A COMPARATIVE QUANTITATIVE MICROSPECTROPHOTOMETRIC STUDY OF NUCLEAR SIZE VERSUS DNA CONTENT IN SEVERAL VARIETIES OF *DIDYMIUM IRIDIS*

by

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ABSTRACT

A COMPARATIVE QUANTITATIVE MICROSPECTROPHOTOMETRIC STUDY OF NUCLEAR SIZE VERSUS DNA CONTENT IN SEVERAL VARIETIES OF DIDYMIUM IRIDIS

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The possibility of a relationship between DNA content and nuclear volume of nine clones of the myxomycete Didymium iridis was investigated. The Feulgen-nucleal reaction followed by quantitative microspectrophotometric analysis for DNA determination was employed in conjunction with nuclear volumetric measurements. In order to determine if any relationship existed, statistical analysis of DNA contents and nuclear volumes was carried out by use of linear regressions with volume being the dependent Y variable. It was concluded that there is no relationship between DNA content and nuclear volume in D. iridis. While DNA contents vary, nuclear volume remains relatively constant.

ACKNOWLEDGEMENTS

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INTRODUCTION

Studies concerning nuclear volume and DNA content are abundantly found in the literature. As early as 1925, Jacobj postulated that nuclear volumes progress in a geometric fashion and are related to chromatin content. Later, investigators of this hypothesis were not in complete agreement. Schrader and Leuchtenberger (1950), Leuchtenberger and Schrader (9151), and Leuchtenberger et. al. (9152) found no correlation between nuclear volume and DNA content while Smith (1943), Becak et. al. (1967), Baetcke et. al. (1967), Price et. al. (1973), and Edwards and Endrizzi (1975) believed that a direct relationship existed between nuclear volume and DNA content. The purpose of this investigation was to determine the relationship, if any, between the nuclear volume and DNA content of myxamoeba and plasmodia of Didymium iridis. This is of primary importance in studies involving the cell cycle kinetics in this organism and related species.

In 1950 Schrader and Leuchtenberger determined, by using quantitative cytochemical methods, that change in the nuclear volume of spermatocytes of the pentatomid Arvelius were not due to differing amounts of DNA, but due to nuclear protein. In 1951, using rat tissue and employing the same methods, Leuchtenberger and Schrader determined that "in contrast to the nearly exact multiple relationship of DNA in

nuclei (1, 2, 4), the nuclear volumes do not show a multiple relationship, but vary over a large range with intermediate values." Thus, the nuclear size appeared to be independent of the amount of DNA found within the nucleus. Later, in 1952, Leuchtenberger et. al. found that although the amount of DNA remained rather constant in beef and rat tissues, nuclear volumes varied considerably. Thus, no correlation was evident between nuclear volume and DNA content.

The literature does, however, contain studies that are in agreement with Jacobj. For example, Smith (1943) discovered that polyploid plants have larger nuclei than diploid plants. The recent studies of Baetcke et. al. (1967), Price et. al. (9173), and Edwards and Endrizzi (1975) using plant tissue tend to be in agreement with Jacobj. By extracting the DNA of various plant tissue and taking measurements of nuclei on longitudinal sections of corresponding tissues, Baetcke et. al. (1967) and Price et. al. (1973) determined that the nuclear volume and DNA content relationship is linear. In 1975, Edwards and Endrizzi, using DNA contents reported earlier for Gossypium (Edwards et. al., 1974), measured nuclei and found that DNA increases as do nuclear volumes. This relationship proved to be linear.

Becak et. al. (1967), in a follow-up study using animal tissues including erythrocyte, liver, pancreas, and kidney of three separate species of South American frogs, supports the studies of Jacobj, as well as, those of Baetcke. In these studies, Feulgen-DNA content was determined with a

histophotometer. Nuclear measurements were taken at the same time. Furthermore, this study supports the contention of Edwards and Endrizzi (1975) who state that the relationship between nuclear volume and DNA content is linear.

As mentioned previously, the purpose of this paper was to determine the relationship between nuclear volume and DNA content of the true slime mold Didymium iridis using quantitative cytophotometric methods. This organism was chosen because of its relatively short life cycle (Plate 1) which is of primary importance in studies involving cell cycle kinetics and the ease with which cultures can be maintained. Nuclei of the myxamoeba and plasmodia were stained by the Feulgen method and measured in arbitrary units with the microspectrophotometer. At the same time nuclear dimensions were taken in arbitrary units. This method lessens experimental error which was considerable in earlier methods, such as the plug method of Caspersson (1936).

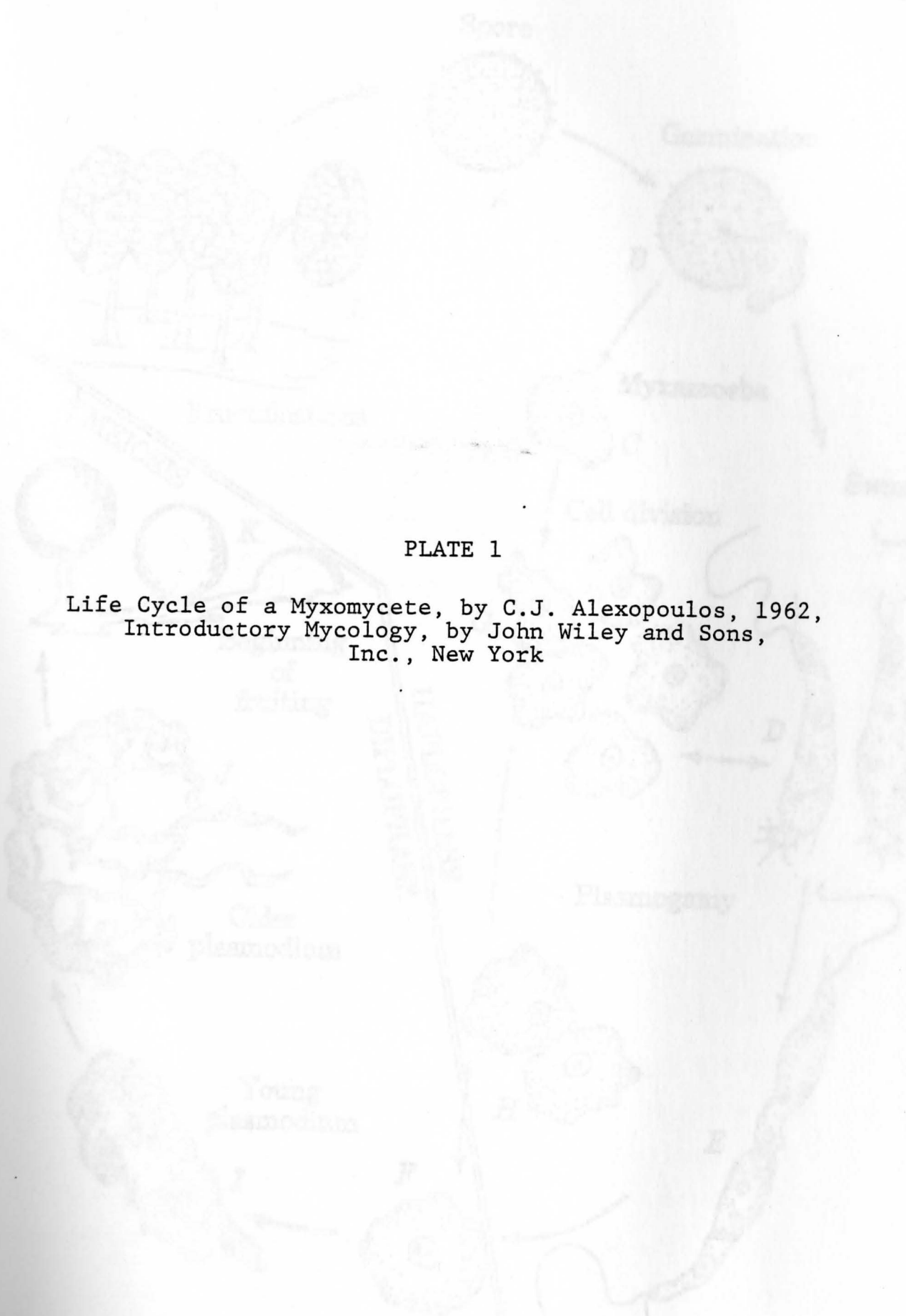
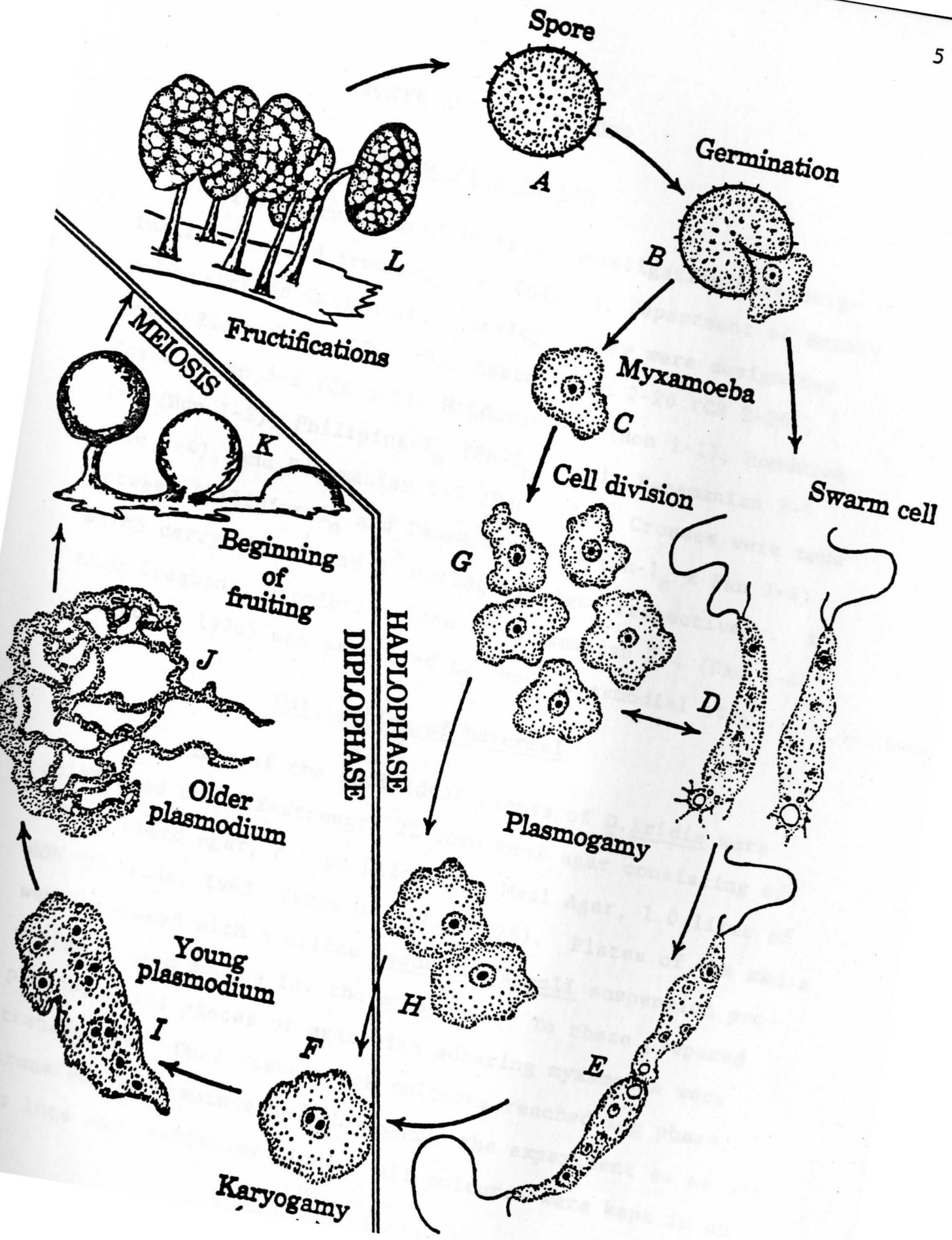


