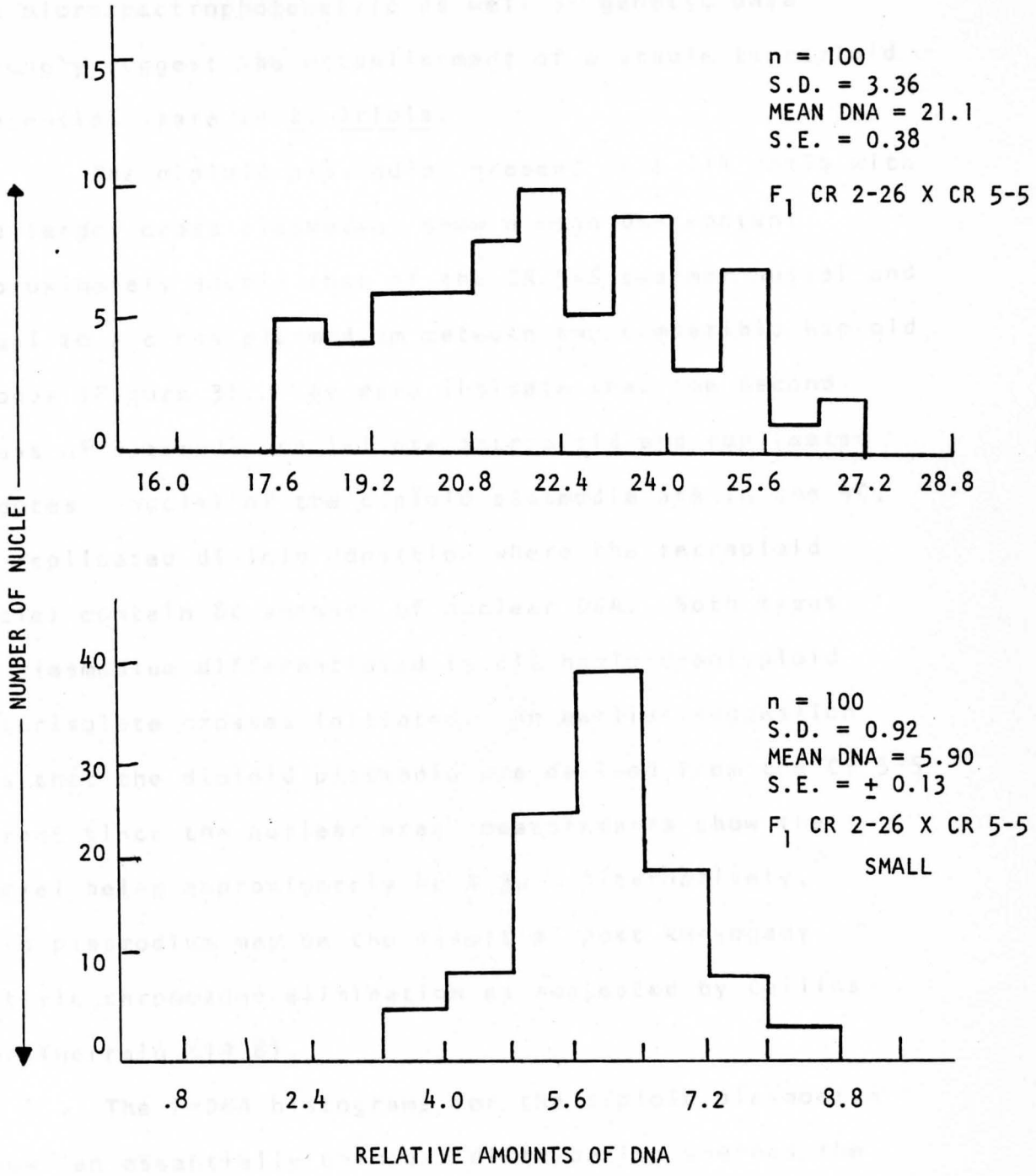

30
20
10
0
n = 50
MEAN DNA = 6.07
S.E. = 0.07
Ph-1 MYXAMOEBA
1.2 2.8 4.4 6.0 7.6 9.2 10.2
NUMBER OF NUCLEI
15
10
5
0
n = 50
S.D. = 1.21
MEAN DNA = 7.61
S.E. = ± .10
CR 2-26 A6
.8 2.4 4.0 5.6 7.2 8.8 10.4
RELATIVE AMOUNTS OF DNA

approximately 86% of the mean nuclear DNA content of the CR 2-26 swarmers. The CR 2-26 swarmers were never observed in the 1C or 2C categories.

2. Analysis of F-DNA in F_1 Plasmodial Nuclei

An interisolate cross between the CR 5-5 and CR 2-26 amoeba consistently produced F_1 plasmodia of two distinct types. Since these types did not exhibit somatic cell fusion they are presumed to be of differing phenotype, hence genotype. No plasmodium containing nuclei of both sizes were observed. Measurements of nuclear DNA content in these plasmodia are shown in Figure 5. The mean DNA content of the diploid plasmodia is similar to a haploid-haploid cross plasmodia. Nuclear DNA values are equal to the mean arithmatic sum of the CR 5-5 swarmer nuclei mean DNA contents. Most of the nuclei are 4C (replicated G_2), however, there exists a small number of higher ploidy nuclei characteristic of the Myxomycete plasmodial stage. The polyploid cross nuclei are also characterized by multinucleoli, each nucleus showing from 2-5 nucleoli. Their presence is highly suggestive of actively metabolizing cells with polyploid nuclei, as found in other eukaryotes. Of considerable interest here is the approximation of these nuclei to tetrapolid amounts of nuclear DNA. While the existence of a small sub-population of tetraploid (and higher ploidy) nuclei are natural occurrences in the plasmodium (Koevenig & Jackson, 1966) of the Myxomycetes; strictly tetraploid plasmodia are rare occurrences.

Fig. 5.--Histograms representing feulgen-DNA values for F_1 CR 2-26 X CR 5-5 plasmodial nuclei of Didymium iridis.


n = 100
S.D. = 3.36
MEAN DNA = 21.1
S.E. = 0.38
F1 CR 2-26 X CR 5-5
15
10
5
0
16.0
17.6
19.2
20.8
22.4
24.0
25.6
27.2
28.8
n = 100
S.D. = 0.92
MEAN DNA = 5.90
S.E. = ± 0.13
F1 CR 2-26 X CR 5-5
SMALL
40
30
20
10
0
.8
2.4
4.0
5.6
7.2
8.8
NUMBER OF NUCLEI
RELATIVE AMOUNTS OF DNA

The microspectrophotometric as well as genetic data strongly suggest the establishment of a stable tetraploid plasmodial state in D. iridis.

The diploid plasmodia, present in a 1:4 ratio with the larger cross plasmodia, show a mean DNA content approximately double that of the CR 5-5 swarmer nuclei and equal to a cross plasmodium between two compatible haploid clones (Figure 3). The data indicate that the second class of plasmodia nuclei are tetraploid and replicated zygotes. Nuclei of the diploid plasmodia are in the 4C, or replicated diploid condition where the tetraploid nuclei contein 8C amounts of nuclear DNA. Both types of plasmodium differentiated in all haploid-polyploid interisolate crosses initiated. An earlier suggestion was that the diploid plasmodia are derived from the CR 5-5 parent since the nuclear area measurements show the nuclei being approximately 4u X 3u. Alternatively, this plasmodium may be the result of post karyogamy mitotic chromosome elimination as suggested by Collins and Therrein (1978).

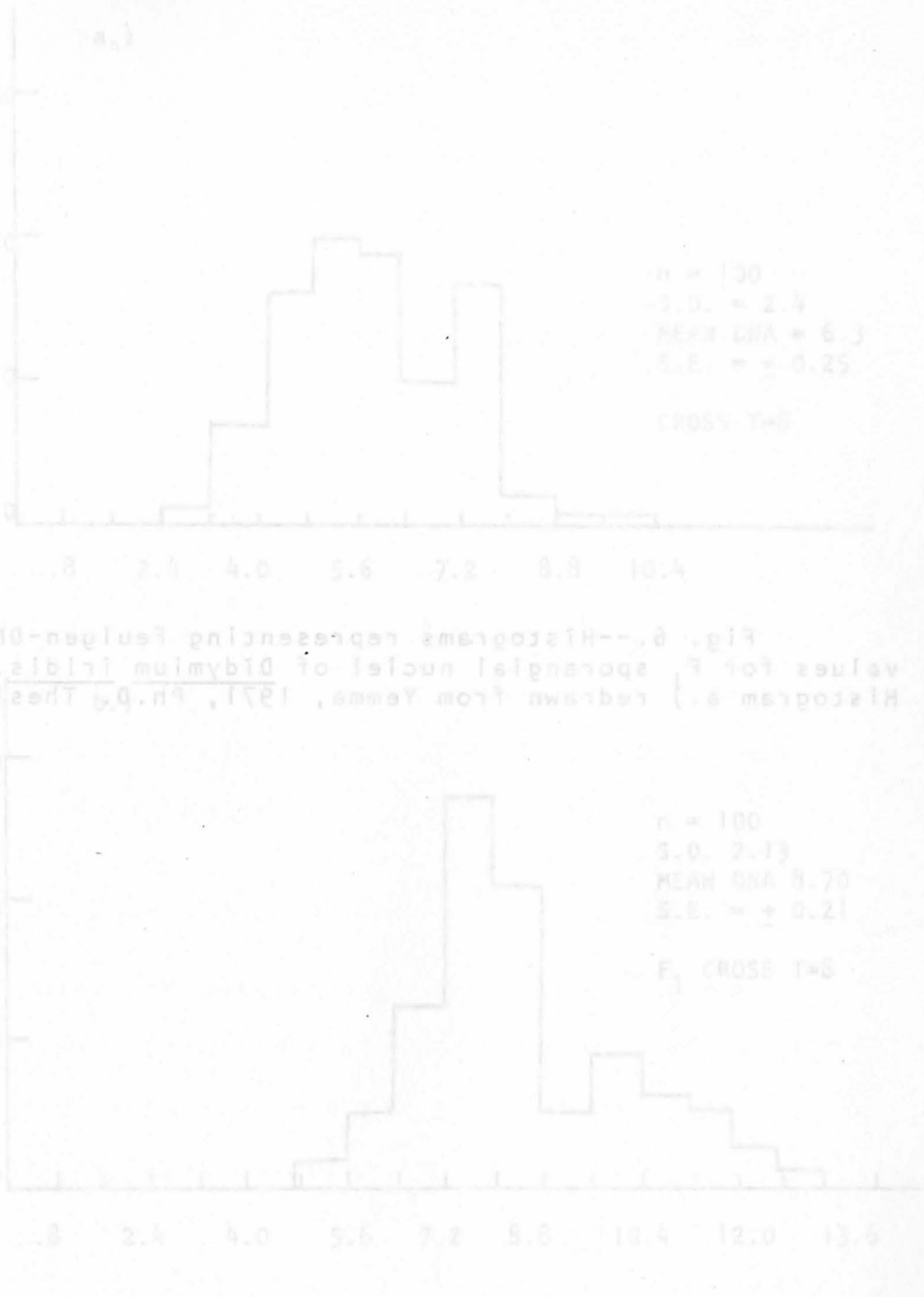
The F-DNA histograms for the diploid plasmodium shows an essentially unimodal distribution whereas the tetraploid plasmodium indicates an active nuclear cycle. There is a skewness to the left in both profiles which may further indicate, as previously suggested by Yemma (1971), then Bradley and Yemma (1975) that plasmodial DNA synthesis in D. iridis is not a completely synchronous event.

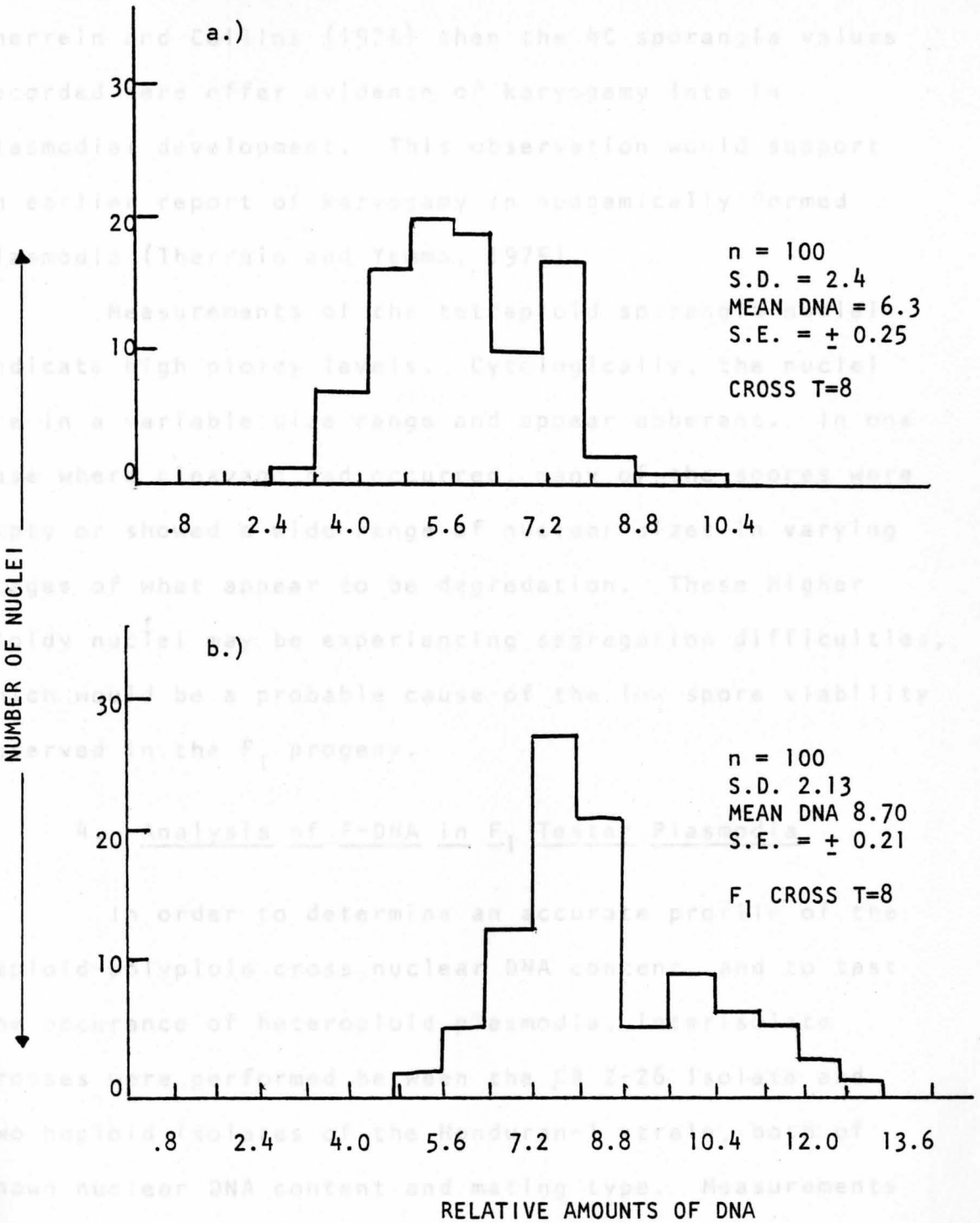
3. Analysis of F-DNA in F_1 Sproangial Nuclei

The occurance of a round of DNA synthesis during sporogenesis, resulting in 4C diploid nuclei in the sporangia has been reported by Sauer, Babcock and Rusch (1969), Yemma (1971) and Bradley (1975). In a polyploid-haploid cross the replicated "diploid" value will always show amounts greater than the 4C haploid-haploid cross; hence it should be pointed out that the term diploid may be better replaced by simply zygote. Although the CR 2-26 is designated a stable polyploid with a mean nuclear DNA content two times that of a haploid cell, its chromosome karyotype is at this time unsure. Thus its designation as a diploid (with the cross being a triploid) is premature. It is possible that some of the chromosomes may be polytene, fragmented or multisomic, thus accounting for an increased nuclear DNA value.

Sporangia were collected at various time intervals during sporogenesis, however a laboratory accident resulted in the loss of several time periods. Measurements of the nuclear DNA content of the diploid sporangium are presented in Figure 6. Consistent with previous studies, these profiles show that DNA replication of 2C nuclei occurs prior to spore cleavage. During this pre-cleavage period nuclei are in the process of DNA synthesis.

Comparison of F_1 histograms shows that the pre-cleavage sporangium is essentially the same as that of the plasmodium. Both indicate the diploid 4C condition. If


a.)
n = 100
S.D. = 2.4
MEAN DNA = 6.3
S.E. = ± 0.25
CROSS T×B
0
10
20
30
.8
2.4
4.0
5.6
7.2
8.8
10.4
n = 100
S.D. 2.13
MEAN DNA 8.70
S.E. = ± 0.21
F1 CROSS T×B
0
10
20
30
.8
2.4
4.0
5.6
7.2
8.8
10.4
12.0
13.6
RELATIVE AMOUNTS OF DNA

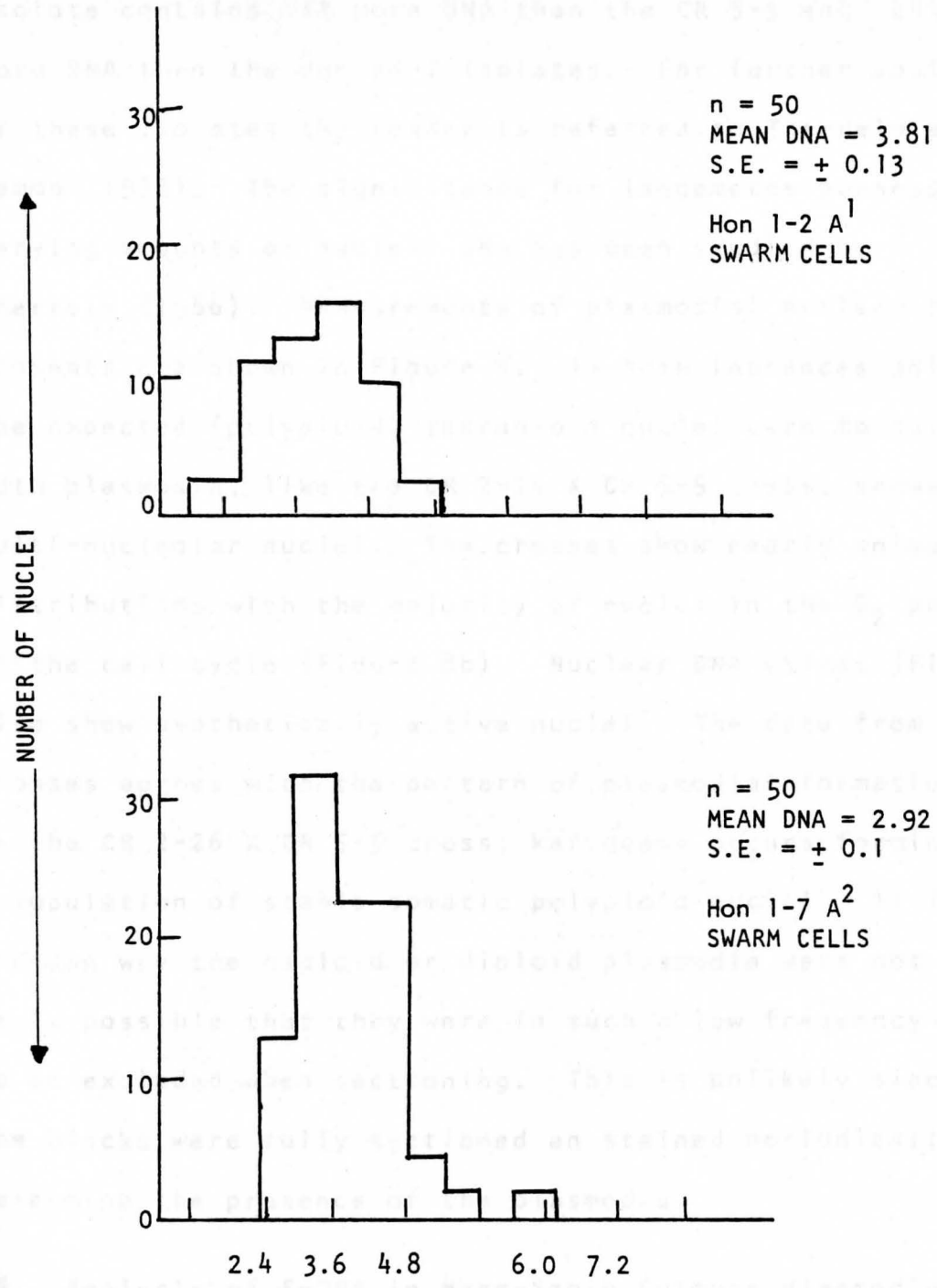

a.)
30
20
10
0
n = 100
S.D. = 2.4
MEAN DNA = 6.3
S.E. = ± 0.25
CROSS T=8
.8
2.4
4.0
5.6
7.2
8.8
10.4
b.)
30
20
10
0
n = 100
S.D. 2.13
MEAN DNA 8.70
S.E. = ± 0.21
F1 CROSS T=8
.8
2.4
4.0
5.6
7.2
8.8
10.4
12.0
13.6
NUMBER OF NUCLEI
RELATIVE AMOUNTS OF DNA

this is the same apogamic induction phenomenon observed by Therrein and Collins (1976) then the 4C sporangia values recorded here offer evidence of karyogamy late in plasmodial development. This observation would support an earlier report of karyogamy in apogamically formed plasmodia (Therrein and Yemma, 1975).

Measurements of the tetraploid sporangia nuclei indicate high ploidy levels. Cytologically, the nuclei are in a variable size range and appear abberant. In one case where cleavage had occurred, many of the spores were empty or showed a wide range of nuclear sizes in varying stages of what appear to be degredation. These higher ploidy nuclei may be experiencing segregation difficulties, which would be a probable cause of the low spore viability observed in the F_1 progeny.

