

Ultrastructural studies on nuclear division during gametogenesis in *Caulerpa* (Chlorophyceae)*

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The ultrastructural features of mitosis in the latter stages of male gametangial differentiation in the siphonous green alga, *Caulerpa brachypus* HARVEY, is described. The mitotic spindle is acentric and completely closed. No spindle microtubule nucleating material is visible, either inside or outside the nuclear envelope. A typical equatorial plate is formed and the chromosome separation towards the opposite poles proceeds synchronously.

The separation of the chromosomes in early anaphase is promoted by the shortening of chromosomal microtubules, concomitant with the extension of the nuclear envelope along the division axis. At late anaphase the nucleus continues to elongate, followed by the sharp constriction of the nuclear envelope, resulting in the formation of a long interzonal spindle connecting two daughter nuclei. The interzonal spindle contains many interzonal microtubules.

Key Index Words: *Caulerpa*; Chlorophyceae; *coenocyte*; *gametogenesis*; *green alga*; *nuclear division*.

The ultrastructure of cell division during vegetative and reproductive differentiation in various green algae has received much attention (see PICKETT-HEAPS 1975, STEWART *et al.* 1975 for reviews), but until recently comparable studies of the coenocytic green algae have been rather meager (BURR and WEST 1970, MUGUHAL and GODWARD 1973, HUDSON and WAALAND 1976, SCOTT and BULLOCK 1976, McDONALD and PICKETT-HEAPS 1976, HORI and ENOMOTO 1978 a, b, c).

A study of mitotic ultrastructure in the coenocytes is important since it is now clear that the ultrastructure of cell division is a valid indicator of phylogenetic affinities among the green algae (PICKETT-HEAPS

1975, STEWART and MATTOX 1975). Comparison of mitosis in the siphonous forms with that of other green algae could help resolve the relationship of the coenocytic algae to the other lines of chlorophycean evolution. Thus, as part of a series of investigations of the mitotic ultrastructure in the siphonous green algae, mitosis in the coenocytic alga *Caulerpa* was studied.

Materials and Methods

Caulerpa brachypus HARVEY was collected at Nemoto, Chiba in May, 1977 and was maintained under the following conditions: material was placed in 200 ml culture dishes in plain, filtered seawater and the seawater was changed daily. Dishes were kept on a table in the laboratory. After 15 days, the cytoplasm began to differentiate into networks (Fig. 1). Blades were immediately

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fixed overnight in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.0) containing 0.25 M sucrose. Next, the material was rinsed in buffer solution for 4 h, and at this time the sucrose content was gradually decreased in one-fifth step reductions by successive changes of the buffer. After this the blades were post-fixed for 20 h in 2% OsO₄. All steps were performed in a cold chamber (1–3°C).

Cultured materials of *C. racemosa* W. v. BOSSE var. *laete-virens* W. v. BOSSE were also preliminarily fixed for electron microscope observation (Figs. 12, 13). However the dividing telophase nuclei were observed only in the material fixed in 2% OsO₄ solution dissolved in seawater. This material was supplied by Dr. S. ENOMOTO (Kobe University), who originally collected it at Ayamaru-Misaki, Amami-Oshima Island, and maintained it in PROVASOLI'S Enriched Medium (McLachlan 1973) at 25°C and under a 16:8 h LD regime.

Observations

Gametes: Gamete formation takes place primarily in the blade portion of *Caulerpa brachypus*. As seen in Fig. 1, gametangial cytoplasm is characterized by a network formation and eventually produces biflagellated gametes. About a day before the release of gametes, the cytoplasm of the female

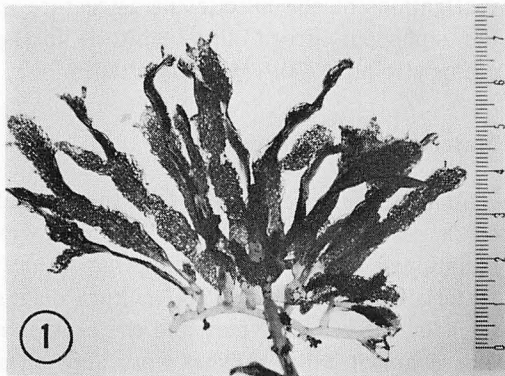


Fig. 1. Light micrograph of living specimens of *C. brachypus* showing the cytoplasm net-work which is differentiating to gametes.

