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Light- and Electron-Microscopic Studies of Growth and Reproduction in *Cutleria* (*Phaeophyta*)

II. Gametogenesis in the Male Plant of *C. hancockii*

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Summary

Development of the plurilocular male gametangium in *Cutleria hancockii* Dawson is fundamentally similar to that of the female gametangium. However, the sequence of mitoses is less regular and the number of divisions is more variable in the male structure. During mitosis the nucleolus disappears and the nuclear envelope breaks down into vesicles and cisternae. No well-defined chromosomal kinetochores were observed. The spindle does not persist during telophase. At least two types of vesicles, but no microtubules, are associated with cytokinesis. After cleavages are completed, each of the cells develops an eyespot and two flagella. The flagellar rootlet system consists of 4–5 bands of 5–10 microtubules radiating posteriorly from the basal bodies. Flocculent material surrounding the gamete at maturity may be involved with liberation. Prior to release, a pore is formed in each locule when the outermost layers of the surficial wall break, and the innermost layers expand out through this weakened region. The inner wall eventually bursts, releasing the gamete and flocculent material through the pore. The liberated gamete has a long, pleuronematic anterior flagellum, and a short, acronematic posterior flagellum which has a swollen base appressed to the plasmalemma.

Keywords: *Cutleria;* Gametogenesis; Morphogenesis; *Phaeophyta;* Developmental ultrastructure.

1. Introduction

The ultrastructural investigation of gametogenesis in the brown algae has been limited to a very few representatives of even fewer orders (see La Claire and West 1978, for a brief review). The genus *Cutleria* was chosen for a comparative ultrastructural study of growth, reproduction and cell division because members possess well-defined meristems and motile anisogametes. Various fine-structural features of isogamete and oogamete development have been

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examined in a few brown algae, but the formation of anisogametes in the *Phaeophyta* has been neglected ultrastructurally. Caram (1975) investigated some stages of male gametogenesis in the European species *Cutleria adspersa*. Therefore, this paper will deal with information complementing some of her work, our previous paper on female gametogenesis (La Claire and West 1978), and the detailed cytological studies of *C. multifida* by Yamanouchi (1909, 1912).

2. Materials and Methods

Male plant material was collected at Puerto Peñasco (Sonora), Mexico (March 4, 1977) and placed in culture. Plants were fixed in the field (and later from cultured material) for 90 minutes at room temperature with 4% glutaraldehyde in either sea water or culture medium. After rinsing briefly in sea water or in 0.15–0.20 M sodium cacodylate (+ 0.075–0.125 M NaCl) with decreasing concentrations of salt, the tissue was postfixed overnight at 0 °C with 2% OsO_4 in 0.1–0.2 M cacodylate. Subsequent methods of preservation, embedding, microtomy, *etc.*, were identical to those previously described (La Claire and West 1977, 1978). Thick sections (0.5 µm) were stained with a modified Polychrome procedure (Hayat 1975) and photographed through an orange filter.

In culture, plants were maintained on a shaker at 17 °C in Provasoli's enriched sea water (McLachlan 1973) (33‰, 16 : 8, 1,500 lux cool-white fluorescent lighting). Vegetatively propagating filaments bearing male gametangia were obtained in a manner similar to that used by Müller (1974) for clones of *C. multifida* males. For whole-mount preparation, fertile filaments of *C. hancockii* and *C. multifida* were transferred to hanging drops of fresh medium during the dark period, and antherozoids were released *en masse* at the onset of the following light period. Gametes in the hanging drops were then fixed with OsO_4 vapors for 5–15 minutes and placed on formvar-coated, carbon-stabilized grids, and the cells were allowed to settle for 10–15 minutes. A drop of 4% formalin-sea water was added to each grid for a few minutes to help preserve mastigonemes, and the grids were then flooded with distilled water for 15 minutes to wash out the sea water. After air drying, the grids were shadowed with platinum-carbon (30° angle) in a Mikros VE 10 vacuum evaporator and examined with a Zeiss EM 9 A electron microscope.

3. Results

Male gametangia are localized in concentric sori on both surfaces of the blade (arrow, Fig. 1). The gametangia are borne laterally (arrow, Fig. 2) or occa-

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Fig. 2, light micrograph; Figs. 3–30, electron micrographs.

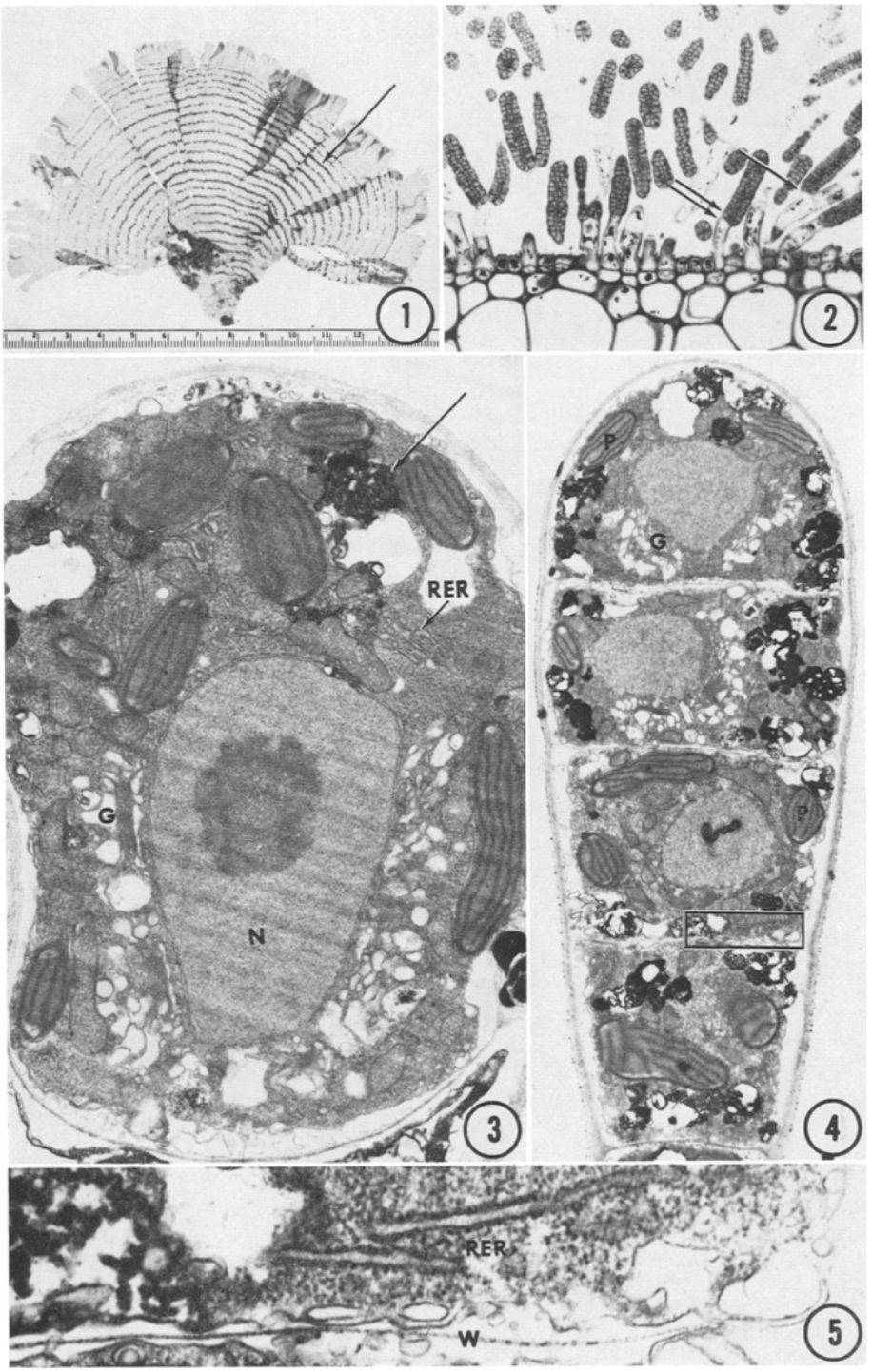
Fig. 1. Large male *Cutleria hancockii* thallus with concentric sori (arrow). ×0.45

