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The use of fluorescence staining to study nucleus development in the multinucleate dasycladalean green algae

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Numbers and sizes of nuclei were established for vegetative and reproductive regions of the large cells of the three multinucleate siphonous algae, *Cymopolia van bossei*, *Halicoryne wrightii* and *Bornetella sphaerica* (Chlorophyceae, Dasycladales). Nuclei were treated with 4, 6 diamidino-2-phenylindole (DAPI) which, when combined with DNA, fluoresces under ultraviolet light. All species were characterized by having a variety of size classes of nuclei in the central siphons, and single large nuclei which migrate from the siphon into the putative gametangia where, through subsequent divisions, 1000's of small gametic nuclei are formed. DAPI staining is a useful tool to study nuclei in a variety of cell types, one of the most unusual of which is illustrated by the multinucleate members of the Dasycladales.

Key Index Words: *DAPI-staining*; *Dasycladales*; *fluorescence staining*; *nucleus development*; *siphonous green algae.*

Nucleus development in association with thallus differentiation and sexual reproduction in members of the Dasycladales has been studied in *Acetabularia* (BERGER *et al.* 1975, GREEN 1975, KOOP 1979 and SPRING *et al.* 1978), *Batophora* (LIDDLE *et al.* 1976) and *Cymopolia* (LIDDLE *et al.* 1982). However, even in these genera meaningful details are lacking. In the uninucleate *Acetabularia* and *Batophora* different stages of nuclear development have been described from light and electron microscopic observations (BERGER *et al.* 1975 and LIDDLE *et al.* 1976) and critical stages which affect the ploidy of nuclei such as mitosis and meiosis have been ovserved. Recently the site of meiosis has bee verified in *Acetabularia* by electron microscopy, genetic analysis and direct observations (KOOP 1979). In *Batophora* synaptonemal complexes were observed in the mature primary nuclei shortly before they divided to form the smaller, morphologically simpler secondary nuclei (LIDDLE *et al.* 1976).

The multinucleate members of the Dasycladales have been studied very little. Certain features of nucleus development have been reported for *Cymopolia barbata* (LIDDLE *et al.* 1982, WERZ 1953) but mitosis and meiosis are still not understood in this or any of the multinucleate genera. The siphonous structure and complex morphology of these organisms offer unique mechanical problems of distribution, localization, development and division of nuclei. In *Cymopolia* the heterogeneous population of nuclei in the large central siphon includes characteristic nuclei that migrate into lateral branches to localize in the putative gametangia (LIDDLE *et al.* 1982). One nucleus is established in each gametangium.

Electron microscopy has been an inefficient tool to study siphonous cells, which can reach lengths up to 26 cm., because of their large central vacuoles, the relatively small amount of cytoplasm and the vast amount

of cell wall material that defines several, often lime-encrusted, compartments of the thallus. Staining with DNA-specific fluorescent dyes is a useful technique that can be applied to study nuclei during differentiation (COLEMAN 1982). Stained nuclei in cytoplasm which has been expressed from the central siphon and other structures of the thalli of giant unicellular green algae can be readily counted to estimate their population sizes. Also, the sizes of individual nuclei and the intensity of their fluorescence as a function of the amount of DNA per nucleus can be measured accurately. The purpose of this preliminary study is to assess nucleus development in several genera of the Dasycladales in order to understand the basic processes which nuclei undergo during cellular differentiation.

