ULTRASTRUCTURE AND DIFFERENTIATION OF HYDRODICTYON RETICULATUM

V.* DEVELOPMENT OF POLYHEDRA

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Abstract

The fine structure of mature zygospores and azygospores of *H. reticulatum* is briefly discussed before their differentiation to form biflagellate zoospores is described. These large zoospores are liberated through a rupture in the parental wall and after a brief period of feeble motility, retract their flagella and secrete a cell wall themselves. This wall is deposited unevenly, producing the characteristic spines of polyhedra.

INTRODUCTION

In a previous paper (Marchant and Pickett-Heaps 1972b), we discussed conjugation of the gametes of H. reticulatum and the subsequent development of thick-walled zygospores. We also mentioned that some of the gametes which fail to conjugate retract their flagella and secrete a wall to become azygospores. This paper describes the ultrastructure of mature spores, their germination to form zoospores, and subsequent deposition of a wall by the zoospores, after a brief period of motility, to produce polyhedra. Differentiation of these cells will be related to similar processes elsewhere in the life cycle of this alga and in other organisms. The position of polyhedra in the life cycle of H. reticulatum is illustrated in an earlier paper in this series (Marchant and Pickett-Heaps 1971).

II. MATERIALS AND METHODS

The Australian strain of H. reticulatum (Marchant and Pickett-Heaps 1970) was used for the work described here. Differentiating spores, zoospores, and polyhedra were cultured and processed for transmission electron microscopy as previously described (Marchant and Pickett-Heaps 1970, 1971, 1972a). Some cells, fixed in glutaraldehyde and osmium tetroxide as usual, were collected on solvent-resistant Millipore filters, dehydrated in ethanol and n-amyl acetate, and dried in a CO₂-critical point drying apparatus (Anderson 1951; Horridge and Tamm 1969). These dried specimens were omnidirectionally shadowed with carbon and gold and examined in a Cambridge Stereoscan scanning electron microscope.

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Fig. 1.—Zygotes of *H. reticulatum*. Note the nuclei (n) and pyrenoids (p). Nomarski optics. $\times 1000$.

Fig. 2.—Zygote cleaving to form zoospores. The chloroplast obscures other cytoplasmic components. The smaller spore is probably an azygospore. Nomarski optics. $\times 1000$.

Fig. 3.—Pattern of settling of zygotes and azygotes corresponding to writing (19/5) on the top of another Petri dish which was subjacent to the dish in which the spores had settled. $\times 1.4$. Fig. 4.—Part of the wall (w) and cytoplasm of a zygospore. Note the lipid droplets (l) and poorly preserved chloroplasts. $\times 18,000$.



Fig. 5.—Zygospores adhering to one another by their outer layer of wall (w) (arrowhead). Note the basic similarity in appearance of this junction with that between mature vegetative cells. $\times 20,000$.

Fig. 6.—Initial cleavage of zygospores to produce zoospores. Note the wall weakening and the preponderance of lipid droplets in the cytoplasm. $\times 4,700$.

III. RESULTS

Of all stages in the life cycle of H. reticulatum, zygospores, azygospores, zoospores, and polyhedra derived from the latter have proved to be the most difficult to preserve satisfactorily for electron microscopy, the main reasons being:

- (1) Zygotes accumulated lipid droplets which came to occupy most of the cytoplasm (Figs. 4, 5, 6). This lipid was difficult to fix and embed, and structures surrounding it were rarely adequately preserved and usually disintegrated when being sectioned (Fig. 5).
- (2) Zoospores were extremely fragile, being easily disrupted by mechanical damage and slight changes in osmotic pressure. In addition, they were only active for a short period of time after release from the spore, and were thus difficult to collect. They too contained much lipid (Fig. 7).
- (3) Polyhedra initially contained lipid droplets (Figs. 20, 21). As they matured they became vacuolate, but the individual cells were too small to cut into segments, the procedure that we adopted to obviate disruption during processing of cylindrical coenobia (Marchant and Pickett-Heaps 1970). Hence it was difficult to avoid collapse when processing polyhedra for electron microscopy.