Fig. 2. Thick (0.5 µm) transection of a sorus with fertile filaments bearing lateral (single arrow) and terminal (double arrows) gametangia (modified Polychrome stain). ×228

Fig. 3. Lateral gametangial initial containing many plastids, mitochondria, rough endoplasmic reticulum (*RER*) and a well-developed Golgi apparatus (*G*) around the prominent nucleus (*N*). Note the osmiophilic material already present (arrow). ×11,700

Fig. 4. Four-cell filament resulting from two series of mitoses. Note peripheral osmiophilic masses and plastids (*P*). Also, the Golgi bodies (*G*) are very dilated. ×5,060

Fig. 5. Greater magnification of inset in Fig. 4. Rough endoplasmic reticulum (*RER*) is especially evident near the new cross walls (*W*). ×43,750



Figs. 1–5

sionally terminally (double arrows, Fig. 2) on filaments within each sorus. These fertile filaments develop from epidermal cells of the blade (Fig. 2). The gametangium usually originates as a lateral protrusion from a cell in the fertile filament. After a basal septum is formed, the gametangial initial consists of a distinct cytoplasmically dense cell (Fig. 3). This initial contains a dilated perinuclear Golgi apparatus, several plastids, mitochondria, and rough (*i.e.*, ribosome-studded) endoplasmic reticulum (Fig. 3). Osmiophilic material also begins to accumulate (arrow, Fig. 3). A four-cell filament differentiates from this initial by successive transverse cell divisions (Fig. 4). The Golgi apparatus remains hypertrophied at this stage, and there is a continued production of osmiophilic material, especially in the cell peripheries (Fig. 4) and in regions near the cleavage furrows (arrows, Figs. 6 and 7). The presence of rough ER is especially evident near the cross walls (Figs. 4 and 5). A third set of transverse divisions follows, resulting in an 8-cell filament (Fig. 6). These divisions usually are acropetal in sequence, starting with the basal cell (Fig. 6). Afterward, the mitoses are asynchronous and occur in both transverse and longitudinal planes (Figs. 7 and 8). There is at least one additional series of transverse divisions resulting in a 16-tier gametangium. The longitudinal divisions occur before (arrows, Fig. 7; single arrow, Fig. 8), during (double arrows, Fig. 8) and after the final transverse divisions. Generally each tier undergoes two longitudinal divisions, but occasionally additional cleavages follow. The result is a 16-tier structure with 4–6 locules in each tier.

Mitosis in the gametangium begins with duplication and migration of centrioles to opposite poles of the nucleus (arrows, Fig. 9). The Golgi apparatus is still hypertrophied in prophase, and the Golgi bodies segregate toward the nuclear poles by metaphase. All microtubules are polar and extranuclear during prophase (not shown). The nucleolus disappears at early to mid-prophase. Although the nuclear envelope breaks down during late prophase, the nuclear outline remains evident throughout mitosis. During metaphase and anaphase, the spindle appears to be simple with respect to microtubule number and arrangement (Fig. 10). No kinetochores are evident and during chromosome movement the chromatin seems to separate into two masses (Figs. 11 and 12). The chromosome-to-pole distance decreases during anaphase and the pole-to-pole distance increases a little, but usually not significantly until telo-

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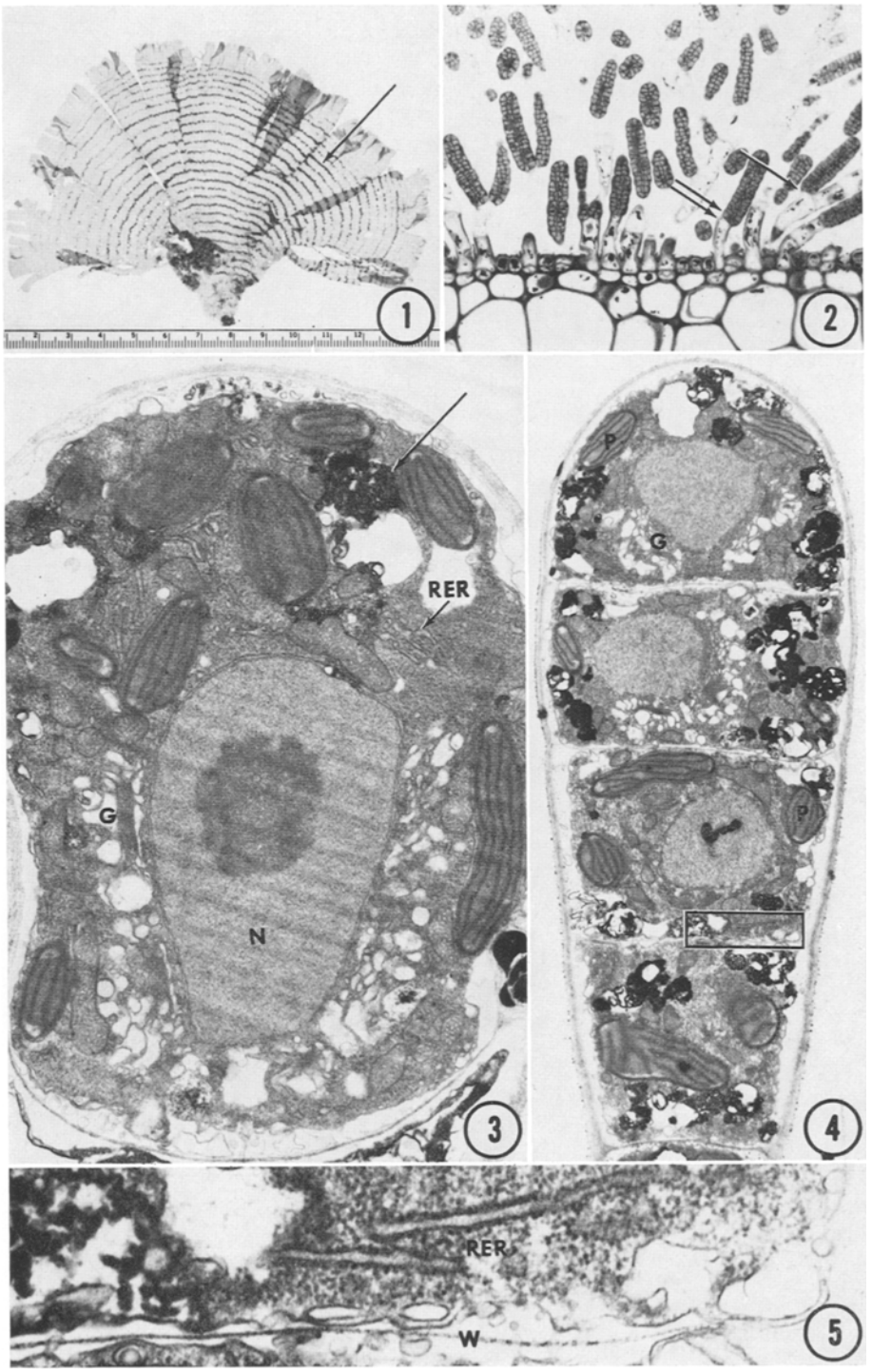
Fig. 6. Four-cell filament undergoing cytokinesis in two basal cells (arrows). Note the association of osmiophilic material with the cleavage plane. ×6,670

