Protoplasma 97, 93—110 (1978)

PROTOPLASMA

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

I. Gametogenesis in the Female Plant of *C. hancockii*

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Received February 20, 1978
Accepted May 17, 1978

Summary

Differentiation of the female gametangium in *Cutleria hancockii* Dawson is described. Four series of mitoses result in a 16-locule structure (four tiers of four cells each). The organelles in each locule become polarized after partitioning is complete, with the mitochondria lying near the longitudinal axis of the gametangium. The nucleus and plastids are centrally located, with abundant osmiophilic material present in the cytoplasm subjacent to the gametangial surface. Both electron density and Toluidine Blue O staining of the material increase. Two flagella are then produced: one becomes tightly appressed to the plasmalemma near its base, and the other is free. A prominent eyespot forms in the plastid nearest the developing flagella. Golgi and endoplasmic reticulum vesicles are prolific in this region and seem to be involved with mastigoneme production and deposition on the free flagellum. Immediately beneath the plasmalemma, flagellar rootlet tubules emanate from amorphous masses near the basal bodies. Some of these tubules are associated with the eyespot. Most of the osmiophilic material is then secreted into the extracytoplasmic spaces while the gametes are rounding up. Granular-cored vesicles may be involved with pore formation and gamete release.

1. Introduction

The order *Cutleriales* (*Phaeophyta*) has been of interest to phycologists for well over a century. The type genus, *Cutleria*, is characterized by having a trichothallic meristem (*i.e.*, cell divisions are at the base of the marginal rows of hairs), marked anisogamy and a putative alternation of heteromorphic generations (Fritsch 1952). Since the cytological and culture studies of the early part of the century, little additional work has been published on the group (see Fritsch 1952, for review). Most of these early investigations involved either *C. adspersa* or *C. multifida*. More recently, Müller (1974) has isolated a sex attractant from the female gametes of the latter species and Caram (1975, 1977 a, b) has done ultrastructural and culture work with

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C. adspersa. Also, Niklas (1977) applied finite element analysis to the morphology and growth of *Cutleria.* However, no investigations have been made with *C. hancockii* since Dawson (1944) first described the plant.

Detailed ultrastructural study of gametogenesis in the brown algae has been limited to the filamentous alga, *Pilayella* (= *Pylaiella*) *littoralis* (Markey and Wilce 1975, 1976 a). Some stages of gamete formation were reported in *Macrocystis* (Gherardini and North 1972), *Zonaria* (Liddle and Neushul 1969), *Fucus* and *Ascophyllum* (Bouck 1969). Sporogenesis has been studied in several brown algae including *Chorda* (Toth 1974), *Ectocarpus* (Baker and Evans 1973 a, b, Lofthouse and Capon 1975), *Macrocystis* (Chi and Neushul 1972, Gherardini and North 1972), *Pilayella* (Loiseaux 1973, Markey and Wilce 1976 b), *Zonaria* (Liddle and Neushul 1969), *Elachista* and *Hecatonema* (Loiseaux 1973). Caram (1975, 1977 b) reported the fine structure of some stages of male and female gametogenesis in *Cutleria adspersa,* and both Hori (1972) and Evans (1966) included *Cutleria* in their ultrastructural surveys of phaeophycean pyrenoids.

The genus *Cutleria* occupies a unique position among the brown algae. Members possess a basically filamentous construction initially with intercalary growth, which is characteristic of the least advanced orders. However, these filaments coalesce and longitudinal divisions produce a truly parenchymatous thallus. This feature coupled with their having anisogamous reproduction and a general alternation of morphologically dissimilar generations, allies the group with the more advanced orders (Fritsch 1952). Aside from these intriguing phylogenetic relationships, the trichothallic meristem is very poorly understood in general. Therefore, a comparative ultrastructural examination of growth, reproduction and mitosis was undertaken with *Cutleria.* This first paper is concerned with differentiation of the female gametangium and gametes in *C. hancockii.*

2. Materials and Methods

Female plant material collected from Puerto Peñasco (Sonora), Mexico (March 4, 1977), was fixed in the field (for 90 minutes at room temperature) with 2% glutaraldehyde and 1% paraformaldehyde in 0.15 M sodium cacodylate buffer. After rinsing briefly in a buffer series with decreasing salt concentrations, the tissue was postfixed overnight at 0 °C in 2% OsO_4 in the same buffer. Thorough rinsing in the cold buffer was followed by stepwise dehydration with acetone (in 10% increments), and slow infiltration and embedding with Mollenhauer's epon-araldite mixture #1 (Dawes 1971). Ultramicrotomy and staining techniques were identical to those previously described (La Claire and West 1977). Thick (0.5 μm) sections were stained with Toluidine Blue O for light microscopy.

3. Results

3.1. Light Microscopy

Concentric bands of gametangial sori (Fig. 1) are present on both surfaces of the blade-like thallus. Each sorus is composed of fertile filaments and

paraphyses (sterile hairs) (Fig. 2). The female gametangia typically develop laterally on the fertile filaments, and mature acropetally. The gametangial initial arises as a protuberance from a cell of the fertile filament, becomes filled with cytoplasm and is then septated at its base (Fig. 3). While the initial enlarges, it divides transversely to form a two-cell (Fig. 4), and then a four-cell filament (Fig. 5). These cells divide twice longitudinally to produce the 16-locule gametangium composed of four tiers of four locules each. Following the cleavages, globular material (Fig. 7) which stains green or blue with Toluidine Blue O (TBO) becomes increasingly evident (Figs. 8 and 9) in the peripheral cytoplasm, especially just beneath the gametangial surface. All this material stains dark blue at maturity.

Gametogenesis requires from four to eight days, depending on the culture conditions. Gamete release can be artificially stimulated by changing the medium.

Flagella develop while the protoplasts are withdrawing from the locular walls. Shortly thereafter, a pore forms in the wall of each locule which faces the medium, and the gametes exit leaving the gametangium partially collapsed (Fig. 6).

3.2. Electron Microscopy

The wall protrusion develops and becomes filled with numerous elongated mitochondria (each with tubular cristae), a prominent nucleus and a Golgi apparatus (Fig. 10). There are also several small (2.5 μm long) plastids which have only 2–5 thylakoid triplets in each. Some osmiophilic material can already be seen (arrow, Fig. 10). After the nucleus has migrated past the base of the elongating initial, the basal wall is formed.

