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THE LIFE HISTORY OF *COLEOCHAETE SCUTATA* (CHLOROPHYCEAE) STUDIED BY A FEULGEN MICROSPECTROPHOTOMETRIC ANALYSIS OF THE DNA CYCLE[1, 2]

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SUMMARY

The life history of Coleochaete scutata *Bréb. was analyzed by Feulgen microspectrophotometry, a technique measuring DNA content in individual nuclei. By correlating nuclear DNA content with morphological structures or stages in the life history, changes in ploidy level are revealed.*

The microspectrophotometric study confirmed the earlier reports of a haploid vegetative thallus with mitotic division restricted primarily to the margin of the thallus. In the mitotic cycle the G_1 (pre-synthesis) phase is longer in duration than the synthesis and G_2 (post-synthesis) phases. Oogamous sexual reproduction results in resistant oospores which attain DNA levels of 2C–8C (1C being the DNA level of gamete nuclei).

Key index words: Chlorophyta; Coleochaete*; DNA; Feulgen staining; microspectrophotometry*

INTRODUCTION

The life history of *Coleochaete scutata* Brébisson was described by Strasburger in 1894 (24) as an example of an organism in which the sexual or haploid generation is the dominant stage with the diploid generation being represented only by the oospore. Allen (1) observed meiosis in the first 2 divisions of the oospore of *C. scutata*, thus supporting Strasburger's proposed life history. The techniques used by Strasburger and Allen in these studies (chromosome counting, observation of gamete fusion, observation of meiosis) are still the dominant techniques for elucidation of algal life histories. These techniques, however, are inadequate for many studies as algal chromosomes typically are very small and often stain poorly, making chromosome counts difficult. In other instances, wall or storage materials obscure the nuclei.

These problems led to the search for techniques which give life history information by other means. The appearance of synaptonemal complexes has been correlated with meiotic divisions (11,16). These complexes are used as evidence of meiosis in the life histories of red algae (10)—a group in which chromosome observations are especially difficult. The present study examines the technique of Feulgen microspectrophotometry as a useful tool in studying algal life histories. Feulgen microspectrophotometry reveals changes in the quantity of DNA (C level) during nuclear cycles rather than changes in chromosome number (N level) (12,31). Measurements can be made on nuclei at any stage in the mitotic or meiotic cycles and are particularly precise on interphase nuclei. With this technique, problems of discovering the timing of nuclear divisions and spreading of chromosomes are circumvented. By comparing

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DNA levels of cells in various morphological stages, the timing of changes in nuclear DNA quantity can be determined; hence, changes in ploidy level during the life history are revealed.

Feulgen microspectrophotometry has been used for many years as a technique for determining DNA content. In plant material, it was first used to quantitatively follow DNA changes during mitosis in *Allium* and *Rhoeo* root tips (18,19). Since then, this technique has been widely used in medical and experimental studies concerning variability of DNA quantity and is generally considered one of the most accurate estimates of DNA quantity (12,31). More recently, Feulgen microspectrophotometry has been successfully applied to life history studies. Bryant and Howard (2) used the technique to follow DNA changes during the life history of the oomycete *Saprolegnia terrestris* Cookson. Through this study, the concept of the life history of this fungus has been changed from that of zygotic meiosis to gametic. Feulgen microspectrophotmetry has also been used to identify the timing of meiosis in myxomycete life histories (29,32). An attempt to use this technique for algal life history studies, involved the determination that meiosis occurs in the Chantransia-stage of the red alga *Batrachospermum* (9).

Since the life history of *Coleochaete scutata* was one of the earliest investigated in the algae and since the pattern of zygotic meiosis as described by Allen (1) has been widely confirmed, this species was chosen as a test organism for the use of Feulgen microspectrophotometry in algal life history studies. The sensitivity of this technique to changes in DNA level in both the mitotic cycle and the life history of *C. scutata* is revealed in this study. Hopefully this will stimulate application of this technique to the study of algae in which the life history is unknown or in dispute.