Fig. 7. Portion of an eight-cell filament beginning longitudinal cleavage (arrows). Osmiophilic masses indicate planes of cleavage in lower two cells. ×6,500

Fig. 8. Collage of near-median longisection of gametangium approaching the 16-tier stage. Often, longitudinal divisions occur before (single arrow) and during (double arrows) the final set of transverse divisions. ×4,800



Figs. 6–8

phase. The nuclear envelope begins to reconstitute at the poles and the chromatin disperses in mid- to late telophase. The spindle does not persist at telophase (Fig. 13). Small translucent vesicles are present throughout the nucleoplasm and the chromatin (Fig. 11). Osmiophilic masses begin impinging on the nucleoplasm as early as late metaphase and anaphase (Fig. 11) and appear to be associated with the centripetally-developing cleavage furrow at telophase (arrow, Fig. 13). Both progeny nuclei are surrounded by several Golgi bodies, and cytokinesis seems to involve the Golgi apparatus, since Golgi vesicles coalesce with both the osmiophilic masses (double arrows, Fig. 14) and with multivesicular bodies which in turn fuse with the developing cleavage furrow (single arrows, Fig. 14). No microtubule systems are observed associated with cytoplasmic cleavage. The centrioles are present throughout all nuclear phases including interphase in *C. hancockii*.

Following cell divisions, the relative ages of the walls can be determined by comparing thicknesses. The older transverse walls are much thicker than younger transverse ones or longitudinal walls (arrows, Fig. 15). Before flagellar formation, a polarity of organelles becomes noticeable. Proceeding from the gametangial axis outward, each locule houses a few mitochondria, a nucleus and Golgi apparatus, 2 centrioles, 1–2 plastids and many osmiophilic vesicles (Fig. 15). Flagellar initiation is preceded by the appearance of two basal bodies near the cytoplasmic surface (Fig. 16). Flagella grow outward from the surface above the basal bodies while an eyespot forms in a region of the plastid near the basal bodies (Fig. 17). Rootlet microtubules (22–25 nm diameter) also become evident radiating from amorphous masses near the proximal ends of the basal bodies (Fig. 17). The anterior flagellum then becomes decorated with mastigonemes. The other flagellum is smooth and is appressed to the plasmalemma at its swollen base. Granular-cored vesicles

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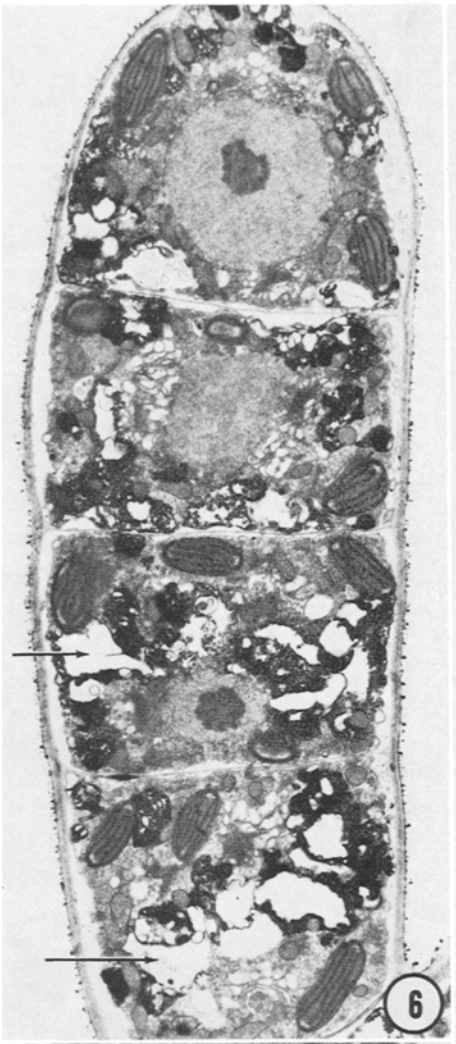
Fig. 9. Mid-prophase nucleus in developing gametangium. One centriole of each pair (arrows) can be seen at each pole, and the chromatin is beginning to condense. The nuclear envelope is still mostly intact, but no nucleolus is present. Note the Golgi body (*G*) which appears rather hypertrophied. ×24,000

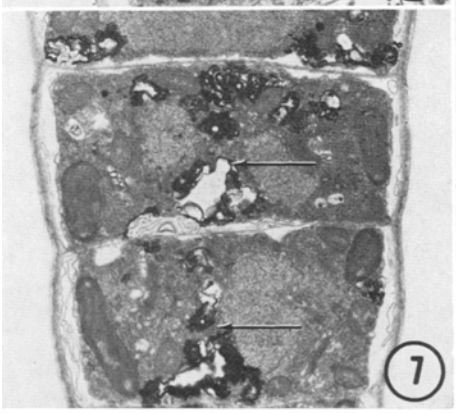
Fig. 10. Portion of a late metaphase/early anaphase nucleus showing one pair of polar centrioles (*C*) with spindle tubules (arrows) radiating toward the chromatin (*CH*). The nuclear envelope is broken into vesicles and cisternae. ×30,625