In the 16-cell gametangium, plasmodesmata are present in both the periclinal and anticlinal walls (Fig. 31). Throughout initiation and cleavage of the gametangia, the cytoplasm is very electron dense and ultrastructural details are difficult to discern.

The Golgi apparatus appears very active (hypertrophied distal face and several cisternae in each, with many peripheral vesicles), after the divisions are completed. There is a concurrent accumulation of what appear to be two types of osmiophilic material: smaller droplets of high electron density and larger amorphous masses of lesser opacity (Figs. 11 and 12). Occasionally, Golgi vesicles are seen to coalesce with these masses (Fig. 12). The electron density of both materials increases as development proceeds until they are no longer discernible from each other. Subsequently, most of the osmiophilic material becomes polarized in the cells (as are all the organelles) occupying the cytoplasm immediately subjacent to the gametangial surface, with the rest of the organelles lying internal to it (Fig. 11).

The plastids are very well developed, with 4–8 bands of thylakoid triplets characteristically seen in section. They are twice the average length (5 μm)

that the plastids were in the gametangial initial. The plastid genophore is visible in the periphery of each plastid (Fig. 23), and plastidglobuli are common in the stroma (double arrows, Fig. 23). Pyrenoids are not present in either reproductive or vegetative cells of *C. hancockii*.

A pair of centrioles is occasionally found at this stage, near the nuclear envelope (Fig. 13). These disappear concurrently with the appearance of two basal bodies at the axial surface of the protoplast. The cytoplasm near the axis of the gametangium pulls away slightly from the wall during flagellar exsertion (Figs. 14 and 15). Both flagella develop simultaneously in each locule, and each flagellum has a distinct base plate (single arrow, Fig. 15). One flagellum is closely appressed to the plasma membrane just beyond the point of its origin (Fig. 15). Both the flagellar membrane and the plasmalemma are distinctly more electron opaque in this region of contact (double arrows, Fig. 15; arrow, Fig. 17), and the flagellar base is usually swollen here.

Throughout the process of gametogenesis, the cytoplasm is replete with ribosomes (Fig. 13), especially prior to, and during flagellogenesis.

An eyespot first appears during flagellar exsertion in a plastid near the basal bodies. Each eyespot is composed of numerous osmiophilic droplets clustered beneath the plastid envelope (Fig. 15).

Concurrently, there is a proliferation of endoplasmic reticulum (ER) and Golgi bodies (Figs. 16–18). The ER is mostly smooth (*i.e.*, ribosome-free). Hypertrophied Golgi bodies contain up to 20 or more cisternae and are surrounded by multivesicular bodies and vesicles containing fibrillar contents (Fig. 16). The proximal faces of the Golgi apparatus appear to be derived from coalescing vesicles arising from the nuclear envelope between the nuclear pores (Fig. 16; arrow, Fig. 18). Because the ER and its associated vesicles are so prevalent near the flagellar bases, it is difficult to determine whether vesicles

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Figs. 2—9, light micrographs; Figs. 10—31, electron micrographs.

Fig. 1. Small female *Cutleria hancockii* thallus with concentric bands of sori (arrow). ×0.9

Fig. 2. Transection through a sorus, with filaments bearing gametangia (single arrows), and paraphyses (double arrows). ×210

Fig. 3. Lateral wall protrusion on a fertile filament filled with cytoplasm (arrow). Above it is an initial with a basal septum. ×860

Fig. 4. An initial cleaved into a two-cell filament. ×860

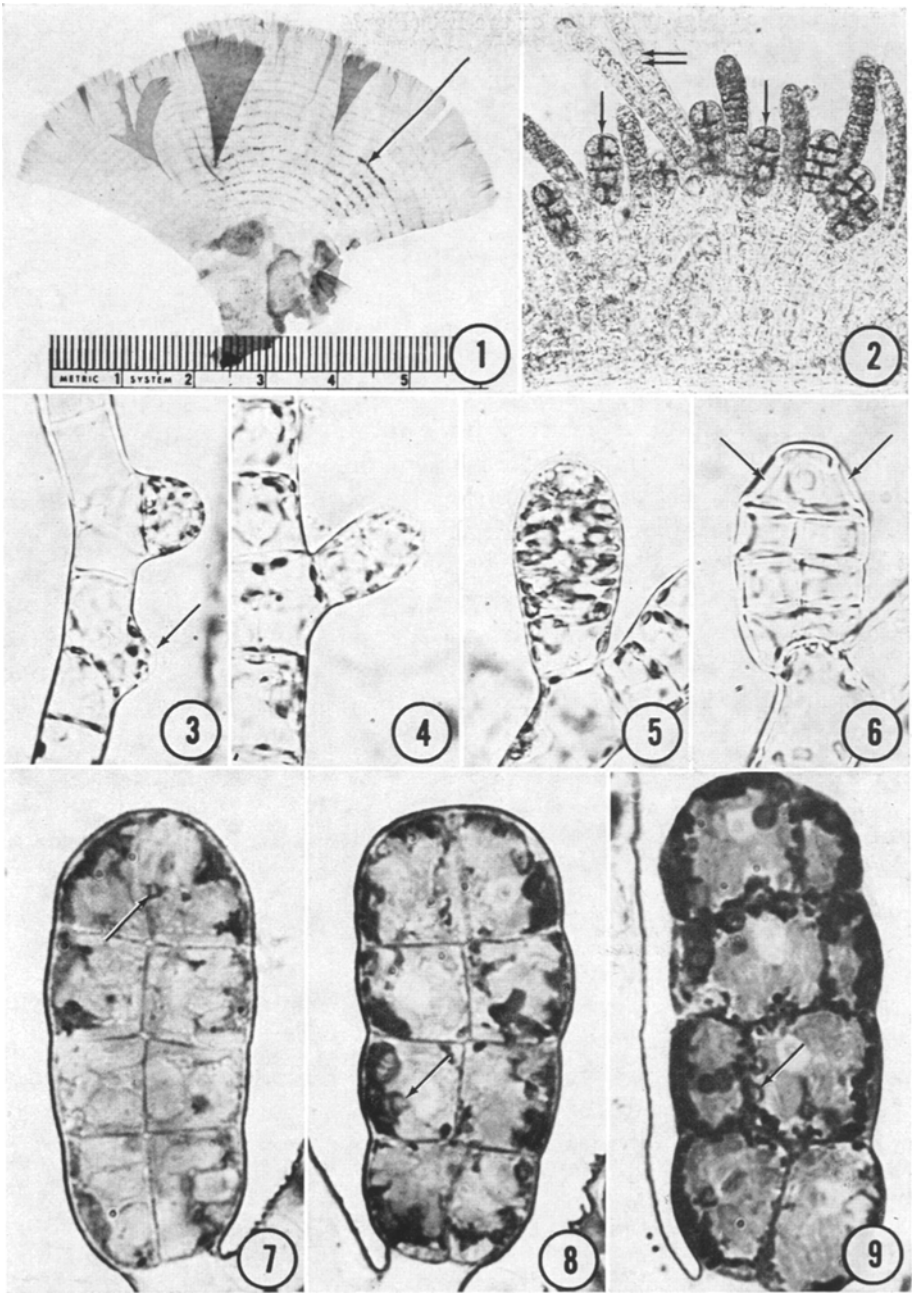
Fig. 5. Four-cell initial on a fertile filament. ×860