MATERIALS AND METHODS

Coleochaete scutata was obtained from the Indiana University collection (LB610). Stock cultures were grown on 1 × 3 in glass slides in 8 oz culture jars containing soilwater medium (21). Material intended for microspectrophotometric study was inoculated into petri dishes containing soilwater and 22 mm #1 cover glasses. The transfer stimulated zoospore production and new colonies were established on the cover glasses, which were used to transfer the specimens through the fixing, hydrolysis, and staining solutions. Cultures were grown in controlled environment chambers at 20 C, with a 16:8 LD regime, at an intensity of 1–4 $\times 10^3$ erg·cm^{-2}·s.

Preparation for Feulgen microspectrophotometry was: a) fix in 3:1 95% ethanol:glacial acetic acid at room temperature; b) wash with running distilled water (*ca.* 3 min); c) hydrolyze in 1 N HCl at 60 C for 20 min; d) repeat b); e) stain for 2 h in dark at room temperature in Schiff's reagent (12); f) repeat b); g) destain in 3 changes of sulfurous bleach (12), 10 min each; h) repeat b); i) dehydrate with ethanol series (30, 50, 70, 95, 100%), 15 min each; j) transfer to xylene, 15 min; k) embed in Permount (Fisher Scientific Co.). The optimum hydrolysis time of 20 min was determined experimentally for *Coleochaete* and will vary from organism to organism.

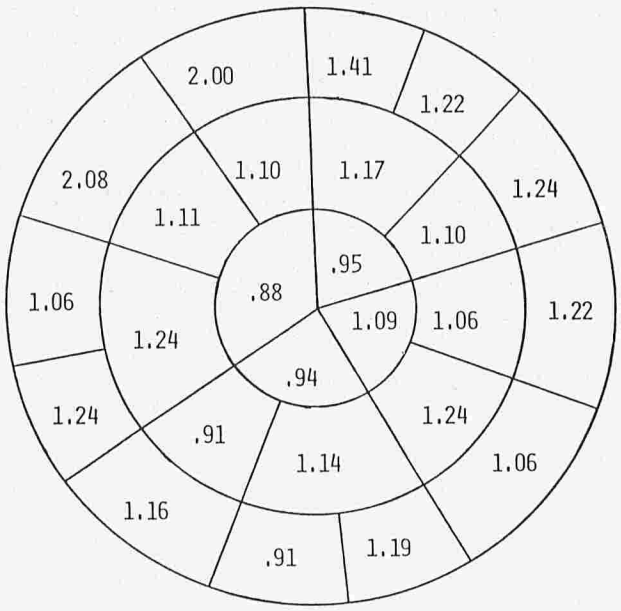

FIG. 1. Representation of microspectrophotometrically determined relative DNA levels for each nucleus of one thallus of *Coleochaete scutata*.

Quantitative DNA measurements were made using a Leitz Ortholux microscope equipped with: a) current regulated tungsten bulb; b) Zeiss monochrometer; c) Leitz MPV photometer; and, d) end window EMI photomultiplier. Voltage was regulated by an Aminco solid state photometer, also used as a read-out.

Relative DNA values were calculated using the 2 wave length method (17) and conversion tables prepared by Mendelsohn (15). By plotting absorbance against the wave length spectrum, the appropriate wave lengths to be used were determined experimentally. These were the wave lengths of maximum absorbance (565 nm) and 1/2 maximum (510 nm); wave lengths with extinction values standing in a 1:2 relationship.

The Feulgen procedure selectively stains for DNA—the quantitative relationship between stain intensity and DNA quantity being well established (12,19,31). The microspectrophotometer was used to determine the amount of specific wave lengths of light absorbed by a nucleus. This is then converted to a relative DNA amount using the Mendelsohn tables. It is possible to convert these figures to absolute values by comparing with a bull sperm standard (31). However, since our goal was to compare the DNA level in various stages of the life history, a conversion to absolute values was unnecessary. The above procedure results in a relative DNA value for each nucleus. These values can then be compared to reveal patterns of changes in ploidy level during the life history.