PLATE 1

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 cell
G
Beginning of fruiting
J
DIPLOPHASE
HAPLOPHASE
D
Older plasmodium
Plasmogamy
H
Young plasmodium
I
F
E
Karyogamy

MATERIALS AND METHODS

Tissue Preparation

The cultures used in this investigation were originally obtained from Dr. O.R. Collins, Department of Botany, University of California, Berkley. They were designated Costa Rican 2-25 (CR 2-25), Costa Rican 2-26 (CR 2-26), Costa Rican 5-5 (CR 5-5), Honduran 1-1 (Hon 1-1), Honduran 1-2 (Hon 1-2), Philipine-1_n (Ph-1_n), A-1, Panamanian 2-4 (Pan 2-4), and Panamanian 2-7 (Pan 2-7). Crosses were made between Philipine-1_n and Panamanian 2-4 (Ph-1_n x Pan 2-4) which carry the A^1 and A^7 mating alleles, respectively. A high frequency apomictic clone of Panamanian 2-4 (Pan 2-4) (Ventura, 1974) was permitted to reach plasmodial stage.

Cultivation of Material

Cultures of the individual clones of D.iridis were cultivated on half-strength 2% corn meal agar consisting of 8.0 gm Difco Agar, 8.5 gm Difco Corn Meal Agar, 1.0 liter of HOH (Collins, 1963; Yemma et. al., 1974). Plates of the media were streaked with a dilute Escherichia coli suspension providing a food source for the myxamoeba. To these prepared plates, small pieces of agar with adhering myxamoeba were transferred. Once these stock cultures reached log phase, transfers were maintained throughout the experiment so as not to lose any particular clone. All cultures were kept in an

incubator at 21°C (Yemma and Therrien, 1972; Yemma et. al., 1974).

In order to obtain plasmodia, Philippine-1_n (Ph-1_n) and Panamanian 2-4 (Pan 2-4) amoeba were mixed on half-strength corn meal agar (Collins, 1963; Therrien, 1966). Being of compatible mating types, the amoeba initiated the plasmodial stage by sexual fusion (Yemma et. al., 1974).

The high frequency apomictic clone of Panamanian 2-4 (Pan 2-4) was grown on half-strength corn meal agar and permitted to reach plasmodial stage.

Preparation and Treatment of Material for Cytochemical Studies

Twenty plates of each clone used were washed with sterile 0.25M phosphate sucrose buffer (0.75gm KCl, 0.58gm NaCl, 2.24gm $NaPO_4$ dibasic, 4.6gm KPO_4 monobasic, 1.0 liter HOH, 85.575gm sucrose). The amoeba-PSB suspension were then pipetted into 50ml conical centrifuge tubes and centrifuged at 70X gravity for 20 minutes. The supernatant, containing mostly bacteria, were discarded. The resulting pellets of amoeba and bacteria were centrifuged again using a layered sucrose gradient of 10% and 30% sucrose solutions to remove any remaining bacteria.

The bacteria-free pellets resulting from the gradient were then fixed in 10% neutral buffered formalin for 12-18 hours (Kasten, 1959; Swift, 1950). The 10% neutral buffered formalin consisted of 250ml 40% formaldehyde, 750ml HOH, 4.0gm NaH_2PO_4 HOH, 6.5gm Na_2HPO_4 (Humason, 1972). After the

allotted time, the amoebae-formalin suspensions were centrifuged at 70X gravity for 20 minutes in order to remove the formalin. The supernatants were discarded and the pellets were washed several times in 70% ethanol. Following centrifugation, the amoebae were post-fixed in 70% ethanol for 12 hours. After the 12 hour post-fixation, the suspensions were again centrifuged. To the resulting pellet 1ml of 70% ethanol was added. This facilitated the making of amoeboid smears on the albuminized slides.

The plasmodia were fixed by flooding the culture plates with 10% neutral buffered formalin. After 12 - 18 hours, the loosened plasmodia were transferred into a beaker. The formalin was then removed and the plasmodia were resuspended in 70% ethanol. Following a 12 hour treatment, the 70% ethanol was removed and replaced by 95% ethanol. After 2 hours, the 95% ethanol was replaced by 100% ethanol for 2 hours. The 100% ethanol was then removed and replaced by xylene. After two exchanges of xylene, each lasting 1 hour, the plasmodia were parafin embedded and sectioned by an A.O. microtome at 4 microns. Slides were mounted in Preservaslide.

Cytochemical Methods

For the localization and the qualification of nuclear deoxyribonucleic acid (DNA), the Feulgen reaction was employed (Feulgen and Rossenbeck, 1924; as modified by Therrien, 1966; and Bryant and Howard, 1969). Hydrolysis was carried

out in 5N HCl for 43 minutes at room temperature. This method proved to yield optimum staining results (Fand et. al., 1967; Yemma and Therrien, 1972). The Feulgen reaction procedure is as follows:

(1) 70% ethanol - 2 minutes
(2) 5N HCl - 43 minutes
(3) HOH - rinse
(4) Schiff's Reagent - 1 hour (Lillie, 1951; The basic fuschin used was supplied by Fischer Scientific Company - C.I. #42500)
(5) KMB - 5 minutes (A freshly prepared solution of potassium-meta-bisulfite rinse consisting of 5ml 10% K-meta-bisulfite, 5ml 1N HCl, 100ml HOH)
(6) KMB - 5 minutes
(7) HOH - rinse
(8) 70% ethanol - 2 minutes
(9) 95% ethanol - 2 minutes
(10) 100% ethanol - 2 minutes
(11) Xylene - 2 minutes
(12) Xylene - 2 minutes
(13) Mount in Preservaslide

The amoeba smears were placed directly into 70% ethanol while the plasmodia slides underwent deparafinization in two xylene changes, transferred to 100% ethanol, and then to 90% and finally to 70% ethanol. Duration in each fluid was for 30 minutes.