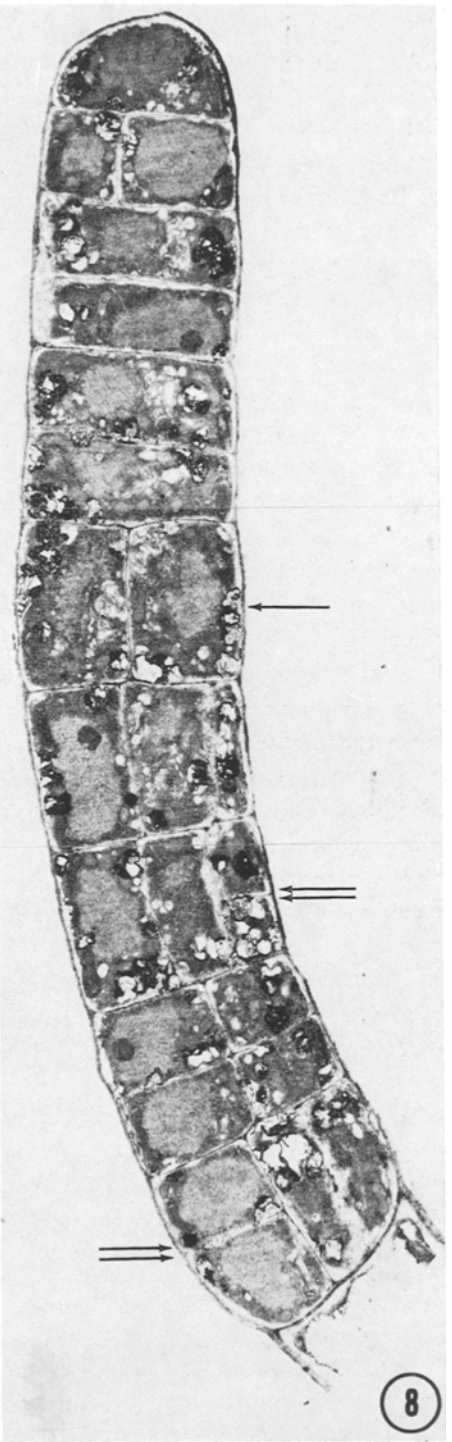
Fig. 11. Late anaphase nucleus with polar centrioles (*C*) evident, and chromatin (*CH*) is accumulated at the poles. The nucleoplasm (*NP*) appears devoid of spindle tubules, and is being encroached upon by osmiophilic masses (*O*). Tiny vesicles (arrows) are present in the nucleoplasm and in the chromatin masses. ×26,250

Fig. 12. Greater magnification of upper nuclear pole in Fig. 11. Spindle fibers (arrows) from the centriolar region (*C*) pass through the chromatin masses (*CH*). ×52,500

Fig. 13. Telophase nuclei (*N*) exhibit dispersing chromatin, while internuclear osmiophilic masses (*O*) are associated with the plane of cleavage (arrow). Nuclear envelopes are reforming, especially in the vicinity of the centrioles (*C*). ×17,250







Figs. 9–13

arise from the Golgi apparatus (single arrows, Fig. 18), and these appear to empty their contents into the spaces around each gamete. Simultaneously, a flocculent matrix appears around each future gamete as the cell rounds up and pulls away from the walls (Fig. 19). Much of the osmiophilic material is also secreted at this time into the extracytoplasmic spaces (double arrows, Figs. 18 and 19).

Prior to gamete release, a pore is formed in the surficial wall of each locule (arrow, Fig. 20). Where the pore forms, the outermost wall layers break first (single arrows, Figs. 21–24) and the inner wall layers bulge outward through this area (double arrows, Figs. 21–23), becoming thinner as they expand. Eventually, this large "vesicle" bursts and the gamete is released with its flagella trailing behind (Fig. 24). The entire process of gametogenesis takes from 4–8 days, and release requires from 30–60 seconds. Usually the gametes remain immobile for a few minutes before swimming off.

Mature gametes reveal several interesting features. The nucleus and plastid are often tightly intertwined (Fig. 24) or very closely juxtaposed (Fig. 27). The mitochondria are clustered at the anterior end and near the flagellar basal bodies. They contain tubular intracristal inclusions (Fig. 26; inset). The eyespot is composed of numerous hexagonally-packed osmiophilic droplets (Fig. 25) and is closely associated with bands of rootlet microtubules (not shown). There is a total of at least four bands of rootlet microtubules, each with 5–10 tubules/band. One band of 6 tubules runs from the basal body of the pleuronematic flagellum (*i.e.*, the one bearing mastigonemes), anteriorly into a small papilla (single arrows, Figs. 26 and 27). Two bands of 6 and

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Fig. 14. Cytoplasmic cleavage between two progeny nuclei (*N*) is complete, and osmiophilic material (O) is associated with the plane of cleavage. Multivesicular bodies seem to empty into the region of partition (single arrows), and these seem to be derived from, or modified by the Golgi bodies (*G*). The Golgi also appears to contribute to the osmiophilic masses (double arrows). ×13,800

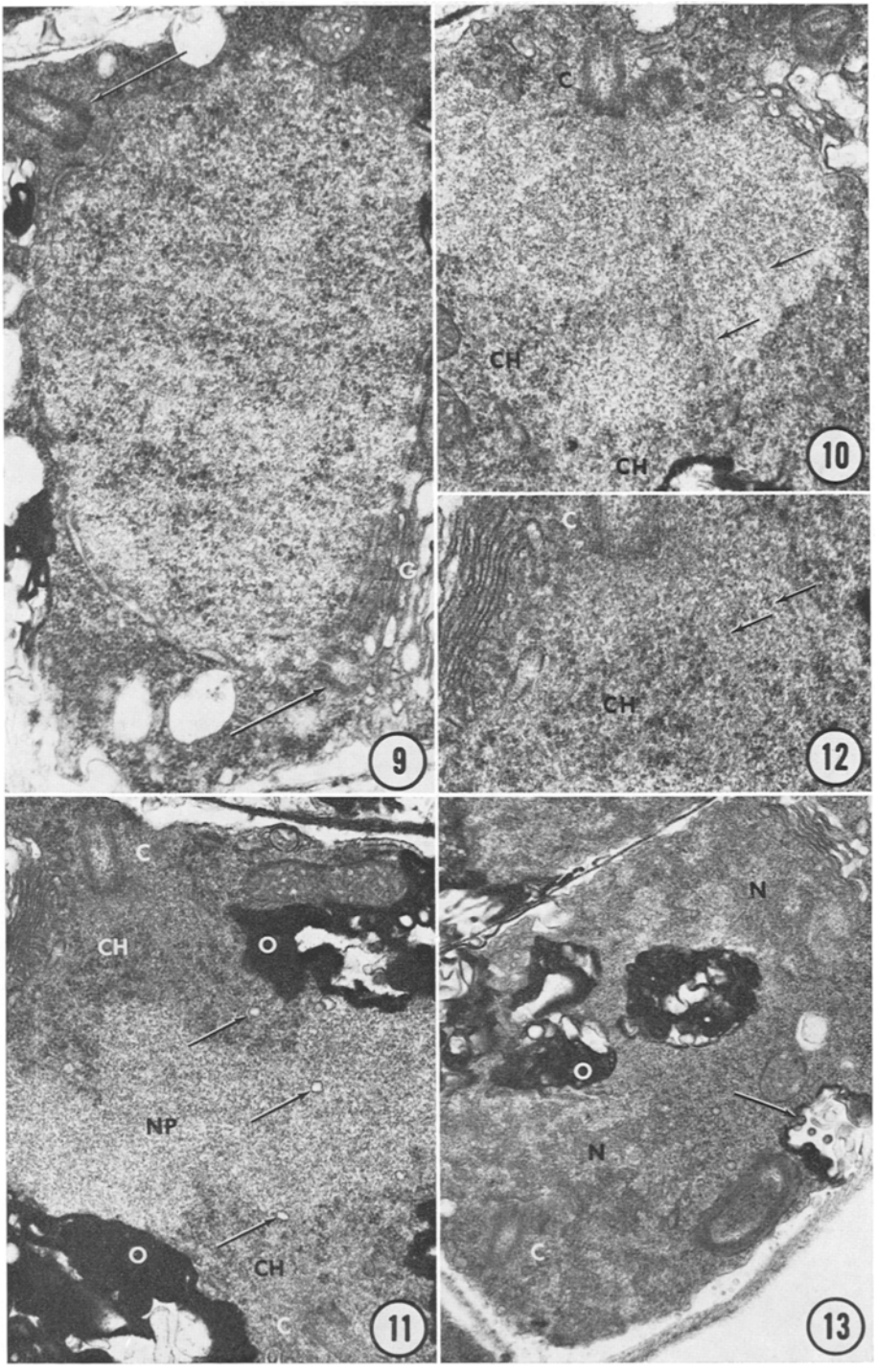
Fig. 15. Longisection of a gametangium after all divisions are completed. Each locule contains a single nucleus (*N*), 1–2 plastids (*P*), a few mitochondria (*M*), Golgi bodies (*G*) and peripheral osmiophilic material (O). Also, one member of a centriolar pair (*C*) is shown. Older walls (arrows) are thicker than more recently formed ones. ×9,750