RESULTS

Figure 1 is a schematic representation of a *Coleochaete scutata* thallus indicating the nuclear DNA content of each cell as measured by Feulgen microspectrophotometry. Individual nuclei on this thallus have relative DNA values varying from .88 to 2.08. Figure 2 shows a thallus grown on a glass slide and stained by the Feulgen technique. The cells along the margin of the thallus are referred to as "marginal cells" and the interior cells are called "internal cells."

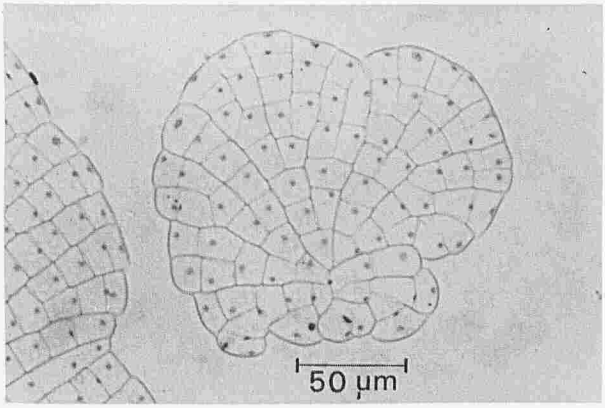

FIG. 2. Photomicrograph of a *Coleochaete scutata* thallus stained by the Feulgen technique. Each cell exhibits a single stained nucleus. × 250.

By determining DNA levels of a number of nuclei, a DNA profile for a population of cells can be plotted as a histogram. In Fig. 3–5, 7, the horizontal axis represents intervals of relative DNA values with the vertical axis giving the number of nuclei with DNA values in that interval. To facilitate comparisons of the internal vegetative cells with other specific cell types, the mean value for the internal cells was each time adjusted to 1.00 relative DNA units. Since cells compared in each histogram represent only cells processed in a single batch, multiplying by a constant adjusts the values but does not affect their relative position.

In Fig. 3, DNA levels of nuclei of internal cells are compared with those of the marginal cells. Nuclei of 126 internal cells were measured and values fell into a normal curve around the statistical mean of 1.00. The marginal cells also show a peak near 1.00, with a second lower peak near 2.00. The marginal cells appear to undergo DNA synthesis frequently; however, during most of their mitotic cycle, they stay at the lower DNA level. The lower or pre-synthesis DNA level will be referred to as the G_1 phase; and the higher or post-synthesis DNA level, the G_2 phase of the mitotic cycle. G_1 and G_2 refer, respectively, to the time gaps preceding or following DNA synthesis (S), and D refers to the division or mitosis stage (26). By observing DNA level changes of marginal cells during the mitotic cycle, the G_1- and G_2-phase DNA levels become evident (Fig. 4). Nuclei in pre-synthesis stages (telophase and anaphase) have mean DNA values of .88 whereas nuclei in post-synthesis stages (late interphase and prophase) have mean DNA values of 1.91. A comparison of these to DNA levels in internal vegetative cells gives further evidence that the internal vegetative cells are at the G_1 phase of the mitotic cycle.

To determine if the vegetative cells represent the haploid or diploid generations, the vegetative cells were compared to DNA levels in male gametes (Fig.