4. Analysis of F-DNA in F_1 Tester Plasmodia

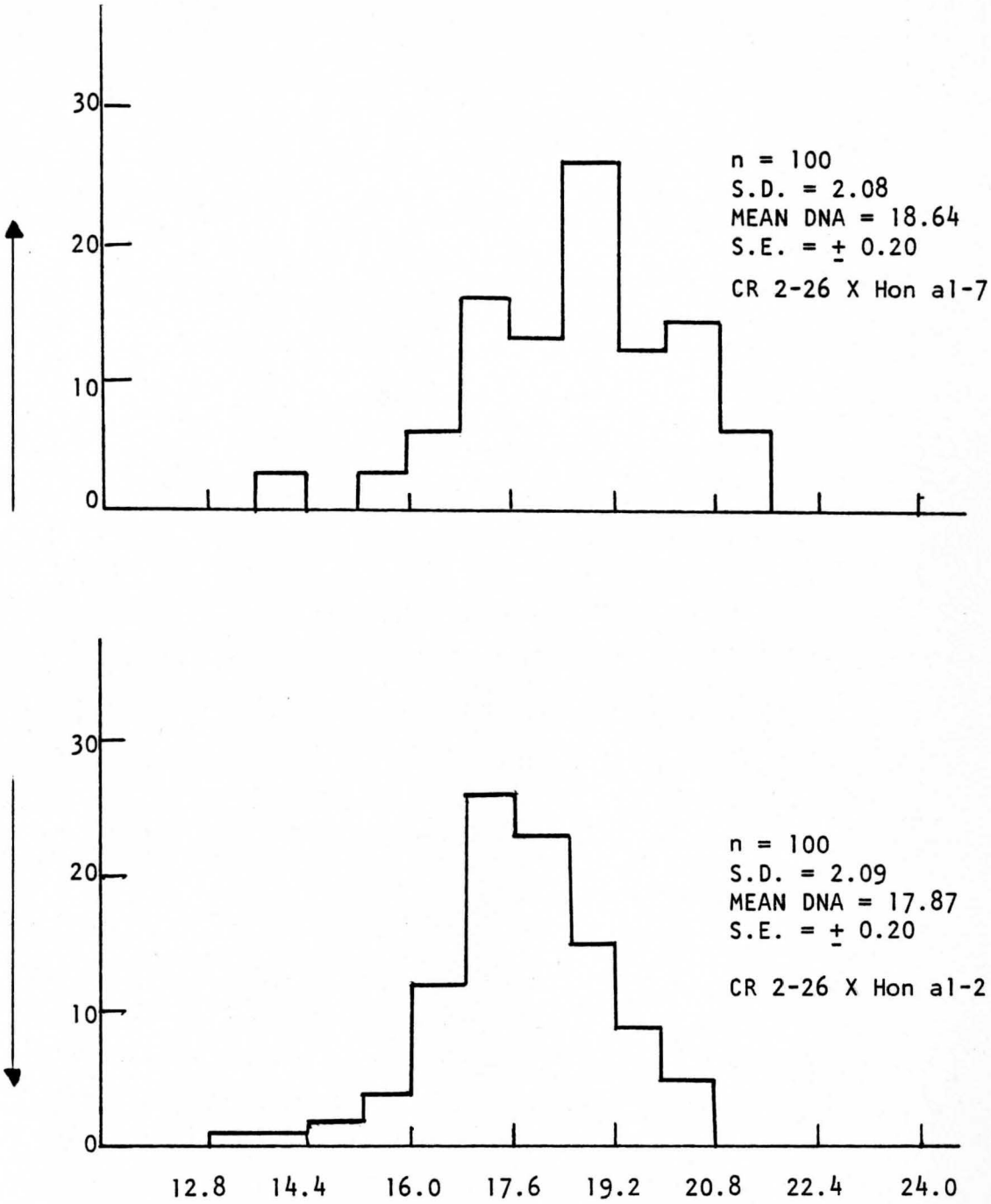
In order to determine an accurate profile of the haploid-polyploid cross nuclear DNA content, and to test the occurance of heteroploid plasmodia, interisolate crosses were performed between the CR 2-26 isolate and two haploid isolates of the Honduran-1 strain, both of known nuclear DNA content and mating type. Measurements of the nuclear DNA content of these clones are presented in Figure 7. Each isolate is in the 2C replicated haploid condition (G_2). There is, however, a variation in DNA content with the Hon al-$7A^2$ isolate possessing 91% of the DNA found in the CR 5-5 isolate. The Hon al-$2A^2$

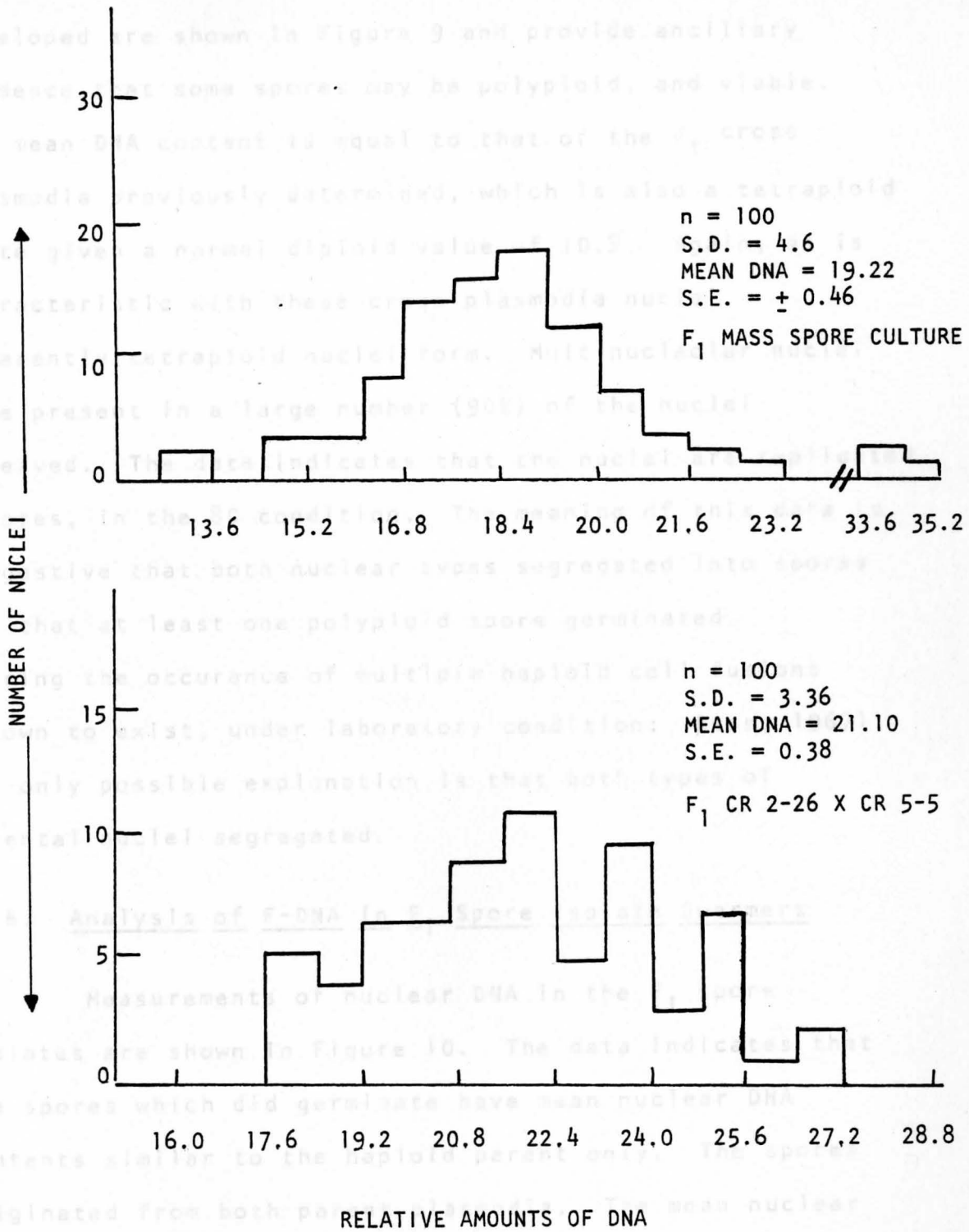

n = 50
MEAN DNA = 3.81
S.E. = ± 0.13
Hon 1-2 A1
SWARM CELLS
n = 50
MEAN DNA = 2.92
S.E. = ± 0.1
Hon 1-7 A2
SWARM CELLS
NUMBER OF NUCLEI
30
20
10
0
2.4
3.6
4.8
6.0
7.2

isolate contains 11% more DNA than the CR 5-5 and 24% more DNA than the Hon al-7 isolates. For further analysis of these isolates the reader is referred to Therrein and Yemma (1975). The significance for isogametes possessing varying amounts of nuclear DNA has been studied by Therrein (1966). Measurements of plasmodial nuclear DNA contents are shown in Figure 8. In both instances only the expected (polyploid) tetraploid nuclei were found. Both plasmodia, like the CR 2-26 X CR 5-5 cross, showed multi-nucleolar nuclei. The crosses show nearly unimodal distributions with the majority of nuclei in the G_2 phase of the cell cycle (Figure 8b). Nuclear DNA values (Figure 8a) also show synthetically active nuclei. The data from these crosses agrees with the pattern of plasmodial formation in the CR 2-26 X CR 5-5 cross; karyogamy occurs forming a population of stable somatic polyploid nuclei. It is unknown why the haploid or diploid plasmodia were not formed; it is possible that they were in such a low frequency as to be excluded when sectioning. This is unlikely since the blocks were fully sectioned an stained periodically to determine the presence of the plasmodia.

5. Analysis of F-DNA in Mass-Spore Culture Plasmodium

To determine if polyploid spores were viable in low frequency a mass-spore culture was made. Here, the entire sporocarp was streaked on a culture plate, inocullated with 2 ml of bacterial suspension then allowed

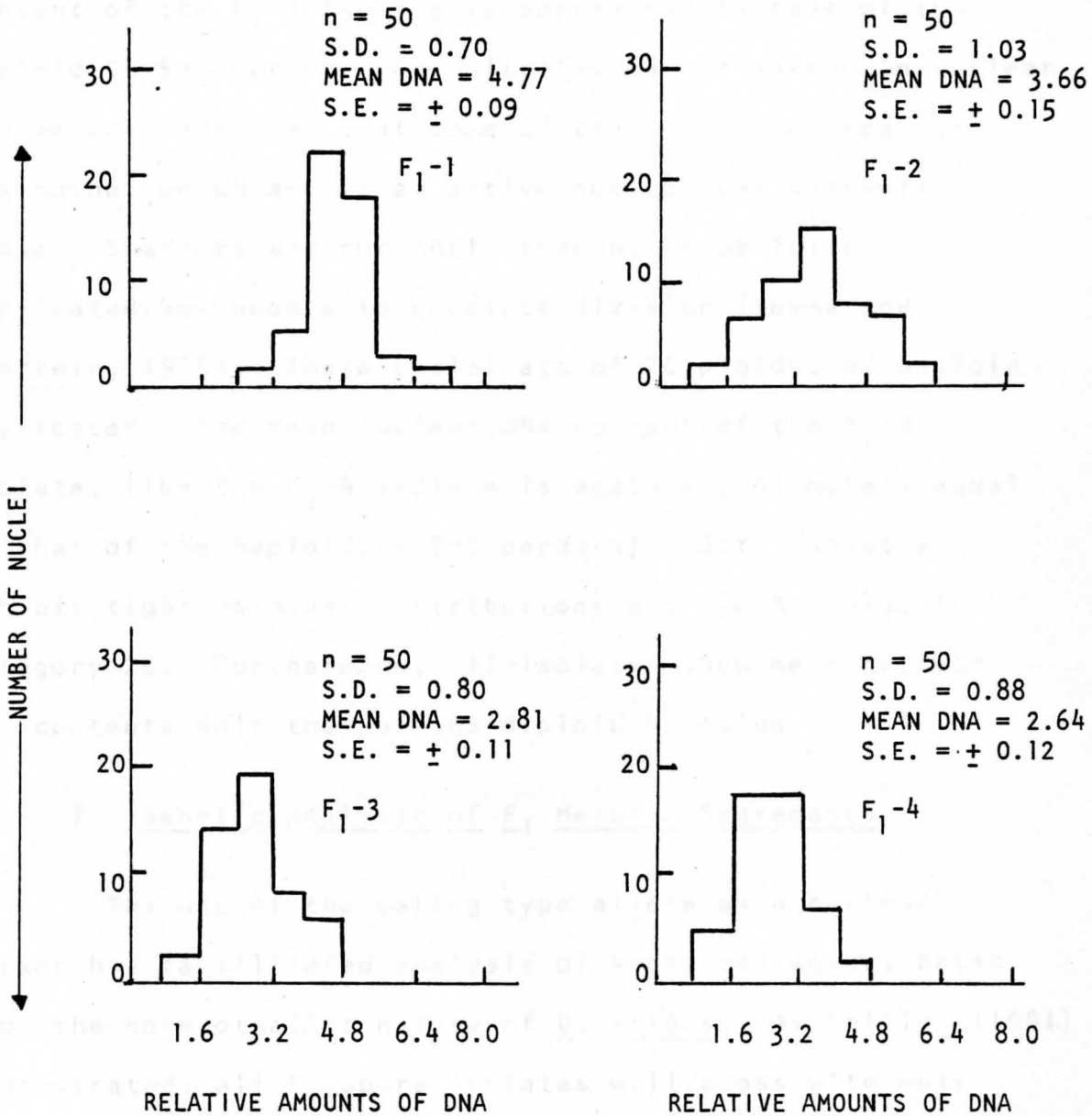

30
20
10
0
n = 100
S.D. = 2.08
MEAN DNA = 18.64
S.E. = ± 0.20
CR 2-26 X Hon a1-7
30
20
10
0
n = 100
S.D. = 2.09
MEAN DNA = 17.87
S.E. = ± 0.20
CR 2-26 X Hon a1-2
12.8
14.4
16.0
17.6
19.2
20.8
22.4
24.0
RELATIVE AMOUNTS OF DNA


30
20
10
0
n = 100
S.D. = 4.6
MEAN DNA = 19.22
S.E. = ± 0.46
F1 MASS SPORE CULTURE
13.6
15.2
16.8
18.4
20.0
21.6
23.2
33.6
35.2
NUMBER OF NUCLEI
15
10
5
0
n = 100
S.D. = 3.36
MEAN DNA = 21.10
S.E. = 0.38
F1 CR 2-26 X CR 5-5
16.0
17.6
19.2
20.8
22.4
24.0
25.6
27.2
28.8
RELATIVE AMOUNTS OF DNA

to incubate. Measurements of plasmodial nuclei which developed are shown in Figure 9 and provide ancillary evidence that some spores may be polyploid, and viable. The mean DNA content is equal to that of the F_1 cross plasmodia previously determined, which is also a tetraploid state given a normal diploid value of 10.9. Again, as is characteristic with these cross plasmodia nuclei apparently tetraploid nuclei form. Multinucleolar nuclei were present in a large number (90%) of the nuclei observed. The data indicates that the nuclei are replicated zygotes, in the 8C condition. The meaning of this data is suggestive that both nuclear types segregated into spores and that at least one polyploid spore germinated. Barring the occurance of multiple haploid cell fusions (known to exist, under laboratory condition: Ross, 1967) the only possible explanation is that both types of parental nuclei segregated.

6. Analysis of F-DNA in F_1 Spore Isolate Swarmers

Measurements of nuclear DNA in the F_1 spore isolates are shown in Figure 10. The data indicates that the spores which did germinate have mean nuclear DNA contents similar to the haploid parent only. The spores originated from both parent plasmodia. The mean nuclear DNA content of the F_1-1 isolate is 14% greater than that of the CR 5-5 parental and also greater than half the value of a diploid plasmodium. Most swarmer nuclei fall into the 2C category, being replicated. It is evident


n = 50
S.D. = 0.70
MEAN DNA = 4.77
S.E. = ± 0.09
F1-1
n = 50
S.D. = 1.03
MEAN DNA = 3.66
S.E. = ± 0.15
F1-2
n = 50
S.D. = 0.80
MEAN DNA = 2.81
S.E. = ± 0.11
F1-3
n = 50
S.D. = 0.88
MEAN DNA = 2.64
S.E. = ± 0.12
F1-4
30
20
10
0
NUMBER OF NUCLEI
1.6 3.2 4.8 6.4 8.0
RELATIVE AMOUNTS OF DNA

that this clone, with such a small spread in DNA distribution (S.D. = .70) is both unimaodal, fully replicated, and hyper-haploid. The mean nuclear DNA content of the F_1-2 isolate is approximately that of the haploid CR 5-5 parent. The slightly larger spread in nuclear DNA amount indicates that some of the cells measured were myxamoeba, which are in an active nuclear DNA synthetic phase. Swarmers are currently thought to be fully replicated but unable to complete division (Yemma and Therrein, 1975). These nuclei are of 2C ploidy, or haploid replicated. The mean nuclear DNA content of the F_1-3 isolate, like the F_1-4 isolate is again approximately equal to that of the haploid CR 5-5 parental. Both isolates exhibit tight unimodal distributions and are haploid, in category 2C. Furthermore, all isolates show mean nuclear DNA contents half that of the diploid 4C value.

7. Genetic Analysis of F_1 Meiotic Segregants

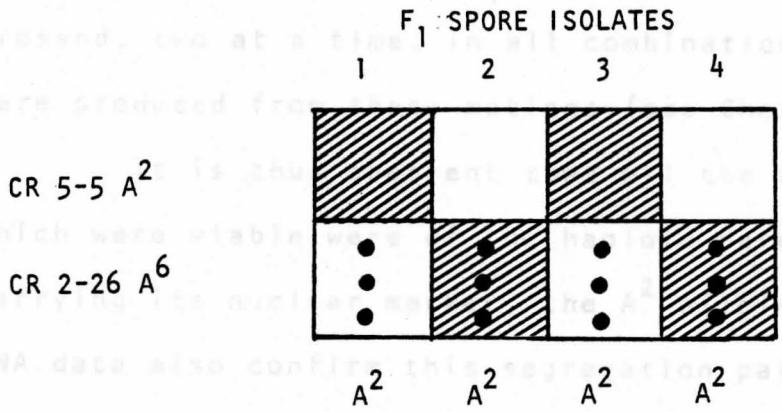
The use of the mating type allele as a nuclear marker has facillitated analysis of spore segregants based upon the heterothallic nature of *D. iridis*. As Collins (1961) demonstrated, all F_1 spore isolates will cross with only one parent - but never both. Thus by simply backcrossing the spore isolate clones to both parental clones and scoring plasmodial production, the mating type, and origin, of each progeny isolate may be deduced.

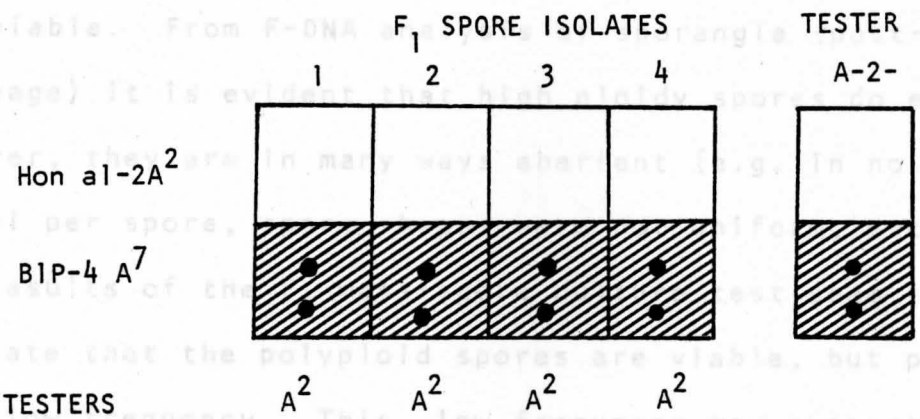
The low germination rate which occured (8%) is suggestive of sterility problems associated with segregation

difficulties in haploid-polyploid crosses. To test a representative sample, spores were isolated at random from both diploid and tetraploid sporangia. According to basic Mendelian segregation and the F_1 analysis first carried out in detail for D. iridis by Collins (1961, 1963), a 1:1 ratio of parental types would be expected.

The results of the F_1 isolates X parentals backcross are presented in Chart 1. Each test consisted of crossing the spore isolate myxamoeba to each parent in 20 plate multiples. Each test was repeated three times. While it was expected to obtain spores in a 1:1 ratio (with respect to both nuclear DNA concentration and mating type allele) spores of only one mating type and nuclear DNA content were viable. In all cases the cross of each spore isolate to the CR 2-26 A^6 polyploid parent produced large, active plasmodia in 5-7 days. Conversely, all crosses of each spore isolate to the haploid CR 5-5 A^2 parent failed to produce plasmodia.

To further validate the mating type of the F_1 spore isolates, each isolate was crossed against clones of known mating types. The results of these tester crosses are presented in charts 1-b. Each of the F_1 spore isolate clones did cross 100% with the tester Bip-4, mating type A^7. No F_1 clones crossed with either of the tester Hon al-$2A^2$ or A-2- clones, both carrying the A^2 allele. A cross between the testers showed that they were not cross-fertile, hence of like mating type. The A^2 isolates, when crossed, were


F1 SPORE ISOLATES
1
2
3
4
CR 5-5 A2
CR 2-26 A6
A2
A2
A2
A2


F1 SPORE ISOLATES
TESTER
1
2
3
4
A-2-
Hon a1-2A2
BIP-4 A7
TESTERS
A2
A2
A2
A2

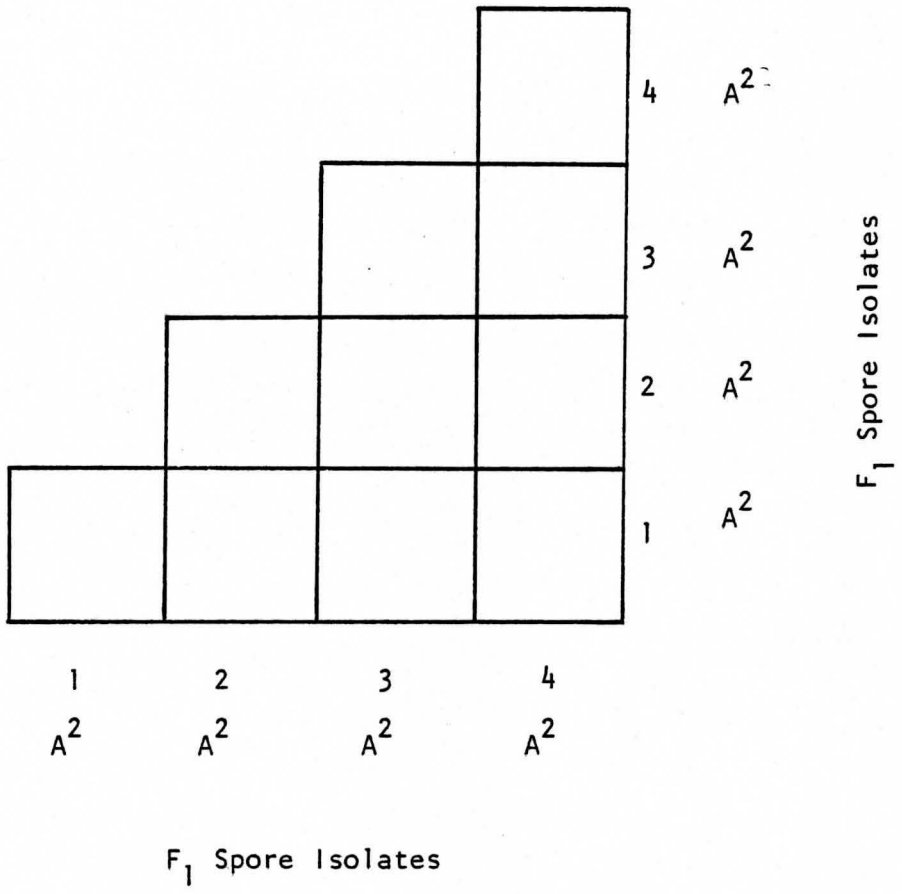
self-fertile; however, both A^2 isolates did produce plasmodia with the Blp-4A^7 isolate. As an additional test of mating type, the F_1 spore isolate clones were crossed, two at a time, in all combinations. No plasmodia were produced from these matings (see Chart 2).