Fig. 6. Empty gametangium with two pores in profile (arrows). ×860

Fig. 7. Section of a 16-cell gametangium stained with TBO, showing slightly-stained peripheral amorphous material and some globular masses (arrow). ×1,340

Fig. 8. Slightly older gametangium than Fig. 7 (TBO-stained section) with peripheral dark staining material and a few metachromatic globules (arrow). ×1,340

Fig. 9. Nearly mature structure (TBO-stained section). Note that the peripheral material stains very darkly and that many globules are still present (arrow). ×1,340



Figs. 1–9

arise from the Golgi apparatus or the ER (Figs. 14 and 17). The multivesicular bodies (Fig. 19; double arrow, Fig. 20) and the fibrillar vesicles (arrow, Fig. 20; double arrows, Fig. 21) empty their contents into the basal region of the flagella at the same time mastigonemes appear on the free flagellum (Figs. 18–22). The mastigonemes are somewhat clumped at first, occupying only one-third of the flagellar circumference in section (Fig. 18). In the region of mastigoneme insertion, amorphous material occurs between the flagellar membrane and the axoneme (arrows, Fig. 19).

The mature gametes have a fairly well-developed plastid-ER (PER) system (arrow, Fig. 24), and occasionally, connections between the nuclear envelope and the PER can be found (arrow, Fig. 23).

Organelle polarity (which is first established shortly after the cleavages) is very evident just prior to gamete maturity. The flagella lie along the central, longitudinal gametangial axis, with many mitochondria, Golgi bodies, ER and their associated vesicles (Fig. 26). Moving outward, one sees the nucleus surrounded by many plastids and finally the osmiophilic material just beneath the gametangial surface.

After flagellar formation and decoration are nearly complete, the protoplasts begin to withdraw from the axial walls (Figs. 25 and 26) breaking protoplasmic connections. There is a concomitant secretion of most of the osmiophilic material into the surrounding spaces and finally into the medium (arrows, Figs. 25 and 26). Many granular vesicles can be found lining the wall in the vicinity of where the pore will be formed (Figs. 27 and 28). They fuse with the plasmalemma, dispersing their contents into the wall (arrows, Figs. 27 and 28). Often, the wall fibrils seem to be unravelling in these areas (Fig. 28).

The flagellar rootlet system is composed of tubules beneath the plasmalemma, radiating from amorphous masses near the basal bodies (Fig. 29). Some of these rootlet tubules lie very close to the eyespot.

A fibrous matrix appears around each gamete as it is rounding up and the pores are forming (not shown). The gametes are then released through the pores and the wall is recurved around the pores in empty locules (arrows, Fig. 30). Plasmodesmata are very evident in the empty gametangia (arrows, Fig. 31), as is the hollow nature of the longitudinal axis (Fig. 30).

4. Discussion

Cutleria provides a good system for examining gametogenesis. The gametangia are borne externally and therefore are more easily preserved and examined in living material. They are localized in sori, and several developmental phases are present sequentially on each fertile filament, so various stages can be found readily by sectioning through a sorus. Finally, it is useful to investigate an anisogamous (non-oogamous) system for comparison with the isogamous and oogamous brown algae which have been explored. This is especially true