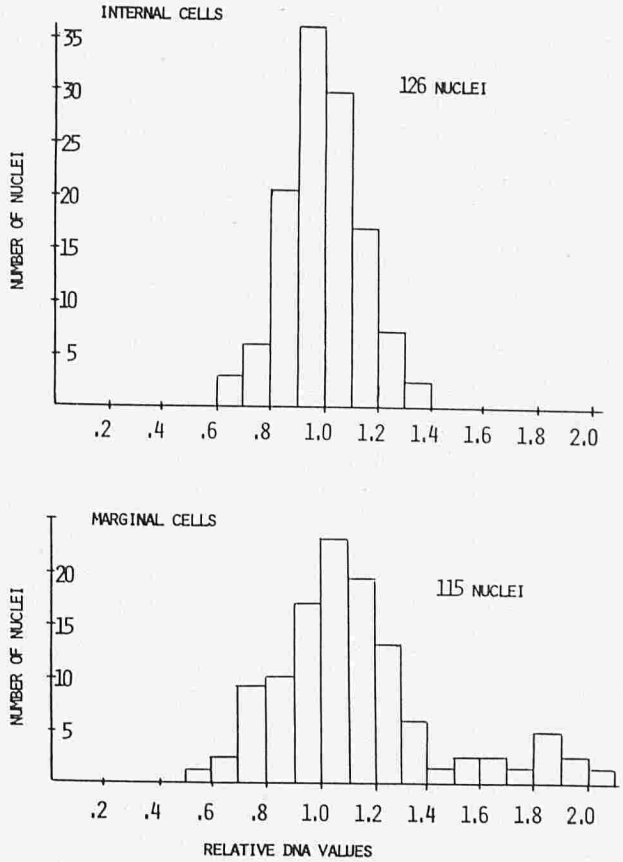

FIG. 3. Comparison of the frequency distribution of relative DNA values of nuclei of internal vegetative cells versus nuclei of cells on the margin of the same 8 thalli.

5). The 1C DNA level or haploid DNA level for a species is defined as the DNA level of the gametes (26). C levels refer to DNA quantity whereas N levels refer to chromosome number (26). Male gametes of *C. scutata* are produced by subdivision of internal cells and can be readily identified in a sexually mature thallus (Fig. 6). Nuclei of 33 male gametes were measured and a mean relative DNA level of 1.09 was determined. A comparison of this with the vegetative cells indicates the haploid nature of the thallus. The computed t statistic for the mean DNA value of male gametes versus internal cells is 2.25, and at the 2% significance level, the hypothesis of equal means may be accepted.

In Fig. 7, DNA levels of oospores located internally on thalli were plotted and compared to internal haploid vegetative cells. DNA levels in the 40 post-fertilization oospores measured varied from 1.81 to 8.33 which corresponds approximately to a 2C–8C range. Following division of the oospore nucleus, structures containing 16–32 nuclei were observed. Due to background interference, measurements could not be made on these.

Table 1 summarizes the DNA data obtained from

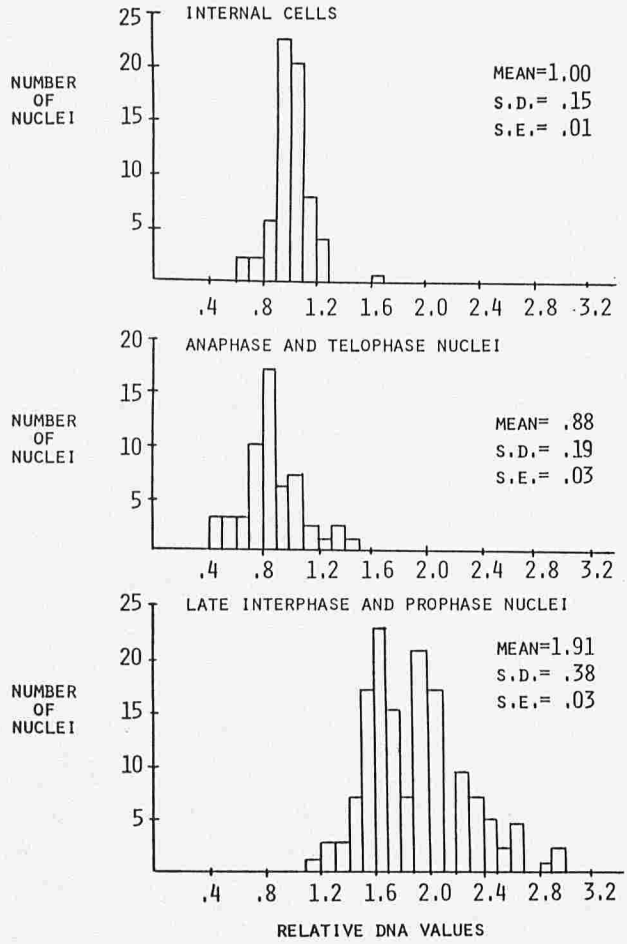

FIG. 4. Comparison of nuclear DNA profiles in internal vegetative cells at interphase (top); marginal cells at anaphase or telophase (middle); and, marginal cells in late interphase or prophase (bottom).

microspectrophotometric measurements of approximately 458 nuclei of *C. scutata* cells. Mean values, standard deviations, and standard errors for various nuclear stages are included where appropriate.