plants changed to a greenish-yellow colour, in contrast to a lack of color change in the male plants. The female gametes produced are 6.0–8.5 μm long and 2.5–3.5 μm wide and have an eyespot (Fig. 2). The male gametes are 5.0–6.5 μm long and 2.0–2.5 μm wide and have no eye-spot (Fig. 3). These observations agree with those of MIYAKE and KUNIEDA (1937). The gametes of *C. brachypus* have cruciate flagellar roots with a 4–3–4–3 pattern (Fig. 4). As nuclei may undergo several successive divisions before gamete formation, the following account of mitosis is based on dividing nuclei in a small portion of the blade at the same stage of cytoplasmic differentiation.

Interphase: The interphase nuclei of *Caulerpa brachypus* are variously shaped, and usually contain a nucleolus which is composed of granular material. The chromatin material is not discernible, because of lack of electron density at interphase (Fig. 5). A single membrane-bound body, probably a microbody (ROTH and FRIEDMANN 1980), is usually present at the depression over the nuclear envelope (Fig. 6).

Prophase: As seen in Fig. 2, the prophase nucleus is spherical and gradually increases its volume, accompanying by the reduction of electron density in the nucleoplasm due to the disappearance of nuclear ribosomes. At this stage the intranuclear microtubules are not found, though cytoplasmic microtubules are still present in the cytoplasm (Fig. 5).

Metaphase: In the metaphase nucleus, the chromosomes line up at the equator and many microtubules connect the chromosomes and the poles (Fig. 8). The microtubules converge at the opposite poles in the nucleus and are attached or lie close to the inner surface of the nuclear envelope (Figs. 8–10). No special polar substance occurs here. No distinctly differentiated kinetochore is found on the chromosomes, but microtubules penetrate into a slightly less dense, homogeneous material in the granular chromosomal substance (Figs. 8, 9). During metaphase and anaphase, the spindle microtubules are never

observed to pass through the nuclear pores or nuclear envelope, the latter remaining completely intact during the nuclear division. Centrioles were not detected in the present material observed.

Anaphase-Telophase: At anaphase the nuclei begin to elongate and chromosomes migrate simultaneously towards the opposite poles (Fig. 9). Microtubules do not proliferate to any degree in the region between the separating sets of chromosomes as compared with the proliferation occurring between the chromosomes and poles (Fig. 9). At a later stage of division, the distance between the pole and chromosomes clearly decreases and many microtubules appear in the region between two separating sets of chromosomes (Fig. 11). It is noted that a significant extension of the nuclear envelope along the division axis occurs during this stage in contrast to the lack of a decrease of nuclear dimensions perpendicular to the long axis of division (Fig. 15).

An extension of the nuclear envelope towards the opposite poles appears to be important in the separation of chromosomes. After the chromosomes draw near to the opposite poles, the nucleus changes its form dramatically. The nuclear envelope of the two daughter nuclei becomes constricted and the long narrow cylindrical interzonal spindle develops between them (Figs. 12, 13). The interzonal spindle contains numerous long microtubules (Fig. 14). The last stage of abscission of the reforming daughter nuclei from the interzonal spindle is made by the sharp constriction of the inner nuclear membrane concomitant with the late synthesis of the outer membrane (Fig. 13). Information from the electron micrographs is schematically presented in Fig. 15.

