

## STRUCTURAL EVOLUTION IN THE FLAGELLATED CELLS OF GREEN ALGAE AND LAND PLANTS\*

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### 1. Introduction

Comparative morphological studies employing the electron microscope and modern improvements of light microscopy have recently provided a more substantial basis for interpreting the phylogeny of green algae (Mattox and Stewart, 1977; Molnar, Stewart and Mattox, 1975; Pickett-Heaps and Merchant, 1972; Pickett-Heaps, 1975; Stewart and Mattox, 1975a). The events of cell division and the micromorphology of zoospores or flagellated gametes have been the most revealing features, and it is now clear that there are at least 2 distinct groups of advanced (i.e., colonial, filamentous or parenchymatous) green algae, the Chlorophyceae and Charophyceae (Pickett-Heaps, 1975; Stewart and Mattox, 1975a), and that the ancestors of both lines were scale-covered green flagellates (Mattox and Stewart, 1977; Stewart and Mattox, 1975a; Stewart, Mattox and Chandler, 1974).

Although the features of the 2 groups are distinct and concerned with such reliable and conservative features as mitosis and the flagellar apparatus, a very perplexing problem has been our ignorance of the function and evolutionary origin of some of the most important characteristics. The flagellar apparatus of one group, the Chlorophyceae, usually has 4 or more relatively narrow microtubular rootlets descending from the region of the basal bodies (Fig. 2D) in a manner first described for zoospores (Manton, Clarke and

Greenwood, 1955; Manton, 1964) and later, in more detail, for *Chlamydomonas* by Ringo (1967). This arrangement is often referred to as a "cruciate" rootlet system.

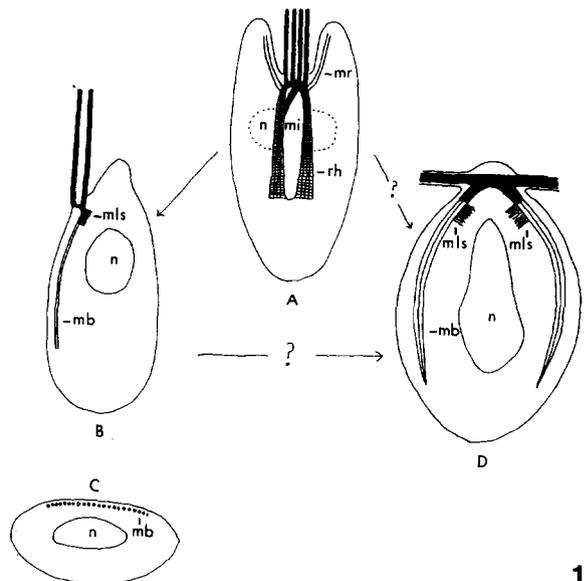


Fig. 1. The diagram outlines how a *Pyramimonas*-like cell (A) might have given rise to a *Klebsormidium* or *Coleochaete*-like zoospore (B) by "halving," the conversion of one rhizoplast to an MLS, and the loss of both the pit and the microtubular rootlets that ascend the sides of the pit. C represents a cross section of the cell shown in B. A zoospore of *Trentepohlia* (D) could have arisen either by a doubling of the cell shown in B or from a "whole" cell by the conversion of both rhizoplasts of A to MLS's. The diagrams are not exact representations because a single plane would not include 2 microtubular rootlets and both rhizoplasts of *Pyramimonas*. In *Pyramimonas* the nucleus is shown in its prophase position. D was drawn after Graham and McBride (1975). mb, microtubular band; mi, microbody; mls, multi-layered structure; mr, microtubular rootlet; n, nucleus; rh, rhizoplast.

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The Chlorophyceae are further characterized by a mitotic spindle whose interzonal region collapses during telophase and is replaced by a phycoplast, a system of microtubules which lies between the daughter nuclei in the plane of cytokinesis. Members of the Charophyceae have a rootlet system consisting of a single broad band of microtubules (2 in *Trentepohlia*, Graham and McBride, 1975), associated at its proximal end with a complex lamellate structure (Fig. 1B). The broad band of microtubules and the associated structure are commonly called the "multilayered structure" (MLS). Although the taxonomic value of the mentioned characteristics has not yet been questioned, they have been difficult to explain for the following reasons: (1) an MLS is not known to occur in the scaly green flagellates presumed to be relatively primitive and related to the ancestry of the Charophyceae and other green algae; (2) a descending rootlet system comparable to that of *Chlamydomonas* and other chlorophycean algae does not occur in *Platymonas*, a scaly green flagellate considered to be a primitive chlorophycean alga because it has a phycoplast; (3) there has been no detailed explanation or hypothesis regarding the circumstances leading to the evolution of the phycoplast, a structure unknown in organisms other than chlorophycean algae.

We have very recently presented evidence and a rationale for the concept that the phycoplast and collapsing telophase spindle evolved in response to a restriction of cell elongation during mitosis caused by the evolution of cell walls from scales in the flagellated unicellular ancestors of the Chlorophyceae (Mattox and Stewart, 1977). The details cannot be repeated here, and the body of this paper will be concerned with the first 2 of the 3 problems mentioned above and with some other aspects of the structure and evolution of green algal flagellated cells.