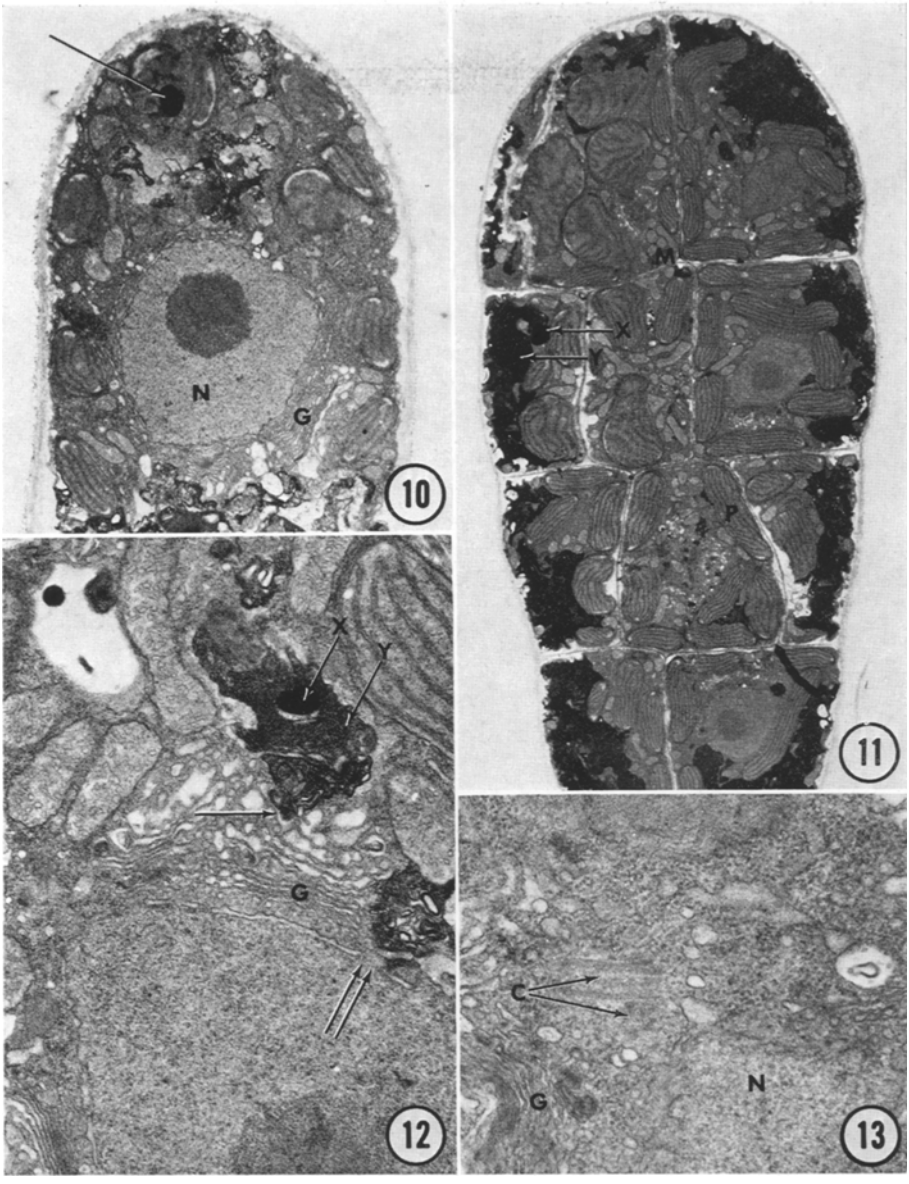


Fig. 10. Initial with basal septum after migration of the nucleus (*N*) and the perinuclear Golgi apparatus (*G*). Note the osmiophilic droplet already present (arrow). ×5,200

Fig. 11. Near-median longisection of a 16-cell gametangium. Note the arrangement of organelles in the locules: peripheral osmiophilic material, central plastids (*P*) and nuclei, and mitochondria (*M*) near the longitudinal gametangial axis. Smaller opaque globules (*X*) and larger amorphous masses (*Y*) are present. ×2,800

Fig. 12. Vesicles (single arrow) from the Golgi apparatus (*G*) apparently fusing with the osmiophilic material composed of opaque globules (*X*) and more translucent masses (*Y*). Prominent openings (double arrows) exist in the nuclear envelope. ×16,250

Fig. 13. A pair of centrioles (*C*) (one in longisection, other in oblique transection) near the nucleus (*N*) and Golgi apparatus (*G*). Note the abundance of cytoplasmic ribosomes. ×28,000

regarding phylogenetic relationships, since reproduction is usually considered an evolutionarily conservative characteristic. Because the brown algae possess all three major forms of reproduction, along with simple to very elaborate tissue types, a clear understanding of these relationships may help elucidate the evolution of more sophisticated reproductive systems in higher plants and animals.

4.1. Nucleus

The only detailed electron-microscopic study of brown algal mitosis is that of *Pilayella* during gametogenesis (Markey and Wilce 1975). Many of the events are similar in *C. hancockii*, and the details of both reproductive and vegetative mitosis will be described in later papers of this series.

4.2. Plastids

The formation of the eyespots occurs rather quickly, as stages during their development were not observed. Their morphology and location are typically phaeophycean (Dodge 1973).

The absence of pyrenoids in *C. hancockii* is similar to what has been reported in European species of *Cutleria* by Evans (1966) and Simon (1954). Thus far, only one species of this genus has been shown to possess pyrenoids: *C. cylindrica* from Japan (Hori 1972, La Claire, unpublished observations).

Connections between the PER and the nuclear envelope have been seen before in other brown algae by Bouck (1965) and various other workers (see Dodge 1973, for review), and are considered an important fine structural characteristic of the chlorophyll *c* containing groups of algae.

Fig. 14. Transverse section showing cytoplasm slightly withdrawn from the gametangial axis at inception of flagellar exsertion. Note the abundance of vesicles in regions near the axis. ×5,525

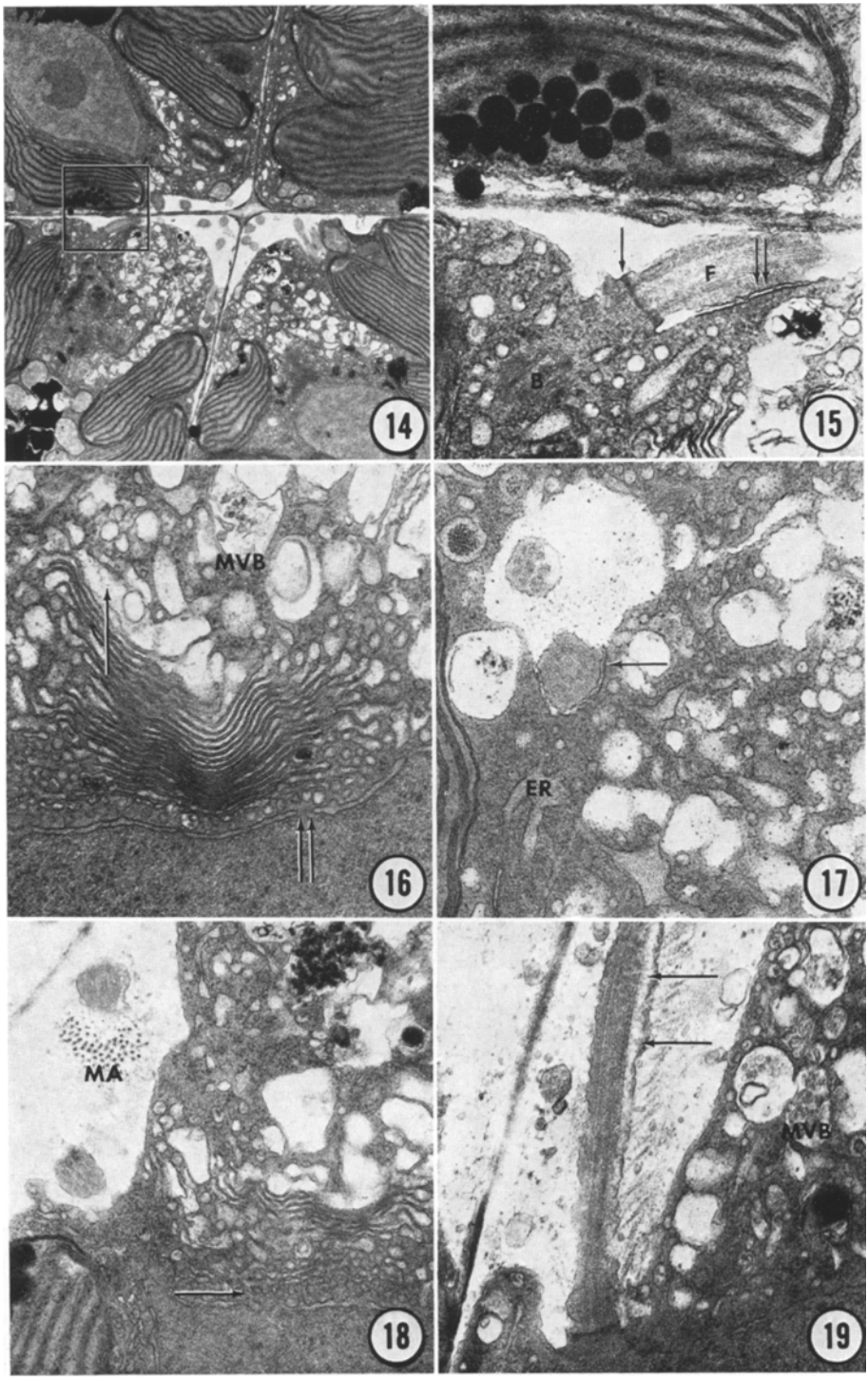
Fig. 15. Greater magnification of inset in Fig. 14. The eyespot (*E*) is composed of several aggregated osmiophilic droplets. The flagellum (*F*) has a distinct base plate (single arrow) and a basal body (*B*). The flagellar sheath and the plasmalemma are more osmiophilic in the region of juncture (double arrows). ×29,750