Discussion

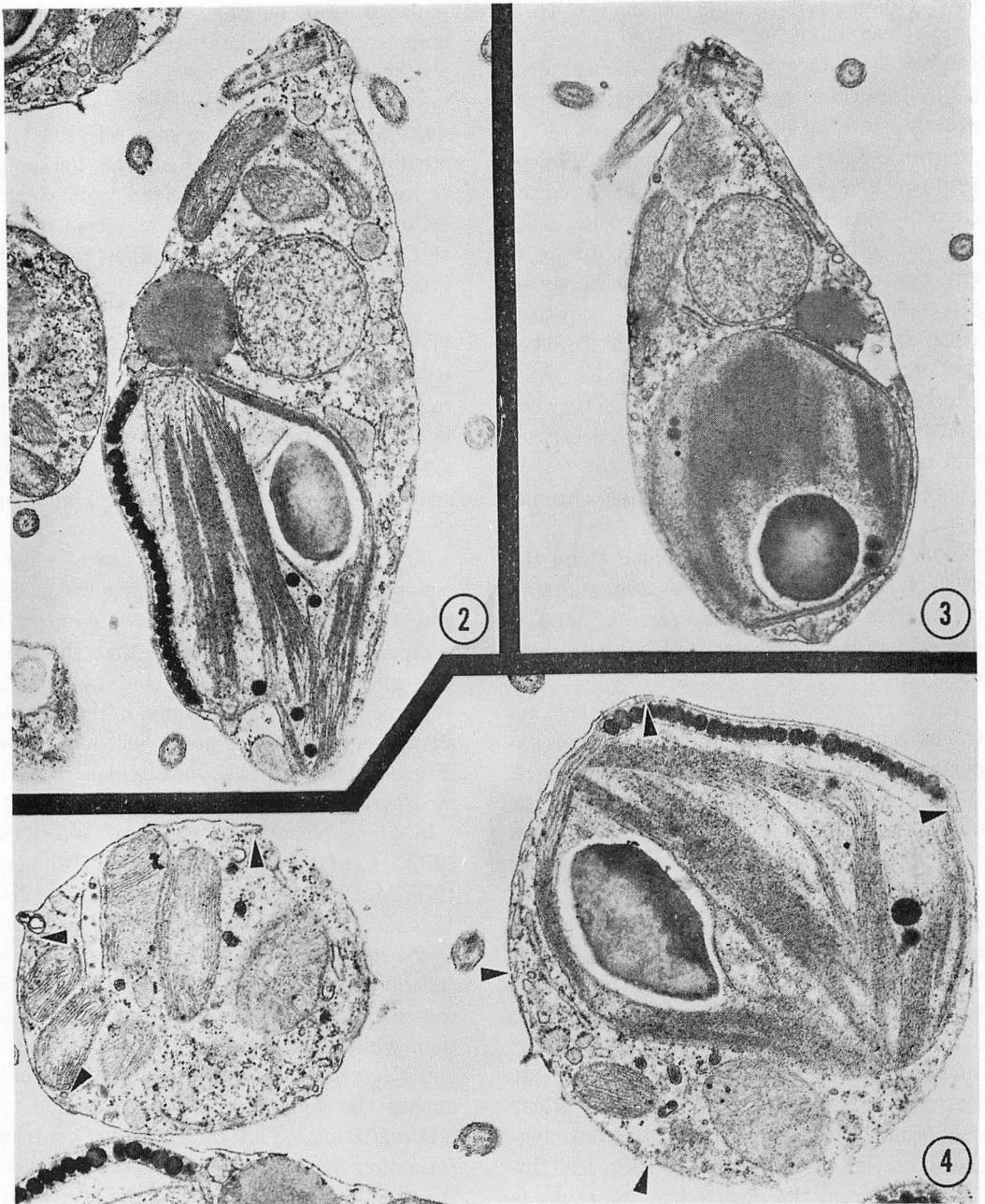
Almost all stages of nuclear division, exclusive of the latest telophase, were observed in nearly all sections cut from an EM block containing a small piece of blade where all areas of cytoplasm was differentiating into

gametes (Fig. 1). The development of remainder of the thallus fixed for the present observation was followed with the light microscope, and it was noted that male biflagellated gametes were later released from other living blades on the same horizontal rhizome from which the fixed blade arose.