Fig. 16. Grazing section of a cell beginning to produce flagella. Two basal bodies (*B*) are seen in transection near a Golgi cisterna (*G*). ×36,750

Fig. 17. Longisection through basal bodies (*B*) and one of the developing flagella (*F*). An eyespot (*E*) forms in the plastid and is composed of many osmiophilic granules. Rootlet microtubules (*R*) are also becoming evident, emanating from amorphous masses near the centrioles. ×36,750

Fig. 18. Gamete showing the Golgi origin of granular-cored vesicles (single arrows) and excretion of osmiophilic material (double arrows). ×22,750

Fig. 19. Mature antherozoids prior to release. As the gametes round up, osmiophilic material is excreted (double arrows) to the spaces surrounding each cell (single arrow). Flocculent material encompasses each gamete before release. ×8,450



Figs. 14–19

10 microtubules radiate from the basal body of the appressed flagellum to the stigma. Another band of 6–7 tubules traverses a region from the eyespot up into the aforementioned papilla and back again (partly seen in Fig. 26, double arrows).

Whole-mount preparations demonstrate that the longer anterior flagellum (*ca.* 20 μm) is covered with mastigonemes and bears a terminal extension that is not decorated (double arrows, Fig. 28). Each mastigoneme consists of a thicker base (*ca.* 1.0 μm long) and a terminal fiber (0.7 μm) (Fig. 30). The shorter posterior flagellum (*ca.* 10–12 μm) is smooth and has a long (2 μm) terminal extension (single arrow, Fig. 28). When the base of the shorter flagellum is pulled away from the body of the gamete, the basal swelling can be seen (Fig. 29).

4. Discussion

Many details of cytoplasmic events leading to gamete formation have been described previously for female gametes of *C. hancockii* (La Claire and West 1978). Since most of these occurrences are fundamentally identical to those found in male gametogenesis, we will limit discussion to those which differ and to various other aspects of gametogenesis not previously dealt with in *Cutleria*.

4.1. Reproductive Mitosis

Brown algal mitosis has received very little attention from electron microscopists, although a few papers have appeared on the fine structure of phaeophycean meristems (*e.g.*, Berkaloff 1963, Davies *et al.* 1973). There has been one exhaustive study of mitosis in reproductive cells of *Pilayella* (= *Pylaiella*)

Fig. 20. Oblique transection through mature gametangium showing antherozoids before, during (single arrow) and after (double arrows) release. ×4,225

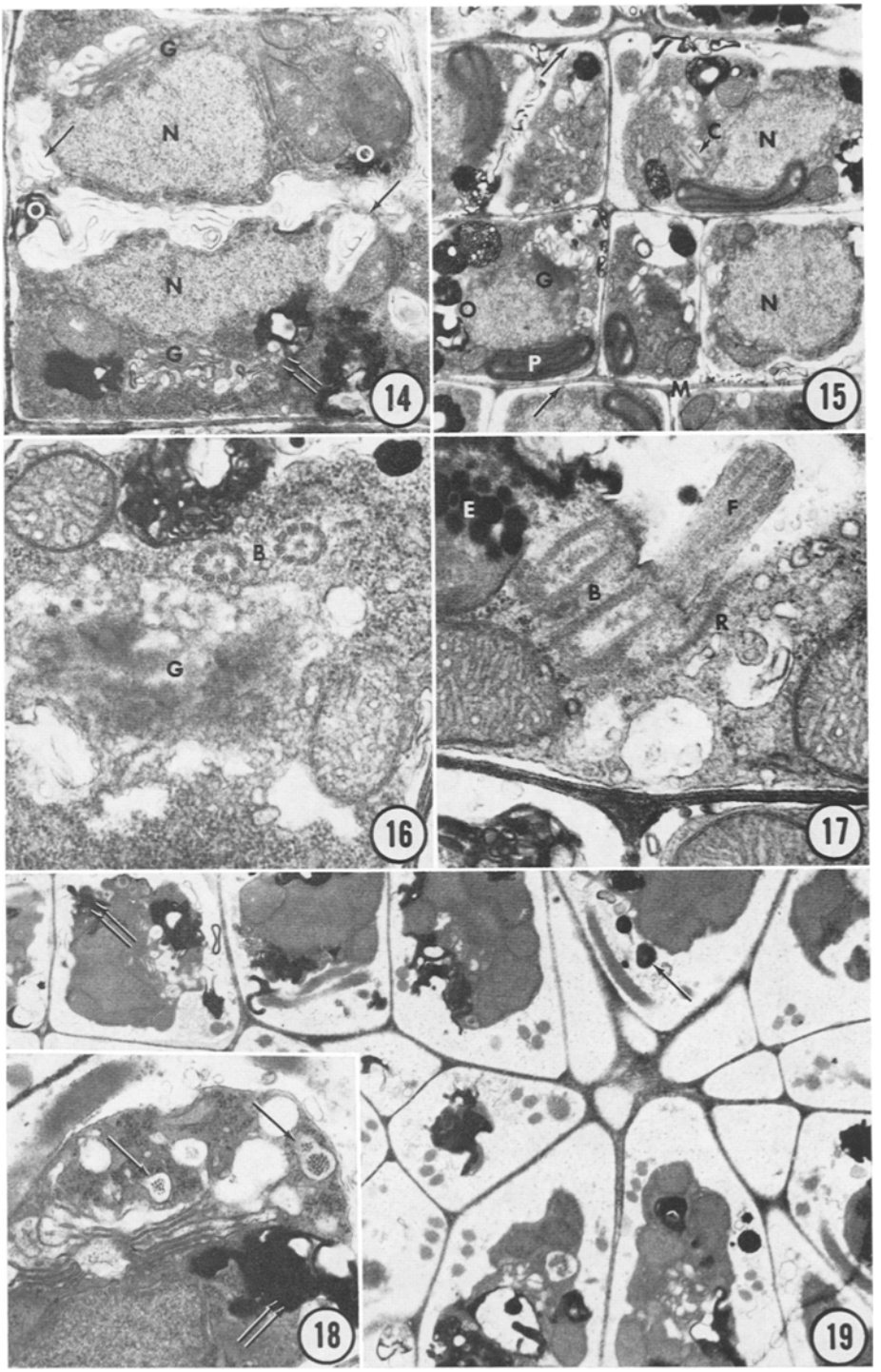
Fig. 21. Earliest stage of release. Outermost wall layers (single arrows) break apart while inner layers (double arrows) bend outward and remain intact. ×14,300