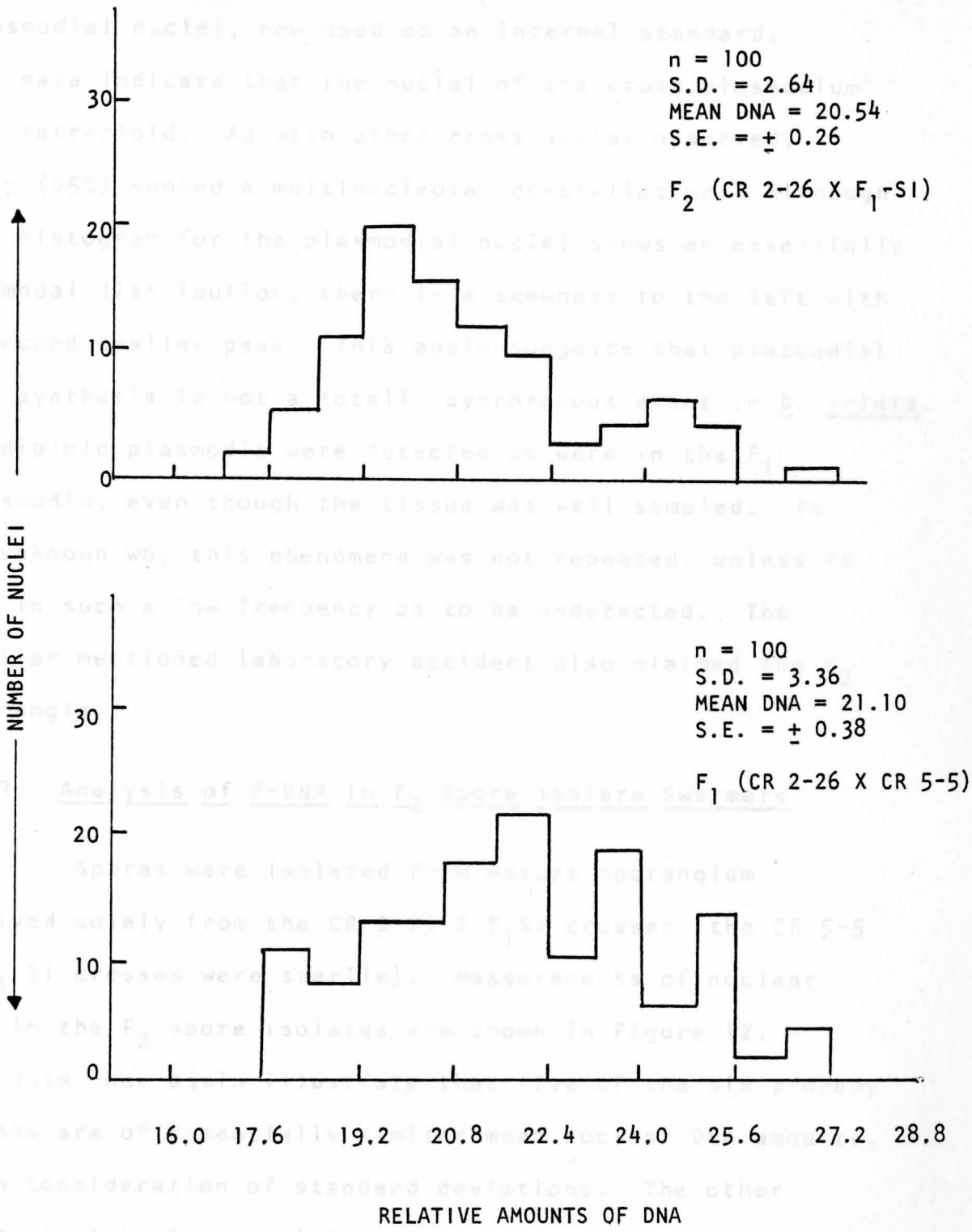
It is thus apparent that all the F_1 spore isolates which were viable were of the haploid parental type, carrying its nuclear marker, the A^2 allele. Mean nuclear DNA data also confirm this segregation pattern. No selfing or other non-heterothallic mating behavior in these isolates was ever noticed during the duration of this study.

It is not known why the polyploid spores were not viable. From F-DNA analysis of sporangia (post-cleavage) it is evident that high ploidy spores do exist, however, they are in many ways aberrant (e.g. in no. of nuclei per spore, spore shapes were not uniform, etc.). The results of the F_1 mass-spore culture test seem to indicate that the polyploid spores are viable, but probably in a low frequency. This low frequency may support the fact that of the spores isolated randomly, the probability of selecting polyploid spores which are viable would be low.

8. Analysis of F-DNA in F_2 (CR 2-26 X F_1 SI) Plasmodia

Measurements of nuclear DNA content in the pooled F_2 cross plasmodial nuclei are presented in Figure 11. The mean DNA value for the cross plasmodial nuclei is


4
3
2
1
A2
A2
A2
A2
F1 Spore Isolates
1
2
3
4
A2
A2
A2
A2
F1 Spore Isolates

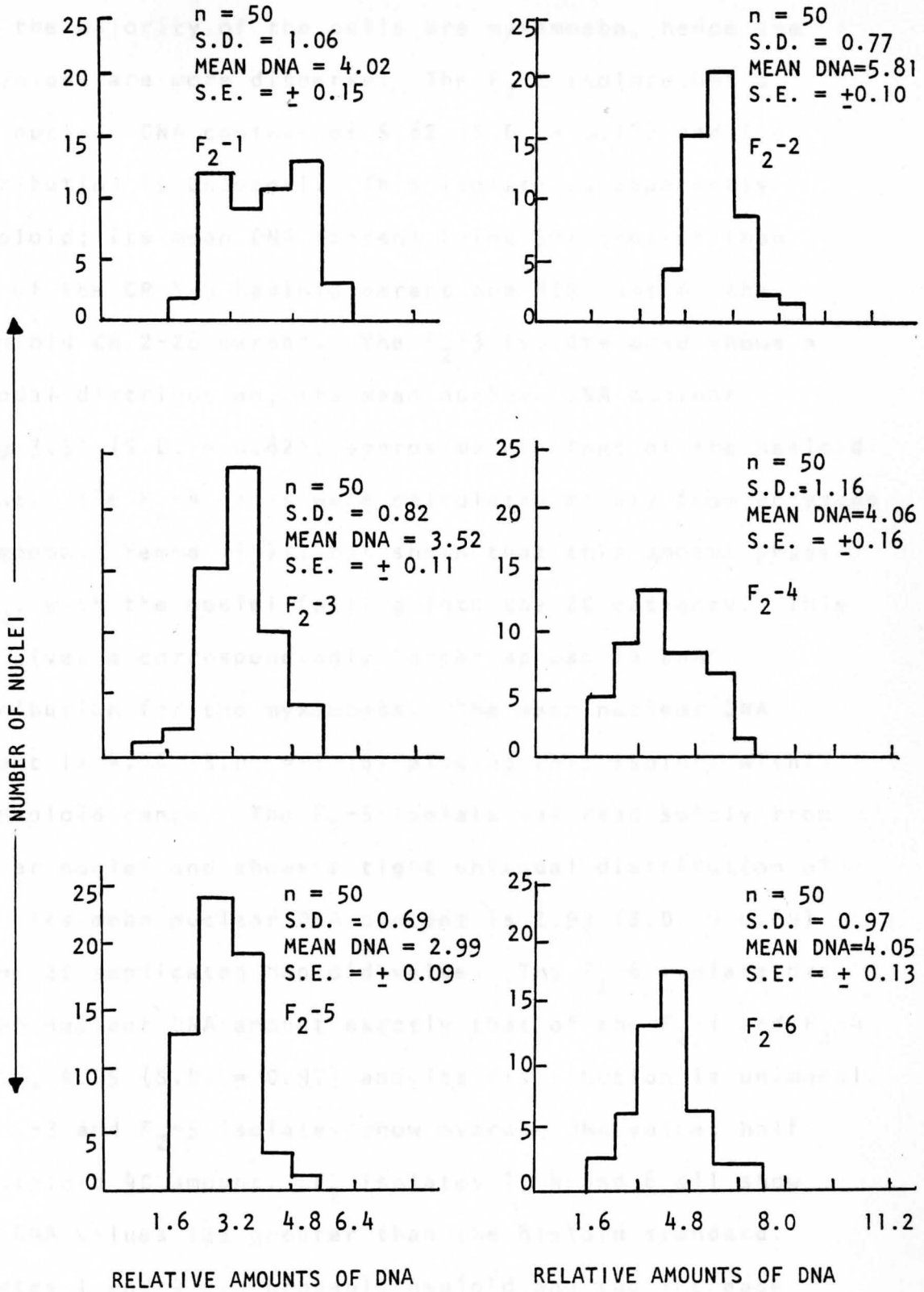

n = 100
S.D. = 2.64
MEAN DNA = 20.54
S.E. = ± 0.26
F2 (CR 2-26 X F1-S1)
n = 100
S.D. = 3.36
MEAN DNA = 21.10
S.E. = ± 0.38
F1 (CR 2-26 X CR 5-5)
NUMBER OF NUCLEI
0
10
20
30
16.0 17.6 19.2 20.8 22.4 24.0 25.6 27.2 28.8
RELATIVE AMOUNTS OF DNA

approximately equal to that of the F_1 polyploid (tetraploid) plasmodial nuclei, now used as an internal standard. The data indicate that the nuclei of the cross plasmodium are tetraploid. As with other cross nuclei observed, many (85%) showed a multinucleolar constellation. Although the histogram for the plasmodial nuclei shows an essentially unimodal distribution, there is a skewness to the left with a second smaller peak. This again suggests that plasmodial DNA synthesis is not a totally synchronous event in D. iridis. No diploid plasmodia were detected as were in the F_1 plasmodia, even though the tissue was well sampled. It is unknown why this phenomena was not repeated, unless it was in such a low frequency as to be undetected. The earlier mentioned laboratory accident also claimed the F_2 sporangia.

9. Analysis of F-DNA in F_2 Spore Isolate Swarmers

Spores were isolated from mature sporangium derived solely from the CR 2-26 X F_1SI crosses (the CR 5-5 X F_1 SI crosses were sterile). Measurements of nuclear DNA in the F_2 spore isolates are shown in Figure 12. The data once again illustrate that five of the six progeny clones are of essentially similar mean nuclear DNA amounts, with consideration of standard deviations. The other spore isolate is aneuploid.

The F_2-1 isolate has a mean nuclear DNA content of 4.02 (S.D. = 1.06) and its frequency distribution indicates


n = 50
S.D. = 1.06
MEAN DNA = 4.02
S.E. = ± 0.15
F2-1
n = 50
S.D. = 0.77
MEAN DNA=5.81
S.E. = ±0.10
F2-2
n = 50
S.D. = 0.82
MEAN DNA = 3.52
S.E. = ± 0.11
F2-3
n = 50
S.D.= 1.16
MEAN DNA=4.06
S.E. = ±0.16
F2-4
n = 50
S.D. = 0.69
MEAN DNA = 2.99
S.E. = ± 0.09
F2-5
n = 50
S.D. = 0.97
MEAN DNA=4.05
S.E. = ± 0.13
F2-6
NUMBER OF NUCLEI
0
5
10
15
20
25
1.6 3.2 4.8 6.4
1.6 4.8 8.0 11.2
RELATIVE AMOUNTS OF DNA
RELATIVE AMOUNTS OF DNA

that the majority of the cells are myxamoeba, hence the DNA values are more disperse. The F_2-2 isolate has a mean nuclear DNA content of 5.82 (S.D. = 0.77) and its distribution is unimodal. This isolate is apparently aneuploid; its mean DNA content being 50% greater than that of the CR 5-5 haploid parent and 82% that of the polyploid CR 2-26 parent. The F_2-3 isolate also shows a unimodal distribution, its mean nuclear DNA content being 3.52 (S.D. = 0.82), approximately that of the haploid parent. The F_2-4 cells were calculated mainly from encysted myxamoeba. Yemma (1971) has shown that this amoeba phase is G_2, with the nuclei falling into the 2C category. This also gives a correspondingly larger spread in DNA distribution for the myxamoeba. The mean nuclear DNA content is 4.06 (S.D. = 1.16) placing this isolate within the haploid range. The F_2-5 isolate was read solely from swarmer nuclei and shows a tight unimodal distribution of DNA. Its mean nuclear DNA content is 2.99 (S.D. = 0.69) or the 2C replicated haploid value. The F_2-6 isolate has a mean nuclear DNA amount exactly that of the F_2-1 and F_2-4 clones, 4.05 (S.D. = 0.97) and its distribution is unimodal. The F_2-3 and F_2-5 isolates show average DNA values half the diploid 4C amount. F_2 isolates 1, 4 and 6 all show mean DNA values 12% greater than the haploid standard. Isolates 1 and 4 are probably haploid and the increase in nuclear DNA values may be accounted for as a function of increased template activity, since myxamoeba present more dye binding sites. F-DNA profiles suggest that

isolates 2 and 6 are aneuploid. Since swarmers represent G_2 nuclei it is thought that the varying DNA concentrations shown by these isolates are correlatable to varying chromosome distributions.

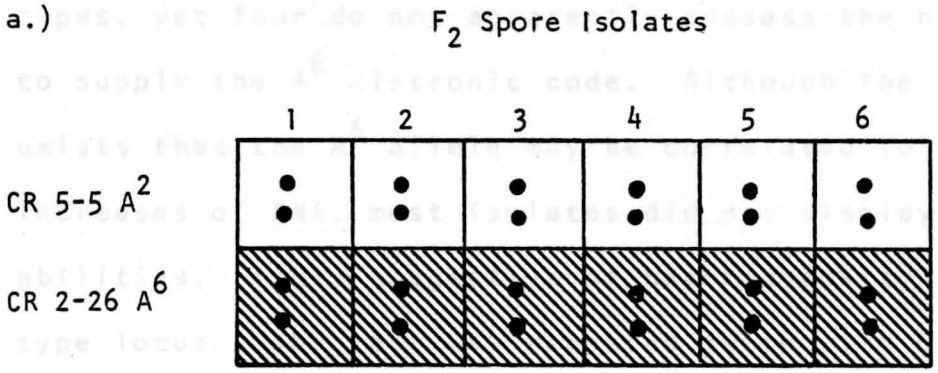
10. Genetic Analysis of F_2 Meiotic Segregants

The results of the F_2-isolate X CR 5-5, CR 2-26 backcrosses are shown in Chart 3a. The F_2 spores exhibited a higher germination rate of 12%. As with the F_1 segregation analysis for a randomly isolated population of spores, a 1:1 ratio of both parental nuclei and associated mating types is expected from an interisolate cross. Inspection of the data indicates that each F_2 isolate consistently produced large, viable plasmodia with both parental mating types. As stated previously, the expected compatability pattern was a 1:1 segregation of both mating type alleles and nuclear DNA content. Analysis of mean nuclear DNA concentration suggests that four isolates are haploid and would, therefore, be expected to carry the CR 5-5 mating type allele (A^2). In the absence of a net increase of nuclear DNA (possibly containing the other mating locus chromosome), these haploid myxamoeba would also be expected to conform to the heterothallic mating system (i.e. self-sterility). The other isolates, possibly aneuploid for some chromosome(s), also behaves like the haploid isolates. Regardless of ploidy, each isolate is able to produce plasmodia regularly with both parental strains. This behavior is not expected in "normal" heterothallic F_2 analysis and was not expected

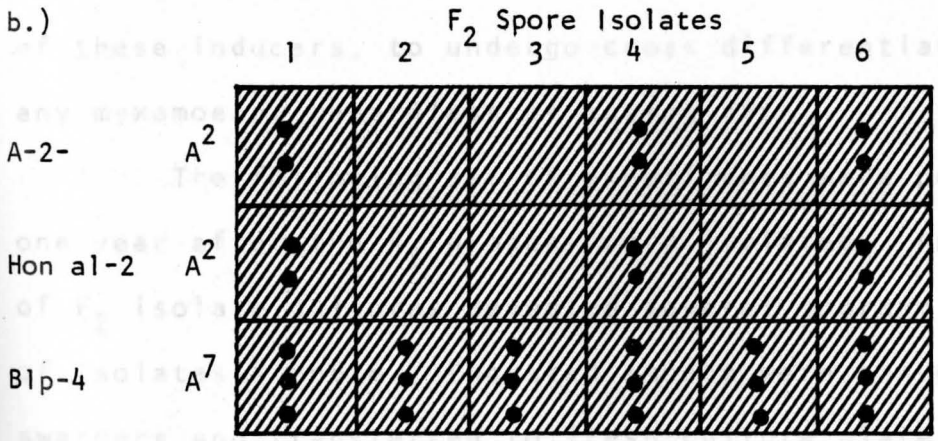
1
2
3
4
5
6
Cn 5-5 A2
UR 2-26 A1


1
2
3
4
5
6
A-2- A2
Non al 2 A2
Blp-4 A1

a.)


F2 Spore Isolates
1
2
3
4
5
6
CR 5-5 A2
CR 2-26 A6

b.)

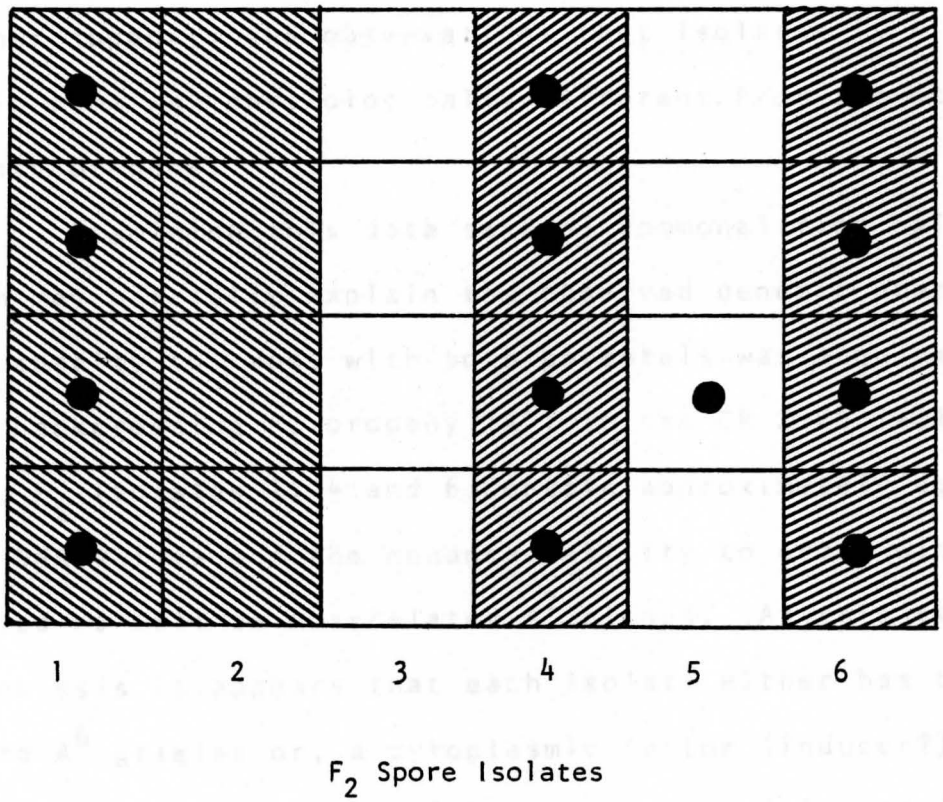

F2 Spore Isolates
1
2
3
4
5
6
A-2- A2
Hon a1-2 A2
Blp-4 A7

based upon the F_1 results. Even more intriguing is how each isolate can function as if expressing both mating types, yet four do not apparently possess the nuclear DNA to supply the A^6 cistronic code. Although the possibility exists that the A^6 allele may be correlated to even slight increases of DNA, most isolates did not display selfing abilities, highly suggestive of heterozygosity at the mating type locus. The test was repeated twice with 50 plates per cross made. Confusion of parental isolates was ruled out since each strain was tested for mating type with known testers. Also, F-DNA analysis of F_3 plasmodia (Section II) indicates two distinct differentiation values, dependent upon the parent used. The data demonstrate that the F_2 isolates possess the ability, due to a nuclear gene, a cytoplasmic influence, a membrance factor, or a combination of these inducers, to undergo cross differentiation with any myxamoeba, regardless of mating type.

The following set of experiments were performed one year after the F_2 progeny were isolated. An examination of F_2 isolate cultures revealed several sporangia on plates of isolates 4 and 6. Encysted amoeba were converted to swarmers and transferred to fresh culture plates. Upon continued passage-cloning these isolates produced plasmodia clonally. F_2-4 was unusual in that plasmodia were vigorous, unlike the general characteristics of selfers (Yemma, 1971).

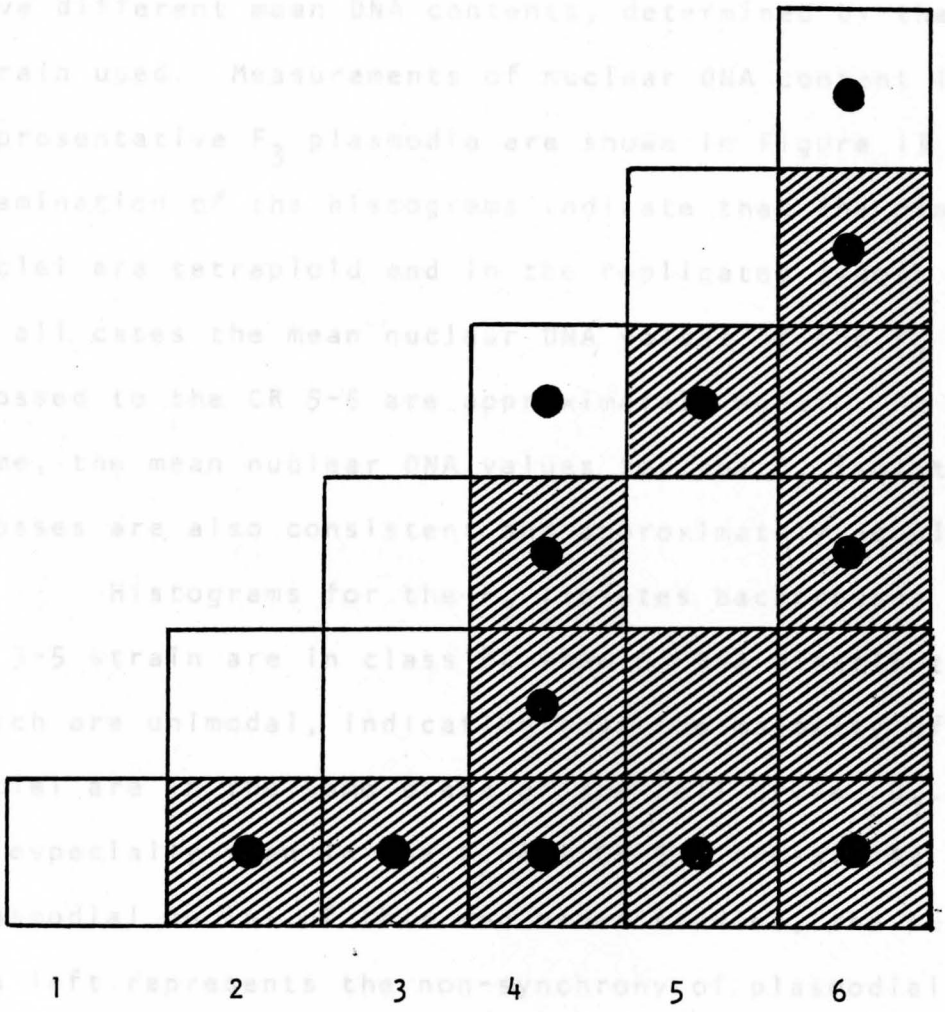
Since each F_2 isolate behaves compatibly with both parentals it was decided to determine their mating types

by use of testers of known mating alleles. The results of these tests are shown in Chart 3b. Each F_2 isolate, like the F_1 isolates were crossed to the Bip-4 A^7, Hon a1-2A^2 and A-2- strains to determine if they would cross with non-related strains of like mating type. Since the F_1 isolates were sterile when mated to their CR 5-5A^2 parent it was hypothesized that the F_2 isolates, able to cross with their A^2 parent would also cross with any genetically related A^2 strain. As a control, each F_2 isolate was crossed to itself, to insure against cross contamination. Each test comprised 25 plates. Inspection of the results shows that all F_2 isolates cross with the Bip-4 A^7 isolate. This was expected since the A^7 mating type is foreign to the system under study and would be compatible with both the A^2 and A^6 alleles. The outcome of the crosses to the A^2 testers presents an interesting pattern. Here, in both tests conducted, only the F_2-1, 4 and 6 isolates yielded plasmodia when crossed to the tester A^2 isolates. In all cases these three isolates produced large viable plasmodia upon crossing with the A^2 tester, an indication of a diploid cross plasmodium. As to why only 3 of the 6 isolates (a 1:1 ratio) were able to participate in plasmodial differentiation is unknown. To further examine this 1:1 pattern of mating behavior each of the F_2 isolates were crossed to the F_1 isolates, previously determined as being haploid & bearing the A^2 mating type allele. The results of this test are presented in Chart 4. Again, the


4
3
2
1
F_1 Spore Isolates
1
2
3
4
5
6
F_2 Spore Isolates

same F_2 isolates, 1, 4 and 6 consistently produced plasmodia with all four of the F_1 isolates. F_2-1 was an exception though, it failed to cross with F_1-2 and F_1-3. The F_2-5 isolate was not expected to yield plasmodia in any of these crosses, however, it did with the F_1-2 clone. It is speculative as to why these events occured and no reason is known. As a final test of F_2 compatibility the F_2 isolates were crossed, two at a time, in all possible combinations. The results are complex and shown in Chart 5. This test further supports the observations that isolates 1, 4 and 6 are in some way physiologically different from the other F_2 isolate progeny.