Fig. 16. A prolific Golgi body surrounded by vesicles with fibrous inclusions (arrow) and multivesicular bodies (*MVB*). Nuclear pores (double arrows) interrupt the envelope. ×26,250

Fig. 17. Transverse section near the flagellar bases. A network of ER occupies the flagellar region. The osmiophilic membranes of the swollen flagellar base of the appressed flagellum and the plasmalemma (arrow) shown in a plane perpendicular to that in Fig. 15. ×28,000

Fig. 18. Slightly clumped mastigonemes (*MA*) present on the free flagellum. The nuclear envelope appears to produce the perinuclear Golgi apparatus (arrow). ×26,250

Fig. 19. Multivesicular bodies (*MVB*) prevalent in the vicinity of mastigoneme deposition. Amorphous material is present between the axoneme and the flagellar membrane beneath the mastigonemes (arrows). ×26,250



Figs. 14–19

4.3. Golgi Apparatus

Although the perinuclear Golgi apparatus exhibits little activity before the developing gametangia have reached the 16-cell stage, at least three types of vesicles are associated with the Golgi bodies after the cleavages are completed, suggesting that this complex may be involved with a few different processes at once. The multivesicular bodies (Fig. 16) and some osmiophilic vesicles (Fig. 12) may be Golgi-derived or modified. Whether the vesicles with fibrillar contents bear mastigonemes/mastigoneme precursors, and whether the mastigonemes are of Golgi, ER or nuclear origin is not clear (see Section 4.5.).

Formation of the Golgi complex from the nuclear envelope has been reported by Bouck (1965) and subsequently, in various brown algae including *C. adspersa* (Caram 1975).

4.4. Endoplasmic Reticulum

Since the ER becomes especially well developed in the region of a locule nearest the gametangial axis (the future anterior end of the free gamete), and ER vesicles are particularly prominent during flagellogenesis, they may be directly involved with flagellar formation and decoration. However, since the ER is smooth, it is unlikely that it is actually synthesizing any of the proteins necessary for the construction of the flagella.

4.5. Flagella

The osmiophilic membrane region where the swollen base of the appressed flagellum and the plasmalemma are contiguous has been reported elsewhere, including in male gametes of *C. adspersa* (Caram 1975). It was described as being smooth ER in *Pilayella* gametes (Markey and Wilce 1976 a). In some

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Fig. 20. Vesicles with fibrous inclusions (single arrow) present near the flagella during mastigoneme attachment, and multivesicular bodies emptying their contents (double arrows). ×29,750

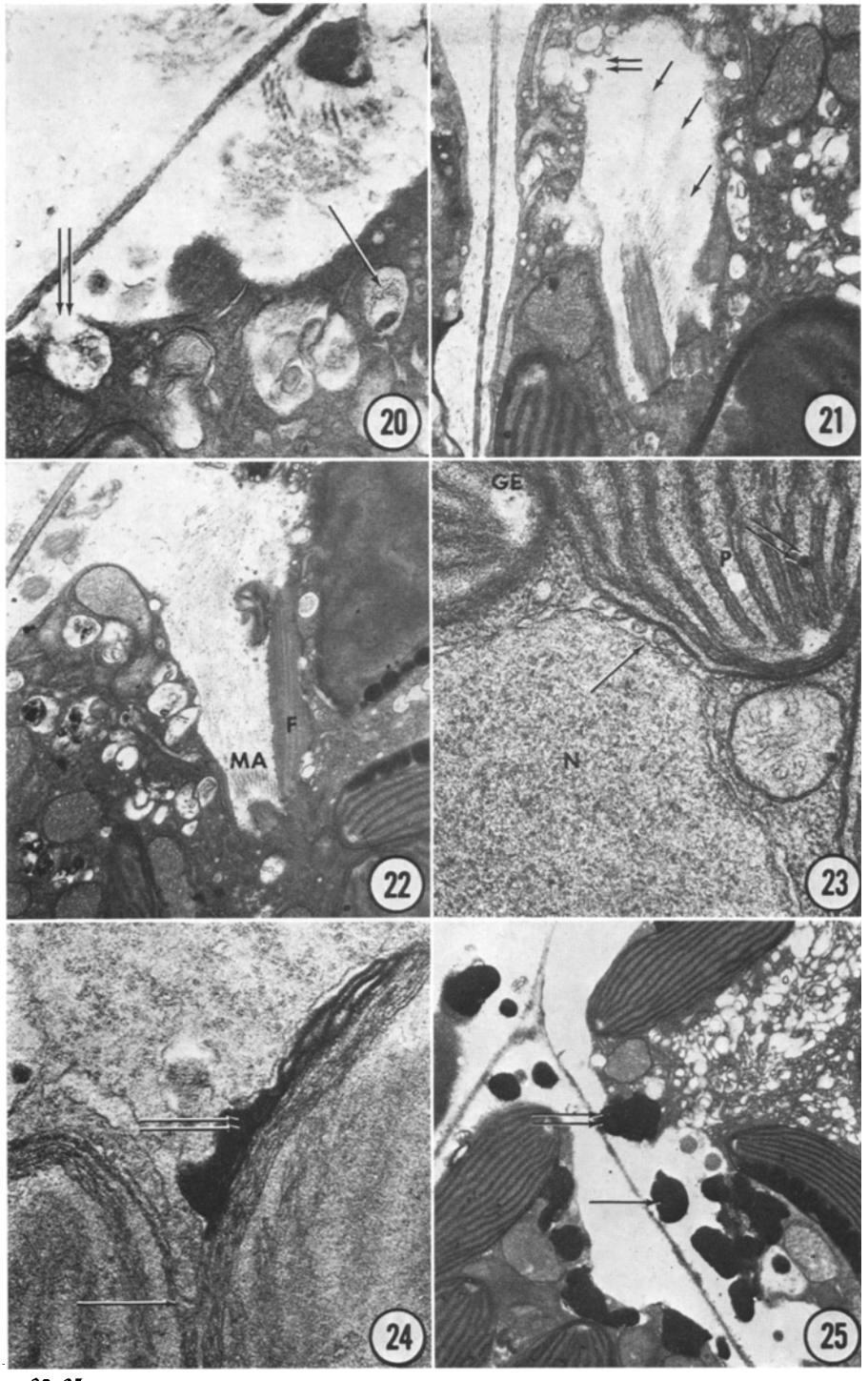
Fig. 21. The fiber-containing vesicles empty their contents during mastigoneme appearance (double arrows). Mastigonemes (single arrows) pass in and out of the section plane in a wave-like fashion. ×17,875