Fig. 22. Slightly later stage of release than Fig. 21. Inner wall (double arrows) continues to bulge outward as outer layers (single arrows) separate further. ×15,000

Fig. 23. Just prior to gamete liberation, the inner wall layers (double arrows) swell out into medium and appear as a "vesicle" surrounding the cell. Note that the thickness of these wall layers seems to be much less than in Figs. 21 and 22. The outer wall layers (single arrows) are present at the edges of the future pore. ×14,300

Fig. 24. Antherozoid exiting as the "vesicle" (*V*) bursts. The intimate association of the nucleus (*N*) and plastid (*P*) is noticeable, and one flagellum (*F*) is shown trailing the exiting gamete. The outer wall layers (single arrows) border the pore. ×15,000

Fig. 25. Grazing section of a released gamete showing the hexagonally-packed nature of the eyespot granules (*E*). Both the pleuronematic anterior flagellum (*AF*) and the swollen base of the posterior flagellum (*PF*) are seen. ×19,500



Figs. 20–25

(Markey and Wilce 1975), and Bouck (in Leedale 1970) reported on cell division in *Fucus* antheridia. Also, certain stages of vegetative mitosis were represented in *Fucus* (Brawley *et al.* 1977) and *Zonaria* (Neushul and Dahl 1972). We will describe details of vegetative cell division in *Cutleria cylindrica* elsewhere, and reproductive cell division is similar to it in most respects. Therefore, we only briefly discuss reproductive cell division here.

4.1.1. Prophase

Yamanouchi (1912) did not observe centriole duplication and migration with the light microscope in *C. multifida*, but did describe "kinoplasmic accumulations at the poles" during prophase in male gametangia (Yamanouchi 1909). In fact, in these reports, he found "neither centrosomes nor central bodies with or without radiations" in vegetative cells (except at metaphase, see Sec. 4.1.2.). Clearly this is not the case in nearly all the brown algae examined with electron microscopy, since paired centrioles were shown surrounded by amorphous material at opposite poles of dividing nuclei in *Pilayella* (Markey and Wilce 1975), *Fucus* (Bouck, in Leedale 1970), *Zonaria* and *Dictyopteris* (Neushul and Dahl 1972). However, no centrioles were reported in the single metaphase nucleus see in *Hormosira* (Forbes and Hallam 1979). Amorphous matter is also present around most centrioles in *C. hancockii* (unpublished observations) and this material is probably the microtubule-organizing center (MTOC) (Pickett-Heaps 1969).

Extranuclear microtubules do not seem to form an aster in our study, but rather seem to be directed toward and around the nuclear envelope during prophase. Therefore, this agrees with Yamanouchi (1909, 1912) finding a lack

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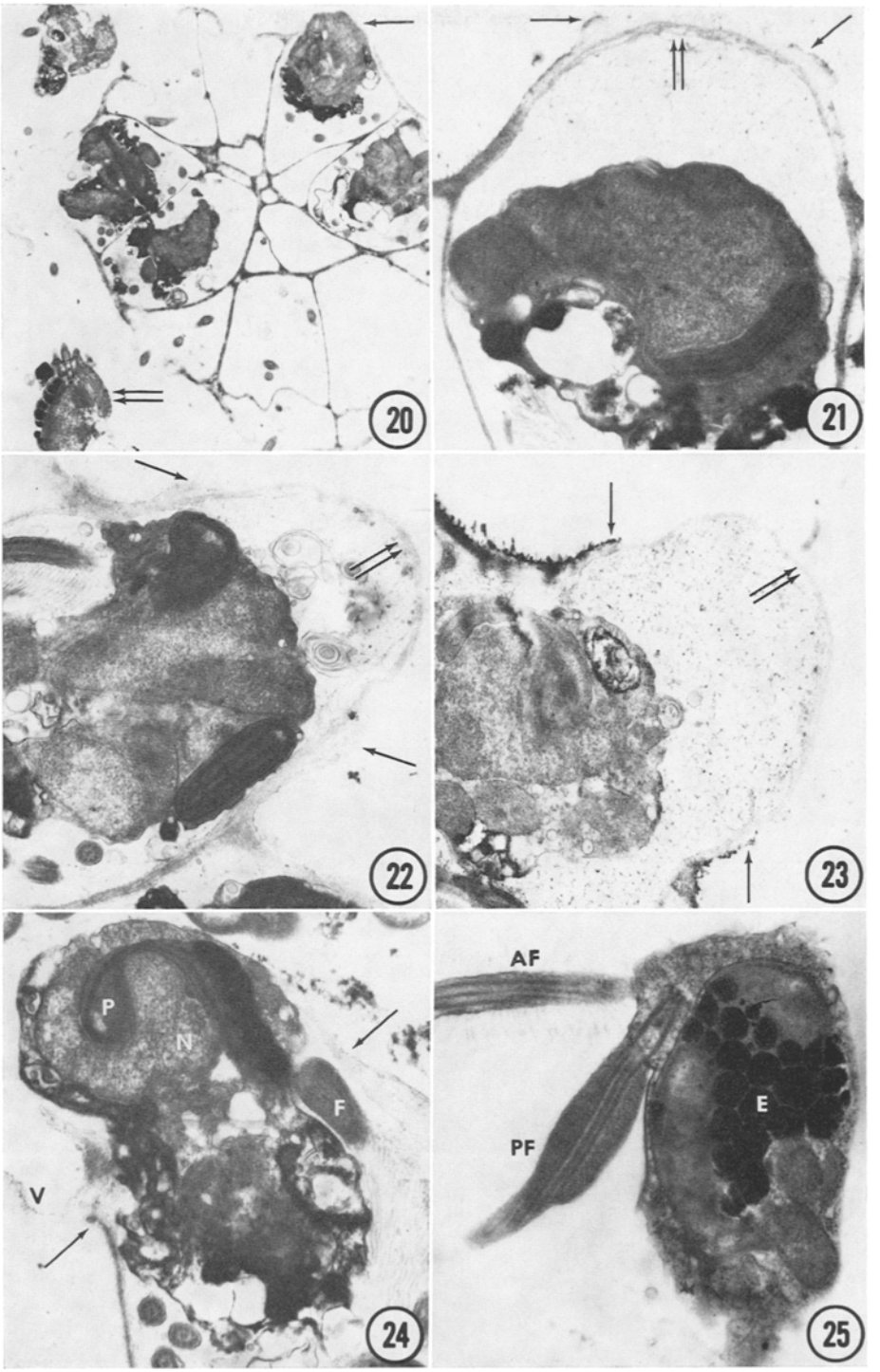
Fig. 26. Released antherozoid revealing mastigonemes (*MA*) on the free anterior flagellum (*AF*) and the swollen base of the smooth posterior flagellum (*PF*). Two rows of rootlet microtubules are shown: one band of tubules from the basal body of the anterior flagellum runs outward into a small papilla (single arrow) (*cf.*, Fig. 27), and a band of 6–7 tubules (double arrows) is seen perpendicular to these in a portion of the papilla. Intracristal inclusions (*Z*) are present in the mitochondria. The eyespot (*E*) is associated with the flagellar apparatus. ×28,000 Inset: greater magnification of intracristal inclusions (*Z*). ×59,360