Materials and Methods

Cymopolia van bossei SOLMS (Fig. 1) was collected from Amami-oshima and Okinawa

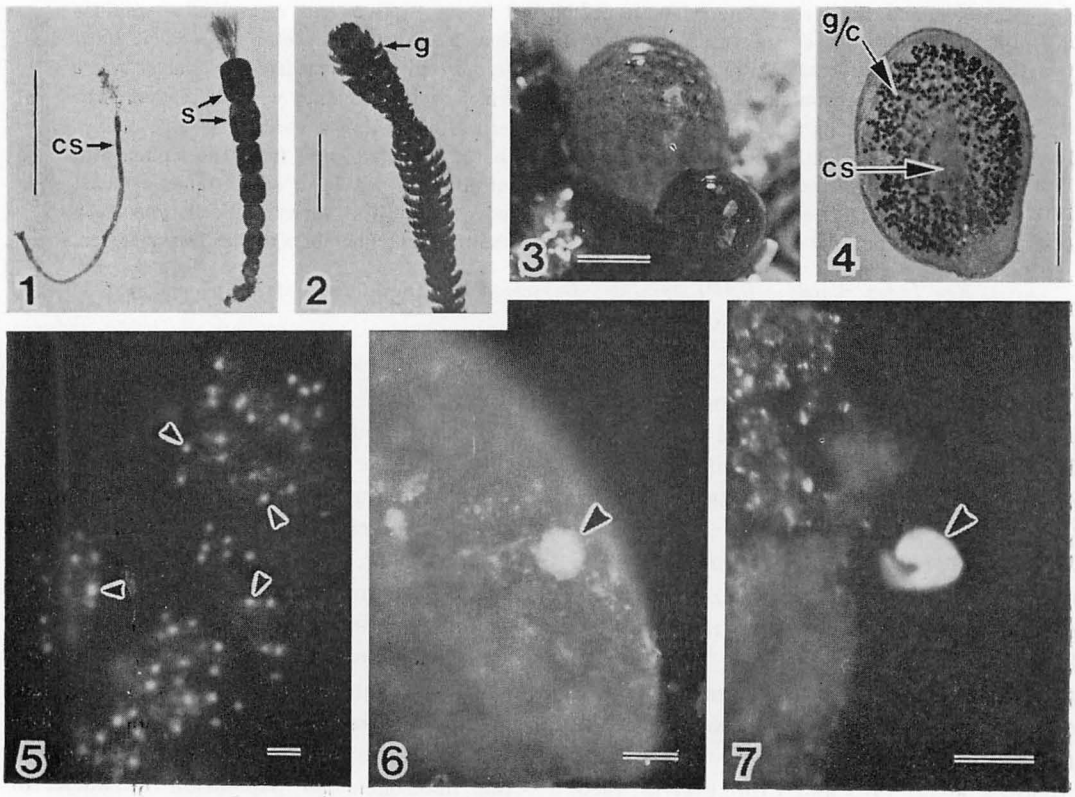


Fig. 1. *Cymopolia van bossei* SOLMS. Whole plant. Left: central siphon (c. s.) with segments (s) stripped off. Right: intact plant. Scale=1 cm.

Fig. 2. *Halicoryne wrightii* HARV. Upper part of whole plant. Lateral gametangia (g) can be removed to bare central siphon. Scale=1 cm.

Figs. 3 and 4. *Bornetella sphaerica* SOLMS. Whole plants. Fig. 3. Scale=0.5 cm. Fig. 4. Mid-longitudinal section. c. s.=central siphon, g/c=Gametangia with cysts. Scale=0.5 cm.

Fig. 5. Cytoplasm from central siphon of *C. van bossei*. DAPI-stained nuclei (arrows). Scale =10 μm.

Fig. 6. Young gametangium of *C. van bossei*. DAPI-stained single nucleus (arrow). Scale= 10 μm.

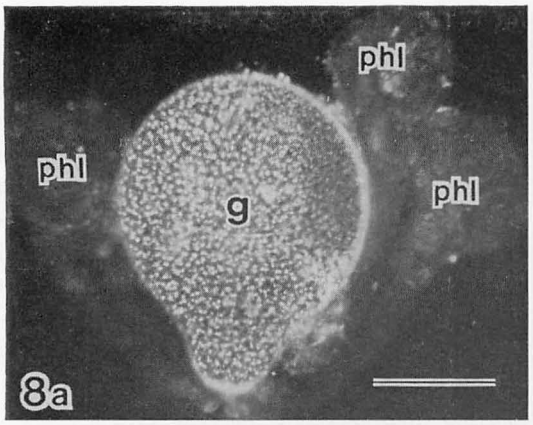
Fig. 7. Cytoplasm of young gametangium of *C. van bossei*. Single nucleus (arrow). Scale= 10 μm.

Islands throughout the year. *Halicoryne wrightii* HARVEY (Fig. 2) and *Bornetella sphaerica* SOLMS (Figs. 3, 4) were collected or sent sporadically from both islands. Plants were maintained in sterile seawater at 24°C under a 14 : 10 LD cycle with ca. 2000 lux illumination from cool white fluorescent bulbs or in an aerated aquarium at room temperature (20-25°C) under constant cool white fluorescent illumination of ca. 1500 lux.

Central siphons, cysts and gametangia were isolated from healthy thalli by gently stripping off the whorls of laterals (Fig. 1). The cytoplasm was pressed out from the cut ends of fragments or whole siphons into a drop of seawater. Cysts and gametangia were washed repeatedly to remove lime and cell wall debris. If they were calcified as in *Halicoryne*, the cysts were rapidly rinsed in sterile seawater acidified with acetic acide to pH 5.7 in order to dissolve heavy calcification without damage. Cytoplasm or whole

Table 1. Fluorescens of nuclei of *Cymopolia*, *Halicoryne* and *Bornetella* in fluorescence units (F.U.).