DISCUSSION

In *Coleochaete scutata* the vegetative thallus is composed of 2 areas which are distinctive with regard

TABLE 1. *Relative DNA Levels in* Coleochaete scutata *as determined by Feulgen Microspectrophotometry.*

Cell type	Number measured	Mean	SD	SE
Vegetative cells				
internal cells	126	1.00	.15	.01
anaphase-telophase	52	.88	.19	.03
late interphase-prophase	150	1.91	.38	.03
Male gametes	33	1.09	.18	.02
Oospores	40	(range 1.81–8.33)		

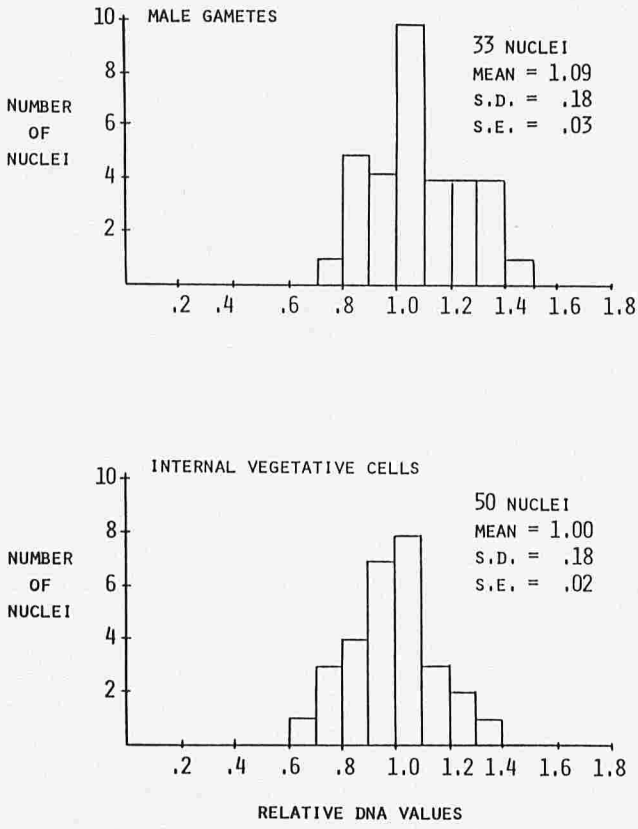

FIG. 5. Comparison of DNA profiles of male gametes and internal vegetative cells.

to DNA levels. The internal cells have a constant DNA level whereas the marginal cells undergo changes in DNA level characteristic of mitotic cycles.

The internal cells of the thalli, when measured microspectrophotometrically, exhibit a normal distribution of DNA values (Fig. 3). The mode, mean and median are all *ca.* 1.00, and the frequency distribution is highly symmetrical about this point. Swift (28) observed that DNA levels of nuclei of a population of "uniform" cells in mouse, rather than

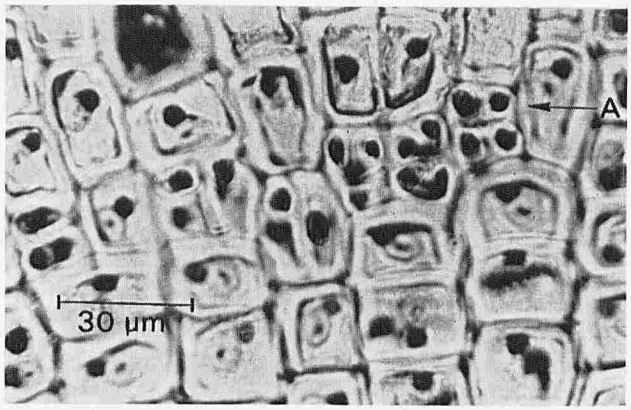

FIG. 6. Photomicrograph of male gametes prior to their release from the antheridia (*A*). ×750.