Comparison of the mitotic ultrastructure of *Caulerpa* with that of other siphonous green algae so far investigated shows some interesting features characteristic of *Caulerpa*: 1) the absence of centrioles at the spindle pole, at least until the later stage of gametogenesis, 2) no appearance of visual spindle microtubule nucleating material either inside or outside the nucleus, and 3) the total complete retention of the nuclear envelope throughout nuclear division.

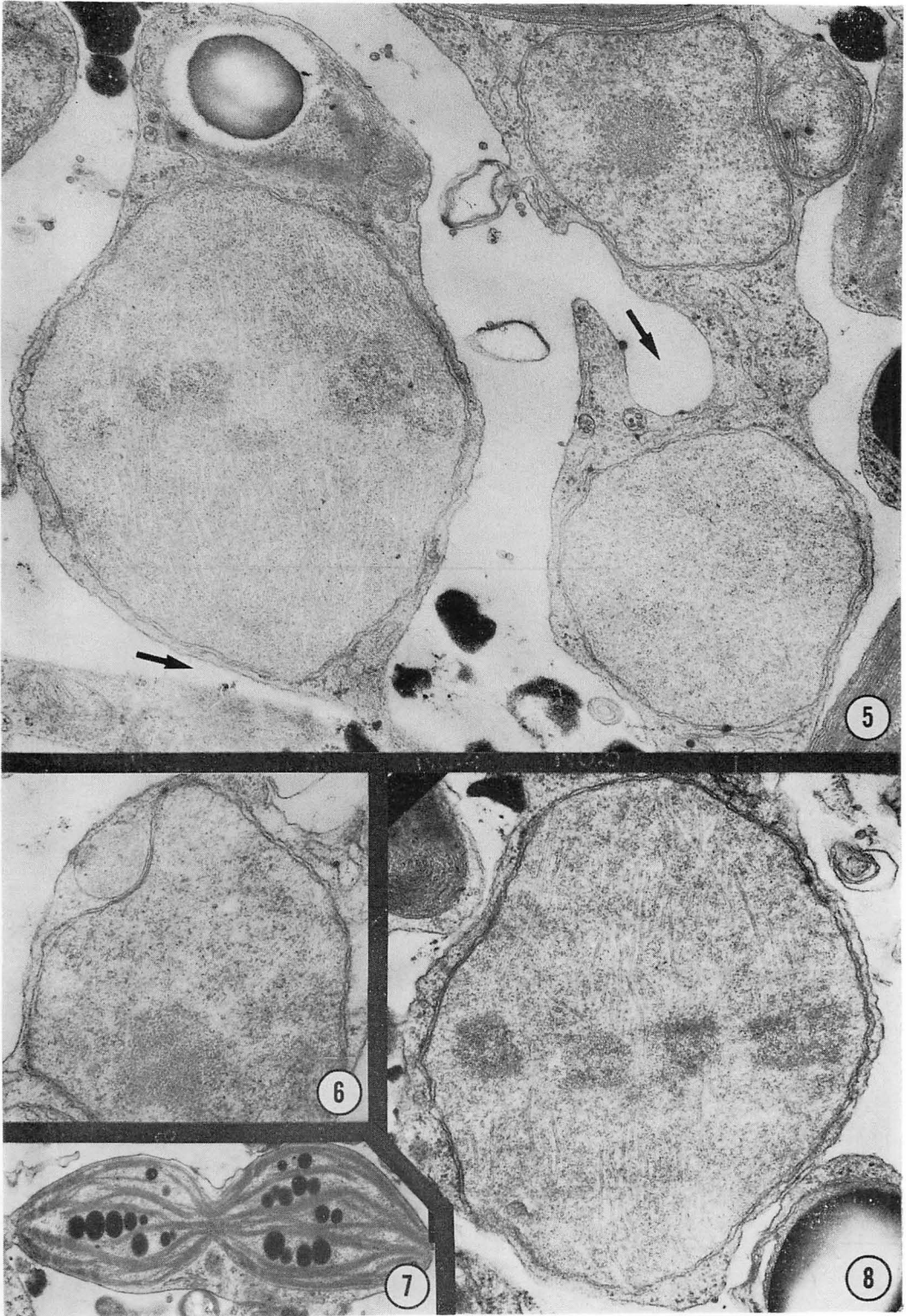
The great variation in the size of chloroplasts found in the differentiating cytoplasm, and the formation of a few number of papilla on the blade suggest that the blade was in a rather early stage of gamete development. As cytoplasmic differentiation advances, the chloroplasts divide many times (Fig. 7) into smaller ones which will be incorporated in the forming gametes.