Cytophotometric Methods

Employing a Planachromat oil immersion objective, N.A. 1.30 x 100, all cytophotometric determinations were made with a Zeiss Type 01 microspectrophotometer. A continuous inference-filter monochromator (Zeiss No. 47 43 10) was used to isolate chosen wavelengths of light. Before each reading session, the alignment of the instrument and linearity of the phototube were checked.

By applying absorption photometry to individual cells, the relative amounts of DNA were able to be measured (Ris and Mirsky, 1949, Swift, 1966, Kasten, 1967). In this study the two-wavelength method of chromaphore measurement was used (Patau, 1952; Ornstein, 1952; Mendelsohn, 1961). This method consisted of circumscribing the entire nucleus within the photometric field and measuring the absorbing molecules by transmission at two reference wavelengths. These wavelenths were chosen by making several absorption curves for the Feulgen stained material. The absorption curves should be at a 2:1 ratio since all the material examined was hydrolized and stained at the same time (Swift and Rasch, 1956). Therefore, this two-wavelength method was desirable because it minimized the distributional error and eliminated the need for direct measurement of nuclear area (Mayall and Mendolsohn, 1970).

The amount of chromophore (M) determined within a given area (A) was calculated from the equation $M = KAL_1Q$. In this study K, being a constant, was eliminated since only relative

values of M and not absolute values were needed. Transmissions (T_1 and T_2) were taken at wavelengths 1 and 2 for each nucleus. $T_1 = I_s/I_o$ and $T_2 = I_s/I_o$, where I_s is the transmitted flux and I_o is the flux of photons on chromophore. From these values, L_1 and L_2 were calculated by the equations $L_1 = (1 - T_1)$ and $L_2 = (1 - T_2)$. The ratio Q, $Q = L_2/L_1$, which corresponds to the 2:1 ratio of the absorption curves was used to determine the correction factor C, where $C = (2 - Q)^{-1}\ln(Q - 1)^{-1}$ (Swift and Rasch, 1956; Leuchtenberger, 1958). The C values and corresponding Q values were found in a table formulated by Patau (1952).

All calculations were done on a Mandahl Model 470V5 computer to ensure accuracy.

Nuclear Volume Determinations

Nuclear dimensions were taken at the same time as the dye-concentrations. The formula $\frac{4}{3}\pi r^3$ was used to determine the volumes, r being the radius of a sphere. Since the fraction and π are constants and the shape of the D.iridis nucleus is spheroid, volume was calculated as r^3. All calculations envolving nuclear dimensions were carried out on the Mandahl Model 470V5 computer. The results are presented in arbitrary units throughout this study.

RESULTS

The results of this investigation are presented in the form of frequency histograms, tables, and graphs. Frequency histograms of DNA measurements facilitate the observation of DNA distributions within a population of cells. In this manner, major and minor shifts in the nuclear DNA are detectable. The tables aid in the characterization of data while the graphs aid in the determination of any relationship between DNA content and nuclear volume.

Feulgen Spectral Absorption Curve

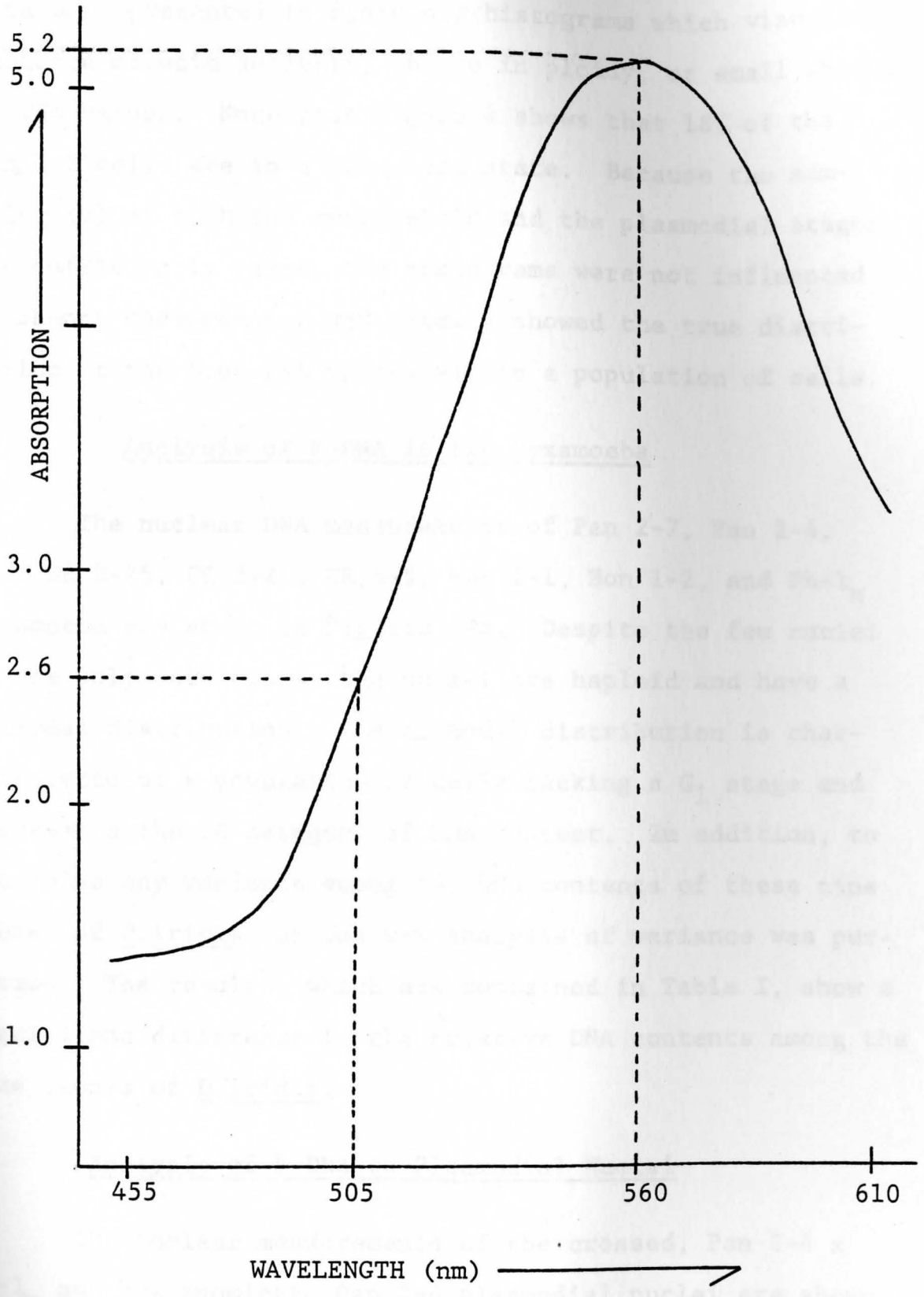
Since the two-wavelength method of microspectrophotometry was utilized, it was necessary to establish an absorption curve for the dye-molecule complex under investigation (Figure 1). Feulgen stained plasmodial nuclei were used to establish such a curve. The wavelengths representing the maximum absorption and the half-maximum absorption were found to be 560 nm and 505 nm, respectively.

Microspectrophotometric Analysis of Nuclear DNA in *D. iridis*

Nuclear DNA measurements were taken in the myxamoeboid stage of Pan 2-7, Pan 2-4, A-1, CR 2-25, CR 2-26, CR 5-5, Hon 1-1, Hon 1-2, and Ph-1_n which are characteristically haploid cells and the plasmodial stage of a cross of Pan 2-4 x Ph-1_n which is characteristically diploid, as

FIGURE 1

Absorption spectrum of Feulgen stained plasmodial nuclei of *Didymium iridis*


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

well as, an apomictic Pan 2-4. The resulting quantitative data were presented in frequency histograms which visually indicate mitotic activity, change in ploidy, or small shifts in DNA values. Note that Figure 4 shows that 18% of the Hon 1-2 cells are in a polyploid state. Because the sampling (n) of both the myxamoeboid and the plasmodial stages was sufficiently large, the histograms were not influenced by chance measurements and readily showed the true distribution of the mean DNA values within a population of cells.