Fig. 27. Section nearly perpendicular to that of Fig. 26. The band of 6 rootlet tubules going up into the papilla is shown (arrow). The tight juxtaposition of the nucleus (*N*) and the plastid (*P*) is seen, along with a Golgi body (*G*). ×45,500

Fig. 28. Negative image of entire antherozoid (whole-mount preparation). The shorter posterior flagellum (*PF*) is smooth and terminates in a long projection (single arrow). The longer anterior flagellum (*AF*) is decorated with mastigonemes and also bears a terminal projection (double arrows). ×4,950

Fig. 29. Negative image of swollen base of posterior flagellum (*PF*) where it has pulled away from the body of the gamete. ×32,480

Fig. 30. Negative image of a mastigoneme displaying the thicker basal portion (single arrow) and the thin distal thread (double arrows). ×43,750



Figs. 26–30

of "radiations" from the nuclear poles during prophase in *C. multifida*. The presence of extranuclear microtubules near the centrioles in a less organized array has also been reported in vegetative mitosis in *Fucus* (Brawley *et al.* 1977), *Zonaria* and *Dictyopteris* (Neushul and Dahl 1972), and in reproductive prophase of *Pilayella* (Markey and Wilce 1975). Extranuclear microtubules are absent during later stages of mitosis in *Cutleria*, so whether these are later incorporated into the spindle is not yet clear. However, this does not seem to be the case (directly) in vegetative mitosis of *C. cylindrica* (unpublished observations).

The exact timing of nucleolar disappearance seems to be somewhat variable in phaeophycean systems investigated so far. Nucleolar dispersal was evident from early to mid-prophase in *C. hancockii* male cells, but occurred during late prophase in *Fucus* embryos (Brawley *et al.* 1977). The timing of this event, therefore, does not seem to be crucial for achieving cell division. Similarly, variations in timing and degree of breakdown of the nuclear envelope are evident. Bouck (in Leedale 1970) reported only polar fenestrations in *Fucus* antheridial nuclei, as was the case in vegetative apices of various Dictyotales (Neushul and Dahl 1972) and in *Hormosira* embryos (Forbes and Hallam 1979). Gaps throughout the envelope were noted in *Pilayella* gametangia after prophase (Markey and Wilce 1975), but the nucleus remained essentially intact throughout mitosis. However, the nuclear envelope almost completely disappears from late prophase to early telophase in *Fucus* embryos (Brawley *et al.* 1977) and in our study of *C. hancockii* gametangia. The fact that the nucleoplasm maintains integrity despite envelope breakdown probably accounts for Yamanouchi's (1909, 1912) light microscope observation that reproductive mitosis is intranuclear in *C. multifida*. As yet, there seems to be no correlation between degree of envelope breakdown and present phylogenetic schemes for the brown algae.

The apparent segregation of Golgi bodies toward the poles during prophase was also noted in *Pilayella* (Markey and Wilce 1975).

4.1.2. Metaphase

Metaphase nuclei seem to be difficult to find in most brown algae, probably because this stage is very brief. Fig. 10 is an oblique section through half of a late metaphase/early anaphase nucleus. Here the spindle is well formed and the chromatin is located near the nuclear equator (as revealed by adjacent sections of the same cell). Yamanouchi (1912) reported a great decrease in nuclear diameter at metaphase in *C. multifida* gametangia, but that was not observed in the present study. He did, however, report a "centrosome" at each pole during metaphase, but at no other stage of reproductive mitosis (Yamanouchi 1912), and even a "central body" (= centriole) at metaphase in vegetative mitosis (Yamanouchi 1909). We already stated that both centri-

oles and the surrounding amorphous regions are present in reproductive mitosis in *C. hancockii*.

The apparent absence of structured chromosomal kinetochores was noted in *Pilayella* (MARKEY and WILCE 1975), and appears to be the case in *Fucus* (BOUCK, in LEEDALE 1970, BRAWLEY *et al.* 1977). It is difficult to determine their absolute presence or absence, since the chromatin in brown algae is not usually very distinct, and looks somewhat homogeneous in the electron microscope. Also, spindle structure is not completely clear, since the spindle seems to be less organized in brown algae than in other plant and animal systems. Perhaps high-voltage electron microscopy of thicker sections will help elucidate both chromosome and spindle structure.

4.1.3. Anaphase

The notion that anaphase must be of very brief duration can be demonstrated by the fact that there was previously only one published electron micrograph of a brown algal nucleus clearly in anaphase (BOUCK, in LEEDALE 1970). It seems that there are relatively few pole-to-pole microtubules in *C. hancockii*, because none were seen in the late anaphase nucleoplasm between the separated chromatin masses. However, anaphase was only rarely found, so details of this process must await further information. The association of small vesicles with the metaphase and anaphase spindle was noted in *Pilayella* (MARKEY and WILCE 1975), *Fucus* (LEEDALE 1970, BRAWLEY *et al.* 1977) and *Zonaria* (NEUSHUL and DAHL 1972). A possible role of membrane inclusions in the spindle apparatus has been suggested with respect to Ca^{++} sequestration and to regulation of spindle polymerization and depolymerization (HEPLER and PALEVITZ 1974, HARRIS 1975, HEPLER 1977).

4.1.4. Telophase

The compact nature of the telophase chromatin was previously mentioned by YAMANOUCHI (1912) in *C. multifida*, and is evident in *Pilayella* (MARKEY and WILCE 1975). Also, the lack of a persistent spindle at telophase was found by the aforementioned authors. The reappearance of nucleoli in the progeny nuclei was noted in *Pilayella* (MARKEY and WILCE 1975), and the reaggregation of the nuclear envelope also occurs in *Fucus* zygotes (BRAWLEY *et al.* 1977). It is noteworthy that the envelope first reconstitutes near the poles where centrioles are still present (Fig. 13).

4.2. Cytokinesis

It was mentioned above that densely osmiophilic vesicles and masses begin to impinge upon the dividing nucleus at anaphase or earlier, and predict the future cleavage site. It is not clear whether these vesicles are actually involved

with cytokinesis or are merely pushed toward the center of the cell by the separating progeny nuclei (due to space limitation in the small locules). Markey and Wilce (1975) found that many vacuoles lie between the progeny nuclei, and their Fig. 19 shows similar osmiophilic vesicles associated with cytokinesis. Electron-lucent vesicles were implicated in this process in *Ascophyllum* (Rawlence 1973). However, in *Fucus* (Brawley *et al.* 1977) it was stated that chloroplast arrangement, rather than vesicle location, predicted the site of cleavage.

So far, only one study (Markey and Wilce 1975) has reported any microtubules present near the cleavage region, and then, "only rarely". Therefore, it would appear that brown algae have developed somewhat different cytokinetic mechanisms from those found among many of the other algae investigated, and in metaphytes. Furthermore, there seems to be some variation within the Phaeophyta as to the directionality of cleavage. In *Ascophyllum* (Rawlence 1973) cleavage appeared centrifugal whereas the other systems investigated were centripetally cleaved with vesicle addition (Markey and Wilce 1975, Brawley *et al.* 1977, present study). Unilateral wall formation was seen in *Ascophyllum* and *Pilayella* by these authors, but was never evident in our study.