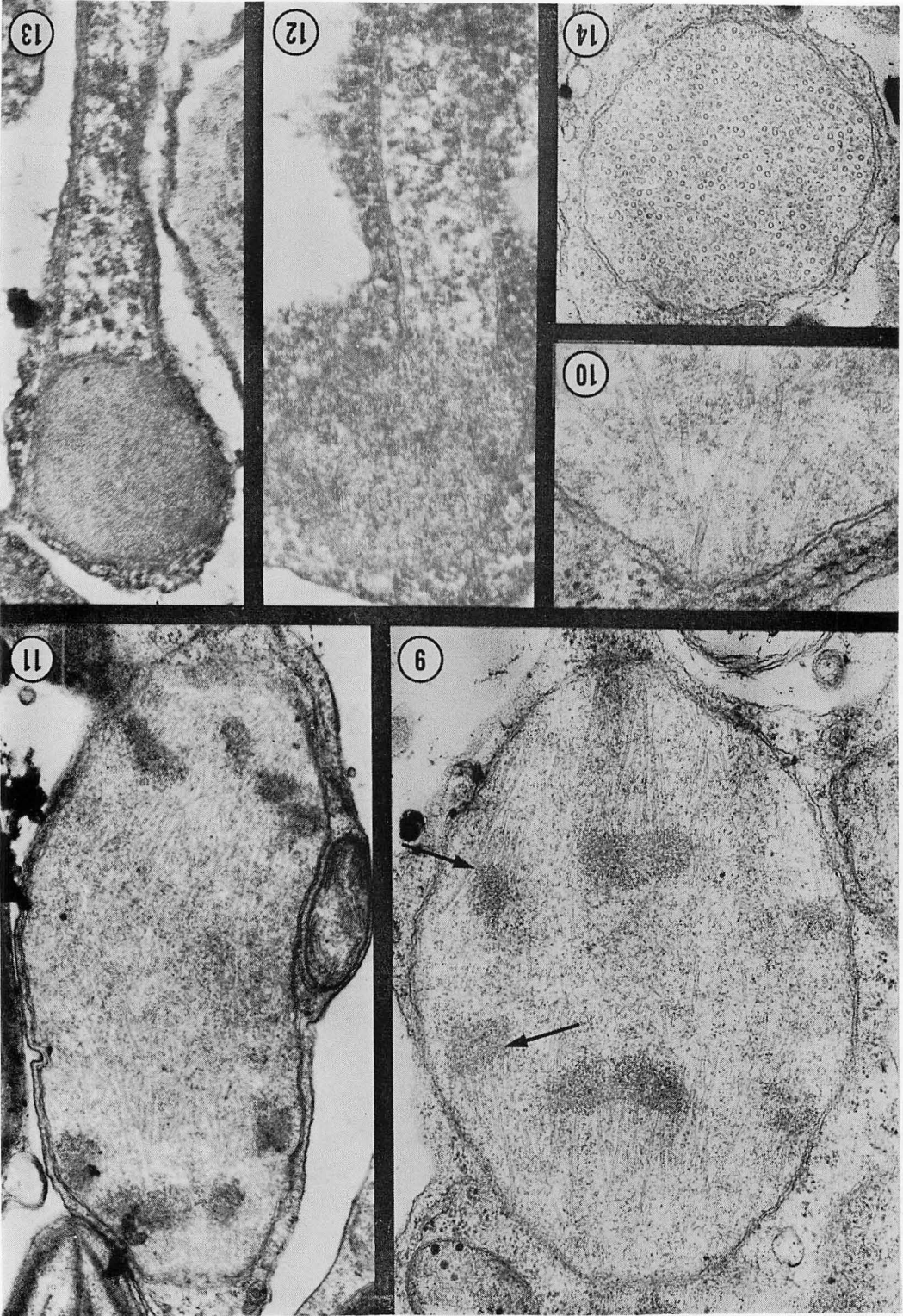
In *Acrosiphonia* (HUDSON and WAALAND 1976), *Cladophora* (SCOTT and BULLOCK 1976, McDONALD and PICKETT-HEAPS 1976), *Valonia* (HORI and ENOMOTO 1978a) and *Dictyosphaeria* (HORI and ENOMOTO 1978 c) centrioles occur near the nucleus even in the early stages of reproductive differentiation, while in *Batophora* (LIDDLE *et al.*, 1976) and *Caulerpa*, centrioles seem to appear only during the final stage of reproductive cell differentiation. This indicates that centriole formation is presumably suppressed until the final stage of swarmer production in some algae and that centrioles are not essential organelles for producing the spindle, but are necessary for flagella production. In *C. brachypus* centrioles are not found in the vegetative cytoplasm and the details of their origin and development during reproductive differentiation is unknown. It is known that in the dividing nuclei of other organisms that electron-dense areas exist in