Analysis of F-DNA in the Myxamoeba

The nuclear DNA measurements of Pan 2-7, Pan 2-4, A-1, CR 2-25, CR 2-26, CR 5-5, Hon 1-1, Hon 1-2, and Ph-1_n myxamoeba are shown in Figures 2-4. Despite the few nuclei in the polyploid class, the nuclei are haploid and have a unimodal distribution. The unimodal distribution is characteristic of a population of cells lacking a G_1 stage and represents the 2C category of DNA content. In addition, to determine any variance among the DNA contents of these nine clones of D.iridis, an one way analysis of variance was performed. The results, which are contained in Table I, show a significant difference in the relative DNA contents among the nine clones of D.iridis.

Analysis of F-DNA in Plasmodial Nuclei

The nuclear measurements of the crossed, Pan 2-4 x Ph-1_n and the apomictic Pan 2-4 plasmodial nuclei are shown in Figure 5. Also, presented for comparison is a represent-

FIGURE 2

Histograms representing Feulgen-DNA values for myxamoeba nuclei of *Didymium iridis*

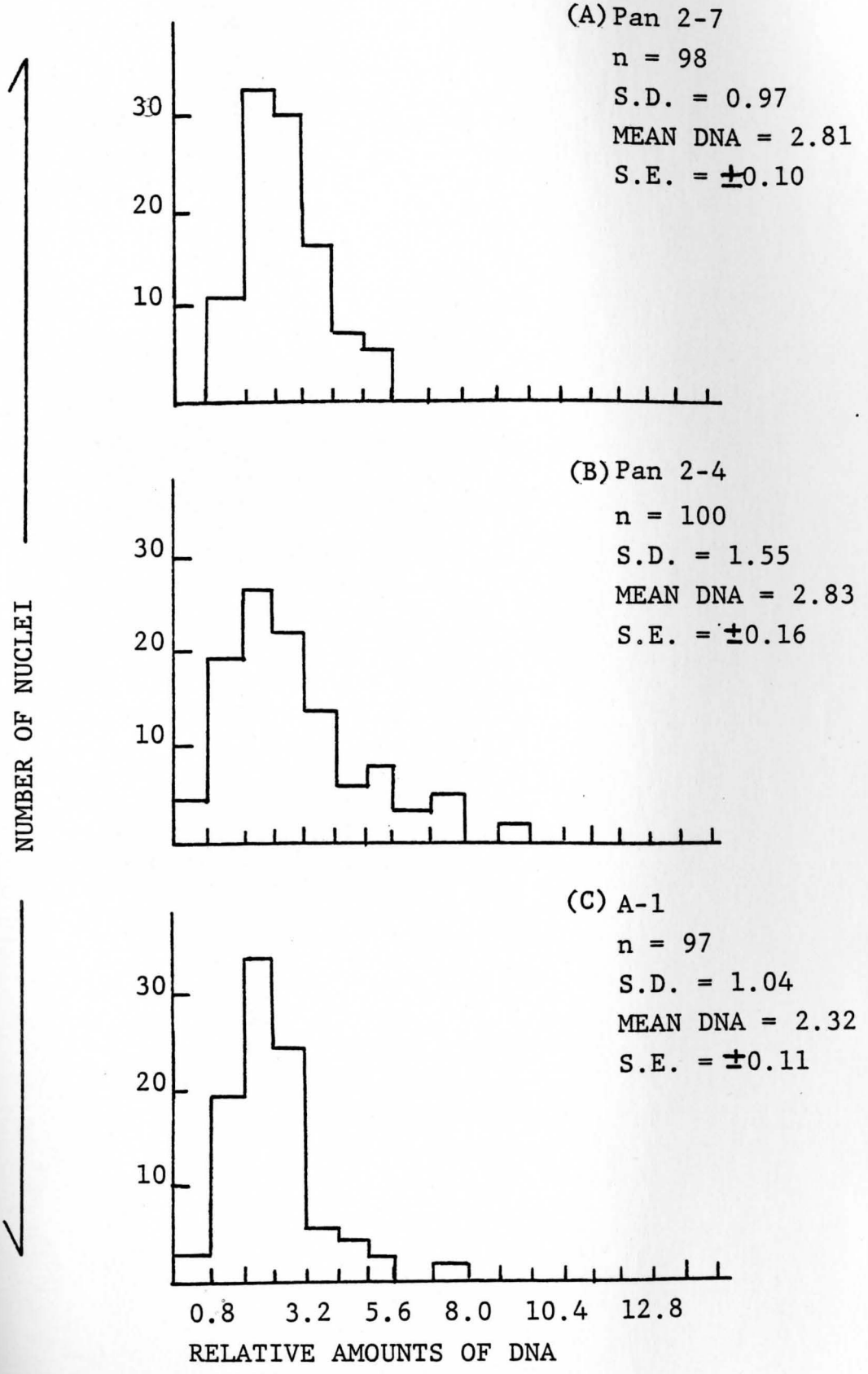

(A) Pan 2-7
n = 98
S.D. = 0.97
MEAN DNA = 2.81
S.E. = ±0.10
(B) Pan 2-4
n = 100
S.D. = 1.55
MEAN DNA = 2.83
S.E. = ±0.16
(C) A-1
n = 97
S.D. = 1.04
MEAN DNA = 2.32
S.E. = ±0.11
NUMBER OF NUCLEI
30
20
10
0.8
3.2
5.6
8.0
10.4
12.8
RELATIVE AMOUNTS OF DNA

FIGURE 3

Histograms representing Feulgen-DNA values for myxamoeba nuclei of *Didymium iridis*