Golgi vesicles have not been implicated previously in cytoplasmic cleavage in brown algae, but the Golgi apparatus is widely believed to be associated with cell wall formation in other organisms. Fig. 14 shows that, although the Golgi bodies are not spatially related to the developing septum, Golgi derived/modified vesicles do coalesce with this region and with the osmiophilic masses associated with cytokinesis (Fig. 7). It is also interesting to compare Fig. 14 with Fig. 3 and with our earlier study on female gametogenesis (La Claire and West 1978). Fig. 3 and the earlier paper show Golgi association with the septum after cleavage; Fig. 14 does not. Since the Golgi apparatus seems to be involved in these cases, spatial association of the apparatus itself does not seem necessary for Golgi involvement in cleavage and wall formation. However, the activity of the Golgi vesicles must be carefully examined. In addition, multivesicular bodies were associated with the cleavage area before (Fig. 14) and during wall development, so the story remains incomplete.

Detailed presentation of cytokinesis and cell wall development will be reported for *C. cylindrica* in a later paper in this series.

4.3. Mitotic Asynchrony

Mitoses are much less synchronous in male gametangia of *C. hancockii* than they are in female gametangia (La Claire and West 1978). However, the females only undergo four series of divisions whereas the males have at least six. Yamanouchi (1912) described, somewhat differently, the sequence of divisions in *C. multifida*, where longitudinal divisions initiate as early as the

four-cell filament stage and are often followed by transverse divisions. Although this seems not to be true for *C. hancockii*, similar gametangia are produced in both species. With respect to asynchrony of mitosis, male gametangia closely resemble plurilocular mitosporangia of *Ectocarpus* (LOFTHOUSE and CAPON 1975).

4.4. Organelle Polarity

The distinct arrangement and polarity of organelles in each locule of the gametangium is similar to that reported in male gametangia of *C. adspersa* (CARAM 1975) and in female structures of *C. hancockii* (LA CLAIRE and WEST 1978). The significance of this phenomenon is unknown.

4.5. Gamete Liberation

Granular-cored vesicles identical to those shown in female gametangia of *C. hancockii* (LA CLAIRE and WEST 1978) were found in male gametes late in development and appear to be Golgi-derived or modified. CARAM (1977) noted vesicles "packed with dark granules" which are polysaccharidic in nature, in male and female gametes of *C. adspersa*. Since these vesicles empty their contents concurrently with the rounding up of gametes and the appearance of the flocculent matrix, they may be secreting this matrix. Similar fibrillar matrices were shown and/or described surrounding both plurilocular and unilocular motile cells prior to release, by many previous authors. These matrices are believed to be mucilaginous polysaccharides (TOTH 1976). A release mechanism involving hydration and swelling of these matrix polysaccharides was proposed for unilocular structures in several brown algae (TOTH 1976). As suggested earlier (LA CLAIRE and WEST 1978), this seems to apply well to the events seen in *C. hancockii* plurilocular organs. As the matrix swells, the outermost wall layers rupture, but the inner wall layers expand greatly and decrease in thickness before they finally break. When this swollen "vesicle" finally bursts, the gamete is liberated. The inner wall layers must be very extensible, whereas the older (outer) layers are not. For *Pilayella*, TOTH (1976) proposed enzymatic digestion of the secondary (inner) wall layers, causing them to weaken and become capable of swelling. Since this doesn't seem to be the case in *C. hancockii*, the mechanism of wall swelling remains unknown.

YAMANOUCHI (1912) stated that the flagella emerge ahead of the gamete when exiting through its pore in *C. multifida*, and even beat actively before release. However, in *C. hancockii*, our studies show that the flagella clearly trail the gamete during release, as if the cell is being passively pushed out. The few minutes of gamete immobility following release might be due to the presence of mucilage upon exiting.

4.6. Mature Gamete

4.6.1. Flagella

Light-microscope observations of swimming antherozoids of both *C. hancockii* and *C. multifida* reveal that the longer flagellum is directed anteriorly, and its undulations appear to provide much of the propelling force (La Claire, unpublished observations). This has also been stated for both male and female gametes of *C. multifida* (Thuret 1850, Yamanouchi 1912, Kuckuck 1929), *Zanardinia,* also in the order *Cutleriales* (Yamanouchi 1913), and depicted in *C. adspersa* (Janczewski 1883). We have demonstrated here that the longer flagellum is pleuronematic and unrestrained, whereas the shorter flagellum is acronematic and has a basal swelling appressed to the plasmalemma of the antherozoid. Similar observations were made on the female gametes of *C. hancockii* (La Claire and West 1978) and on antherozoids of *C. multifida* (La Claire, unpublished observations). Therefore, the flagellation of the gametes in these species of *Cutleria* appears to be that typical of biflagellate swarmers in the *Phaeophyta* (Scagel 1966). Caram (1975) reported that the shorter acronematic flagellum of *C. adspersa* antherozoids is anteriorly directed, with the longer pleuronematic flagellum being posterior and propelling the gamete forward with undulating movements. Her paper is the only report of a pleuronematic posterior flagellum in all the brown algae.

The anterior flagellum of *C. hancockii* appears to be terminated by only a short tip. However, in *Chordaria* zooids (Petersen *et al.* 1958) the anterior flagellum ends in a long, fragile extension in many preparations. Müller (1965) noted that this extension was removed from living *Ectocarpus* and *Sorocarpus* swarmers during osmication. This may also be true for *C. hancockii*, since osmium vapors were utilized for fixation. In a few of our preparations this tip was up to 1 μm long. Similarly, Loiseaux and West (1970) found delicate hairs on the posterior flagellum of *Giffordia* zoospores, and perhaps these were also removed in our preparations of *C. hancockii*, as they were never observed.

Mastigoneme-like tubules (11–13 nm diameter) were observed within the nuclear envelope and in membrane-bound vesicles in developing male gametes (not shown). Thus, mastigonemes are apparently formed in a manner similar to that described for other brown algae (*e.g.*, Bouck 1969).

4.6.2. Flagellar Rootlets

The existence of a flagellar rootlet system in *Cutleria* spermatozoids was first shown in de-flagellated preparations of *C. adspersa* (Caram 1975). In the present study, it can be seen that these rootlets are actually microtubular bands. Bouck (1970) suggested that rootlet tubules associated with the eyespot may actually be involved in the alignment of eyespot granules in developing *Fucus* antherozoids. Also, Manton and Clarke (1951) noted "strands" from the

basal bodies radiating into the proboscis of *Fucus* sperm, and these may be similar to those running into the small papilla of *Cutleria* antherozoids. The implications of this similarity between *Fucus* and *Cutleria* rootlets are unknown. Serial sectioning is necessary to determine the complete array of flagellar rootlets in the gametes.

4.6.3. Mitochondria

Mitochondrial cristae contain tubular inclusions in male gametes, in accord with numerous reports on phaeophycean antherozoids. In contrast, fibrous inclusions were found in the mitochondria of female gametes of *C. hancockii* (La Claire and West 1978). However, the importance of this difference and the overall significance of such inclusions are not known.

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