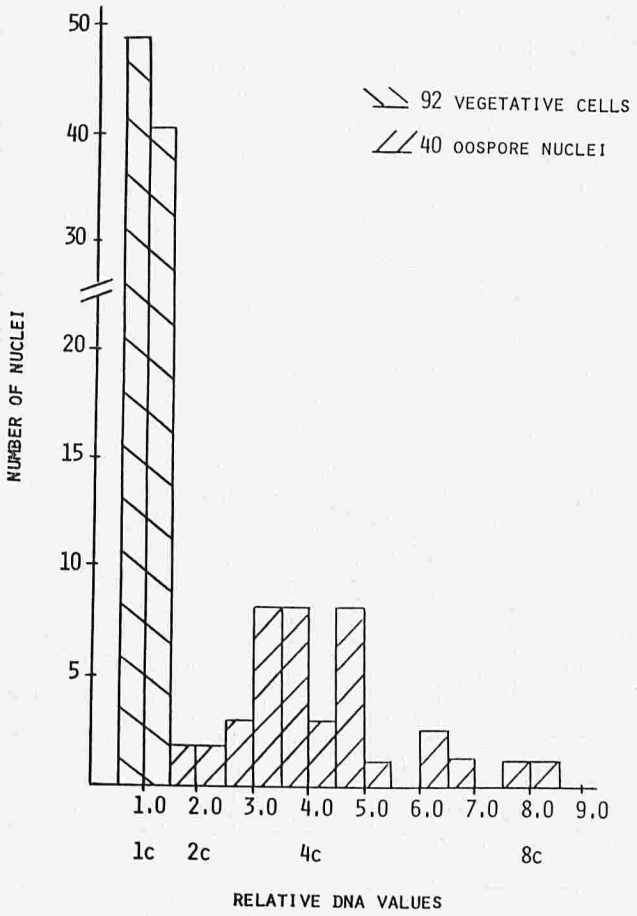

FIG. 7. Comparison of nuclear DNA profiles of internal vegetative cells and oospores.

giving a single DNA value, give values that form a normal curve around a mean and vary up to 15% from the mean. This phenomenon has been supported in similar studies on a variety of biological material (2,7,14,20). In instances such as these which exhibit a normal curve with a low standard deviation, it is assumed that the mean DNA value represents the C level for that population of cells.

In contrast to the internal area, the marginal area includes cells undergoing DNA synthesis and mitosis with the resulting asymmetry in the DNA profile (Fig. 3). In the marginal area, cells were observed in all stages of the mitotic cycle. Daughter nuclei in anaphase or telophase were observed to have a corrected mean DNA level of .88 (Fig. 4). A comparison of these to the interphase nuclei (1.00), indicates that these 2 groups of cells have DNA values at the same C level. Although the means are not identical, they are clearly closer to identical than to the interphase cells representing twice the DNA level of the daughter nuclei, or 1.76. The lower mean DNA level of the anaphase-telophase nuclei may also be explained by the widely observed phenomenon of lower Feulgen DNA levels in cells with more compacted nuclei (5,6,25). The similarity of DNA profiles between the internal cell nuclei and the anaphase-telophase nuclei of the marginal cells indicates that the internal cells are at the G_1 phase in the mitotic cycle. Marginal cells with large nuclei, interpreted as late interphase or early prophase nuclei, gave DNA levels that were noticeably higher than the level of the internal cells (Fig. 4). Most of these cells have relative DNA levels in the 1.5–2.4 range, with a mean of 1.91.

The vegetative phase of *C. scutata* is dominated by cells with nuclei in the G_1 or pre-synthesis phase of the mitotic cycle. Cells of the internal area are suspended at this stage but apparently have the potential to undergo mitosis when sexual reproduction is induced or when neighboring cells are destroyed, indicating some contact pressure control (13). In the marginal area, approximately 85% of the cells in this actively growing region were in the G_1 phase. This indicates that in *C. scutata* the synthesis (S) and G_2 phases comprise a much shorter part of the mitotic cycle than the G_1 phase.