Figs. 2-4. Electron micrographs of gametes of *C. brachypus*. 2. Female gamete. $\times 15000$; 3. Male gamete. $\times 15000$; 4. Cross section of female gametes showing the cruciate flagella roots (arrowhead). $\times 18000$.

Figs. 5-8. Electron micrographs of the nuclear division in *C. brachypus*. 5. Three phases of the nuclei, interphase (top right), prophase (lower right) and metaphase (large left) nuclei. Vacuolar invagination (arrows) results in the production of gametes. $\times 28000$; 6. Interphase nucleus. Single membrane bound body is present in a depression of the nucleus. Nucleolus is composed of closely aggregated granular material. $\times 26000$; 7. Dividing chloroplast. $\times 12000$; 8. Metaphase nucleus clearly showing a classical metaphase plate composed of four sets of paired chromosomes. $\times 24000$.





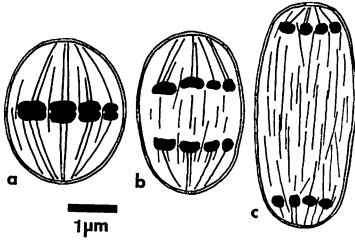


Fig. 15. Diagrammatic comparison of nuclear changes during the division in *Caulerpa*. Note the distinct expansion of the nuclear envelope along the division axis without significant decrease of the nuclear width in dimension. a, metaphase; b, early anaphase; c, late anaphase.

the regions which are in contact with the spindle microtubules (cf. PERKINS 1975, FRANKE and REAU 1973, HINCHEE and HASKINS 1980). In neither *Batophora* (LIDDLE *et al.*, 1976) nor in *C. brachypus* was a specially differentiated polar structure present, either inside and or outside the nuclear envelope, nor was any perinuclear substance from the nuclear envelope found which would suggest participation in spindle microtubule formation. Although nothing can be said at present about the origin of microtubules in the nucleus of *C. brachypus*, it may be reasonable to suspect that the microtubule nucleating substance, if present, is in a very diffusible form in this alga.