## 2. Cell symmetry and the flagellar apparatus

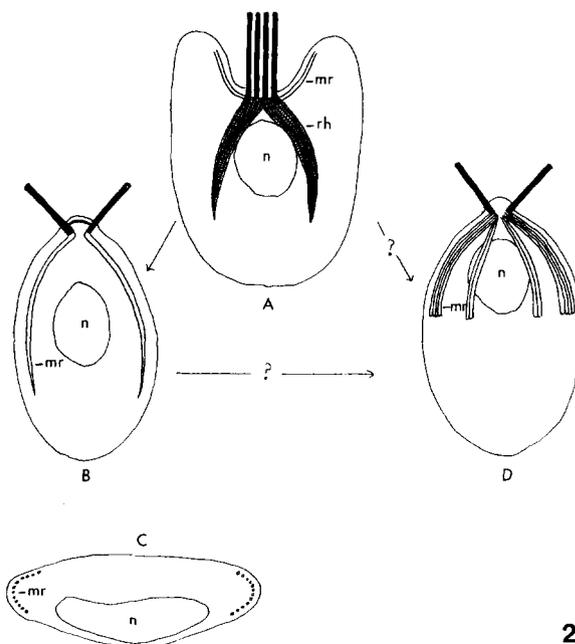
The MLS of the zoospores and flagellated gametes of charophycean algae matches the

structure of the spermatozooids of lower archegoniates and demonstrates that Charophyceae includes the algae most closely related to the ancestry of land plants. Phycologists and other botanists have long exercised their imaginations as to the identity, growth form and life cycle of those green algae which gave rise to the land flora, but modern discussion of the significance of the structure of flagellated cells to this problem began recently with Manton's (1965) paper on the phylogeny of flagellar structure. Of particular interest here is her conviction that the asymmetrical flagellated gametes of lower land plants indicate that the flagellated unicellular ancestors of land plants, and by implication the zoospores of the later filamentous ancestors, were asymmetrical and unlike *Chlamydomonas* and the zoospores of advanced chlorophycean algae. The conspicuously lateral attachment of the flagella in archegoniates also stands in contrast to the anterior insertion of the Chlorophyceae. Manton's study of scaly green flagellates ("prasinophytes") appeared to strengthen this view, because some of those organisms are asymmetrical and appear to be rather primitive green algae. The later discoveries of scales on the asymmetrical spermatozooids of *Chara* (Pickett-Heaps, 1968) and zoospores of *Coleochaete* (McBride, 1971) had a number of significant implications that became clearer with further studies. Most importantly, the scales indicate that the monadal ancestors of *Coleochaete* and *Chara* were scaly rather than walled like *Chlamydomonas*. Furthermore, the presence of a rootlet system like that of the spermatozooids of land plants indicated that that line of descent from scaly flagellates led not only to *Coleochaete* and *Chara* but to land plants as well. The presence of an MLS in *Klebsormidium* (Marchant, Pickett-Heaps and Jacobs, 1973) further demonstrated that some of the relatively primitive filamentous forms of that line are extant. Finally, evidence accumulated that all advanced green algae, both Charophyceae and Chlorophyceae had scaly ancestors. Scales on *Platymonas*, a flagellate with a

phycoplast (Stewart, Mattox and Chandler, 1974), and scales on the zoospores of *Pseudendoclonium*, in which the rootlet system is cruciate (Mattox and Stewart, 1973), point out that it is the MLS, not scales, that truly distinguishes the flagellated cells of the Charophyceae from those of the Chlorophyceae.

The ancestral position of scaly green flagellates and the occurrence of some asymmetrical genera among them have seemed to strongly support Manton's opinion that the unicellular ancestors of land plants were asymmetrical (see Birkbeck, Stewart and Mattox, 1974; Mattox and Stewart, 1973; Pickett-Heaps, 1975, Chap. 8; Stewart and Mattox, 1975b). The fact remains, however, that no known scaly green flagellate has an external form that resembles the zoospores of *Klebsormidium* and *Coleochaete*, where the flagella are inserted a very short distance from the acute anterior of the cell (see Fig. 1B); in scaly green flagellates the flagella are usually inserted in a pit of depression and in the asymmetrical forms, proportionately farther from the cell's anterior. Even more serious are the facts that no known scaly green flagellate has a rootlet system even remotely similar to the MLS of charophytes and archegoniates and that the zoospores of *Trentepohlia* are symmetrical by virtue of having 2 MLS's (Fig. 1D). With the information available, it seems to be a reasonable inference that the distinctive characteristics of charophycean zoospores never existed in unicellular flagellated organisms but arose with the transition from unicellular flagellated organisms to flagellated reproductive cells of filamentous forms. If so, the questions to be answered are: (1) what happened to the flagellar pits during the transition? (2) how and from what did the MLS evolve? and (3) were the ancestral forms distinctively asymmetrical scaly flagellates like *Heteromastix*, *Micromonas* and *Mesostigma*, as following Manton's argument, or were they nearly symmetrical and with anterior flagella like the scaly flagellate *Pyramimonas*?