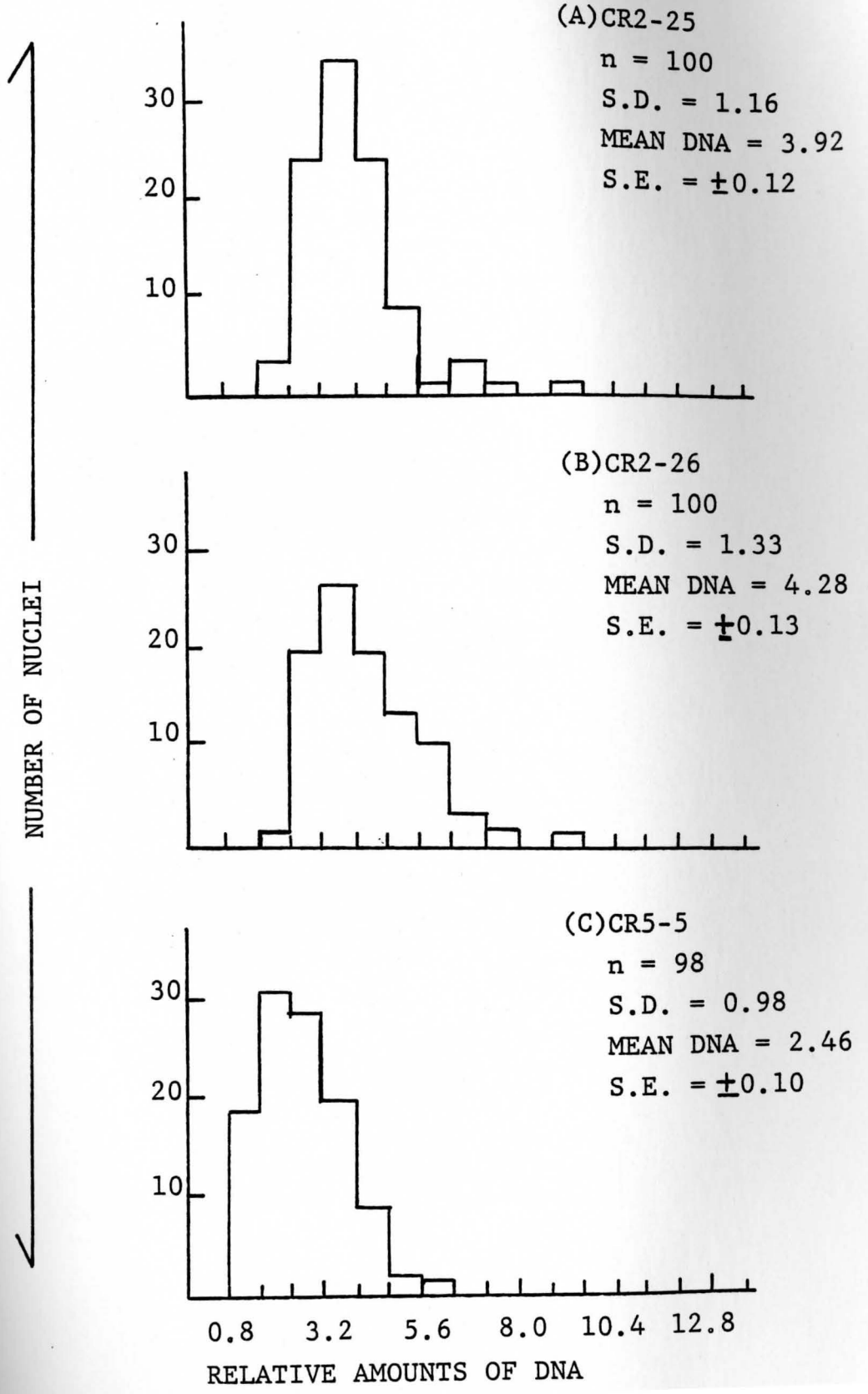

(A)CR2-25
n = 100
S.D. = 1.16
MEAN DNA = 3.92
S.E. = ±0.12
(B)CR2-26
n = 100
S.D. = 1.33
MEAN DNA = 4.28
S.E. = ±0.13
(C)CR5-5
n = 98
S.D. = 0.98
MEAN DNA = 2.46
S.E. = ±0.10
NUMBER OF NUCLEI
30
20
10
0.8
3.2
5.6
8.0
10.4
12.8
RELATIVE AMOUNTS OF DNA

FIGURE 4

Histograms representing Feulgen-DNA values for myxamoeba nuclei of *Didymium iridis*

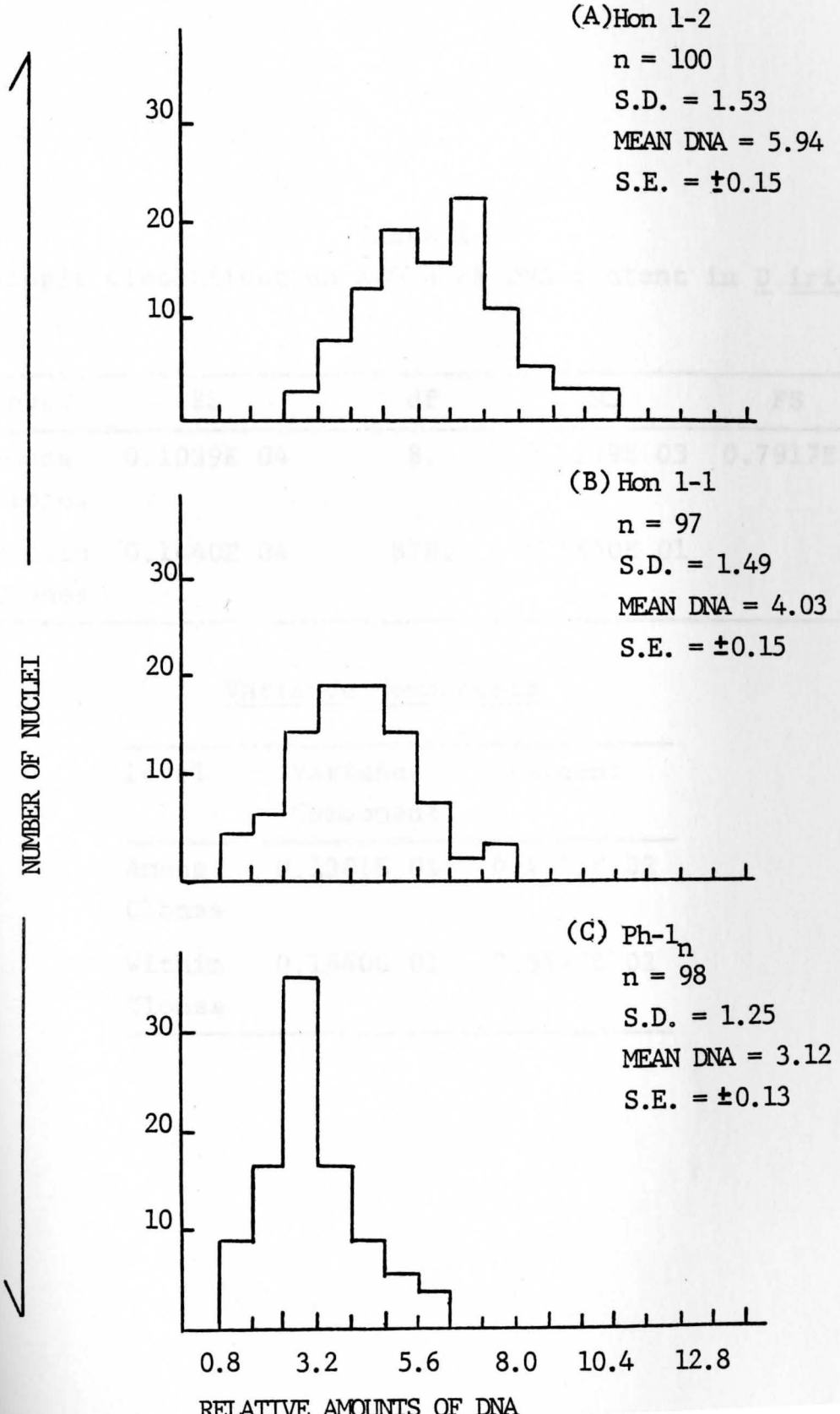

(A)Hon 1-2
n = 100
S.D. = 1.53
MEAN DNA = 5.94
S.E. = ±0.15
(B) Hon 1-1
n = 97
S.D. = 1.49
MEAN DNA = 4.03
S.E. = ±0.15
(C) Ph-1n
n = 98
S.D. = 1.25
MEAN DNA = 3.12
S.E. = ±0.13
NUMBER OF NUCLEI
30
20
10
0.8
3.2
5.6
8.0
10.4
12.8
RELATIVE AMOUNTS OF DNA

TABLE I

Single Classification ANOVA of DNA-content in *D.iridis*

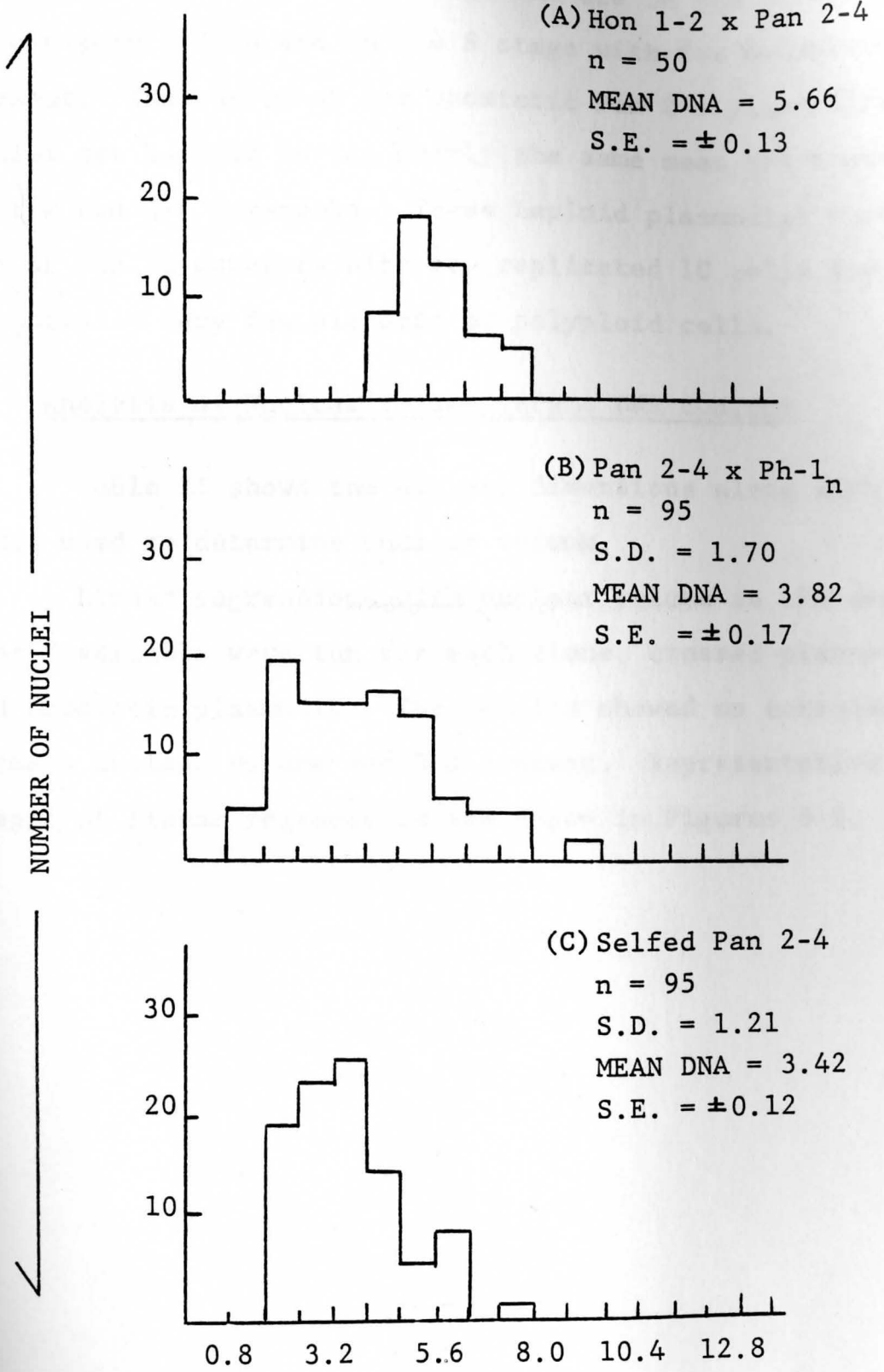
Level	SS	df	MS	FS
Among Clones	0.1039E 04	8.	0.1299E 03	0.7917E 02
Within Clones	0.1440E 04	878.	0.1640E 01	