Examining this data several commonalities exist which may partially explain the observed genetic phenomena. The capacity to cross with both parentals was acquired upon backcrossing F_1 progeny (A^2) to the CR 2-26 isolate. Also, F_2 isolates 1, 4 and 6 contain approximately equal mean DNA values and the enhanced ability to cross with other related as well as non-related A^2 clones. At this stage of analysis it appears that each isolate either has both A^2 and A^6 alleles or, a cytoplasmic factor (inducer?) was acquired with F_2-1, 4 and 6 being more sensitive to its action. That the aneuploid F_2-2 isolate was unable to cross with any A^2 isolates (except its parent CR 5-5) may indicate the retention of chromosomes or chromosomal segments not linked to mating type.



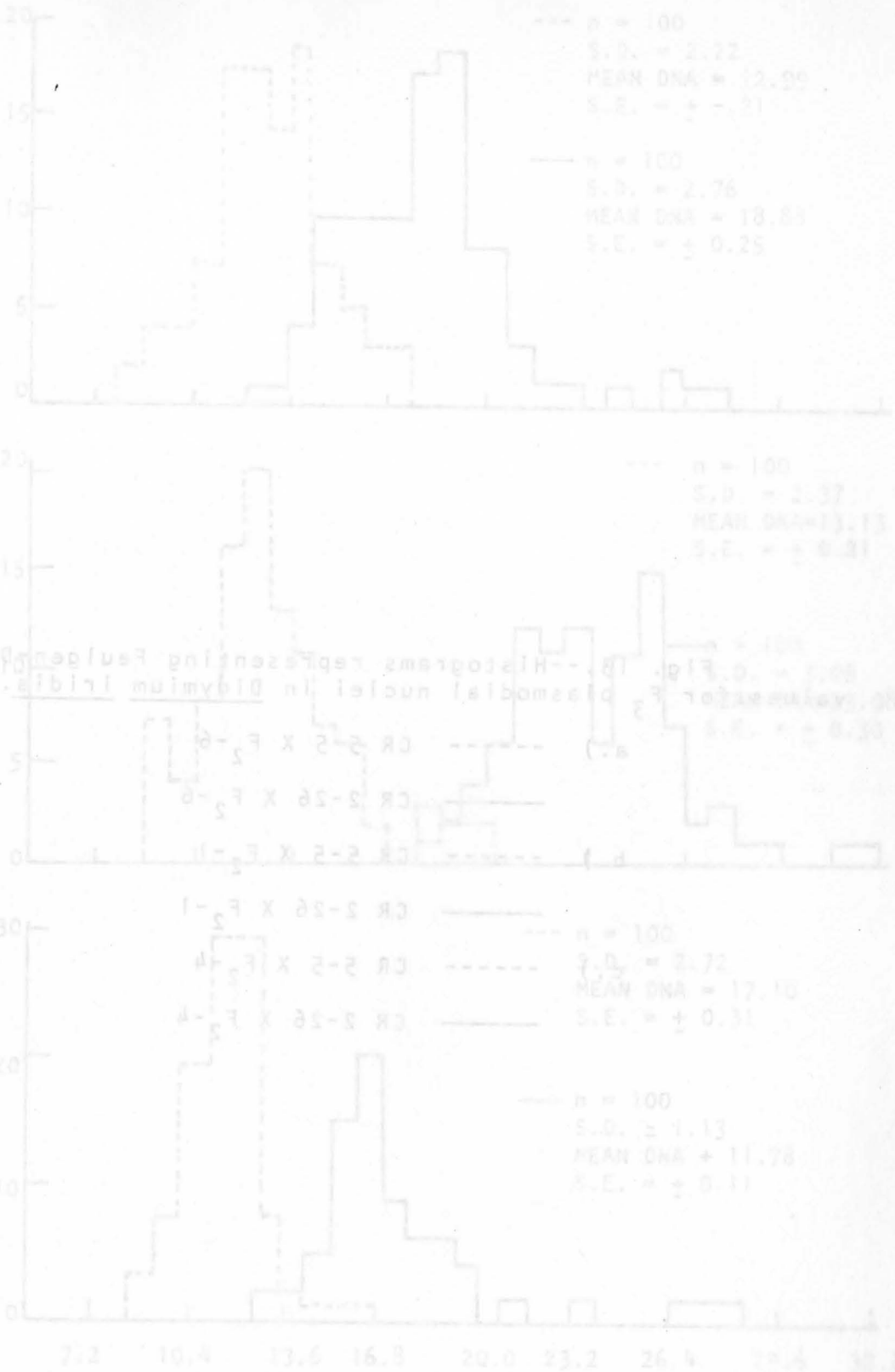
F_2 Spore Isolates

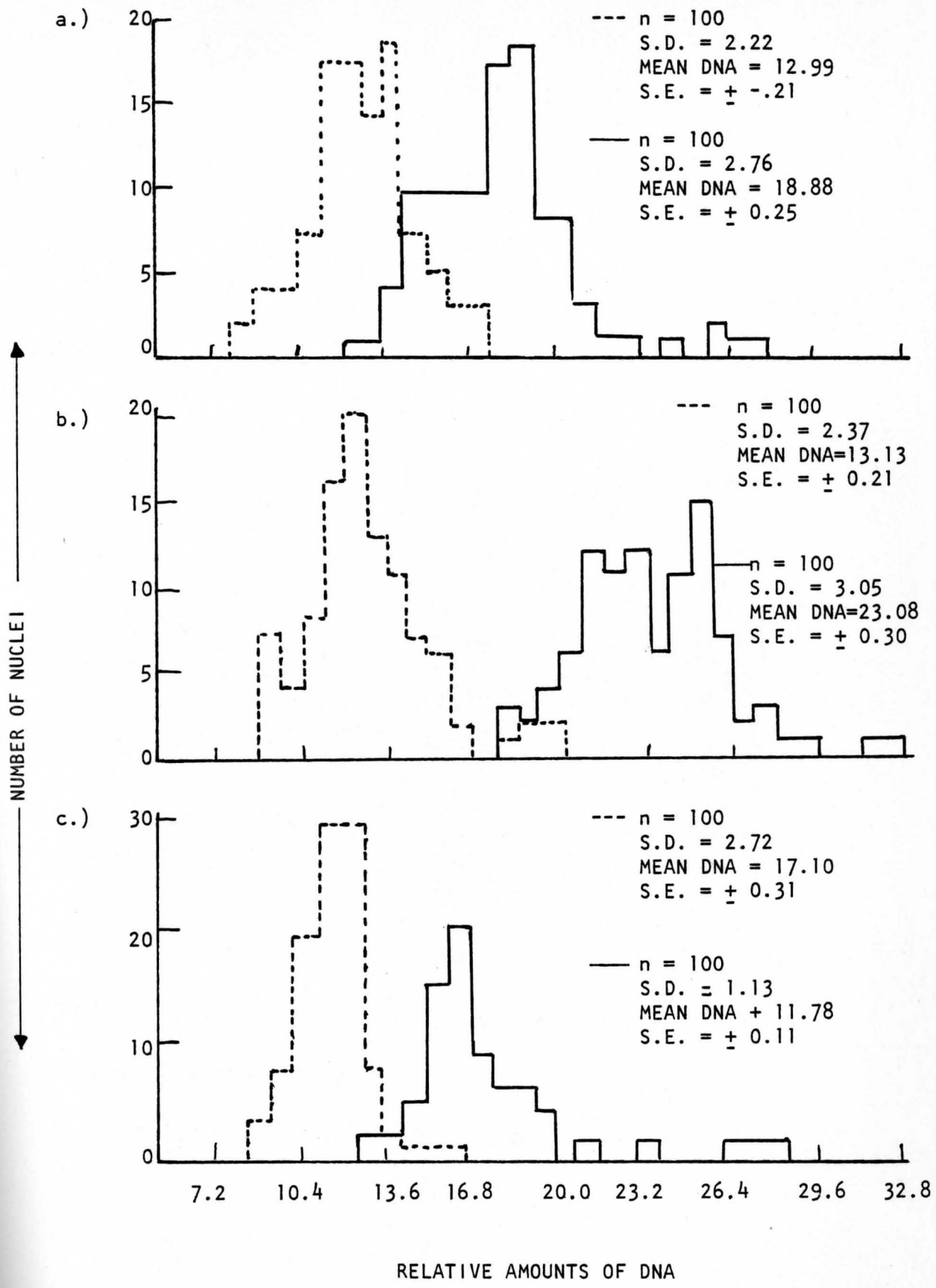
11. Analysis of F-DNA in F_3 Plasmodial Nuclei

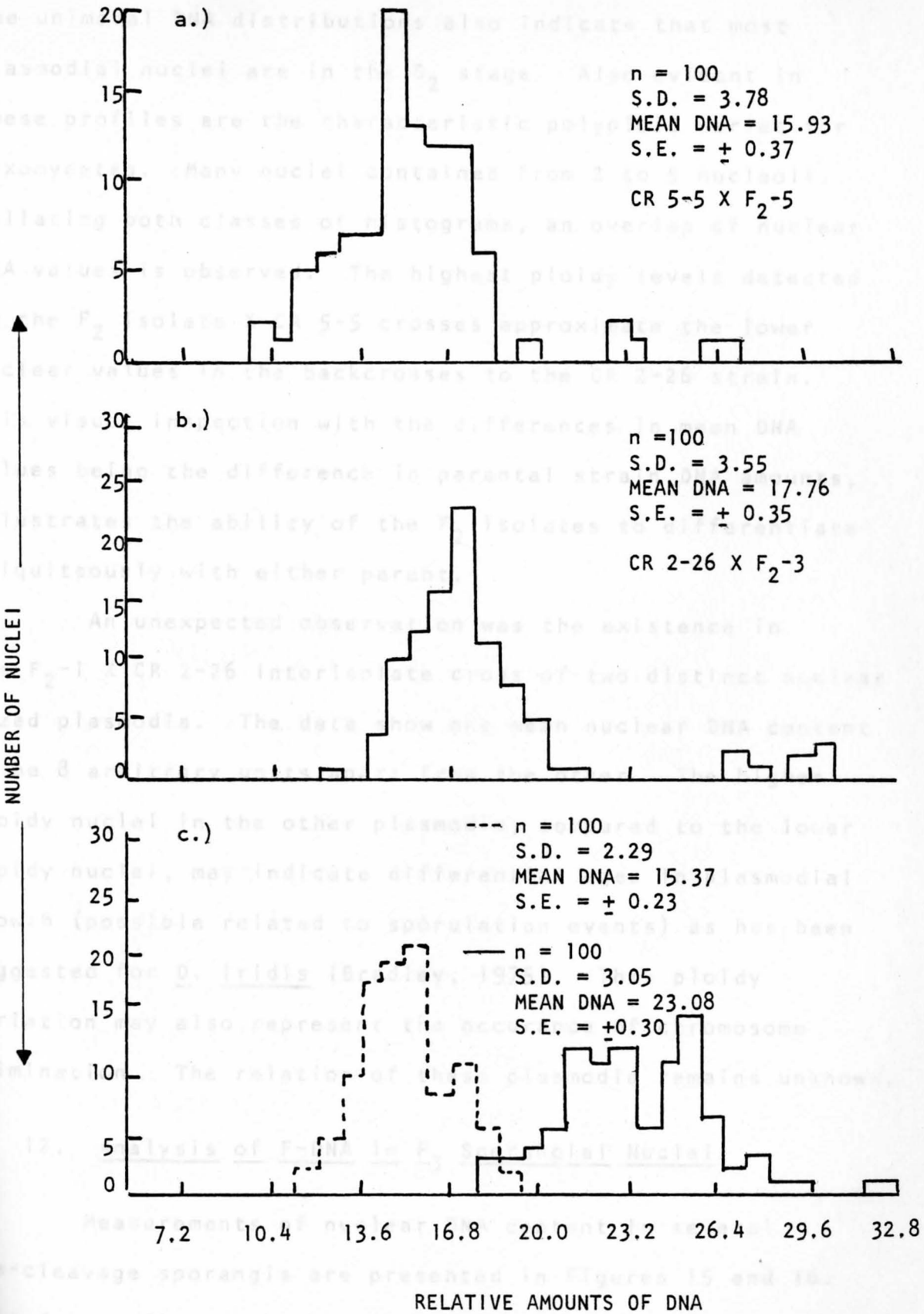
As has been shown in section 10, the F_2 isolates produced plasmodia with both parental strains. The F-DNA analysis confirms this event in that the resultant plasmodia have different mean DNA contents, determined by the parental strain used. Measurements of nuclear DNA content in representative F_3 plasmodia are shown in Figure 13 and 14. Examination of the histograms indicate that the plasmodia nuclei are tetraploid and in the replicated zygote class. In all cases the mean nuclear DNA values of the F_2 isolates crossed to the CR 5-5 are approximately equal. At the same time, the mean nuclear DNA values for the F_2 isolate X CR 2-26 crosses are also consistent and approximately equal.

Histograms for the F_2 isolates backcrosses to the CR 5-5 strain are in class 8C showing DNA distributions which are unimodal, indicating that the majority of the nuclei are in the same stage of the cell cycle, G_2. This is especially seen in the F_2-4 X CR 5-5 cross. As in other plasmodial nuclei of this and other studies, the peak to the left represents the non-synchrony of plasmodial DNA synthisis.

Upon comparison, the histograms of F_2 isolate backcrosses to the CR 2-26 parental represent a similar state. Plasmodial nuclei are tetraploid replicated zygotes. Mean nuclear DNA contents are greater than those involving backcrosses to the haploid strain, corresponding to the increased amount of nuclear DNA in the CR 2-26 strain.




a.)
--- n = 100
S.D. = 2.22
MEAN DNA = 12.99
S.E. = ± -.21
— n = 100
S.D. = 2.76
MEAN DNA = 18.88
S.E. = ± 0.25
b.)
--- n = 100
S.D. = 2.37
MEAN DNA=13.13
S.E. = ± 0.21
n = 100
S.D. = 3.05
MEAN DNA=23.08
S.E. = ± 0.30
c.)
--- n = 100
S.D. = 2.72
MEAN DNA = 17.10
S.E. = ± 0.31
— n = 100
S.D. = 1.13
MEAN DNA + 11.78
S.E. = ± 0.11
NUMBER OF NUCLEI
RELATIVE AMOUNTS OF DNA
7.2
10.4
13.6
16.8
20.0
23.2
26.4
29.6
32.8

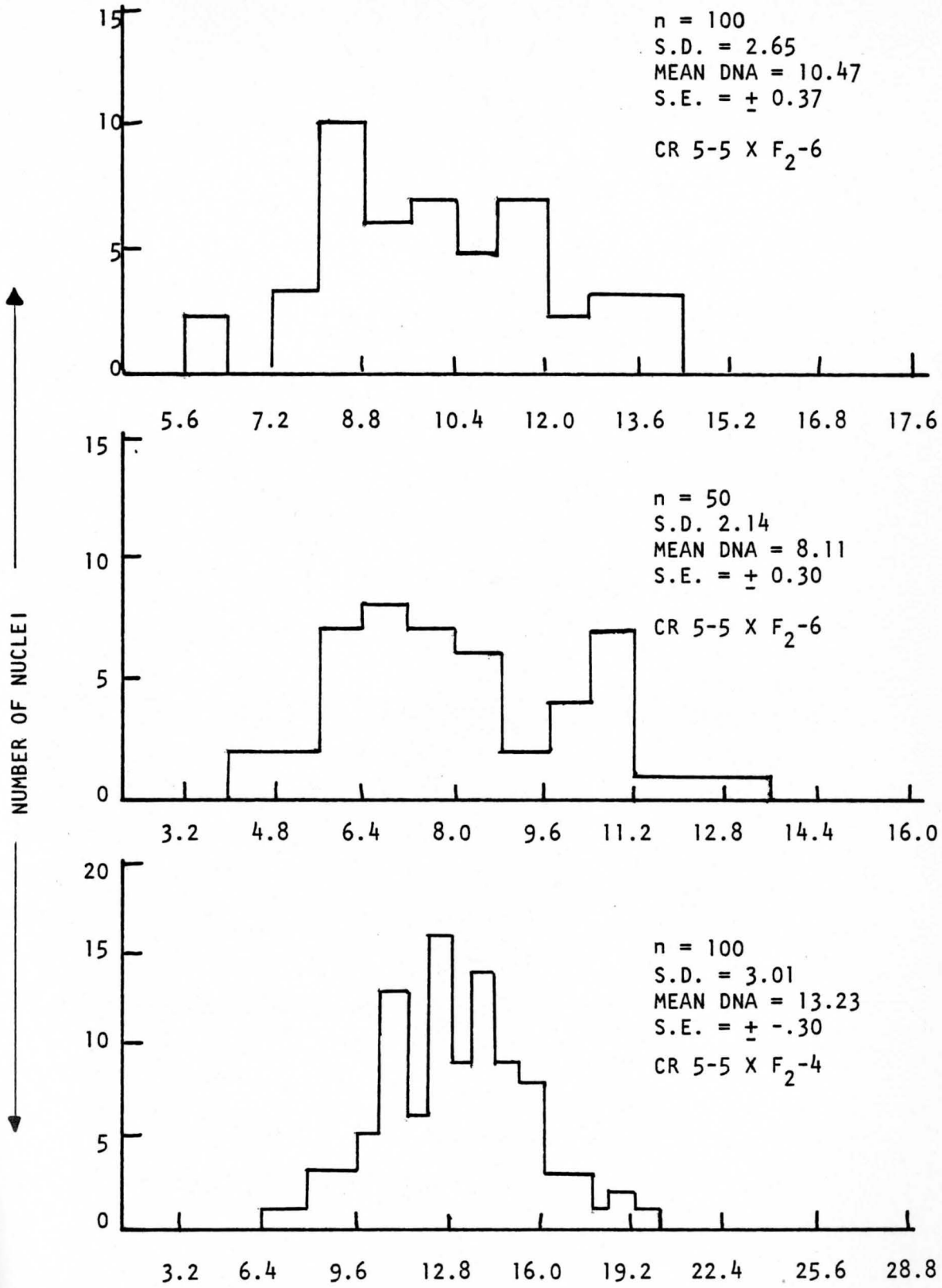

a.)
n = 100
S.D. = 3.78
MEAN DNA = 15.93
S.E. = ± 0.37
CR 5-5 X F2-5
b.)
n =100
S.D. = 3.55
MEAN DNA = 17.76
S.E. = ± 0.35
CR 2-26 X F2-3
c.)
--- n = 100
S.D. = 2.29
MEAN DNA = 15.37
S.E. = ± 0.23
—— n = 100
S.D. = 3.05
MEAN DNA = 23.08
S.E. = ±0.30
NUMBER OF NUCLEI
0
5
10
15
20
25
30
7.2
10.4
13.6
16.8
20.0
23.2
26.4
29.6
32.8
RELATIVE AMOUNTS OF DNA

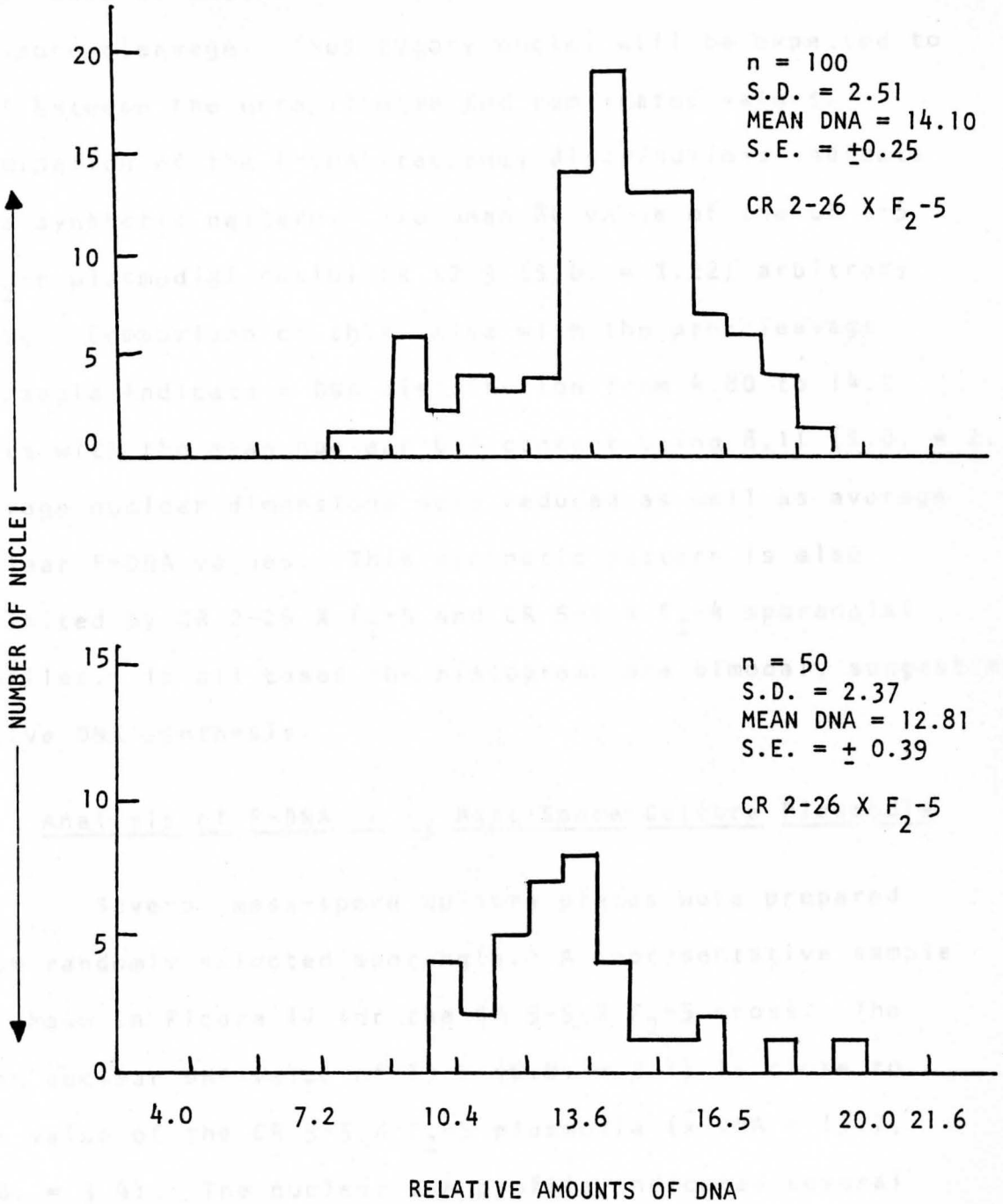
The unimodal DNA distributions also indicate that most plasmodial nuclei are in the G_2 stage. Also evident in these profiles are the characteristic polyploid series for myxomycetes. Many nuclei contained from 2 to 5 nucleoli. Collating both classes of histograms, an overlap of nuclear DNA values is observed. The highest ploidy levels detected in the F_2 isolate X CR 5-5 crosses approximate the lower nuclear values in the backcrosses to the CR 2-26 strain. This visual inspection with the differences in mean DNA values being the difference in parental strain DNA amounts, illustrates the ability of the F_2 isolates to differentiate ubiquiteously with either parent.

An unexpected observation was the existence in the F_2-1 X CR 2-26 interisolate cross of two distinct nuclear sized plasmodia. The data show one mean nuclear DNA content to be 8 arbitrary units apart from the other. The higher ploidy nuclei in the other plasmodia, compared to the lower ploidy nuclei, may indicate differential ages in plasmodial growth (possible related to sporulation events) as has been suggested for D. iridis (Bradley, 1975). This ploidy variation may also represent the occurance of chromosome elimination. The relation of these plasmodia remains unknown.