Fig. 22. Longisection through region in which the smooth flagellum (*F*) is tightly apposed to the plasmalemma. Mastigonemes (*MA*) are only present on the free flagellum. ×13,000

Fig. 23. Mature cell with connections between a plastid (*P*) and nucleus (*N*) via the PER and nuclear envelope (arrow). Regions of plastid genome (*GE*) and osmiophilic inclusions (double arrows) are present also. ×35,750

Fig. 24. Mature cell showing PER connecting two plastids (arrow). Some osmiophilic material is associated with one plastid (double arrows). ×70,000

Fig. 25. Some osmiophilic material prior to, during (double arrows) and after (single arrow) secretion from the withdrawing cytoplasm. ×9,100



Figs. 20–25

longisections (Fig. 22) the juncture does resemble smooth ER, but closer examination shows that it is clearly a tight juxtaposition of the two cytomembranes (Figs. 15 and 17). No connecting bridges (*"ponts très ténus"*) were seen in this junction, as Caram (1975) reported in *C. adspersa* male gametes.

The flagella of *C. hancockii* clearly develop on the surface rather than internally in a large vesicle as was reported for *Pilayella* gametes (Markey and Wilce 1976 a) and zoospores (Markey and Wilce 1976 b). Some transverse sections near the flagellar bases (Fig. 17), and grazing sections (Fig. 21), give the appearance that they are enclosed by cytoplasm, but this is definitely a result of the plane of sectioning.

Bouck (1969) first demonstrated that mastigoneme elements in *Fucus* and *Ascophyllum* spermatozoids originate in vesicles from the nuclear envelope. A similar phenomenon has been seen in several other organisms including *Pilayella* gametes (Markey and Wilce 1976 a). In our study, no tubular structures were observed in the cytoplasm, but ER cisternae and vesicles containing fibrillar materials were common near the flagellar bases (Fig. 20). As was mentioned above, Golgi vesicles were also found carrying similar material (Fig. 16), and Loiseaux (1973) reported mastigonemes in a specialized Golgi body during zoosporogenesis in three brown algae. Caram (1975) saw fibers apparently in Golgi vesicles in spermatozoids of *C. adspersa*. But it cannot be stated with certainty that these substances in *C. hancockii* are, in fact, mastigoneme precursors, nor from where the mastigonemes originate. Perhaps both the Golgi and ER are involved in mastigoneme development here, and some stages of formation were missed due to the apparent speed of the process.

The initial clumping of mastigonemes (Fig. 18) was noted in *Pilayella* zoospores (Markey and Wilce 1976 b), but it is not as obvious in female gametes of *C. hancockii*.

It is believed that the mastigonemes are arranged helically on the decorated flagellum because they pass in and out of the plane of section in a wave-like fashion (Fig. 21). This arrangement has been seen in other brown algal gametes including *C. adspersa* (Caram 1975), *Pilayella* (Markey and Wilce 1976 a), *Fucus* and *Ascophyllum* (Bouck 1969). The amorphous masses

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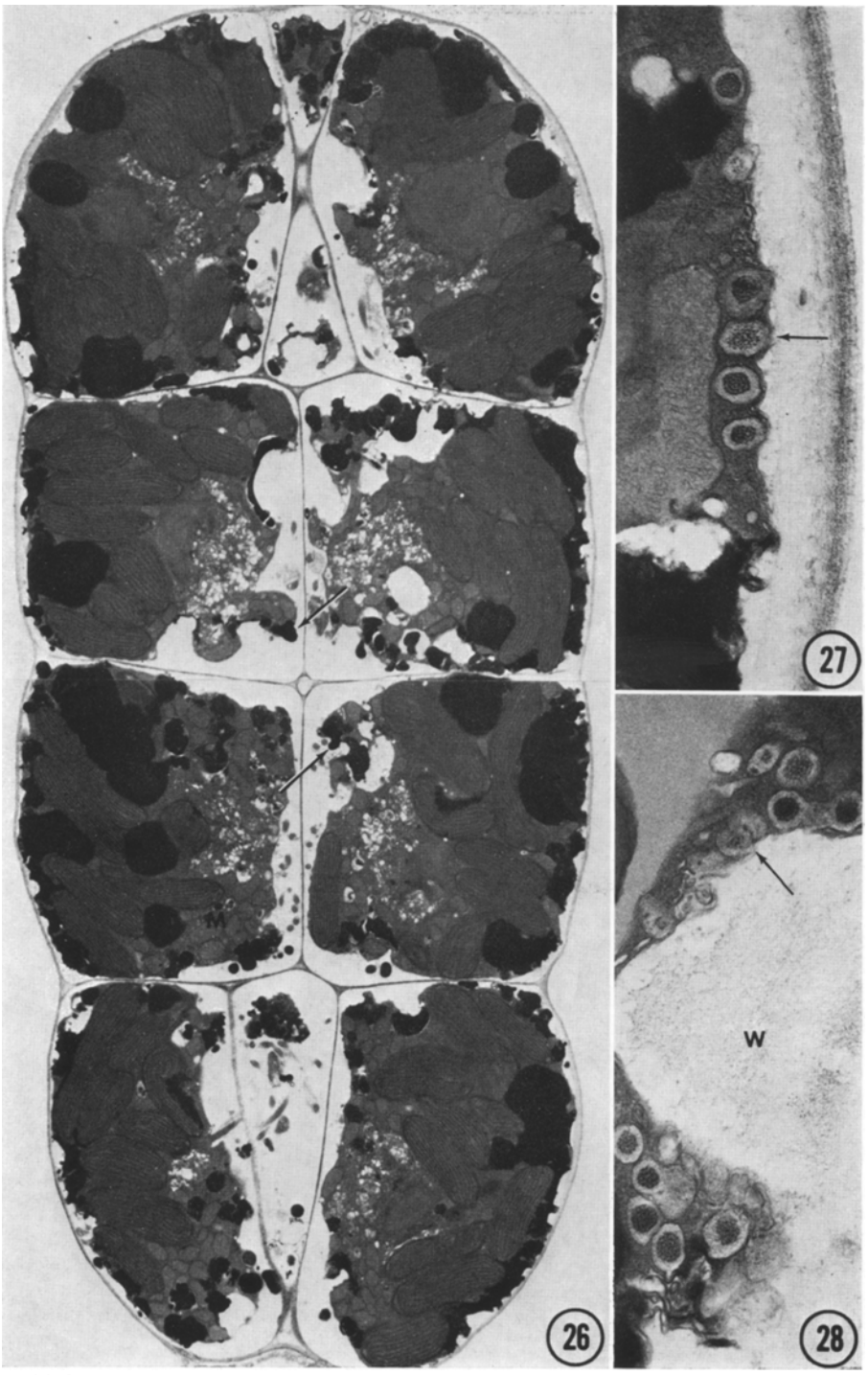
Fig. 26. Collage of near-median longisection through entire gametangium just prior to gamete release. Note that the polarization of organelles is still present. Mitochondria (*M*) are primarily in the axial region near the flagella. At this stage, flagella have been formed and decorated, and osmiophilic material is being secreted (arrows). Note that gametes are starting to round up. ×3,040