(a) Mature Zygospores and Azygospores

Zygospores were usually considerably larger than azygospores (Fig. 2), although the size of both varied widely. As these resistant spores aged, they lost their green colour and became orange and could be stored dry for several weeks. No attempt was made, however, to investigate how long they could survive desiccation. Both zygospores and azygospores had a thick bilayered wall (Figs. 4, 5), similar to that of cylindrical coenobia; sometimes, however, the layering of the wall appeared to be reversed, i.e. they had a thin inner layer (Fig. 25, Marchant and Pickett-Heaps 1972b). The spores were often attached to one another in irregular aggregates, or to the substrate, by their outer layer of cell wall (Fig. 5). What induces their aggregation is unknown. That zygotes and azygotes are negatively phototactic before losing their motility and secreting a wall is illustrated in Figure 3. Here, spores had aggregated and adhered to one another and to the bottom of a Petri dish in a pattern corresponding to the date written with a black felt pen on the *top* of another Petri dish immediately subjacent to the dish containing these spores (cultures were illuminated from below).

Fig. 7.—Zoospores formed by cytoplasmic cleavage of zygospores. At the wall weakening the inner layer of wall has disappeared and the thin outer layer (arrowhead) is becoming greatly distended. Note the transverse section of a flagellum (arrow). l, lipid droplet; n, nucleus. $\times 5,700$.

Fig. 8.—Light micrograph of section adjacent to Figure 7, showing the distension of the outer layer of wall and the other zoospores. $\times 1,100$.

Fig. 9.—Cleavage of zygotic cytoplasm. Note the microtubules (arrow) adjacent to the developing cleavage furrow. $\times 32,500$.

ULTRASTRUCTURE AND DIFFERENTIATION OF H. RETICULATUM. V 1191





Fig. 10.—Scanning electron micrograph of a zoospore showing the flagella and distortion of the plasmalemma by lipid droplets. $\times 2,300$.

Fig. 11.—Quadriflagellate zoospore (cf. Fig. 10). Attachment of flagella shown at higher magnification in Figure 12. $\times 2,300$.

Fig. 12.—Scanning electron micrograph of part of a quadriflagellate zoospore. Note the projections on the zoospore from which the flagella arise. $\times 12,000$.

Fig. 13.—Four zoospores within a highly distended zygotic wall. $\times 1,300$.

ULTRASTRUCTURE AND DIFFERENTIATION OF H. RETICULATUM. V 1193

(b) Developing Zoospores

Whereas zygospores cleaved to form usually four zoospores on germination, azygospores generally only produced a single zoospore. Meiosis was not observed in our material. Cleavage furrows (Fig. 2, cf. Fig. 1) in the zygotic cytoplasm grew along bands of phycoplast (Pickett-Heaps 1972*a*) microtubules (Fig. 9). Following cytoplasmic cleavage, part of the outer layer of zygotic wall expanded (Figs. 6, 7, 8, 13), and the zoospores moved into this space before being liberated through a rupture in the distended wall. If this distended zygotic wall failed to rupture, the trapped zoospores completed their transformation to polyhedra within it (Fig. 21; also see Fig. 5 in Marchant and Pickett-Heaps 1971).

These zoospores bore a pair of flagella (Fig. 10) and were only weakly motile. Occasionally quadriflagellate zoospores (Figs. 11, 12) were observed. In culture, zoospores appeared to lack any phototactic response and rarely moved far from where they were released.

(c) Formation of Polyhedra from Zoospores

After a period of motility which lasted only a few minutes, zoospores retracted their flagella and secreted a cell wall (Figs. 14–19). Wall formation was extremely rapid and not uniform over the surface of the zoospores, so that the characteristic projections of polyhedra (Fig. 22) soon became obvious. The cytoplasm of young polyhedra contained lipid droplets (Figs. 20, 21) which disappeared as the polyhedra aged.