To determine whether the cells of the vegetative thallus represent the haploid or diploid generation they must be compared to the DNA levels of gametes, which define the 1C level (26). Figure 5 compares the DNA profiles of internal vegetative cells in which the nucleus is at the G_1 phase, i.e., the lower DNA level of the mitotic cycle, with male gametes on the same thalli. The male gametes have a mean value of 1.09 and the internal vegetative cells, 1.00. The similarity of the DNA levels of the 2 populations shows that the vegetative thallus represents a haploid generation. If the vegetative cells were diploid and the cells were suspended at the G_1 phase, the mean would approximate twice the male gamete mean and be about 2.18.

The t test may be used to evaluate the interpretation given regarding DNA levels of the various cell types, since this test compares the means of populations exhibiting normal distributions (3). The hypothesis to be tested is that the means of the 2 populations are equal. A significance level of .02 was used for the test. The computed t statistic for the mean DNA value of the male gametes versus that of the internal cells is 2.25; therefore the hypothesis of equal means for the 2 populations may not be rejected at the 2% significance level. This supports the description of the internal cells as having 1C DNA levels.

The oospores, as resistant structures with thick walls and abundant starch reserves, were difficult to analyze since the nuclei were always partially obscured. To offset the loss of precision due to interference, multiple measurements were made on each of the 40 nuclei and the resulting DNA values were averaged. Values between 1.81 and 8.33 were observed; these represent DNA levels in the oospores