12. Analysis of F-DNA in F_3 Sporangial Nuclei

Measurements of nuclear DNA content in several pre-cleavage sporangia are presented in Figures 15 and 16. This data reflects the previous suggestion that upon sporangial differentiation a mitotic division occurs followed

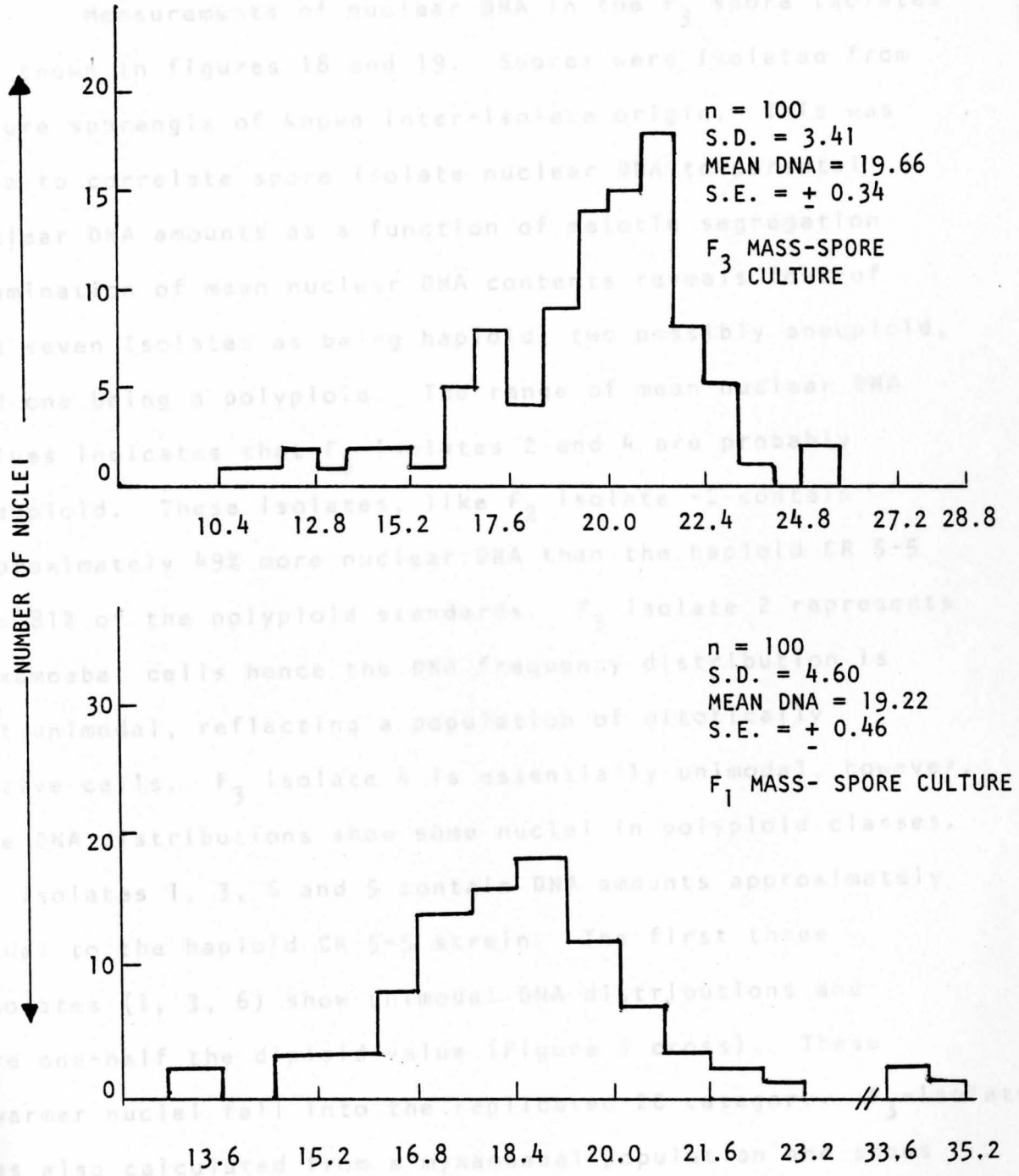

n = 100
S.D. = 2.65
MEAN DNA = 10.47
S.E. = ± 0.37
CR 5-5 X F2-6
15
10
5
0
5.6
7.2
8.8
10.4
12.0
13.6
15.2
16.8
17.6
n = 50
S.D. 2.14
MEAN DNA = 8.11
S.E. = ± 0.30
CR 5-5 X F2-6
15
10
5
0
3.2
4.8
6.4
8.0
9.6
11.2
12.8
14.4
16.0
n = 100
S.D. = 3.01
MEAN DNA = 13.23
S.E. = ± -.30
CR 5-5 X F2-4
20
15
10
5
0
3.2
6.4
9.6
12.8
16.0
19.2
22.4
25.6
28.8
NUMBER OF NUCLEI
RELATIVE AMOUNTS OF DNA


n = 100
S.D. = 2.51
MEAN DNA = 14.10
S.E. = ±0.25
CR 2-26 X F2-5
n = 50
S.D. = 2.37
MEAN DNA = 12.81
S.E. = ± 0.39
CR 2-26 X F2-5
NUMBER OF NUCLEI
RELATIVE AMOUNTS OF DNA
0
5
10
15
20
4.0
7.2
10.4
13.6
16.5
20.0
21.6

by a round of nuclear DNA synthesis, terminating prior to spore cleavage. Thus zygote nuclei will be expected to fall between the unreplicated and replicated values. Examination of the F-DNA frequency distributions indicate this synthetic pattern. The mean 8C value of the CR 5-5 X F_2-6 plasmodial nuclei is 12.9 (S.D. = 2.22) arbitrary units. Comparison of this value with the pre-cleavage sporangia indicate a DNA distribution from 4.80 to 14.0 units with the mean nuclear DNA content being 8.11 (S.D. = 2.14). Average nuclear dimensions were reduced as well as average nuclear F-DNA values. This synthetic pattern is also exhibited by CR 2-26 X F_2-5 and CR 5-5 X F_2-4 sporangial profiles. In all cases the histograms are bimodal, suggesting active DNA synthesis.

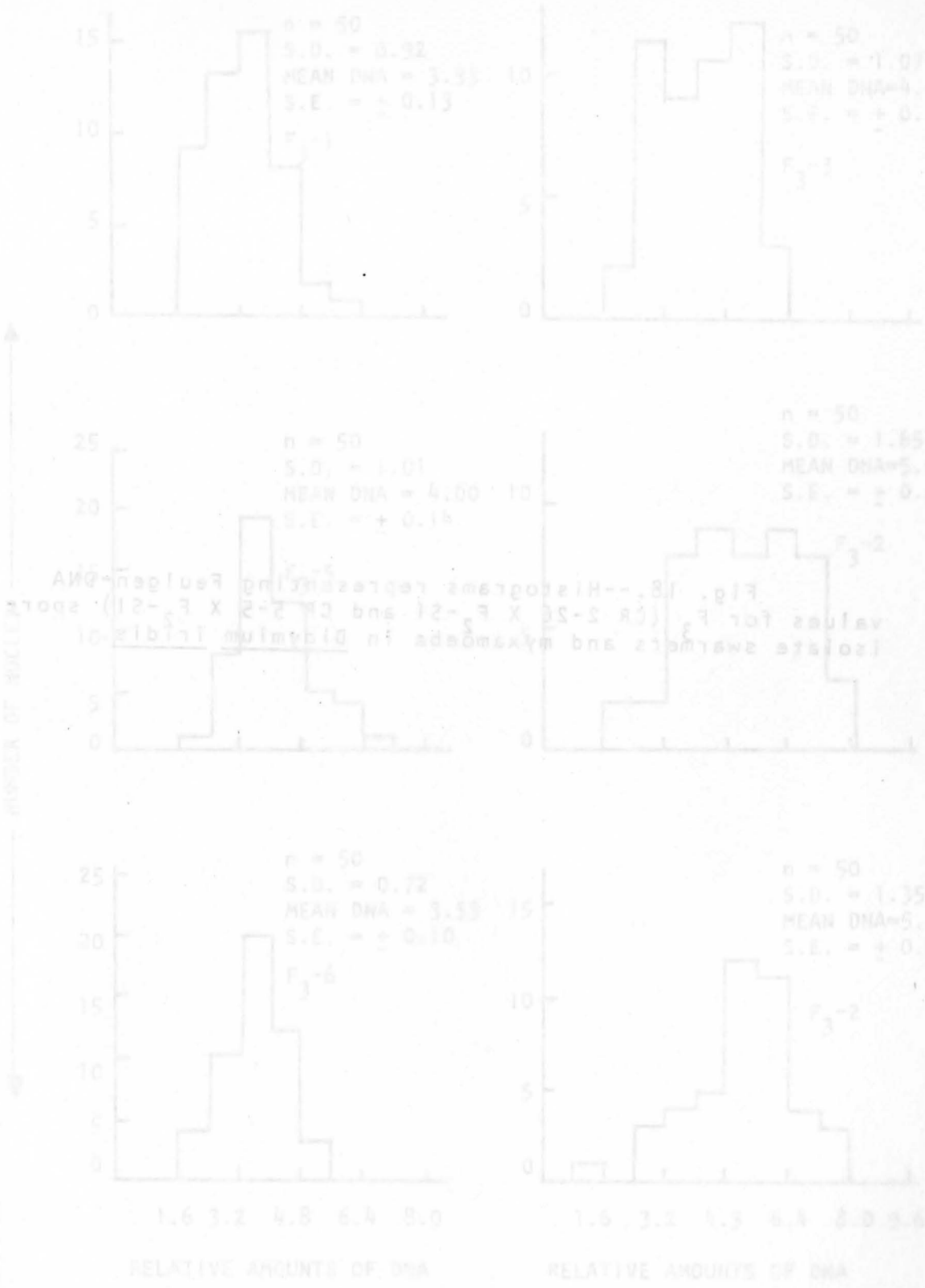
13. Analysis of F-DNA in F_3 Mass-Spore Culture Plasmodia

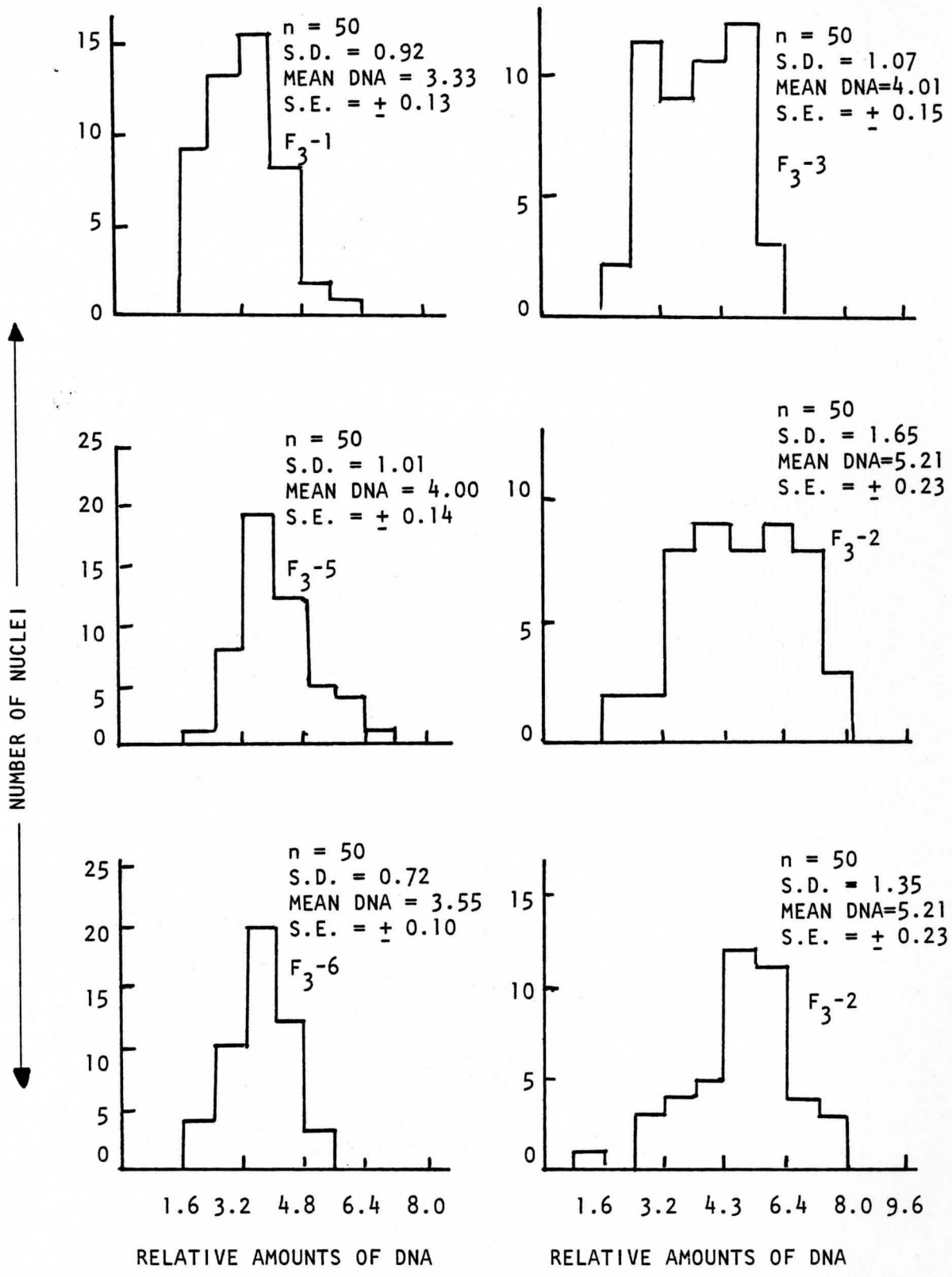
Several mass-spore culture plates were prepared from randomly selected sporangia. A representative sample is shown in Figure 17 for the CR 5-5 X F_2-5 cross. The mean nuclear DNA value of 19.6 (S.D. = 3.1) is close to the value of the CR 5-5 X F_2-5 plasmodia ($\bar{x}$ DNA = 15.7, S.D. = 3.4). The nuclear DNA profile indicates several peaks suggestive of an active nuclear cycle and tetraploidy (polyploidy). This data implies that sporogenesis is producing spores of compatible mating types or that the condition of the F_2 isolates (enhanced cell fusions) is being transmitted to the F_3 generation.

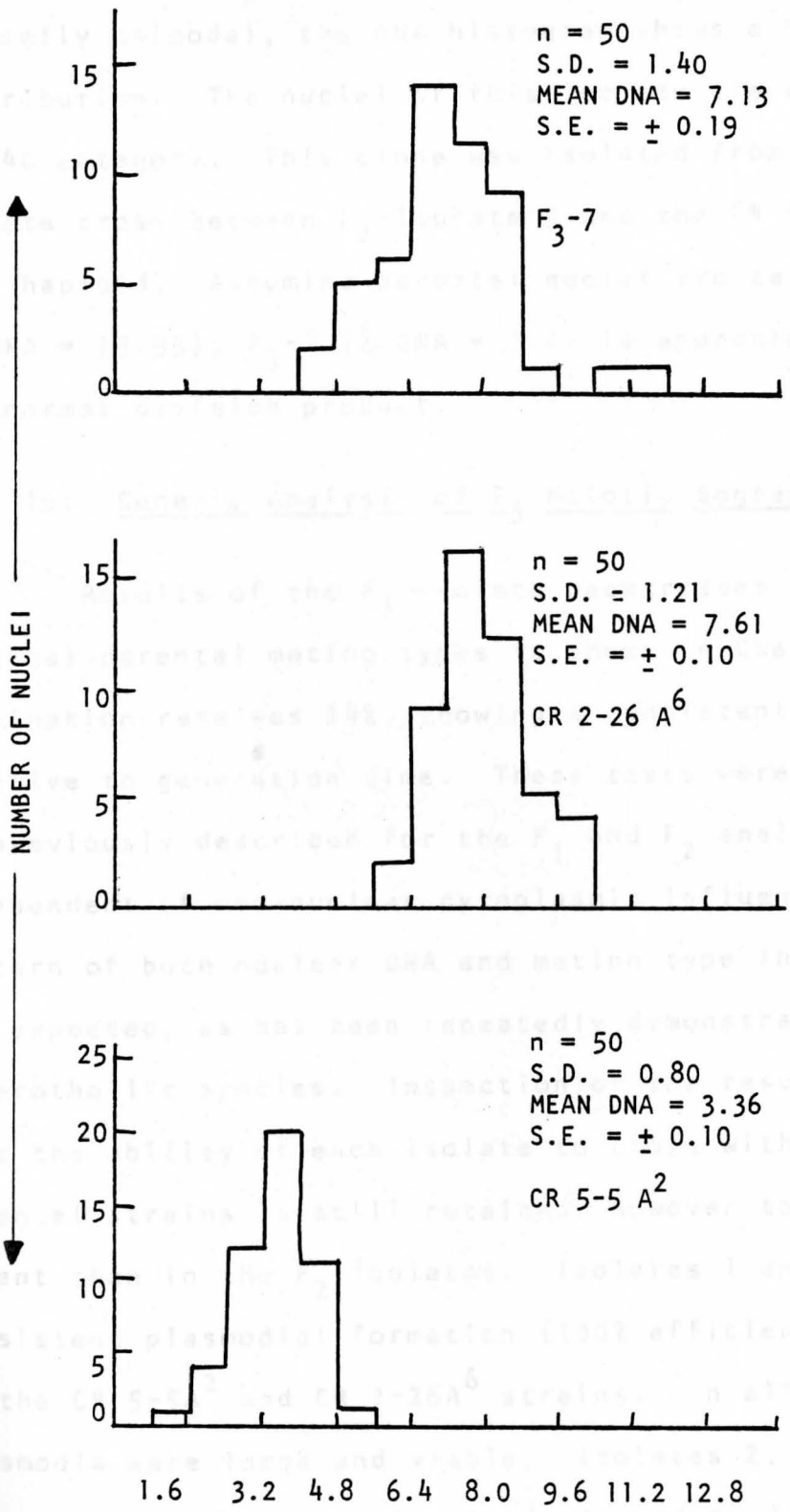

n = 100
S.D. = 3.41
MEAN DNA = 19.66
S.E. = ± 0.34
F3 MASS-SPORE CULTURE
20
15
10
5
0
10.4
12.8
15.2
17.6
20.0
22.4
24.8
27.2
28.8
n = 100
S.D. = 4.60
MEAN DNA = 19.22
S.E. = ± 0.46
F1 MASS- SPORE CULTURE
30
20
10
0
13.6
15.2
16.8
18.4
20.0
21.6
23.2
33.6
35.2
NUMBER OF NUCLEI
RELATIVE AMOUNTS OF DNA

14. Analysis of F-DNA in F_3 Spore Isolate Swarmers

Measurements of nuclear DNA in the F_3 spore isolates are shown in figures 18 and 19. Spores were isolated from mature sporangia of known inter-isolate origin. This was done to correlate spore isolate nuclear DNA to parental nuclear DNA amounts as a function of meiotic segregation. Examination of mean nuclear DNA contents reveals four of the seven isolates as being haploid, two possibly aneuploid, and one being a polyploid. The range of mean nuclear DNA values indicates that F_3 isolates 2 and 4 are probably aneuploid. These isolates, like F_2 isolate -2 contain approximately 49% more nuclear DNA than the haploid CR 5-5 and 81% of the polyploid standards. F_3-isolate 2 represents myxamoebal cells hence the DNA frequency distribution is not unimodal, reflecting a population of mitotically active cells. F_3 isolate 4 is essentially unimodal, however, the DNA distributions show some nuclei in polyploid classes. F_3 isolates 1, 3, 6 and 5 contain DNA amounts approximately equal to the haploid CR 5-5 strain. The first three isolates (1, 3, 6) show unimodal DNA distributions and are one-half the diploid value (Figure 3 cross). These swarmer nuclei fall into the replicated 2C category. F_3-isolate 5 was also calculated from a myxamoebal population and shows a slightly increased spread of DNA values. While showing higher ploidy nuclei, the isolate is haploid replicated or 2C. F_3 isolate-7 has a mean nuclear DNA value of 7.13 (S.D. = 1.40), not appreciably different from the CR 2-26


n = 50
S.D. = 0.92
MEAN DNA = 3.35
S.E. = ± 0.13
n = 50
S.D. = 1.07
MEAN DNA=4.01
S.E. = ± 0.15
n = 50
S.D. = 1.01
MEAN DNA = 4.00
S.E. = ± 0.14
n = 50
S.D. = 1.35
MEAN DNA=5.21
S.E. = ± 0.23
n = 50
S.D. = 0.72
MEAN DNA = 3.55
S.E. = ± 0.10
n = 50
S.D. = 1.35
MEAN DNA=5.21
S.E. = ± 0.23
1.6 3.2 4.8 6.4 8.0
RELATIVE AMOUNTS OF DNA

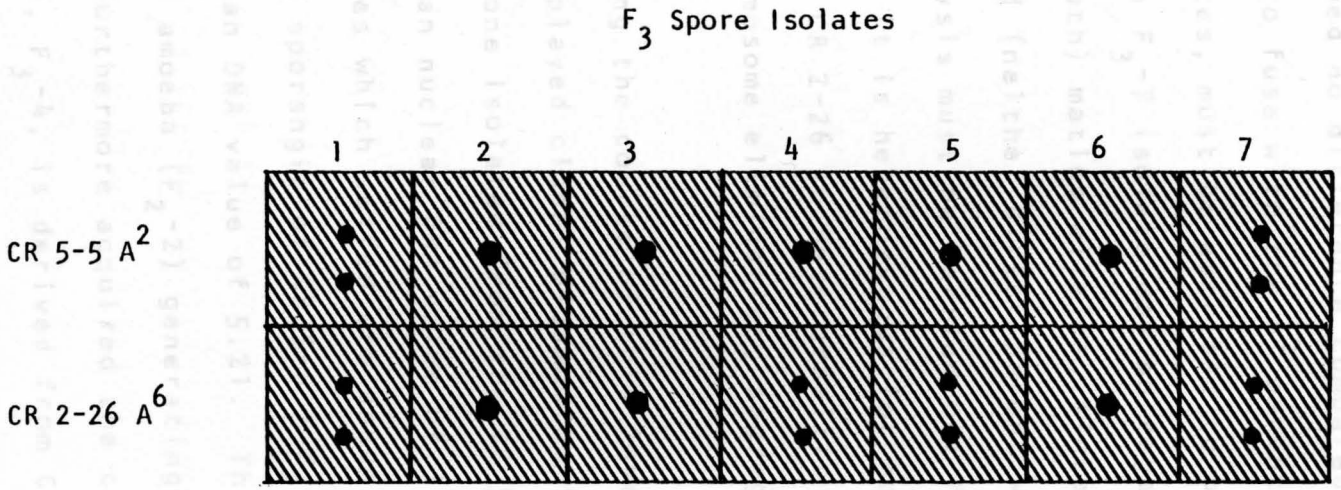

n = 50
S.D. = 0.92
MEAN DNA = 3.33
S.E. = ± 0.13
F3-1
n = 50
S.D. = 1.07
MEAN DNA=4.01
S.E. = ± 0.15
F3-3
n = 50
S.D. = 1.01
MEAN DNA = 4.00
S.E. = ± 0.14
F3-5
n = 50
S.D. = 1.65
MEAN DNA=5.21
S.E. = ± 0.23
F3-2
n = 50
S.D. = 0.72
MEAN DNA = 3.55
S.E. = ± 0.10
F3-6
n = 50
S.D. = 1.35
MEAN DNA=5.21
S.E. = ± 0.23
F3-2
NUMBER OF NUCLEI
1.6 3.2 4.8 6.4 8.0
RELATIVE AMOUNTS OF DNA
1.6 3.2 4.3 6.4 8.0 9.6
RELATIVE AMOUNTS OF DNA


n = 50
S.D. = 1.40
MEAN DNA = 7.13
S.E. = ± 0.19
F3-7
n = 50
S.D. = 1.21
MEAN DNA = 7.61
S.E. = ± 0.10
CR 2-26 A6
n = 50
S.D. = 0.80
MEAN DNA = 3.36
S.E. = ± 0.10
CR 5-5 A2
NUMBER OF NUCLEI
0
5
10
15
20
25
1.6 3.2 4.8 6.4 8.0 9.6 11.2 12.8
RELATIVE AMOUNTS OF DNA

isolate's value of 7.61 (S.D. = 1.21) (Figure 19). Basically unimodal, the DNA histogram shows a polyploid distribution. The nuclei of this isolate are comparable to the 4C category. This clone was isolated from the inter-isolate cross between F_2-isolate 5 and the CR 5-5 isolate, both haploid. Assuming parental nuclei are tetraploid ($\bar{X}$ DNA = 15.93), F_3-7 ($\bar{X}$ DNA = 7.1) is approximately $\frac{1}{2}$ or the normal division product.