IV. DISCUSSION

Proskauer (1952) reported that, as expected, meiosis in H. reticulatum precedes germination of the diploid zygotes and so restores the haploid condition of the rest of the life cycle. Our attempts to confirm his observations have been thwarted: first, fragmentation of the zygotic chloroplast completely obscures the nucleus shortly before the presumed meiotic division and differentiation of the zygote (cf. Figs. 1, 2). Secondly, we have been unable to fix and embed the cytoplasm of mature zygotes satisfactorily for electron microscopy. These zygotes frequently disintegrated when sectioned, and when they are adequately fixed and embedded, they appeared to be electron-dense and ultrastructural detail was obscured.

Zygospores often attach to the bottom of culture dishes or some other substrate within the medium, or they may stick to one another by their outer layer of wall. Many become attached to the underside of the surface film of the culture medium. We do not know whether motile zygotes are weakly attracted to each other or whether their aggregation is a result of being negatively phototactic; Figure 3 suggests that the latter is more likely.

The motility of the large zoospores released by zygospores and azygospores does not appear to be sufficient to constitute an important factor in the dispersal of this alga. Polyhedra, however, are planktonic, aided in floating by their characteristic spiny processes and their lipid content. We suspect that it is these cells which are carried by water currents to disperse the alga in nature. The bristles and spines on *Pediastrum* and *Scenedesmus* (Trainor and Burg 1965) have been considered to be



Figs. 14-19.—Series of micrographs illustrating a zoospore retracting its flagella and developing the first projection to become a polyhedron. 14, Actively swimming zoospore. Arrow indicates the attachment of flagella. 15, Flagellar shortening and commencement of elongation of projection. 16, Flagella much shortened. 17, Considerable elongation of projection and flagella almost completely retracted. 18, Flagella completely retracted. 19, Extension of first projection nearly complete. All phase-contrast.

Fig. 20.—Electron micrograph of part of a young polyhedron showing the remaining lipid droplets (l) in the cytoplasm. *n*, nucleus; *v*, vacuole; *w*, cell wall. $\times 12,000$.



Fig. 21.—Irregular polyhedra produced by zoospores which had not been released from the zygotic wall. Note the new polyhedral wall (arrow). l, lipid droplet; n, nucleus; p, pyrenoid; v, vacuole. $\times 8,600$.

Fig. 22.—Scanning electron micrograph of a mature polyhedron. $\times 2,300$.

adaptations to a planktonic existence (Fritsch 1935); however, Trainor and Burg suggest that they principally serve in spacing out the colonies, thus reducing overcrowding and shading of one another. No bristles were evident on polyhedra of H. reticulatum which was somewhat surprising since they occur on the polyhedra of its close relative, *Pediastrum* (Davis 1967), as well as on the vegetative cells of that alga.

Polyhedra can also be considered to be another resistant stage in the life cycle; although they are not able to withstand desiccation to the same extent as zygospores and azygospores, they will survive drying that would kill cylindrical coenobia. Other members of the Chlorococcales have a polyhedral stage in their life cycle, e.g. Pediastrum (Davis 1967), while the vegetative cells of Tetraedron (Pickett-Heaps 1972b) are polyhedral in shape. In fact, some early phycologists apparently considered polyhedra of Hydrodictyon and Pediastrum to be species of Tetraedron. There are numerous similarities, not only in their gross morphology, but also at the ultrastructural level between these three genera. Vegetative cells of Tetraedron are uninucleate when first formed and become coencytic by repeated mitoses; mitotic nuclei are enclosed by a perinuclear envelope and in the cleavage of the multinucleate cytoplasm phycoplast microtubules are utilized (Pickett-Heaps 1972a); all these phenomena are observed in both Hydrodictyon and Pediastrum (Marchant and Pickett-Heaps 1970, 1971, and unpublished data). However, the cells produced by cleavage of *Tetraedron* are usually non-motile autospores. Only very rarely are motile zoospores formed (Starr 1954; Davis 1966), the usual product of cleavage in polyhedra of Hydrodictyon (Pocock 1960; Marchant and Pickett-Heaps 1972c) and Pediastrum (Davis 1967).

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