As was true for *Vaucheria litorea* (OTT and BROWN, Jr. 1972), *Cladophora glomerata* (MCDONALD and PICKETT-HEAPS 1976), *C. flexuosa* (SCOTT and BULLOCK 1976), *Valonia ventricosa* (HORI and ENOMOTO 1978 a) and *Dictyosphaeria cavernosa* (HORI and ENOMOTO 1978 c), *C. brachypus* prolongs the interzonal spindle after each chromosome set draws near to the opposite poles. This

suggests that the separation of chromosomes towards each pole has been substantially completed at early telophase. The interzonal spindle separation in *Vaucheria littorea* is known to be accomplished both by an invagination of the inner nuclear membrane and by what appears to be an abscission or depolymerisation of the nucleus (OTT and BROWN, Jr. 1972). The rotation of the late telophase nuclei might be instrumental in interzonal spindle separation in *C. flexuosa* (SCOTT and BULLOCK 1976) and *Valonia ventricosa* (HORI and ENOMOTO 1978 a). However the method of separation in *C. brachypus* remain unresolved.

The question arises why it is necessary to produce such a very long spindle in coenocytic nuclear division. Although there has been no reasonable interpretation for this feature, it may be due to the nature of the cytokinetic mechanism. Reproductive cytokinesis in the green coenocytes does not use either a phycoplast or a phragmoplast, unlike many other green algae. During swarmer differentiation in the green coenocytes, cytoplasm is gradually partitioned into small, multinucleate areas by vacuolar intrusions (BURR and WEST 1970, SCOTT and BULLOCK 1976, HORI and ENOMOTO 1978 b). This appears to be a rather unreliable mechanism, because such a form of cytokinesis may not necessarily produce an equal distribution of nuclei into all zooid units. In addition, the final process of cytoplasmic separation leading to the production of uninucleate zooids is also not necessarily by binary partition concurrent with nuclear division, but it appears to be a multiple partition which simultaneously produces

Figs. 9-14. Electron micrographs of anaphase and telophase nuclei in *C. brachypus* (9-11, 14) and *C. racemosa* var. *laete-virens* (12, 13). 9. Anaphase nucleus showing the progressive separation of chromosomes towards the opposite poles. Ill-defined structure of kinetochores (arrows). $\times 31000$; 10. Microtubules either closely approach to or impinge on the inner surface of nuclear envelope. $\times 52000$; 11. Late anaphase nucleus showing chromosomes close to the poles. $\times 21000$; 12. Telophase nucleus. The nuclear envelope of daughter nuclei sharply invaginate and develop a long interzonal spindle between two daughter nuclei. $\times 34000$; 13. Invagination of the inner nuclear membrane of daughter nucleus has just completed. $\times 32000$; 14. Cross section of interzonal microtubules that connect two daughter nuclei. $\times 33000$.

many zooids (HORI and ENOMOTO 1978 b). Thus, a long interzonal spindle may contribute to separating the two daughter nuclei at a great distance within the cytoplasm mass and may prevent the inclusion of more than one nucleus in each zooid unit at the final stage, ensuring the formation of uninucleate zooids.

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堀 輝 三: イワツタ属(緑藻類)における配偶子形成時の核分裂の微細構造

ヘライワツタ (*Caulerpa brachypus*) とスリコギツタ (*C. racemosa* var. *laete-virens*) の雄性配偶子形成過程にある材料を使って、イワツタ属植物の核分裂過程を電顕的に調べた。分裂核の極には中心体は存在せず、核分裂中も核包膜の消散は起らない。したがって紡錘糸微小管の形成中心は核内に存在すると思われるが、その構造的実体を見出すことはできなかった。染色体は典型的な中期核板を形成した後、両極へ分離する。染色体分離の原動力は、中期～後期では染色体微小管の短縮であるが、それ以降は核の変形と核包膜の伸長である。すなわち、各染色体組が染色体微小管の短縮によって核内の両極へ分離完了した後、分裂軸に沿って核は核包膜を伸長させながら長い亜鈴形に変化する。同時に娘核の間には両者の分離を助けるための長い中間紡錘糸微小管が発達する。このことによって、染色体物質相互のより遠い場所への分離と隔離が保証される。この特異な核分裂様式の意義について簡単な考察を行った。(305 茨城県新治郡桜村天王台 1-1-1 筑波大学生物科学系)