15. Genetic Analysis of F_3 Meiotic Segregants

Results of the F_3 isolate backcrosses to the original parental mating types is shown in Chart 6. The germination rate was 14%, showing a consistent increase relative to generation time. These tests were performed as previously described for the F_1 and F_2 analyses. Independent of non-nuclear cytoplasmic influences, a 1:1 pattern of both nuclear DNA and mating type inheritance was expected, as has been repeatedly demonstrated for heterothallic species. Inspection of the results indicated that the ability of each isolate to cross with both parental strains is still retained, however to a lesser extent than in the F_2 isolates. Isolates 1 and 7 exhibited consistent plasmodial formation (100% efficiency) when crossed to the CR 5-5A^2 and CR 2-26A^6 strains. In all cases plasmodia were large and viable. Isolates 2, 3, 4, 5 and 6 showed decreasing percentages of plasmodial differentiation with both parents, but the crossing efficiency was still


F3 Spore Isolates
1
2
3
4
5
6
7
CR 5-5 A2
CR 2-26 A6

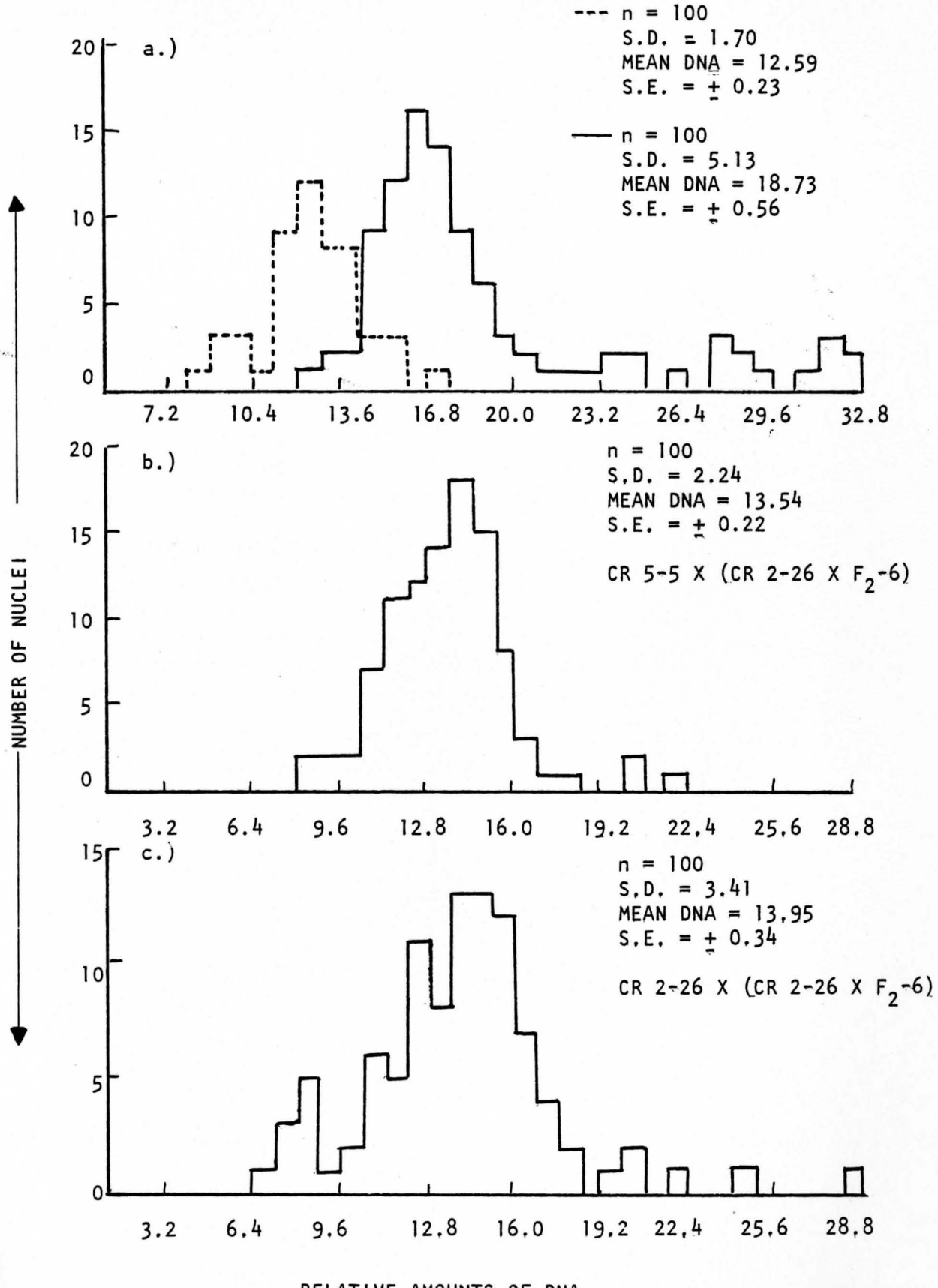
above 60% in each case. It is unknown why these isolates were less successful than the 1 and 7 clones; though a possible contributing factor may have been heavy bacterial growth which could inhibit amoebal growth. With respect to crossing frequency, the behavior of the higher ploidy isolates showed no difference compared to the haploid isolate. The ability to fuse with the A^2 parental tester, as with the F_2 isolates, must be ascribed to other factors as yet unknown. The F_3-7 isolate may be regarded as possessing the A^2 (or both) mating alleles, since it may be an autopolyploid (neither direct parent was the CR 2-26 isolate). Further analysis must be carried out for this isolate to determine if it is heterozygous for mating type and if it behaves like CR 2-26 in heterothallic crosses causing similar chromosome elimination, segregation, and viability problems.

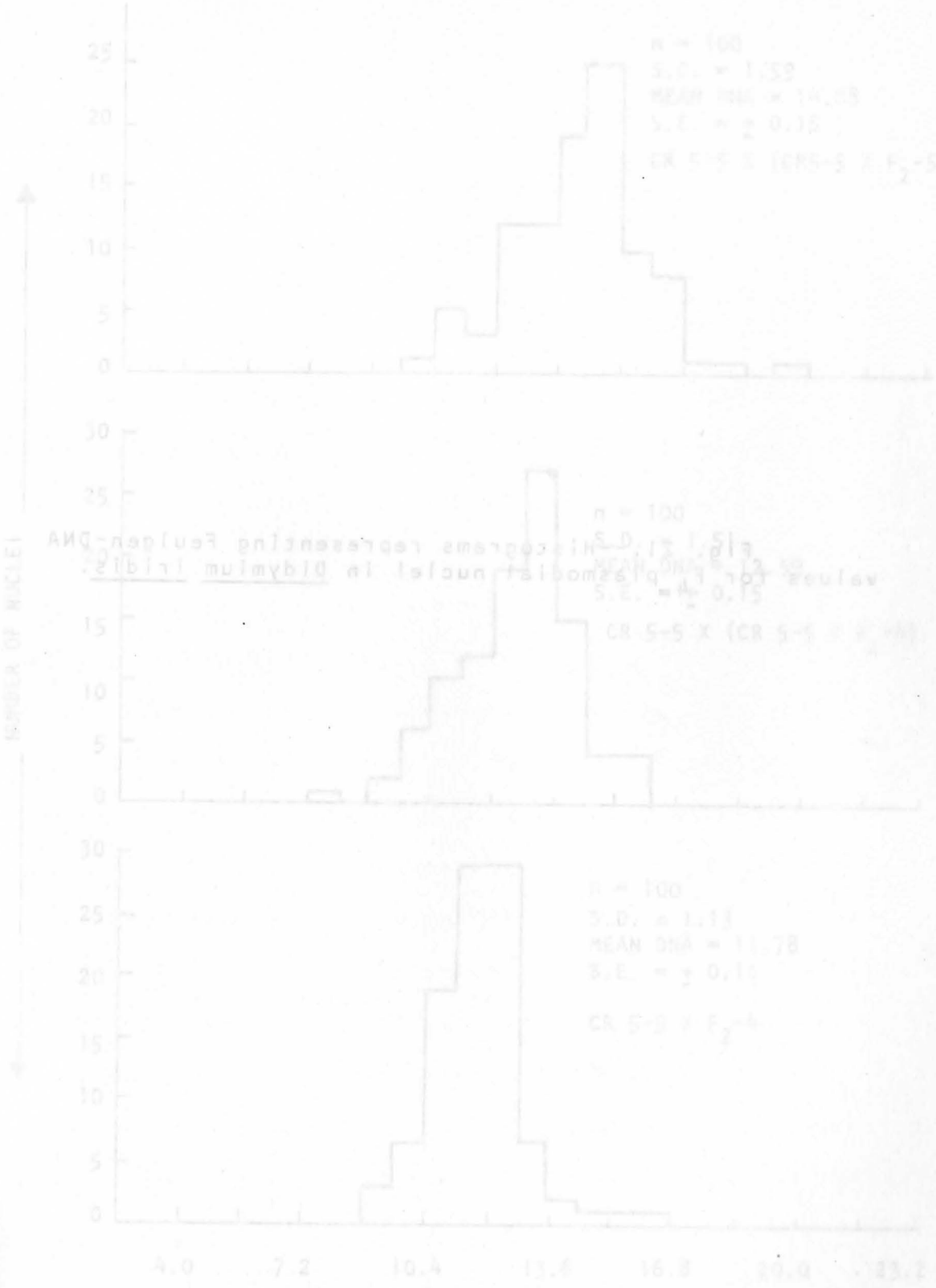
During the course of this investigation several F_3 isolates displayed clonal plasmodium formation. F_3-1 is a haploid clone isolated from the CR 5-5 X F_2-1 sporangium and has a mean nuclear DNA value of 3.34. The other two progeny clones which selfed are aneuploid. F_3-2 was derived from sporangia of CR 5-5 X F_2-2 (F_2-2 $\bar{X}$ DNA = 5.81) and has a mean DNA value of 5.21. This observation demonstrates an aneuploid amoeba (F_2-2) generating aneuploid progeny which have furthermore acquired the capacity to self. The last isolate, F_3-4, is derived from CR 5-5 X F_2-4 and has a mean DNA value of 5.22. Recalling that F_2-4 was haploid and able to self, the net increase in nuclear DNA may not

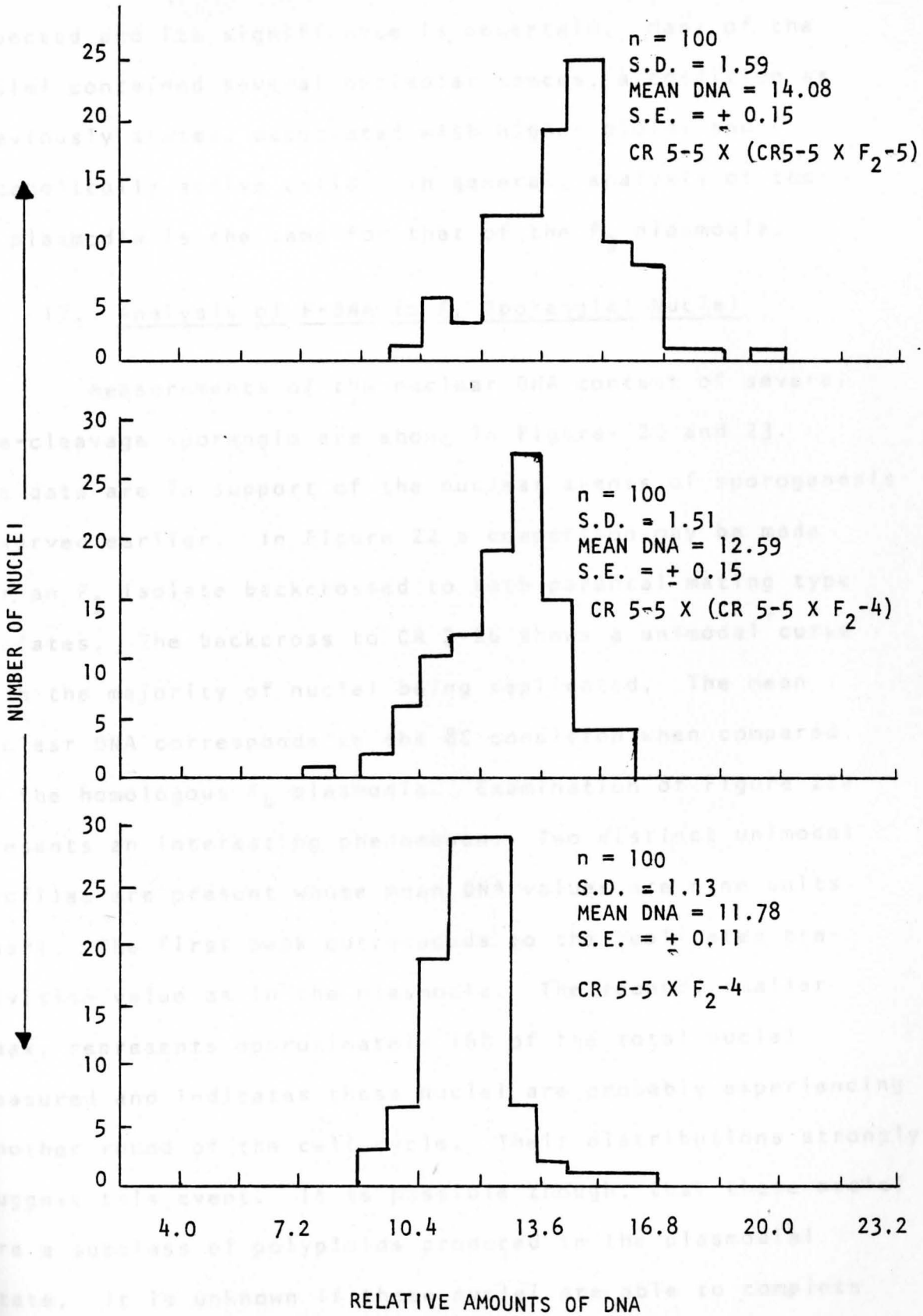
be related to selfing. That F_2-4 and its progeny F_3-4 were both able to self strongly implicates the transmission of a cytoplasmic factor linked to selfing. The evidence for other F_3 isolates selfing is questionable. The results of selfing in F_2 and F_3 isolates are limited; further research into this phenonenon is necessary before a firm conclusion may be drawn relating aneuploidy to selfing.

16. Analysis of F-DNA in F_4 Plasmodial Nuclei

Measurements of nuclear DNA content in plasmodia produced from both F_3 isolate X CR 5-5 and F_3 isolate X CR 2-26 interisolate crosses are shown in Figures 20 and 21. Examinations of representative histograms indicates the majority of nuclei are replicated tetraploid polyploids. For the sake of comparison, as done with the F_3 plasmodia, spore isolates crossed to both parentals are shown. The F_4 plasmodial nuclei show a pattern similar to that observed in the F_3 plasmodia; plasmodial mean nuclear DNA contents are distinguishable based upon which parental the isolate was crossed to. Isolates crossed to the CR 2-26 parent show a net increase of mean DNA as compared to interisolate crosses with the CR 5-5 parent. Examination of Figure 20a indicates an overlap of nuclei with the mean values being separated by six units. F_3 isolate crosses to CR 2-26 all exhibit a definite polyploid series with lesser peaks recorded. Each curve is unimodal and the majority of plasmodial nuclei appear to be replicated. Comparison of the histograms in Figure 20 show that the mean nuclear DNA


--- n = 100
S.D. = 1.70
MEAN DNA = 12.59
S.E. = ± 0.23
—— n = 100
S.D. = 5.13
MEAN DNA = 18.73
S.E. = ± 0.56
a.)
20
15
10
5
0
7.2
10.4
13.6
16.8
20.0
23.2
26.4
29.6
32.8
b.)
n = 100
S.D. = 2.24
MEAN DNA = 13.54
S.E. = ± 0.22
CR 5-5 X (CR 2-26 X F2-6)
3.2
6.4
9.6
12.8
16.0
19.2
22.4
25.6
28.8
c.)
n = 100
S.D. = 3.41
MEAN DNA = 13.95
S.E. = ± 0.34
CR 2-26 X (CR 2-26 X F2-6)
NUMBER OF NUCLEI
RELATIVE AMOUNTS OF DNA


25
20
15
10
5
0
30
25
20
15
10
5
0
30
25
20
15
10
5
0
4.0
7.2
10.4
13.6
16.8
20.0
23.2
RELATIVE AMOUNTS OF DNA

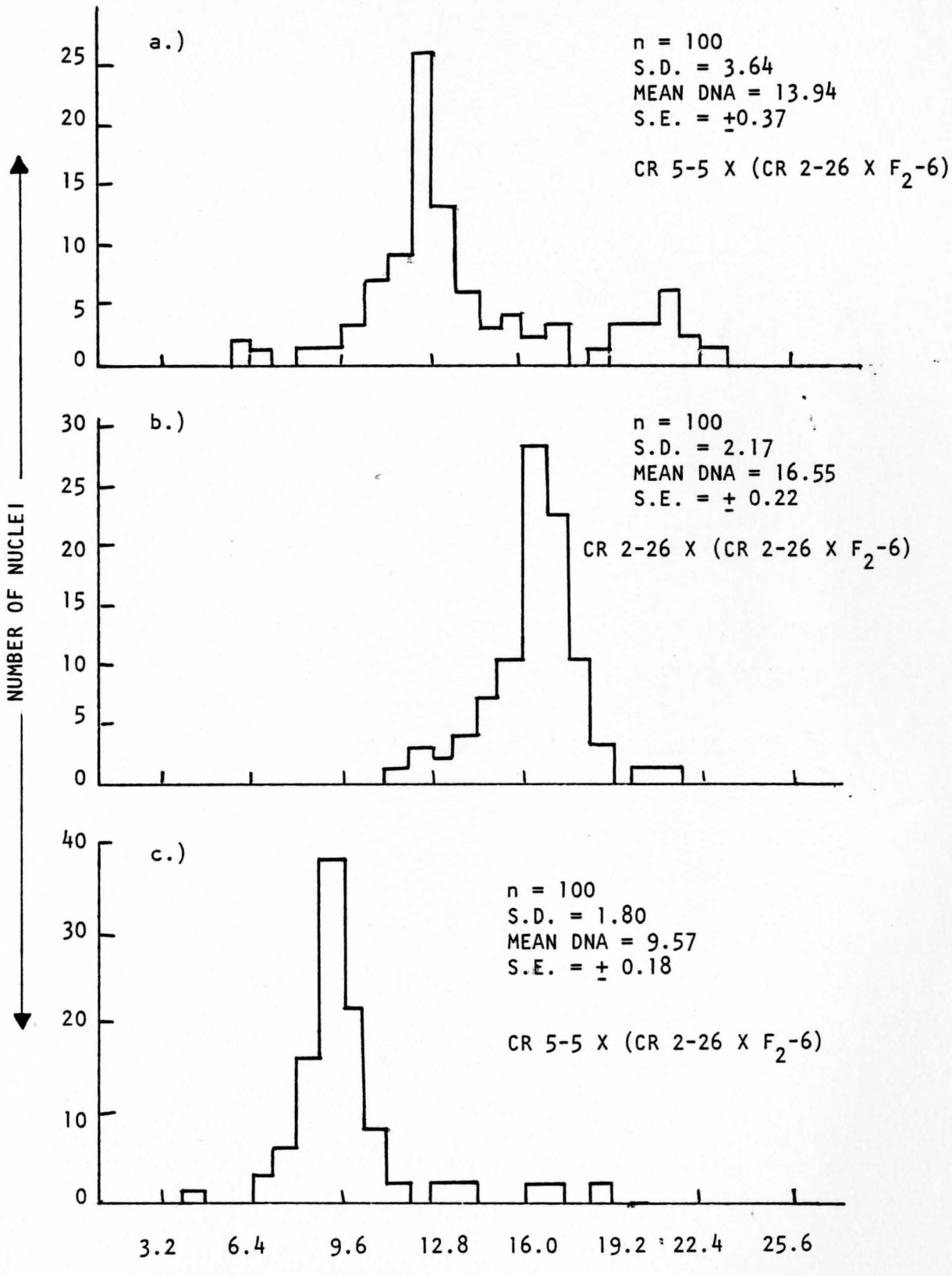

n = 100
S.D. = 1.59
MEAN DNA = 14.08
S.E. = ± 0.15
CR 5-5 X (CR5-5 X F2-5)
n = 100
S.D. = 1.51
MEAN DNA = 12.59
S.E. = ± 0.15
CR 5-5 X (CR 5-5 X F2-4)
n = 100
S.D. = 1.13
MEAN DNA = 11.78
S.E. = ± 0.11
CR 5-5 X F2-4
NUMBER OF NUCLEI
RELATIVE AMOUNTS OF DNA
0
5
10
15
20
25
30
4.0
7.2
10.4
13.6
16.8
20.0
23.2

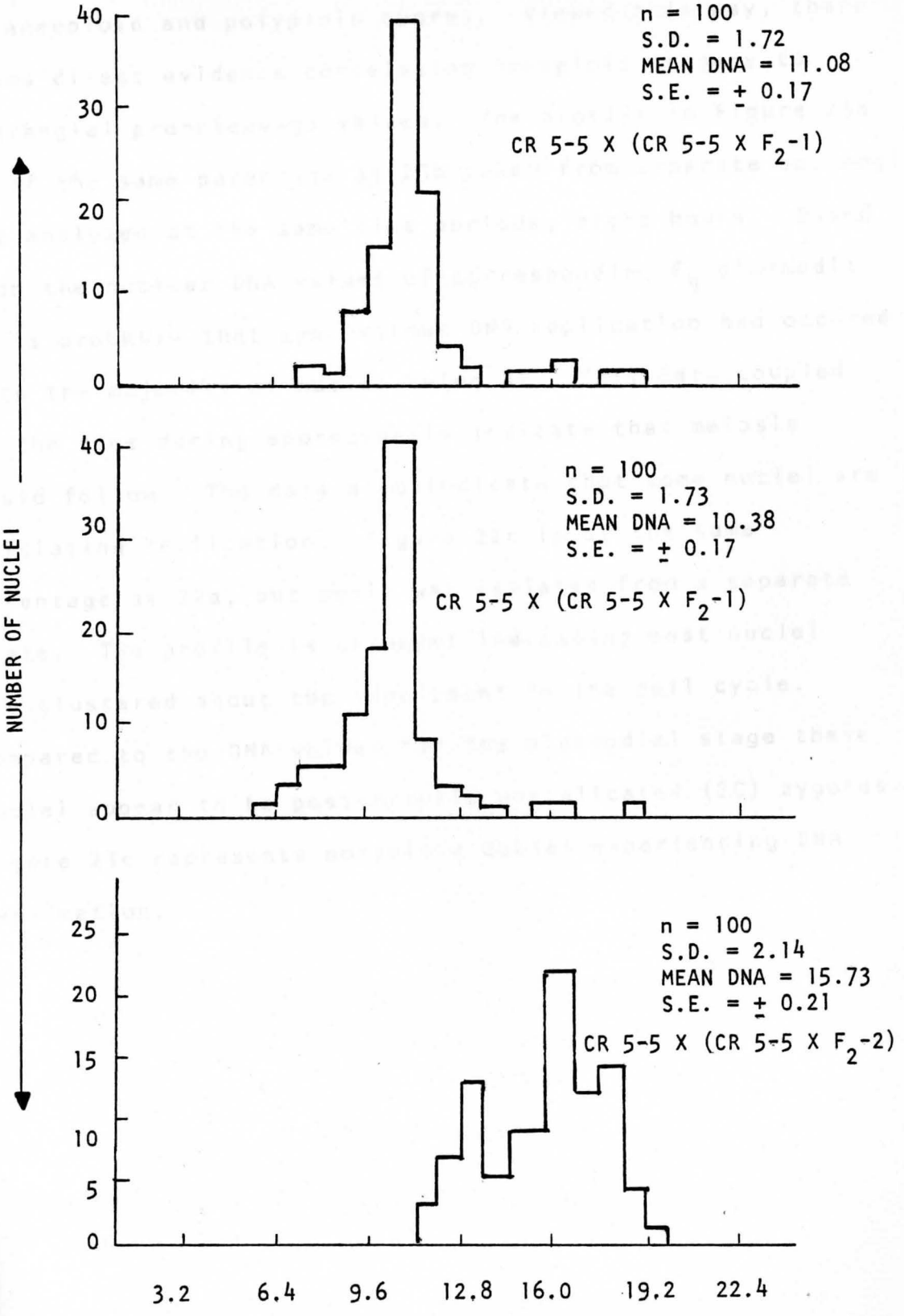
values for these crosses are identical. This is not expected and its significance is uncertain. Many of the nuclei contained several nucleolar spaces, a condition as previously stated, associated with higher ploidy and metabolically active cells. In general, analysis of the F_4 plasmodia is the same for that of the F_3 plasmodia.