Source of nuclei	No. of thalli	Mean size of nucleus (μm in dia.)	Mean no. of nuclei	F.U. (Mean)	F.U. (Range)
Cymopolia van bossei					
Central siphon	20		252		
Apical	16	10.0	25	3.9	3.0-5.0
		5.0	64	2.4	2.0-3.1
		2.5	34	1.8	1.5-2.0
Middle	20	10.0	9	3.9	3.0-5.0
		5.0	28	2.4	2.0-3.1
		2.5	71	1.8	1.5-2.0
Base	15	5.0	6	2.4	2.0-3.1
		2.5	15	1.8	1.5-2.1
		1.75	3	1.6	1.5-1.8
Gametangia					
Young	20	10.0	1	3.6	3.0-4.6
Intermediate	10	5.0	112	2.5	2.1-3.1
		2.5	300	1.7	1.5-2.2
Mature	15	1.25	1180	1.1	0.9-1.2
Halicoryne wrightii					
Central siphon	7	10.0	20	3.7	2.9-4.6
		7.0	192	3.2	2.8-4.0
Gametangia					
Young	10	12.5	1	4.0	3.6-4.1
Mature	9	1.25	2000	1.0	0.9-1.2
Bornetella sphaerica					
Central siphon	10	10.0	10	3.8	3.0-5.0
		5.0	72	2.5	2.1-3.0
		3.5	20	2.0	1.7-2.2
		2.5	90	1.7	1.4-1.5
Gametangia					
Young	0				
Intermediate	15	5.0	240	2.6	2.1-2.9
Mature	8	1.25	1800	1.0	0.8-1.1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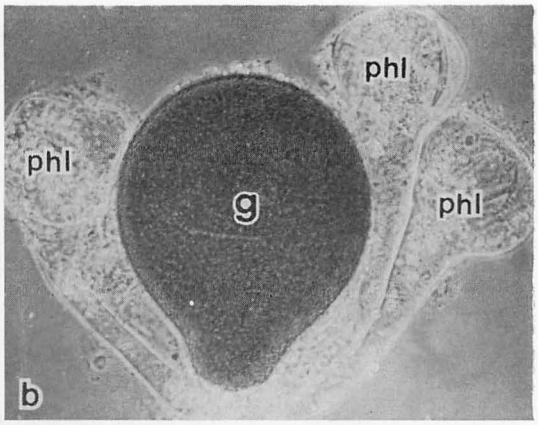
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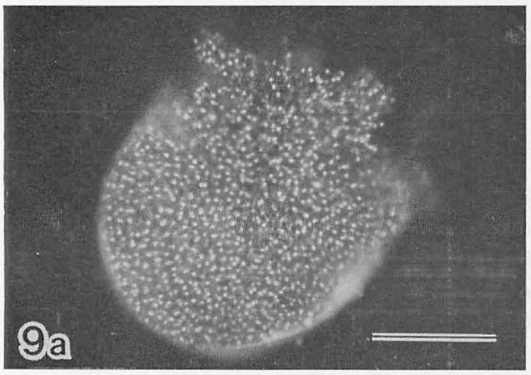
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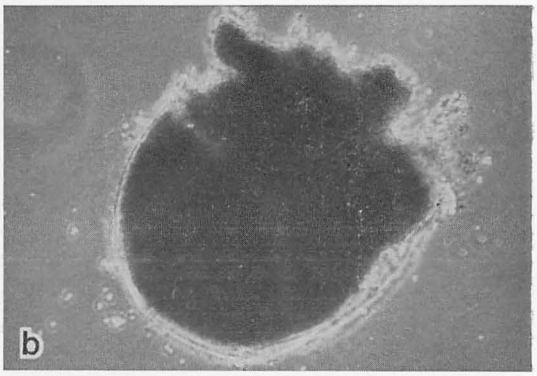
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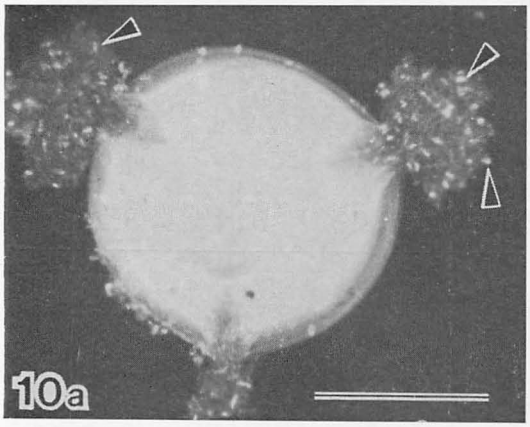
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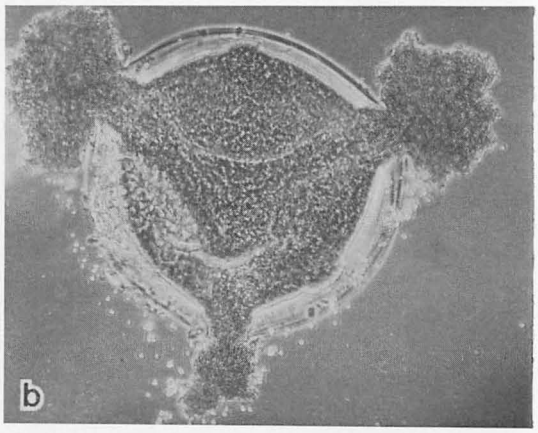
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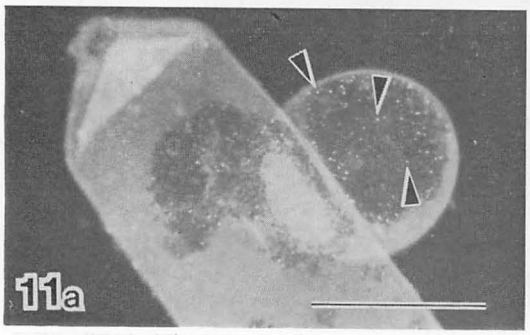
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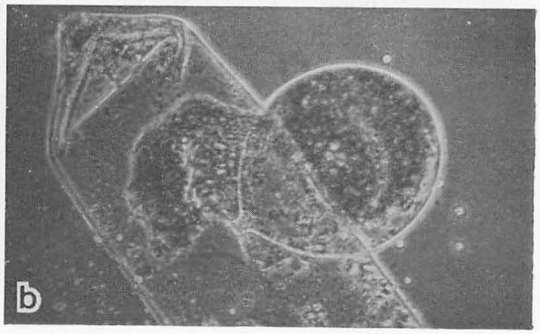
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cysts and gametangia in seawater were stained with an equal volume of 50 μg/ml DAPI (4, 6 diamidino-2-phenylindole) in S buffer (COLEMAN 1982) to a final concentration of 25 μg/ml. Slightly higher or lower concentrations of DAPI did not affect the fluorescence characteristics of the nuclei. Acid and alcohol-cleaned coverslips were used to contain suspensions of stained cytoplasm on clean glass slides or a haemocytometer.