Variance Components

Level	Variance Component	Percent
Among Clones	0.1301E 01	0.4423E 02
Within Clones	0.1640E 01	0.5577E 02

FIGURE 5

Histograms representing Feulgen-DNA values for plasmodial nuclei of *Didymium iridis*


(A) Hon 1-2 x Pan 2-4
n = 50
MEAN DNA = 5.66
S.E. = ± 0.13
(B) Pan 2-4 x Ph-1n
n = 95
S.D. = 1.70
MEAN DNA = 3.82
S.E. = ± 0.17
(C) Selfed Pan 2-4
n = 95
S.D. = 1.21
MEAN DNA = 3.42
S.E. = ± 0.12
NUMBER OF NUCLEI
30
20
10
0.8
3.2
5.6
8.0
10.4
12.8

ative diploid plasmodia in the 4C category. As seen in histogram (B), the diploid nuclei of the Pan 2-4 x Ph-1_1 cross are mainly in the G_1 stage and are in the unreplicated 2C category. Some are in the S stage with few polyploids present. The nuclei of the apomictic Pan 2-4 plasmodial nuclei are haploid having nearly the same mean DNA content as the Pan 2-4 myxamoeba. These haploid plasmodial nuclei are of the 1C category with few replicated 1C cells and essentially very few picnotic or polyploid cells.

Analysis of Nuclear Volume versus DNA Content

Table II shows the nuclear dimensions along with the radii used to determine nuclear volume.

Linear regressions with nuclear volume as the dependent Y variable were run for each clone, crossed plasmodia, and apomictic plasmodia. The results showed no correlation between nuclear volume and DNA content. Representative graphs of linear regresssion are shown in Figures 6-9.

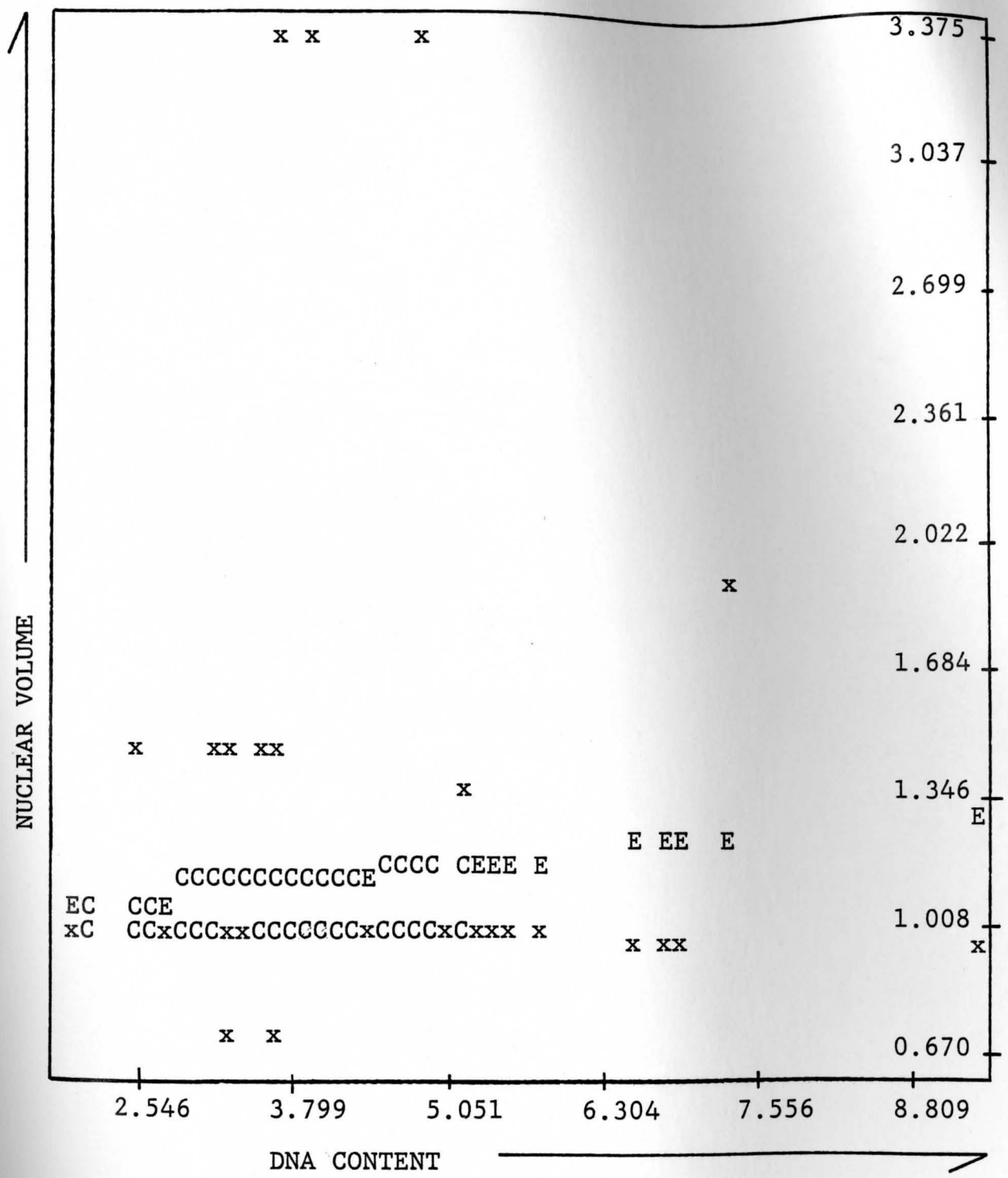
TABLE II

Nuclear Dimensions Used in Determining Nuclear Volumes

Clone	Length	S.D.	Width	S.D.	Ratio L/W	Radius
Pan 2-7	1.990	0.106	1.990	0.011	1.007	0.995
Pan 2-4	1.830	0.197	1.860	0.020	1.001	0.925
A-1	1.345	0.150	1.320	0.015	1.026	0.666
CR 2-25	2.045	0.135	2.065	0.014	0.999	1.029
CR 2-26	2.035	0.179	2.025	0.018	1.014	1.015
CR 5-5	2.071	0.259	2.015	0.026	1.054	1.022
Hon 1-1	2.139	0.066	2,134	0.007	1.004	1.068
Hon 1-2	2.343	0.137	1.353	0.014	1.005	1.174
Ph-1_n	2.091	0.171	2.061	0.017	1.023	1.038
Plasmodia						
Pan 2-4 x Ph-1_n	1.916	0.220	1.942	0.022	1.004	0.965
Pan 2-4	2.074	0.066	2.084	0.007	0.997	1.039