Fig. 27. Granular-cored vesicles lining the wall near future pore site. One vesicle is fusing with the plasmalemma and dispersing its contents into the wall (arrow). ×28,000

Fig. 28. Granular-cored vesicles in the region where the wall (*W*) appears to be unravelling. Some of the vesicles are releasing their contents (arrow). ×28,000



Figs. 26–28

between the flagellar membrane and the axoneme may represent attachments of the mastigonemes to the axoneme as has been previously seen in *Leptonematella* and *Ralfsia* zoospores (Loiseaux and West 1970), and in the chrysophyte *Ochromonas* (Markey and Bouck 1977).

The rootlets were difficult to discern in sectioned material because of the high cytoplasmic density found in most developmental stages. Both the tubular appearance of the rootlets and their association with amorphous masses near

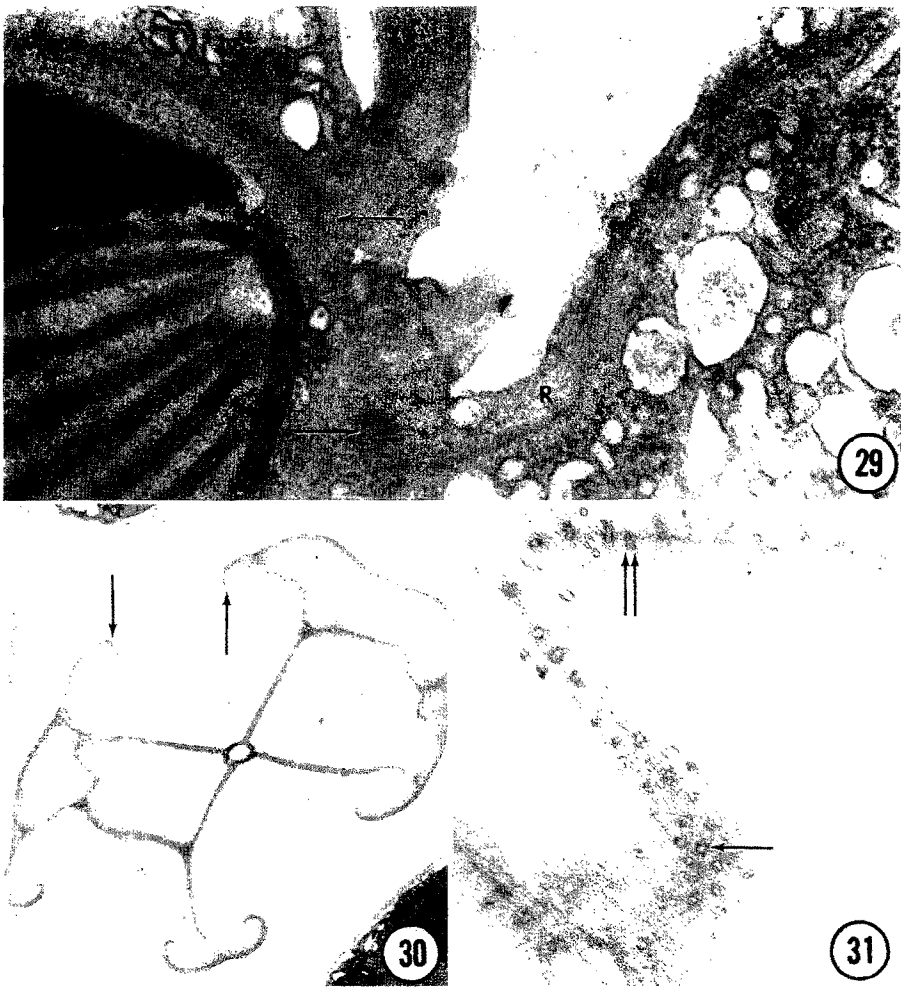


Fig. 29. Tubular rootlet structures (*R*) radiating from basal body region in gamete prior to release. Note that the rootlets appear emanating from amorphous material (arrows), and some tubules are associated with the eyespot. ×52,500

Fig. 30. Oblique transection trough empty gametangium. Walls are curved inward around pores (arrows). Gametangial axis is hollow. ×2,400

Fig. 31. Walls in an empty gametangium with plasmodesmata seen in transection (single arrow) and longisection (double arrows). ×26,250

the basal bodies (Fig. 29) have been reported elsewhere [including diploid zoospores of *Pilayella* (MARKEY and WILCE 1976 a)]. Also, the association of rootlet tubules with the eyespot has been previously seen in motile cells of several members of the *Phaeophyta* and *Chrysophyta.* CARAM (1975) indicated similar rootlets on detached flagella (whole-mount preparation) of *C. adspersa* male gametes.