The preparations were observed by using a Zeiss photomicrosope equipped with phase optics and an epi-illumination system which emitted an excitation beam of approximately 360 nm from an HBO 100 W Hg lamp. Fluorescence was measured by a Zeiss microspectrophotometer system which included a Hamamatsu Photomultiplier attached to a Zonax computer and printer. A range of sizes of measuring diaphragms permitted rapid measurement of fluorescence from various types of nuclei. Comparisons were frequently made with the fluorescence of healthy cultures of *Chlamydomonas sp.* To check for irregularities that might be caused by differences in stain concentration or other factors. Fluorescene of the background nearby the nuclei was subtracted from nucleus reading which were taken immediately after being exposed to excitation illumination.

Photographs were made from Kodak Tri-X using a Zeiss M35 motordriven camera with a Zeiss MC63 automatic exposure meter.

Results

Nuclei of specific size classes were localized in the various compartments of the thallus (Table 1). They were distributed throughout the central siphon of *Cymopolia van bossei* with a decrease in numbers from the apex to the base. Also the size classes of nuclei were differentially distributed. The apical region contained a higher proportion of 10 μm dia. nuclei than the middle and basal regions which contained a higher proportion of 5 and 2.5 μm dia. nuclei (Fig. 5). A single nucleus in a gametangium was also 10 μm in diameter (Figs. 6, 7). After division and just prior to gamete liberation the numerous gametangial nuclei were 1.25 μm dia. (Figs. 8a, 9a). Intermediate stages of gametangial development (Figs. 10, 11) contained nuclei of larger sizes. Each successive division of the original single gametangia nucleus resulted in a smaller product. At any point in gametangial development nuclei were markedly homogeneous in size. The fluorescent DNA content was correlated with nucleus size. Large 10 μm nuclei emitted ca. 4 fluorescent units and 1.25 μm nuclei ca. 1 fluorescent unit.