17. Analysis of F-DNA in F_4 Sporangial Nuclei

Measurements of the nuclear DNA content of several pre-cleavage sporangia are shown in Figures 22 and 23. The data are in support of the nuclear events of sporogenesis observed earlier. In Figure 22 a comparison may be made for an F_3 isolate backcrossed to both parental mating type isolates. The backcross to CR 2-26 shows a unimodal curve with the majority of nuclei being replicated. The mean nuclear DNA corresponds to the 8C condition when compared to the homologous F_4 plasmodia. Examination of Figure 22a presents an interesting phenomenon. Two distinct unimodal profiles are present whose mean DNA values are nine units apart. The first peak corresponds to the replicated pre-division value as in the plasmodia. The second, smaller peak, represents approximately 16% of the total nuclei measured and indicates these nuclei are probably experiencing another round of the cell cycle. Their distributions strongly suggest this event. It is possible though, that these nuclei are a subclass of polyploids produced in the plasmodial state. It is unknown if these nuclei are able to complete meiosis, which, if so, could account for the occurance


a.)
b.)
c.)
NUMBER OF NUCLEI
RELATIVE AMOUNTS OF DNA


a.)
n = 100
S.D. = 3.64
MEAN DNA = 13.94
S.E. = ±0.37
CR 5-5 X (CR 2-26 X F2-6)
b.)
n = 100
S.D. = 2.17
MEAN DNA = 16.55
S.E. = ± 0.22
CR 2-26 X (CR 2-26 X F2-6)
c.)
n = 100
S.D. = 1.80
MEAN DNA = 9.57
S.E. = ± 0.18
CR 5-5 X (CR 2-26 X F2-6)
NUMBER OF NUCLEI
RELATIVE AMOUNTS OF DNA
3.2
6.4
9.6
12.8
16.0
19.2
22.4
25.6


n = 100
S.D. = 1.72
MEAN DNA = 11.08
S.E. = ± 0.17
CR 5-5 X (CR 5-5 X F2-1)
n = 100
S.D. = 1.73
MEAN DNA = 10.38
S.E. = ± 0.17
CR 5-5 X (CR 5-5 X F2-1)
n = 100
S.D. = 2.14
MEAN DNA = 15.73
S.E. = ± 0.21
CR 5-5 X (CR 5-5 X F2-2)
NUMBER OF NUCLEI
RELATIVE AMOUNTS OF DNA
3.2
6.4
9.6
12.8
16.0
19.2
22.4

of aneuploid and polyploid spores. Viewed this way, there seems direct evidence correlating aneuploid progeny to sporangial pre-cleavage values. The profile in Figure 23a is of the same parentage as 23b taken from separate sporangia but analyzed at the same time periods, eight hours. Based upon the nuclear DNA values of corresponding F_4 plasmodia it is probable that synchronous DNA replication had occured with the majority of nuclei being 8C. This data coupled to the time during sporogenesis indicate that meiosis would follow. The data also indicate that some nuclei are initiating replication. Figure 22c is of the same parentage as 22a, but again was isolated from a separate plate. The profile is unimodal indicating most nuclei are clustered about the same point in the cell cycle. Compared to the DNA values for the plasmodial stage these nuclei appear to be post-mitotic unreplicated (2C) zygotes. Figure 23c represents polyploid nuclei experiencing DNA replication.

DISCUSSION

The results of this investigation span four filial generations and support the observations of Collins et al., (1978) that in heterothallic crosses with polyploids of the CR 2 isolate of D. iridis, chromosome elimination after karyogamy produces both heteroploid plasmodia and sporulation myxamoeba. Comparative microspectrophotometry of the nuclear DNA content of major life cycle stages demonstrates that all F_2 and F_3 progeny clones, regardless of ploidy, differentiate into plasmodia with both parental clones. The DNA measurements also indicate the establishment of tetraploid nuclei. At present though, the mechanism of tetraploid formation as well as sporogenesis are unknown. Moreover, the results of both nuclear DNA and genetic analysis suggests that the cell fusion capacity is transmissible and possibly due to a cytoplasmically active factor.

The recent influx of experimental data and theoretical considerations on the behavior of polyploid nuclei during life cycle events and the mechanics of membrane fusions has led to some interesting conclusions regarding this investigation. As previously mentioned, Adler and Holt (1975) working with polyploid cross nuclei of P. polycelphalum have isolated myxamoeba heterozygous for the mating type locus. These myxamoeba have the ability to clonally form plasmodia and

when analyzed for nuclear DNA content the myxamoeba were found to have near-diploid amounts. Ross and Cummings (1970) in an interesting study provided photomicrographic evidence of multiple cell and nuclear fusions in heterothallic myxamoeba of D. iridis. The unusual pattern of fusions is a function of culture age and once established, is highly predictable. A detailed discussion concerning changes in cell architecture as a means of membrane fusion is also presented. The authors state, "The cell behavior described above (multiple fusions) does not conform to any concept of normalcy, but falls into the wide range of cell behavior patterns of which these organisms are obviously capable without apparently permanently disrupting their ability to complete a life cycle."

Some observations made during the course of this investigation, while not giving definitive answers to key queries, do provide added insight into the interpretation of the results. F_2 myxamoeba derived from tetraploid (polyploid) nuclei function as if they contained both parental mating types as demonstrated by nuclear DNA measurements of resultant F_3 plasmodia. The data also show that this capacity for cell fusion is exhibited by F_3 myxamoeba, thus suggesting an inheritable condition. It was originally thought that this behavior could be correlated to increased amounts of nuclear DNA. However, it was found that while some F_2 and F_3 myxamoeba were aneuploid others are haploid. Another manifestation was the reduced effectiveness of F_3 amoeba to participate in cell fusions indicating a diluting

out of the factor involved in the fusions. This interpretation though, assumes the acquisition of a factor derived initially from the CR 2-26 clone which, present at a minimal inter (or intra) cellular concentration, allows membrane apposition, hence fusion. No data at present supports the existence of a factor associated with the CR 2-26 clone; the evidence is indirect. Also, that several F_2 (presumably A^2) isolates displayed an enhanced degree of cell fusions (in both rate and numbers) by forming plasmodia with non-related clones with A^2 mating types suggests a sensitivity to the factor in question.

Further observations show that the F_2 isolates capable of enhanced cell fusions also clonally produce plasmodia. One of these isolates is known to have directly produced an F_3 isolate which selfed. The nuclear DNA contents of these clonal plasmodia have not yet been measured but the finding of a diploid value would indicate intra-clonal cell fusions. Finding a haploid value would of course, support apogamic plasmodial differentiation. This becomes even more perplexing considering that neither parental clone exhibited regular selfing.

These observations suggest the operation of a cytoplasmic or membrane factor(s) able to bring about the primary pre-requisite of cell fusion, membrane contact. It must be pointed out that at present there is no data to indicate if a factor or gene can be derived from either the CR 2-26 clone or the process of chromosome elimination. The normal mode of haplophase cell fusions has been shown

to be under the control of a multiple allelic mating locus system which allows recognition then fusion of compatible cell membranes (Collins, 1963). The mechanism, though largely unknown, is presumed to involve an orderly genetic program of events where appropriate inducers stimulate compatible cells thereby derepressing gene loci. Present evidence suggests the mating type locus functions as a regulator gene whose products re-program or sensitize the plasma membrane to allow contact. As stated earlier (Inbar and Sachs, 1969) this process need not involve genetic transcription since pre-existing cryptic sites can be exposed by extracellular or disturbed intra-cellular conditions. Again, Ross and Cummings (1970) state "...once the cell surface has become modified, whether under the influence of orderly genetic programming or by some abnormal process, the subsequent series of cell behavioral events will be essentially similar and result directly from interactions of the modified cell surfaces without regard to the manner in which these modifications occured." Finally, the work of Yemma et al., (1974) offers firm evidence of both the action and transmission of a cytoplasmic factor involved in major life cycle events.

Since karyotyping and directly monitoring chromosome behavior are not feasible for D. iridis, chromosome distributions were determined by the combined use of nuclear DNA measurements and nuclear marker gentics. Appropriate testing of the hypothesis outlined above requires that relative ploidy levels be determined in the various life

cycle stages. This was accomplished by quantitative miscrospectrophotometry of Feulgen-DNA nuclei in swarmer, myxamoeba, plasmodia and sporangia. Segregation of the mating type locus was also analyzed to further determine distribution patterns. The use of the mating type allele as a nuclear marker is due to its apparent immutability and its functional presence in meiospores. The mating type of progeny clones can be deduced by simply backcrossing to both parental isolates or to testers of known mating type. The nuclear DNA data are presented as frequency histograms which indicate shifts in ploidy distributions within a given population of nuclei. The nuclear DNA profiles are essentially unimodal, in contrast to the bimodal profiles obtained with most experimental organisms (Swift, 1950). The work of Rusch (1969) using P. polycephalum best explains this phenomenon as a reflection of the specialized cell cycle of the plasmodium. Plasmodial mitosis is a nearly synchronous event with approximately 99% of all nuclei entering metaphase within a three minute period. Mitosis spans a fifteen minute period whereupon the nuclei enter a three hour period of DNA replication. The S phase is then followed by a five hour pre-mitotic G_2 period. Like neoplastic cells studied by Rusch, the plasmodial cell seemingly bypasses G_1 in favor of repeated replication-division. The amount of nuclear DNA is then 2C or greater, 99% of the time. When represented as a histogram, nuclear populations describe a unimodal distribution. Swarmers and myxamoeba also have a comparably short or bypassed G_1

period (Olive, 1975; Yemma and Therrein, 1972). Therefore, most nuclear DNA values approximate a 2C figure. As a result, they also describe a unimodal distribution.

Swarmer populations of the CR 5-5 parental clone show F-DNA values of 3.2 units, a figure currently accepted as the 2C haploid value for D. iridis (Collins and Therrein, 1976). These same authors note that the CR 2 isolates show consistently high ploidy values, with the CR 2-26 clone measuring approximately 7.6 units. As has been stated, there is no reliable chromosome karyotype data for this isolate. Cytologically, the CR 2-26 nuclei measured 6u X 4u whereas the CR 5-5 nuclei are 4u X 2u. Since nuclear surface area is proportional to DNA content (Alfert and Das, 1969), these measurements were used as ancillary evidence when determining ploidy levels.

It has been hypothesized by Collins et al., (1978) that after karyogamy, chromosome elimination in polyploid zygotes results in the formation of plasmodia with unexpected ploidy levels. The data do indeed show polyploid F_1 plasmodia and unexpected diploid plasmodia. The polyploid plasmodia show disperse nuclear DNA values appearing to be in various stages of the nuclear cycle. This spread is due to a population of mitotically active nuclei. Conversely, the tight unimodal profile for the diploid plasmodia indicates that most nuclei are 4C. The positive results of an F_1 Mass Spore Culture test demonstrates unquestionably that karyogamy of parental nuclei occured to produce the polyploid nuclei. Another possible source for polyploid

nuclei is multiple cell fusions followed by multiple karyogamy (Ross and Cummings, 1970). While nuclear DNA levels are high, no data exists to verify the operation of this mechanism here.

Of interest was the production of unexpected diploid plasmodia from the CR 2-26 X CR 5-5 parental cross. It is interesting to note that Collins and Therrein (1976) obtained haploid plasmodia from the CR 2-25 X CR 5-5 cross. They also obtained a diploid plasmodium from a CR 2-25 X Hon 1-2 cross; in this study however, only polyploid plasmodia were found in a similar cross of CR 2-26 X Hon 1-2. The origin of the diploid plasmodium poses an interesting problem. They may have arisen by a chromosomal elimination from a polyploid zygote (as Collins & Therrein suggests) or alternatively, the plasmodium may be formed in a homothallic process. Another possibility still is the diploidization of haploid CR 5-5 nuclei which apogamically differentiated into a plasmodium (Ashworth and Dee, 1976). Since there is no record on the sequence of events leading to plasmodium formation, any of the above processes are at least, theoretically possible. Future experiments conducted under more controlled conditions (e.g. micro-cinematography) should provide answers to this intriguing developmental event.

Evidence from F_1 sporangia suggest that polyploid nuclei experience much difficulty sporulating by meiosis. Nuclear DNA values occupied a wide range and were not graphable. In addition cytological examination of post-cleavage

spores showed irregular spore wall structure, spores which were empty, and spores with separate Feulgen positive material co-existant with varying sized nuclei. In the absence of directly following chromosome behavior, these latter spores may represent eliminated chromosomes. The evidence for this interpretation is at present inconclusive. These observations support the general agreement that polyploid nuclei lead to segregation difficulties. Considerable documentation exists demonstrating this problem (Strickberger, 1968). Sterile gametes often result as a consequence of incomplete homologue formation during meiotic prophase I and a feature common to the fungi is spores which are empty or aneuploid. The results of Mohberg et al., (1973) indicate variable germination rates with spore viability being correlated to DNA content. Their results showed that spores with a "low" DNA content (haploid) are more viable than spores with a DNA content twice the "low" value (aneuploid). In some cases F_1 spore viability is apparently lacking (Henney, 1967, 1968; Collins, 1975b). The relationship of polyploid nuclei to low F_1 spore viabilities and this effect on the evolution of the Myxomycetes has been discussed (Clark and Collins, 1976; Collins and Therrein, 1976).

The diploid pre-cleavage sporangial values correspond to the observations of Mohberg et al., (1973), where, for heterothallic and homothallic species comparative chromosome numbers (ploidy) are equivalent in plasmodia and pre-cleavage sporangia. Although the mean DNA value is slightly

larger than the diploid value (due to the presence of a small peak to the right) the main peak corresponds to 4C diploid nuclei. The second peak may represent a small percentage of natural polyploids.

The low germination rate (8%) exhibited by F_1 spores is explainable based upon the previous cytological data and reinforced by other similar reports (Collins, 1975). Analysis of the segregation products of polyploid nuclei suggest abnormalities during mitosis, meiosis, or both division cycles. Collins and Therrein (in press) have reported both complete elimination of genetic markers or highly skewed marker segregation in F_1 progeny of D. iridis. The CR 2-26 clone may contain chromosomal fragments, multisomic sets, or unpairable chromosomes; any of which would result in an unstable chromosome complement upon karyogamy with haploid nuclei. Accordingly, chromosomal instability during meiosis would be reflected in the production of aneuploid progeny. Chromosome elimination as a response to unbalanced chromosome complements has been demonstrated in both plants and animals (Thompson, 1962). A recognized mechanism for the production of aneuploid progeny is chromosomal non-dysjunction of homologues during meiotic anaphases I and II, and somatic anaphase (Strickberger, 1968). The asexual fungi have developed a parasexual cycle (mitotic crossing-over) involving random chromosome elimination by non-dysjunctions which produces aneuploid progeny. Recently, heterozygous diploids of the Myxomycete

Dictyostelium discoideum were demonstrated to undergo parasexuality comparable to Aspergillus (Loomis, 1975). Thus if random chromosome elimination and reassortment were operative, one would expect to find a variety of progeny with respect to ploidy, marker genetics, and viability.

F_1 isolates 2, 3, and 4 are composed of myxamoeba and swarm cells, and are haploid. Their mean DNA values correspond to the 2C content of the life cycle stage. Although the mean DNA content for F_1-2 is 3.66 (as compared to the 3.36 haploid 2C standard) the slightly elevated DNA value is the result of measuring a myxamoebal population of cells. Myxamoeba are believed to register slightly greater F-DNA readings than swarmers due to metabolic differences (Yemma, 1971). F_1-1 is aneuploid and contains approximately 14% more DNA than the haploid clones. All isolates were found to have segregated the A^2 allele from the haploid parent. As previously stated, F_1 Mass Spore Culture data show that spores containing the A^6 allele segregated but probably in low number.

F-DNA analysis of F_2 plasmodia shows only polyploid nuclei were formed; this does not obviate the possibility that plasmodia of diploid or haploid values might be found, only that such plasmodia were not detected here.

Nuclear DNA measurements of F_2 progeny show the segregation of both haploid and aneuploid amoeba. F_2-2 was measured in the swarmer state and is clearly near-diploid displaying 50% more DNA than the haploid clones. F_2-6, also calculated from swarmers shows a 12% increase in

nuclear DNA compared to the haploid standards. F_2-isolates 1 and 4 were calculated from myxamoeba and encysted amoeba respectively. The DNA level in F_2-1 is a reflection of chromatin dispersion in the synthetic amoeba, hence the isolate is probably haploid. F_2-4, being calculated from encysted amoeba, are assumed to be in a G_2 arrest (Yemma, 1971). Being in G_2 the amoeba must have the 2C value, thus they are haploid. All isolates except F_2-2 display 2C or slightly greater amounts of DNA. It should be noted that regardless of distribution, the isolates F_2-1, 4 and 6 show equal amounts of DNA. This fact may be of physiological importance as will be discussed later. Based upon nuclear DNA data the aneuploid clones are though to have resulted from chromosomal elimination in the F_2 polyploid sporangia. This conclusion is supported by the observation that the aneuploid amoeba display ploidys intermediate between the theoretically expected haploid and polyploid segregation products.

The results of F_2 nuclear marker analysis are difficult to interpret. Each F_2 isolate proved cross-fertile with both CR5-5A^2 and CR 2-26A^6 parental clones. This behavior is inconsistent with the expected 1:1 segregation ratio for the paired mating type alleles. F-DNA measurements of the F_3 plasmodial nuclei indicate two distinct values, dependent upon which parental clone was used in each particular cross. Further genetic analysis using related and non-related tester clones with known

mating types produced rather complex data, in part. As was expected all F_2 isolates crossed with the BIP-4 isolate carrying the A^7 allele. However, when crossed to the non-related Hon 1-2 and A-2- clones as well as the related F_1 myxamoeba (all A^2 mating types) the F_2 isolates 4 and 6 consistently co-differentiated into plasmodia. F_2-1 produced plasmodia only with F_1 isolates 1 and 4 and F_2-5 X F_1-2 also yielded plasmodia. As a final test F_2 amoeba were crossed with each other, two at a time, and this produced plasmodia in the same pattern as above; i.e. F_2 isolates 1, 4 and 6 appear to cooperate in plasmodial differentiation with all clones tested against, regardless of mating type or relatedness. It would be desirable to cross all F_2 isolates, especially 1, 4 and 6 with other non-related A^6 clones in addition to other allelic mating types. A remote possibility could lie in mating type mutation as once suggested by Collins (1965). This is however, unlikely.

Another unexpected event (although predicted - Clark and Collins, 1976) was the observation that F_2 isolates 4 and 6 selfed i.e. formed plasmodia clonally. No other F_2 isolate displayed this property. It is unknown if the encystment period may have influenced the behavior of the F_2 isolates. Encysted amoeba, being in G_2 arrest, are thought to divide immediately upon re-animation (Therrein and Yemma, 1972). The F_2-4 isolate, upon recloning was differentiating into plasmodia within 24-36 hours. This capacity appears to be a function of recloning,

similar to the data of Yemma, Therrein and Ventura (1974). Other work in this laboratory (unpublished) indicate certain Ph-1 sublines display the same behavior.

At this point the results of the F_3 progeny analysis offer added information. F-DNA measurements indicate a recapitulation of F_2 segregation, with haploid, aneuploid and polyploid progeny having germinated. F_2 and F_3 germination rates also increased over F_1 (12% and 14% respectively) probably due to the presence of progeny backcrosses to the CR 5-5 parent. Nuclear marker analysis for mating type imply that while each isolate maintains the capacity to cross with both parental clones these crosses occur with reduced frequency. Plasmodia were in general observed to be less vigorous than expected cross plasmodia. Nuclear DNA measurements in representative F_4 plasmodial nuclei again demonstrate two distinct values, indicative that the representative isolates crossed with each parent clone. Clonal plasmodial formation was again displayed by several F_3 isolates. Two of the three isolates are aneuploid and thus strongly suggest a relationship between ploidy and selfing. One of these isolates, F_3-4 was derived from the F_2-4 isolate selfer and implies cytoplasmic inheritance of the capacity to self (Yemma et al., 1974).

Based strictly upon ploidy measurements, all F_2 isolates excluding F_2-2 and F_3 isolates 1, 2, 3 and 6 should predictably carry the A^2 mating allele derived from the haploid CR 5-5. Without further genetic analysis

it is difficult to assign F_2 and F_3 isolates a mating type (they may tentatively be considered as being A^2A^6 heterozygous, or more likely containing respective mating type inducers). Since many of the F_2 and F_3 isolates carry DNA complements greater than the haploid value, it is reasonable to expect that they are disomic for some chromosome(s). Non-dysjunction producing disomy for the part of the chromosome containing the mating type locus would, of course, produce a heterozygous condition for mating type. The observation that F_2 isolates 4 and 6, and F_3 isolates 1, 2 and 4 selfed may be due to mating type heterozygosity. At least three of these isolates contain aneuploid amounts of DNA to supply the extra gene product. The inability of F_2-1 to self may be related to its less efficient capacity to cross with F_1 A^2 amoeba. Heterozygosity could be determined by isolating spores from sporulated selfer plasmodia. If the spores germinated producing clones of both parental heterothallic mating types (A^2 and A^6) and if myxamoeba able to self were all recovered, then the evidence would favor that these isolates are heterozygous. Furthermore, the clonal plasmodia should also be of the same ploidy as the amoeba, since karyogamy need not occur. This model is consistent with the concept of the mating type locus being a regulatory gene. Mutual induction would then lead to an obligatory plasmodium differentiation. Alternative explanations for these myxamoeba would be mating type recombinations or crossing over at these alleles.

Future genetic studies should determine which, if any, of the above mechanisms are responsible for the observed data.

As previously mentioned, F-DNA measurements of F_3 plasmodial nuclei indicate two classes of plasmodia based upon ploidy. Many nuclei are tetraploid and synthetically active. Of considerable interest was the detection of two distinct plasmodia within the same cross, CR2-26 X F_2-1. Mean nuclear DNA content and DNA distributional profiles show that both are polyploid and actively synthetic. The higher ploidy plasmodia may represent nuclear DNA increases due to aging. The difference in mean nuclear DNA amounts (8 units) argues against this though, and they may represent the same heteroploid phenomena of F_1 plasmodia i.e. establishment of a ploidy series 2N:4N:8N etc.

Pre-cleavage F_3 sporangia are consistent with their plasmodial nuclear DNA contents. Nuclei of sampled sporangia indicate an active synthetic period, representing post-mitotic DNA synthesis.