FIGURE 6

Graph of linear regression
for CR 2-25 myxamoeba

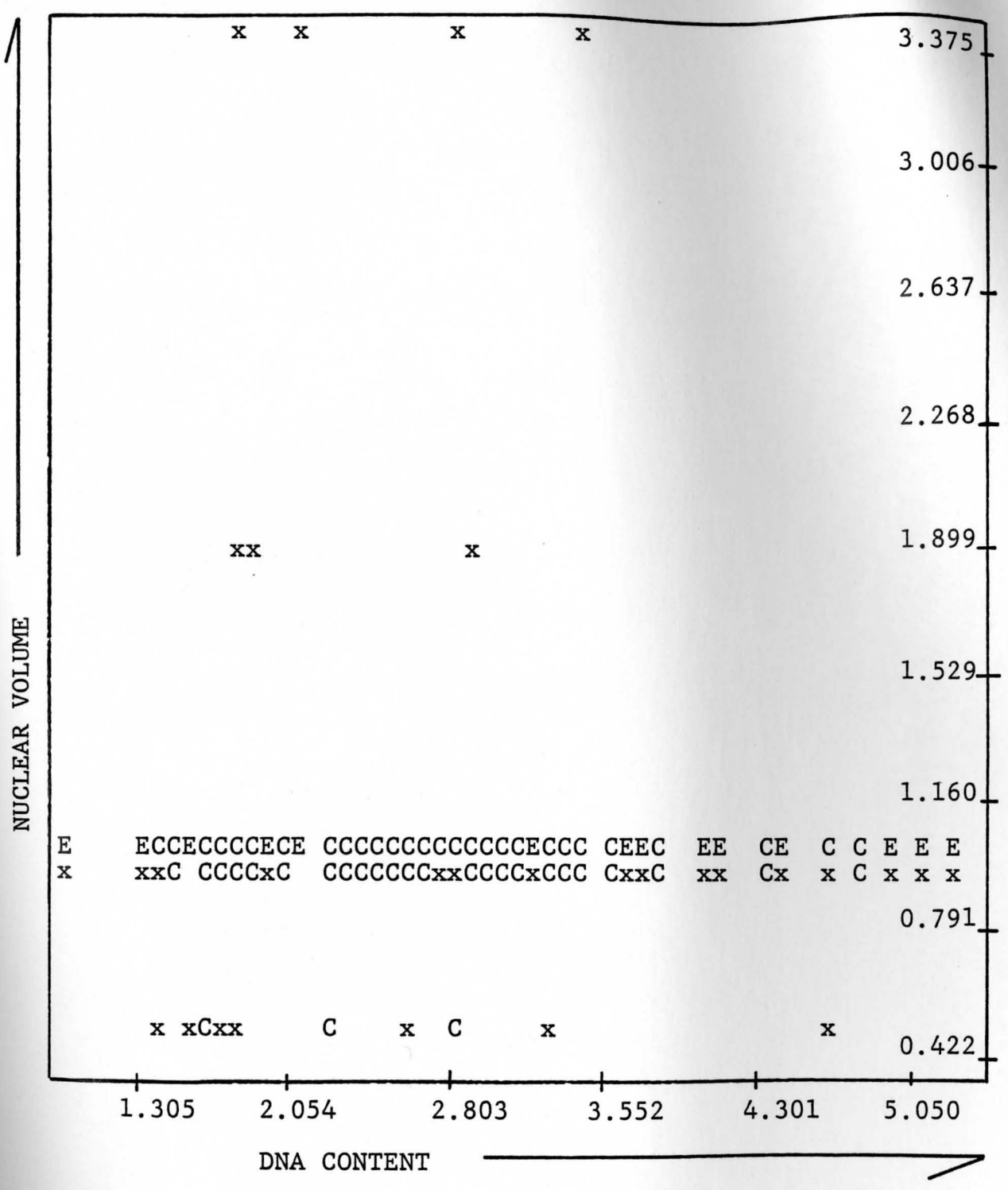


x = Y Observed
E = Y Estimated
C = Common Point

Equation of the Line:

$Y = 0.042X + 0.9597$

Intercept = 0.9597
Regression Coefficient = 0.042
Correlation Coefficient = 0.100



x = Y Observed
E = Y Estimated
C = Common Point

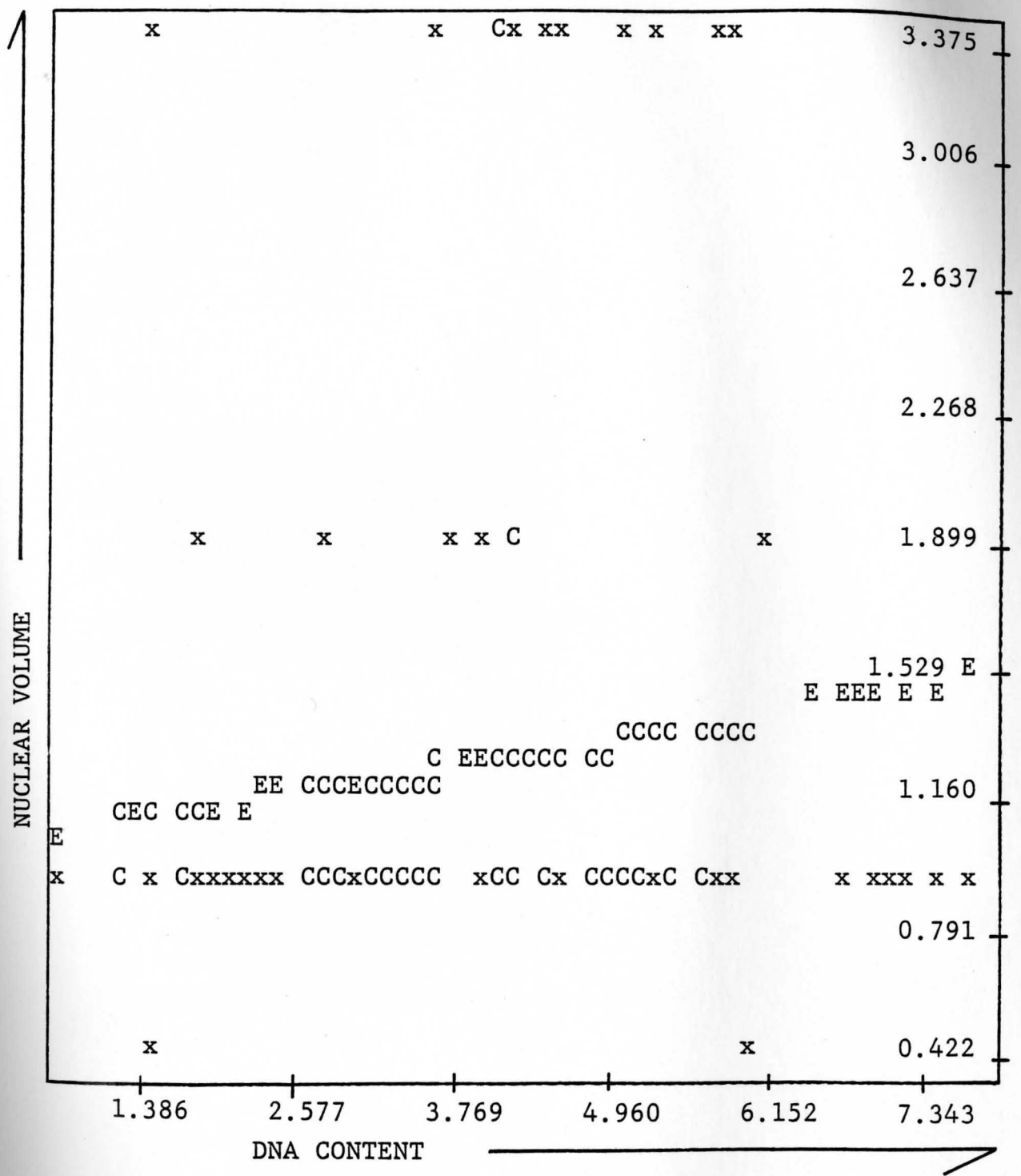
Equation of the Line:

$Y = -0.009X + 1.0758$

Intercept = 1.0758
Regression Coefficient = -0.009
Correlation Coefficient = -0.017