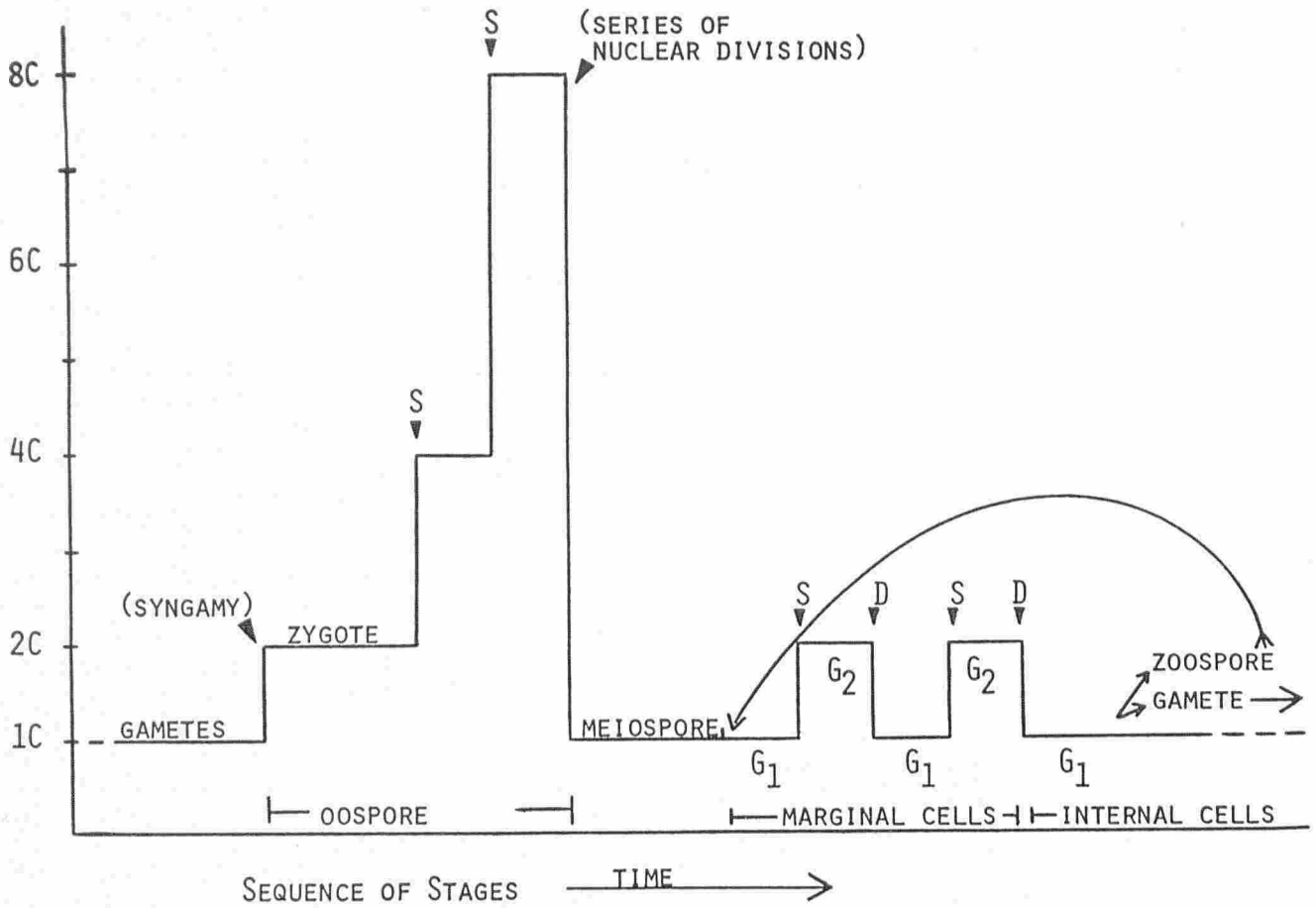

FIG. 8. Representation of changes in DNA content of nuclei during the life history of *Coleochaete scutata* as revealed by Feulgen microspectrophotometry. Stages in the mitotic cycle are represented by G_1 (pre-synthesis gap); S (synthesis); G_2 (post-synthesis gap); and, D (mitosis).

of 2C, 4C, and 8C in addition to the intermediate levels, indicating DNA synthesis. Following the life history as described by Allen (1) and Fritsch (4), we should expect to find DNA levels of 2C representing the zygotic fusion nucleus, and 4C representing the nucleus following synthesis and prepared for meiosis. These earlier observers felt the oospore nucleus was the meiotic center and that its first division was meiotic. The reports by Fritsch (4) and Smith (23), and confirmed in this study, of 8–32 meiospores as products indicate more divisions than a single meiotic one, and do not eliminate the possibility of high C levels in the oospore nucleus.

Interpretation of the 8C DNA levels can proceed in 2 ways. The first would be that these higher values represent errors in measurement and therefore should be disregarded. To accept this position would be to reject measurements of 17 of 40 oospores or nearly 40% of the sample. Granting the added problems of obtaining good measurements on oospore nuclei, this still seems highly unjustified in light of the general wide respect for Feulgen microspectrophotometry and the preceding evidence for accuracy in this *Coleochaete* study. The second possible interpretation is that prior to meiosis, the oospore nucleus undergoes replication of DNA in excess of that of the normal pre-meiotic replication. In fact, bias in these measurements is, if anything, against oospore nuclei with higher DNA levels. As the oospores mature, the nuclei become more and more obscured until microspectrophotometric measurements are impossible in a few instances. Therefore the 8C nuclei appear a reality and not an artifact.

With the exception of Dasycladales (22), the authors have been unable to uncover other reports in the algae of DNA levels in excess of 4C. The lack of reports of high C levels in other algae could reflect the non-existence of this condition, but more likely reflects the lack of easy means of observing the chromosomal story in the algae. Microspectrophotometric studies may uncover other instances in the algae of DNA in excess of the predicted values as has been true in other groups of organisms (2,8,18,27). Since the oospore is the overwintering stage which undergoes nuclear division in the spring to produce 16–32 meiospores, the colonizing units

(1,30); the high DNA levels in the oospore may be competitively advantageous to *Coleochaete* in permitting rapid production of more than 4 propagules without delays of intervening DNA synthesis when conditions are right for establishment of new colonies. Due to the abundance of interfering materials, we were unable to get microspectrophotometric measurements of the meiospore nuclei.

Figure 8 summarizes the changes in nuclear DNA level during the life history of *C. scutata* as revealed by this microspectrophotometric study. The vegetative phase is haploid with most nuclei at the G_1 phase of the mitotic cycle. Oogamous sexual reproduction results in resistant oospores which have DNA levels from 2C to 8C. Nuclear division results in 16 to 32 meiospores. Transformation of these into the first cell of the vegetative thallus without nuclear division suggests that Allen's (1) interpretation of the haploid nature of these cells is valid.

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