F_3 spore isolation, as stated previously, yielded progeny with haploid and aneuploid chromosome complements. In one case, isolate F_3-7 contains 93% as much DNA as the CR2-26 parent. This isolate was derived from sporangia of cross CR5-5 X F_2-5. Since neither direct parent was polyploid, this clone may represent a nearly full non-dysjunction of metaphase chromosomes. It will be interesting to test the hyper-diploid F_3-7 isolate to determine if it behaves

like the CR2-26 isolate. Again, these isolates were cross-fertile with both parental clones, though at a reduced efficiency.

F_4 plasmodial nuclei present the same pattern as that for F_3 plasmodia; mean nuclear DNA values correspond to which parental clone was used in each cross. Representative histograms indicate most nuclei are in tetraploid (polyploid) classes with some nuclei being synthetically active. F_3 isolates crossed to CR2-26 also show a pronounced polyploid series; in one case, both parental crosses to CR2-26 X F_2-6 display approximately equal mean nuclear DNA values. It is uncertain why these values are alike.

Profiles of F_4 sporangial nuclei demonstrate ploidy continuity between plasmodial and pre-cleavage stages. The extent of chromosomal variability generated by polyploid nuclei is again evidenced by the different values obtained from different plasmodia of the same cross. The observation of two separate peaks for CR5-5 X (CR2-26 X F_2-6) suggests a sub-population of polyploid nuclei going through the cell cycle synchronous with the regular cross nuclei. Such polyploid sub-populations may be responsible for the production of diploid spores.

This figure may illustrate the genetic plasticity and natural adaptability of the slime molds. The summation of data in this experiment views the differentiated plasmodial state as a productive source of nuclear (genetic) variability. In normal haploid X haploid crosses plasmodial nuclei are predominantly diploid with a small percentage

showing polyploid multiples (e.g. tetraploidy), a condition which may be correlated to aging. Whether this phenomenon represents a precise control or a non-directed lack of control is unknown, however, its ubiquity indicates that either select nuclei replicate without concomitant division (full non-dysjunction, an established source of polyploidy) or already diploid nuclei undergo karyogamy. Both modes have been documented for the myxomycetes (Ashworth and Dee, 1976). The consistent observation of tetraploid plasmodia resulting from haploid X polyploid interisolate crosses raise many interesting questions concerning this ability. For example, is there a logical pathway allowing for tetraploidy, and if so, is it controlled by key, programmable, nuclear cycle events? Also, could this lead to a state of co-dominant heterozygosity at some loci, which would thereby lead directly to increased survival value literally bypassing sexual prerequisites to life cycle completion (i.e. asexual apogamy). Whether tetraploidy is natural or anomolous, it apparently has advantageous ramifications. While apparently being natural and non-disruptive of life cycle events, tetraploidy can certainly explain the presence of diploid spores. More perplexing though is the observation that tetraploid nuclei eventually yield haploid spores. The mechanism of its reduction division certainly presents fascinating implications.

This study has established that F_1, F_2 and F_3 spore isolates with various chromosome complements are

segregated in polyploid neiosis and that the capacity for the expression of both mating types is acquired by F_2 progeny and is apparently transmitted to F_3 progeny. Further evidence also indicates that several of these F_2 and F_3 progeny clonally form plasmodia. Many questions have been raised which the scope of this study cannot answer. It would be desirable to validate the hypothesized process of chromosome elimination which, after karyogamy has occured, is believed to account for the heteroploid cell lines. The isolation of strains mutant for genetic markers other than mating type would certainly facilitate such a study. The underlying cause for the cell fusion and selfer behavior of progeny myxamoeba must also be solved. Such cell lines could prove invaluable in determining the functioning of the mating type locus in plasmodial differentiations. They may also represent a step in the evolutionary transition from heterothallic strains to apogamic strains as suggested by Collins (1976).

With respect to any evolutionary discussion of the slime molds, the observer must keep in mind that their evolutionary success is due in large part to the maintenance of an enormous amount of genetic variation in the Myxomycetes as a whole. The work of many researchers (e.g. Collins, 1975) is only now, after years of observation, elucidating the details of the mechanisms of their highly successful adaptation.

Two examples, central to this thesis, serve to illustrate this point. The discovery by Collins (1961)

that the heterothallic behavior of Didymium apparently is mediated by a one locus-multiple allele cell fusion programme (the mating type gene complex) established a source of genetic variation for the myxamoeba as a result of sexual meiosis. Further revelation of at least twelve allelomorphs for this single locus (Collins, 1975) suggests an enormous amount of concealed genetic variation. These combinations could then lead to an indefinite range of pre-adapted individuals in the population. Therefore, in a normally diploid, sexually reproducing organism, a great deal of genetic variation is maintained in the heterozygous condition since the operation of one allele per locus is adequate for the expression of a given character. Second, is the recent evidence linking Myxomycete speciation to reproductively isolated polyploids. This phenomenon is well documented in the Plant Kingdom (Stebbins, 1950) and is apparently functional in the fungi as well (Collins and Therrein, 1976). In this model autopolyploidy and allopolyploidy are the two generally recognized mechanisms for speciation. Since one species was used in this study, only autopolyploidy need be mentioned. Briefly, autopolyploids are formed by the somatic doubling of the chromosome number in the plasmodial stage or by the production and union of unreduced gametes. In a normally diploid organism this will lead to a multivalency proportional to the degree of doubling. Within such a system, gametes of varying ploidy are produced, each capable of fusing with other compatible gametes (e.g. the fusion of 2N and 1N gametes

yield a 3N gamete), thereby increasing genetic variation. Mettler and Gregg (1969) point out that, "One feature of autopolyploids that may have some evolutionary significance is that they are quite frequently reproductively isolated." Therefore, the production of polyploid populations (as in the CR 2-26 X CR 5-5 plasmodia) will lead to aneuploid meiospores and in many cases sterility. In some cases the probability of generating a balanced haploid (1N) or diploid (2N) gamete from such non-homologous chromosome complements is a rare event; i.e. $2(\frac{1}{2})^N$, where N equals the number of chromosome pairs. Thus reproductive isolation could occur before genetic divergence begins.

The data presented in this study, as well as other related works, must be analyzed in view of the aforementioned points. While the true ploidy of the CR 2-26 isolate is not known, it is obvious that fusion with a haploid gamete leads to a certain degree of meiotic sterility and especially, aneuploidy. The persistence of tetraploid values could represent a stable autopolyploid condition resulting from either somatic doubling, the fusion of diploid (2N) nuclei or both.

Thus, combining the natural tendencies of Didymium for multiple allelism controlling sexual fusion and, the generation of autopolyploid series in the plasmodial stage of differentiation, one may speculate upon these mechanisms and evolutionary activity. Also, the various heteroploid values obtained in the meiospores, as well as their mating ability (e.g. the F_2 and F_3 spores)

may reflect these mechanisms. This point becomes even more challenging when a correlation is suggested between nuclear-cytoplasmic physiology and cellular behavior.

Determination of the causes for the observed behavior of progeny isolates will necessitate precise genetic analysis of these myxamoeba. An important question here is whether the process of chromosome elimination (due to the polyploid clone, CR 2-26) and the expression of cell fusion properties are related events. This is sure to be a topic of future research in this laboratory. The cell fusion behavior is certainly consistent with the action of an acquired gene, gene product or, cytoplasmic factor able to program the sequence of events involved in heterothallic sexual fusions. Finally, the differential amounts of nuclear DNA involved in the progeny clones requires more detailed analysis concerning a possible relationship between ploidy and selfing. Studies to determine the presence of heterozygosity at the mating type allele will also answer this question. It is hoped that the results of this investigation may both contribute to an understanding of the complex genetics of the acullular slime molds and, generate meaningful areas of study for future research. With the constant input of new experimental data and procedures, the evolutionary view of the myxomycetes as successful organisms becomes increasingly apparent. They can seemingly adapt to a wide range of cell behavior while still completing normal life cycle events. As Collins(1961) first commented, considering the selfer phenomenon,

"...there are obviously other factors besides simple mating type that govern plasmodial production in *Didymium iridis*."

BIBLIOGRAPHY

Abe, F. 1934, Syngamy of some Myxomycetes. FCI. REPT. Tokyo, Bunrika Daigaku. B1: 193-202.

Adler, P.N., and C.E. Holt. 1974a. Genetic analysis in the Colonia Strain of Physarum polycephalum: Heterothallic strains that mate with and are isogenic to the Colonia strain. Genetics. 78: 1051-1062.

Adler, P.N., and C.E. Holt. 1974b. Change in Properties of Physarum polycephalum amoeba during extended culture. J. Bacteriol. 120: 532-533.

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

Alexopoulos, C.J. 1963. The Myxomycetes II. The Bot. Rev. 29: 1-78.

Alexopoulos, C.J., and J. Koevenig. 1962. Slime Molds and Research. Educational Programs Improvement Corp., Colorado.

Alfert, M., and N.K. Das. 1969. Evidence for control of the rate of nuclear DNA synthesis by the nuclear membrane in Eukaryotic cells. Proc. Nat. Acad. Sci. US 63: 123-128.

Anderson, R.W., D.J. Cooke, and J. Dee. 1976. Apogamic development of plasmodia in the myxomycete Physarum polycephalum: A cinematographic analysis. Protoplasma. 89: 29-40.

Ashworth, J.M., and J. Dee. 1976. The Biology of Slime Molds. Crane, Russak and Co., Inc., New York.

Boivin, A., R. Vendrely, and C. Vendrely. 1948. L'Acide desoxyribonucleique deu moyae cellulaire de positaire des caracteres hereditaires; arguments d'orche analytique. Compt. Rend. Acad. Sci. 226: 1061-1063.

Bold, H.C. 1960. The Plant Kingdom. Prentice Hall Inc., New Jersey.

Bradley, L.J. Quantitative cytophotometric and autoradiographic analysis of the nuclear changes in the Myxomycete Didymium iridis during growth and development of the plasmodia. Masters Thesis, Youngstown State Univ., 1975.

Bryant, T.R., and K.L. Howard. 1969. Meiosis in the Oomycetes: A microspectrophotometric analysis of the nuclear deoxyribonucleic acid in Saprolegnia terrestims. Amer. J. Bot. 56: 1075-1083.

Carlile, M.J. 1973. Cell fusion and somatic incompatibility in Myxomycetes. Ber. Deutsch. Bot. Ges. Bd. 86: S. 123-139.

Caspersson, T. 1936. Uber den Chemischen Aufbau der Strukturen des Zellketnes. Scand. Arch. Physiol. 73: Suppl. 8.

Cayley, D.M. 1929. Some observations on Mycetozoa of the genus Didymium. Trans. Brit. Myxol. Soc. 14: 227-248.

Clark, J., and O.R. Collins. 1974. Studies on the mating systems of eight species of Myxomycetes. Amer. J. Bot. 61: 21 (abstr.).

Clark, J., and O.R. Collins. 1976. Studies on the mating systems of eleven species of myxomycetes. Amer. J. of Bot. 63(b): 783-789.

Collins, O.R. 1961. Heterothallism and homothallism in two Myxomycetes. Amer. J. Bot. 48: 674-683.

Collins, O.R. 1963. Multiple alleles at the incompatibility locus in the myxomycete Didymium iridis. Amer. J. Bot. 50: 477-480.

Collins, O.R. 1965a. Evidence for a mutation at the imcompatibility locus in the slime mold, Didymium iridis. Mycologia. 57: 314-315.

Collins, O.R. 1965b. Homothallic behavior in two Costa Rican isolates of the slime mold Didymium iridis. Amer. J. Bot. 52: 634 (Abstr.).

Collins, O.R. 1974. Mating type in 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. Amer. J. Bot. 63: 138-143.

Collins, O.R., and J. Clark. 1966. Inheritance of the brown plasmodial pigment in Didymium iridis. Mycologia. 58: 314-315.

Collins, O.R., and H. Ling. 1964. Further studies in mulitple allelomorph heterothallism in the myxomycete Didymium iridis. Amer. J. Bot. 51 (3): 315-317.

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. Therrein. 1976. Cytophotometric measurements of nuclear DNA in seven heterothallic isolates of Didymium iridis, a Myxomycete. Amer. J. Bot. 63(4): 457-462.

Collins, O.R., and C.D. Therrein, and D.A. Betterley. 1978. Genetical and cytological evidence for chromosomal elimination in a true slime mold, Didymium iridis. Amer. J. Bot. 65(6): 660-670.

Cooke, D.J., and J. Dee. 1974. Plasmodium formation without change in nuclear DNA content in Physarum polycephalum. Genet. Res. 23: 307-317.

Davies, H.G., and P.M.B. Walker. 1953. Microspectrophotometry of living and fixed cells. Progress in Biophys. 3: 195-236.

DeCosse, J.J., and N. Aiello. 1966. Feulgen hydrolysis; Effect of acid and temperature. J. of Histochem. and Cytochem. 14: 601.

Dee, J. 1960. A mating type system in an acellular slime mold. Nature. 185: 780-781.

Dee, J. 1962. Recombination in a myxomycete, Physarum polycephalum. Schu. Genet. Res. 3: 11-23.

Dee, J. 1966. Multiple alleles and other factors affecting plasmodium formation in the true slime mold, Physarum polycephalum. Schu. J. Protozool. 13: 610-616.

DiStefano, H.S. 1948. A cytochemical study of the Feulgen nucleal reaction. Chromosoma. 3: 282.

Fand, S.B., Genco, K.K. and R.H. Holland. 1967. Qualitative and quantitative utility of room temperature 5N Hcl Feulgen hydrolysis. J. Histochem. Cytochem. 14: 778(Abstrs.).

Feulgen, R. and H. Rossenbeck. 1924. Microskoposch-Chemeschen Wachweis einer Nucleinsaure von Typus der Thymo nucleinsaure und die darauf diruhencle elektive Farburg von Zellkernen in Mikroskopischen Praparaten. Z. Physiol. Chem. 135: 203-248.

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

Guttes, E., S. Guttes, and H.P. Rusch. 1961. Morphological observations on growth and differentation of Physarum polycephalum grown in pure culture. Dev. Biol. 3: 588-614.

Haugli, F.B. 1971. Mutagenesis, selection and genetic analysis in Physarum polycephalum. Ph.D. Thesis, Univ. of Wisconsin.

Henney, M.R. 1967. The mating type system of the Myxomycete Physarum flavicomum. Mycologia. 59: 637-652.

Henney, M.R. 1968. Mating type systems in the myxomycetes Physarum globerliferum and Physarum biligramii. Amer. J. Bot. 55: 720(Abstr.).

Howard, A., and S.R. Pelc. 1953. Synthesis of desoxyribonucleic acid in normal and irradicated cells and its relation to chromosome breakage. Heredity Suppl. 6: 261-273.

Inbar, M. and L. Sachs. 1969. Structural differences in sites on the surface of membrance of normal and transformed cells. Nature. 223: 710-712.

Kasten, F.H. 1959. Flourescent Schiff-type reagents for cytochemical detection of polyaldehyde moietes in sections and smears. Nature. 184: 1797-1798.

Kasten, F. H. 1960. The chemistry of Schiffs Reagent. Intern. Rev. Cytol. 10: 1-100.

Kasten, F.J. 1967. Histochemical methods in the study of nucleic acids. In Encyclopedia of Biochemistry. Reinhold Co., New York.

Kerr, S.J. 1967. A comparative study of mitosis in amoeba and plasmodia of the true slime mold, Didymium nigripes. J. Protozool. 14: 439-445.

Kerr, S.J. 1968. Ploidy level in the true slime mold Didymium nigripes. J. Gen. Microbiol. 53: 9-15.

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

Koevenig, J.L., and R.C. Jackson. 1966. Plasmodial mitosis and polyploidy in the Myxomycete Physarum polycephalum. Mycologia. 58: 662-667.

Kraemer, P.M., et al., 1972. DNA constancy despite variability in chromosome number. Advances in cell and molecular biology. Vol. 12. Academic Press Inc., New York.

Lessler, M.A. 1953. The nature and specificity of the Feulgen Nucleal Reaction. Intern. Rev. Cytol. 2: 231-245.

Lillie, R.D. 1951. Simplification of the manufacture of Schiffs Reagent for use in Histochemical Procedures. Stain Technology. 26: 163.

Loomis, W.F. 1975. Dictyostelium discoideium: A developmental System. Academic Press, Inc., New York.

McCullough, C.H.R., et al. 1973. Nuclear DNA content and senescence in Physarum polycephalum. Nature New Biol. 245: 263-265.

Mendelsohn, M.L. 1961. The two-wavelength method of microspectrophotometry. IV. A new solution. Journal of Biophysical and Biochemical Cytology. 11: 509-513.

Mettler, L.E. and T.G. Gregg. 1969. Population genetics and evolution. Prentice-Hall Inc., New Jersey.

Mohberg, J., and H.P. Rusch. 1971. Isolation and DNA content of nuclei of Physarum polycephalum. Exp. Cell Res. 66: 305-316.

Mohberg, J., et al. 1973. Nuclear DNA content and chromosome numbers in the Myxomycete Physarum polycephalum. Dev. Biol. 34: 228-245.

Moore, B.C. 1950-1952. Desoxyribose nucleic acid in embryonic diploid and haploid tissues. Chromosoma 4: 563-576.

Nauman, R.V. et. al. 1960. A spectroscopic study of the Schiff reaction as applied to the quantitative determination of sulfur dioxide. Annals of Chemistry. 32: 1307-1311.

Olive, L.S. 1975. The Mycetozoans. Academic Press Inc., New York.

Oklowski, Z.L. 1976. Microspectrophotometry: potential applications in clinical oncology. Amer. Lab. Vol. 8. No. 4: 13-20.

Ornstein, L. 1952. The distributional error in micro-spectrophotometry. Lab. Invest. 1: 250-265.

Patau, K. 1952. Absorption microphotometry of irregular shaped objects. Chromosoma. 5: 341-362.

Pearse, A.G.E. 1968. Histochemistry: Theoretical and Applied. V.I. 3rd Ed., London: J.A. Churchill Lts.

Pollister, A.W. et al. 1949. The nucleoprotein content of whole nuclei. Proc. Nation. Acad. Sci. USA. 35 (1): 66-71.

Pollister, A.W. and A.E. Mirsky. 1947. The nucleoprotamine of trout sperm. Jour. Gen. Physiol. 30(2): 101-116.

Pollister, A.W., and M.J. Moses. 1949. A simplified apparatus for photometric analysis and photomicrography. J. Gen. Physiol. 32: 567.

Poste, G. 1970. Virus-induced polykarycytosis and the mechanism of cell fusion. Adv. Virus Res. 16: 303-356.

Poste, G., and A.C. Allison. 1971. Membrane fusion reaction: A Theory. J. Theor. Biol. V. 32: 165-184.

Ris, H., and Mirsky. 1949. Quantitative chtochemical determination of desoxyribonucleic acid with the Feulgen nucleal reaction. J. Gen. Physiol. 33: 125-146.

Ross, I.K. 1957. Syngamy and Plasmodium formation in Myxogastres. Amer. J. Bot. 44: 843-850.

Ross, I.K. 1966. Chromosome numbers in pure and gross cultures of Myxomycetes. Amer. J. Bot. 53: 712-718.

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

Ross, I.K. 1967b. Abnormal cell behavior in the heterothallic myxomycete Didymium iridis. Mycologia. 59: 235-245.

Ross, I.K., and R.J. Cummings. 1970. An unusual pattern of multiple cell and nuclear fusions in the heterothallic slime mold Didymium iridis. Protoplasma. 70: 281-294.

Ross, I.K., and G.L. Shipley. 1973. Sexual and somatic fusion in the heterothallic slime mold Didymium iridis. 2. Effects of Acetinomucen D., cyclohexamide and lysosome stabilizers. Microbios. 7: 165-171.

Ross, I.K., and G.L. Shipley, and R.J. Cummings. 1973. Sexual and somatic cell fusions in the heterothallic slime mold Didymium iridis. 1. Fussion assay, fusion kinetics and cultural parameter. Microbios. 7: 149-164.

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

Rusch, H.P. 1973. Revision of the terminology of the G$_1$ phase. Ber. Deutsch. Bot. Ges. Bd. 86: J. 5-10.

Sauer, H.W., K.L. Babcock, and J.P. Rusch. 1969. Sporulation in Physarum polycephalum. Exp. Cell Res. 57: 319-327.

Skupienski, F.X. 1927. Sur le cyde evolutef chez ume espece de myxomycete endosporee Didymium difforme. Etude cytologique. C.R. Acad. Sci. Paris, 184: 1341-1344.

Stebbins, G.L. 1971. Chromosomal evolution in higher plants. Edward Arnold (Publishers) Ltd. London.

Stosch, H.A. von. 1935. Untersuchungen uber die entwicklungsgeschichte der Myxomyceten. Sexualitat und Apogamie bei Didymiaceen. Planta. 23: 623-656.

Stosch, H.A. von. 1937. Uber den Generationwechsel der Myxomyceten, eime Euriderung. Ber, Deutsch. Bot. Ges. 55: 367-369.

Stosch, H.A. von, et al. 1964. Nuclear phase alternance in the myxomycete Physarum polycephalum. Abstracts Xth Intern. Bot. Congr., Edinburgh, August 1964, p. 481-482.

Strickberger, M. W. 1968. Genetics. The Macmillan Co., New York.

Sussman, M. and R.R. Sussman. 1962. Ploidal inheritance in Dictyostelium discoideum : Stable haploid, stable diploid and metastable strains. J. Gen. Microbiol. 28: 417-429.

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

Swift, H. 1966. Microphotometry in Biologic Research. Journal of Histochem. and Cytochem. Vol. 14(11): 842-852.

Swift, H., and E. Rasch. 1956. Microphotometry with visible light. In Physical Techniques in Biological Research. Academic Press Inc., New York.

Taylor, J.H. 1959. Autoradiographic studies of nucleic acids and proteins during meiosis in Lilium Longifolium. Amer. J. Bot. 46: 477-484.

Therrein, C.D. 1966. Microspectrophotometric measurements of nuclear deoxyribonucleic acid content in two Myxomycetes. Can. J. Bot. 44: 1667-1675.

Therrein, C.D., and O.R. Collins. 1976. Apogamic Induction of haploid plasmodia in a Myxomycete, Didymium iridis. Develop. Biol. 49: 283-287.

Therrein, 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. Amer. J. Bot. 61: 400-404.

Therrein, C.D., and J.J. Yemma. 1974. Differential gene activity in myxomycete swarmers and myxamoeba. Amer. J. Bot. 61: 26(Abstr.).

Therrein, C.D., and J.J. Yemma. 1975. Nuclear DNA content and ploidy values in clonally-developed plasmodia of the Myxomycete Didymium iridis. Carylogia, Vol. 28 n.3: 313-320.

Thompson, M.M. 1962. Cytogenetics of Rubus III. Meiotic instability in some higher polyploids. Amer. J. Bot. 49: 575-582.

Vendrely, R., and C. Vendrely. 1948-1949. La Teneur du moyeau cellulaire en acide desoxyribonucleique a trauers des organes des individus et les esperes animales. Experimentia. 4: 434-436, 5: 327.

Wheals, A.E., 1970. A homothallic strain of the myxomycete Physarum polycephalum. Genetics. 66: 623-633.

Wyckoff, R.W.G., and B.J. Luyet. 1932. The effects of X-rays, cathode, and ultraviolet rays on yeast. Radiology. 17(6): 1171-1175.

Yemma, J.J. 1971. Quantitative microspectrophotometry of nuclear DNA in selfing strains of the myxomycete Didymium iridis. Ph.D. Dissertation, Pennsylvania State University.

Yemma, J.J., and C.D. Therrein. 1972. Quantitative microspectrophotometry of nuclear DNA in selfing strains of the myxomycete Didymium iridis. Amer. J. Bot. 59: 828-835.

Yemma, J.J., et al. 1974. Cytoplasmic inheritance of the selfing factor in the myxomycete Didymium iridis. Heredity. 32: 231-239.