FIGURE 8

Graph of linear regression
Hon 1-1 myxamoeba



x = Y Observed
E = Y Estimated
C = Common Point

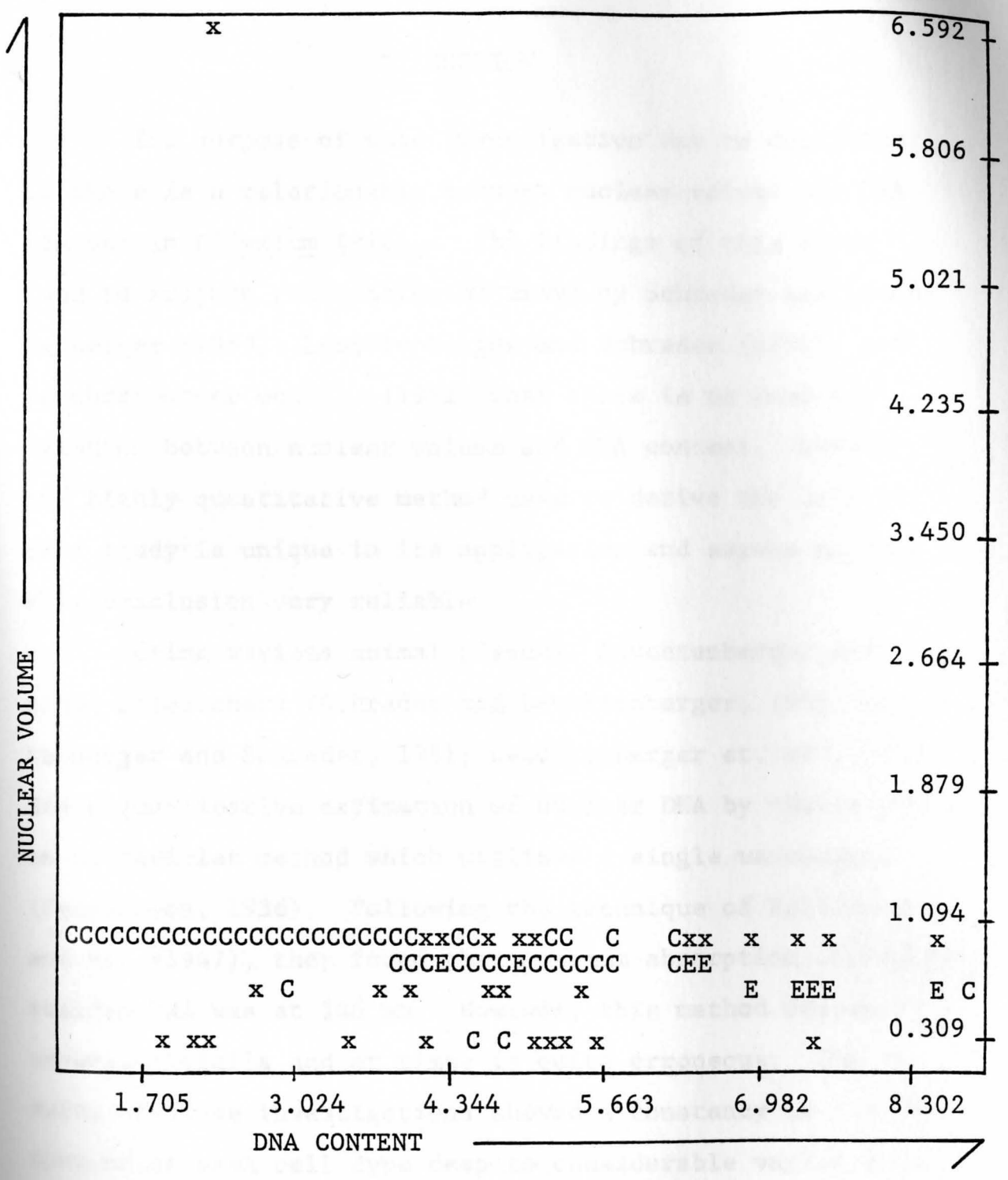
Equation of the Line:

$Y = 0.055X + 1.10415$

Intercept = 1.10415
Regression Coefficient = 0.055
Correlation Coefficient = 0.105

FIGURE 9

Graph of linear regression
for Pan 2-4 x Ph-1_n plasmodia



x = Y Observed
E = Y Estimated
C = Common Point

Intercept = 1.12285
Regression Coefficient = -0.051
Correlation Coefficient = -0.139

Equation of the Line:

$$Y = -0.051X + 1.12285$$

DISCUSSION

The purpose of this investigation was to determine if there is a relationship between nuclear volume and DNA content in Didymium iridis. The findings of this study tend to support the conclusion drawn ny Schrader and Leuchtenberger (1950), Leuchtenberger and Schrader (1951), and Leuchtenberger et. al. (1952) that there is no real correlation between nuclear volume and DNA content. However, the highly quantitative method used to derive the data in this study is unique in its application and serves to make this conclusion very reliable.

Using various animal tissues, Leuchtenberger and the other researchers (Schrader and Leuchtenberger, 1950; Leuchtenberger and Schrader, 1951; Leuchtenberger et. al., 1952) did a quantitative estimation of nuclear DNA by the Caspersson ultraviolet method which utilizes a single wavelength (Caspersson, 1936). Following the technique of Pollister and Ris (1947), they found that maximum absorption of Feulgen stained DNA was at 546 nm. However, this method presents several pitfalls and at times is quite erroneous. The results of these investigations showed a constancy in the DNA content of each cell type despite considerable variance in nuclear volume. Thus, no correlation was apparent between nuclear volume and DNA content.

In contrast, this investigation utilized the two-wavelength method of Patau (1952) and Ornstein (1952) on an

acellular slime mold, D.iridis. This method allows for greater accuracy because it minimizes distributional error and eliminates the direct measurement of the nuclear area which is of considerable importance in the in the plug method of Caspersson (1936).. While the results according to the two-wavelength method show no correlation between nuclear volume and DNA content, there is variability in DNA content and relatively constant nuclear volume within each clone of myxamoeba and plasmodia. It is quite possible that such is the case in other cellular populations.

The variability in DNA content is seen upon examination of the DNA frequency histograms of the myxamoebae. Essentiallly this is attributed "to the high rate of mitotic activity readily demonstrated in this stage of the life cycle" (Yemma and Therrien, 1972). For example, in Figures 2, 3, and 4, the representative clones demonstrate a unimodal distribution of population DNA. This is characteristic of the myxomycetes for these cells demonstrate no appreciable G_1 interphase period, spend about 30 minutes in S phase, and 12 hours in G_2. Therefore, any investigation concerning these cells will almost always result in observing G_2 cells (Rusch, 1969). This appears to be the case in the clonal populations used. However, as it is observed in Figure 2, a population of polyploid cells does exist.. This is most prevelent in histogram B which represents the clone Pan 2-4. Thus, the mean DNA is slightly elevated (2.83). Such an unobvious occurence as this is easily detected by the use of microspectrophotometry. In Figure 3, histogram B, a large

population of polyploid cells is quite evident. This causes a shift to the right of the mean DNA value (4.28), which is common to this clone (Collins and Therrien, 1976) for it contains a large number of polyploid cells. This is, also, true in Figure 4, histogram B, which represents Hon 1-2. Here the mean value is approximately that of a diploid population when compared to the plasmodia resulting from a cross between Hon 1-2 and Pan 2-4 (Figure 5, histogram A). Such DNA variability provided a unique opportunity for testing a hypothesis concerning DNA content of cells and their volumes. The analysis of variance (Table I) done on the actual DNA values of the nine clones of D.iridis resulted in a highly significant F value (0.7917E 02) at the 0.05 probabilty level. Therefore, the nine clones under study not only demonstrate varying amounts of DNA, but also are not of the same population although they are of the same species.

In the plasmodia, which is the diploid stage of the life cycle, less variability in DNA content is generally noted. This can be attributed to the essentially synchronous division of nuclei during development of the plasmodia. However, there are still polyploid cells present (Figure 5, histogram B). Also, note that in the plasmodial stages represented in Figure 5, histograms B and C, many cells are in G_1, unreplicated 2C. This is fortunate for it readily demonstrates subtle changes in DNA as the plasmodial nuclei divide. Daughter cells are in G_1. As they proceed through S and through to G_2, it can easily be observed in histogram B. This, also, explains why the mean DNA values for B and

C are lower than A and similar to the represented clones in Figures 2, 3, and 4. They are G_2 nuclei representing replicated 1C DNA, whereas, the plasmodial nuclei in histograms B and C are G_1, unreplicated 2C. This makes these two crosses appear near equal in mean DNA content. Once again, this lends a unique opportunity for testing the previously mentioned hypothesis. The standard error was calculated along with the mean DNA contents for each plasmodia represented. These standard errors were low enough to indicate sampling of the same population. As in the myxamoebae, variabilty in the DNA content exists in the plasmodial stage.

Unlike the DNA content, nuclear volume is relatively constant. This is evident upon examining the small standard deviations of length and width for each clone and plasmodia in Table II. And graphically, it can be seen in Figures 6 through 9. While DNA content increases, nuclear volume remains relatively constant and is not dependent on the DNA content of the nucleus. Therefore, there is no relationship between DNA content and nuclear volume within the clones and plasmodia of D.iridis.

In summary, nuclear volume within a clone or plasmodia of D.iridis is not dependent upon DNA content. Polyploids and diploids have the same nuclear volume as the haploids. It is not possible to determine ploidy levels of D.iridis nuclei upon visual examination. Therefore, it can only be determined through such methods as microspectrophotometry.

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