Nuclei of the central siphon of *Halicoryne wrightii* were also distributed in a decreasing apico-basal gradient although there were strikingly few in the cytoplasm of the basal one half. Sizes were also more homogeneous with two size classes, 7 μm and 10 μm dia., predominating. Mature gametangial nuclei were 1.3 μm dia. prior to gamete liberation (Table 1). The single progenitor nucleus of

Figs. 8a and b. Reproductive unit of *C. van bossei*. a. Fluorescence photograph; b. Phase contrast photograph. g=mature gametangium, phl=photosynthetic lateral, DAPI-stained nuclei are distributed throughout the gametangium. Scale=100 μm.

Figs. 9a and b. Cytoplasm of mature gametangium of *C. van bossei*. a. Fluorescent photograph. DAPI-stained nuclei are distributed throughout the cytoplasm; b. Phase contrast photograph. Scale=100 μm.

Figs. 10a and b. *H. wrightii*. Double-walled gametangium and cyst in intermediate stage. Cytoplasm being exuded. a. Fluorescence photograph. DAPI-stained nuclei (arrows); b. Phase contrast photograph. Scale 25 μm.

Figs. 11a and b. *B. sphaerica*. Young gametangium before cyst formation. a. Fluorescence photograph. DAPI-stained nuclei (arrow); b. Phase contrast photograph. DAPI-stained nuclei (arrows). Scale=50 μm.

cyts/gametangia was 12.5 μm in diameter. Approximately 2000 nuclei were formed in a mature gametangium. Similar patterns exist for *Bornetella sphaerica*, except, due to the short central siphon, it was not technically possible to make differential counts from the apex to the base. However, 2.5, 5 and 10 μm dia. nuclei were present in the cytoplasm with a lower percentage of 10 μm ones. A mature gametangium contained ca. 1800 nuclei 1.25 μm dia. (Fig. 6).

Nuclei in all species were usually oval to sphaerical although some *Cymopolia* apical regions contained decidedly spindle-shaped nuclei 2.5 μm long as well as other irregular shapes. Presumably these nuclei were undergoing synchronous division. Nuclei were in general sparsely distributed in the cytoplasm of the siphon or evenly spaced in the gametangia (Figs. 5, 8a, 9a, 10a, 11a). However, frequently in all three species nuclei were in clumps of 5-10 in the central siphon. These may have been post-division.

Discussion

The multinucleate species of the Dasycladales seem to conform to a general pattern of a limited variety of sizes of nuclei in the central siphon, large nuclei that migrate into putative gametangia and a standard (1.25 μm dia.) nucleus in the gametes. If measurements of fluorescence of DAPI-stained nuclei are extrapolated to represent amounts of DNA and further to ploidy of nuclei, the general pattern appears to be diploid and polyploid nuclei streaming throughout the siphon, a polyploid nucleus being transported to presumptive gametangia and haploid (by definition) nuclei being formed during gametogenesis. The variety of nucleus sizes can be interpreted as being stages in the formation of large (10-12 μm dia.) nuclei or self-replicating products of nuclei which were established early from the single zygote nucleus. It is clear that multinucleate species of the Dasycladales have mechanisms of nucleus continuity and distribution unique among all organisms. The usual dogma of nuclear and "cell" cycle does not interpret the patterns observed in the giant cells of *Cymopolia*, *Halicoryne* and *Bornetella*. Further investigations of nucleus development beginning with zygotes are required to characterize nuclear cycles.

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L. B. リドル*・堀　輝三: 緑藻類，カサノリ目の多核種における核分化の研究のための蛍光染色法の有用性**

緑藻，カサノリ目に属する種の多核管状種，*Cymopolia van bossei*, *Halicoryne wrightii*, *Bornetella sphaerica*, について，その栄養体および生殖器官にみられる核の数とサイズの変化を測定観察した。核の DNA を DAPI で染色し，紫外線励起蛍光を観察した。調査した全ての種で，中心管状部はいろいろなサイズの核を含む特徴がみられた。また，将来配偶子嚢になる部分には，中心管状部から移動した大きな核が存在する。それらは後に分裂して千個以上の小核になる。DAPI 染色法はいろいろな細胞タイプの核の研究に有用であり，そのことを最も特異な細胞タイプの一つであるカサノリ目の多核種で示した。(* ロングアイランド大学サウザンプトン・カレッジ，ニューヨーク，** 茨城県新治郡桜村天王台 1-1-1，筑波大学生物科学系)