4.6. Basal Bodies

The concurrent disappearance of the centrioles near the nucleus and the appearance of basal bodies near the cytoplasmic surface suggest that the former may migrate to the surface to become the latter. If so, this process must be rapid since no centrioles were ever observed free in the cytoplasm in this study.

4.7. Mitochondria

No tubular inclusions were found in the mitochondrial cristae here, although they were reported in spermatozoids and zoospores of several brown algae [*e.g., Fucus* (POLLOCK and CASSELL 1977), *Pilayella* (MARKEY and WILCE 1976 b), *etc.*]. However, fibrous elements were occasionally found in the cristae of *C. hancockii* female gametes (LA CLAIRE, unpublished observations). The significance of intracristal inclusions is not yet known.

4.8. Osmiophilic Material

Of the two different types of osmiophilic masses evident early in gametogenesis, the smaller, electron-opaque globules correspond to those which stain green with TBO. These are probably physodes. EVANS and HOLLIGAN (1972) reported that physodes usually stain turquoise or green with TBO and are electron transparent (unless fixed with formaldehyde, then they are electron dense). Since the material used in this study was fixed with a glutaraldehyde-paraformaldehyde mixture, an opaque appearance would be anticipated. EVANS and HOLLIGAN (1972) also found that physodes in older cells of *Dictyota* stained darker blue in formaldehyde-treated tissue. Therefore, the larger, amorphous (less electron opaque) masses in *C. hancockii* which stained blue with TBO, may represent physodes of a different age. This correlates well with the *Dictyota* results since all the osmiophilic material becomes more electron dense (and stains darker blue with TBO) as gametangial maturation proceeds. The Golgi apparatus may be involved with the "aging process" of the physodes since Golgi vesicles were seen fusing with these osmiophilic masses (Fig. 12).

The origin of the physodes is not clear in *C. hancockii.* The presence of globular bodies in the plastid stroma (double arrows, Fig. 23) was previously reported in *Dictyota* (EVANS and HOLLIGAN 1972). Also, osmiophilic material was often found close to the plastids or even beneath some of the membranes

surrounding plastids (arrows, Fig. 24). Because of the electron opacity of the material, it is uncertain whether it is located in or beneath the PER, or in the envelope itself. Although Evans and Holligan (1972) implicated plastid involvement in physode production in *Dictyota*, the role of plastids in this process in *Cutleria* is still in question.

In *C. adspersa*, Caram (1977 b) noted the movement of physodes through the walls in female gametangia and mentioned the absence of physodes in liberated spermatozoids (Caram 1975). Loiseaux (1973) also reported a similar excretion of material in developing zoospores of three brown algae, with some of this substance actually being incorporated into the zoosporangial wall. It is noteworthy that the maturing female gametes of *C. hancockii* clearly secrete the bulk of this material into the extracytoplasmic spaces, and it eventually escapes through the walls into the medium. This causes a yellow coloration of the medium when fertile plants are maintained in culture (La Claire, unpublished observations). This secretion may imply that the physodes are a metabolic waste product of little or no use to the future zygote (see Ragan 1976, for review of the roles of physodes). Alternatively, the release of this material may be involved with the gametes rounding up and pulling away from the walls. If this is the case, whether this is an osmotic phenomenon or merely to reduce the cell volume, is not yet known.

4.9. Walls and Pores

The hollow axial supporting column of wall material (seen in cross section, Fig. 30) was previously observed in plurilocular zoosporangia of *Ectocarpus* (Lofthouse and Capon 1975).

The presence of plasmodesmata in the walls of plurilocular structures has also been noted in most of the brown algae examined [*e.g.*, *Pilayella* (Markey and Wilce 1975, 1976 a), *Ectocarpus* (Lofthouse and Capon 1975), *etc.*].

The granular-cored vesicles (Figs. 27 and 28) may have a lytic function in pore production since they aren't usually found until the gametes begin to round up, and they empty their contents into wall areas which appear unravelling (Fig. 28). However, the evidence is merely by association. Caram (1977 b) mentioned polysaccharide-containing vesicles which sound similar in appearance, in female gametes of *C. adspersa*. If these bodies are polysaccharidic, then they may be secreting the matrix material instead. Ultrahistochemical information will be necessary to determine their composition and possible function. Identical vesicles are present in both degenerating female gametangia (La Claire, unpublished observations) and in developing male gametes of *C. hancockii* (La Claire and West, in preparation). The origin of these vesicles is unknown.

The chemical nature of the matrix surrounding the gametes is not known, but it visually resembles that reported in unilocular and plurilocular structures of

several other brown algae [*e.g.*, *Pilayella* (MARKEY and WILCE 1976 a)]. TOTH (1976) has shown that mucilaginous polysaccharides surround mature zoids of *Pilayella* and these may be involved with a release mechanism. Perhaps this matrix is related to gamete liberation in *C. hancockii*, but further speculation is unwarranted at this time.

The recurving of the wall surrounding each pore (Figs. 6 and 30) probably results from differential swelling or contraction of different wall layers upon gamete release.

4.10. Polarity of Organelles

A similar polarity of organelles about the gametangial axis is found here as was seen in *C. adspersa* male structures (CARAM 1975). Also, the peripheral location of osmiophilic material was reported in *Ectocarpus* mitosporangia (LOFTHOUSE and CAPON 1975). However, the significance of organelle polarity in general, remains obscure.

Acknowledgements

We would like to thank Dr. MICHAEL D. GUIRY of Portsmouth Polytechnic for help with the collection of plant material, and Dr. JAMES N. NORRIS of the Smithsonian Institution for tidal and phenological information concerning Puerto Peñasco. We are also grateful to Prof. WILLIAM A. JENSEN and MARY ASHTON for use of the electron microscope facilities, to Dr. PATRICIA SCHULZ and to CAROLINE SCHOOLEY (Electron Microscopy Laboratory) for stimulating discussions, and to LINDA OBATA for typing the manuscript. This work was supported in part by U.S. National Science Foundation Grant GB-40550, U.S. Department of Commerce National Oceanic and Atmospheric Administration Sea Grant NOAA 04-6-158-44021, North Atlantic Treaty Organization Grant #1130, and the University of California, Berkeley, Miller Institute for Basic Research in Science, which provided a one-year research professorship to J.A.W.

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