We believe that a consideration of the position, nature and distribution of some components of the flagellar apparatus in scaly green flagellates provides some strong hints toward an answer to the questions posed above. Although scaly green flagellates have microtubular rootlets similar in structure to those of other green algae except charophytes, their orientation is different. The microtubular rootlets of chlorophytes extend posteriorly from the basal bodies (Fig. 2B) whereas those of some scaly green flagellates do not descend



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Fig. 2. The diagram outlines how a phycoplast-utilizing *Platymonas*-like cell might have given rise to a *Pleurastrum*-like zoospore (B) by the conversion of the rhizoplasts to microtubular rootlets and the loss of both the pit and the microtubular rootlets that lie along the sides of the pit. C represents a cross section of the cell in B. The cell in D has 4 cruciately arranged, microtubular rootlets similar to *Chlamydomonas* and many flagellated reproductive cells of chlorophycean algae; it could have arisen by a doubling of the rootlet system in a cell like B or by a replication of rhizoplasts in a cell like *Platymonas* (A) before the rhizoplasts were transformed to microtubular rootlets. The drawings are not exact representations because a single plane would not include 2 microtubular rootlets and both rhizoplasts of *Platymonas* (A). mr, microtubular rootlet; n, nucleus; rh, rhizoplast.

from the basal bodies (e.g., *Heteromastix*, Manton et al., 1965; Mattox and Stewart, 1977), and in those scaly flagellates with distinct flagellar pits such as *Platymonas* (Fig. 2A) and *Pyramimonas* (Fig. 1A), the rootlets extend anteriorly from the basal bodies along the wall of the pit. *Pyramimonas* has been described as having a cruciate rootlet system similar to that in chlorophytes (Moestrup and Thomsen, 1974), but the difference in orientation is probably important. Aside from consideration of the flagellar apparatus, *Pyramimonas* has more features like those of charophycean algae than any other scaly green flagellate yet studied in detail. Points of similarity are a single microbody which is attached close to the basal bodies and which stains densely in DAB reaction medium at pH 8, basal bodies at, rather than lateral to, the spindle poles; an open spindle; and an interzonal spindle that persists until the completion of cytokinesis (see Norris and Pearson, 1975; Pearson and Norris, 1975). Since *Pyramimonas* differs in symmetry from the flagellated reproductive cells of most charophycean algae and since its flagellar apparatus is very different, it may be profitable to consider how evolutionary changes in symmetry and structure could have occurred in a transition from a *Pyramimonas*-like flagellated ancestor to the zoospores and flagellated gametes of Charophyceae.

As far as we know, the flagella of green algal zoospores and gametes are never inserted in a pit or depression as in scaly green flagellates. It appears that the pit is a primitive characteristic that has been lost in flagellated reproductive cells of filamentous genera. Since the microtubular rootlets of *Pyramimonas* extend anteriorly from the basal bodies along the pit, the loss of the pit would require an evolutionary reorientation of the rootlets, the development of new rootlets, or the assumption of rootlet function by some other part of the flagellar apparatus. We strongly favor the last alternative because of the position and nature of the rhizoplast — the

“striated fiber” or “striated rootlet” present in *Pyramimonas* and many other flagellated cells — and because of the tendency of some scaly green flagellates to “halve” and “double”. To state the entire idea briefly before further explanation, we believe that the zoospores of charophytes like *Klebsormidium* and *Coleochaete* evolved from “half” a cell similar to *Pyramimonas* by loss of the pit and the conversion of the rhizoplast to an MLS. Symmetrical charophycean zoospores like those of *Trentepohlia* could have arisen similarly from a “whole” cell or by a doubling of the flagellar apparatus of a *Klebsormidium*-like zoospore (Fig. 1).

Firstly, there is evidence that the conversion of a rhizoplast to a broad microtubular structure like the MLS is quite possible. Rhizoplasts and microtubules seem to have some chemical similarities (Stewart, Mattox and Chandler, 1974; Stewart and Mattox, 1975b), since in *Platymonas*, the rhizoplasts dissolve to form the granular mass from which the extranuclear spindle microtubules develop. In *Pleurastrum*, which appears to be phylogenetically related to *Platymonas*, bands of microtubules occur at certain times in positions that would be occupied by rhizoplasts in *Platymonas* (Molnar, Stewart and Mattox, 1975). Furthermore, Kubai (pers. comm.) has found microtubules continuous with the fibrous units of a striated rhizoplast-like structure in *Barbulanympha*, a protozoan. Therefore, if a rhizoplast can be converted to microtubules ontogenetically, there would be no particular difficulty for the conversion to be made phylogenetically. The relationship between rhizoplasts and microtubules may prove to be very interesting, because some striated structures resembling rhizoplasts are contractile (see Cachon et al., 1977).

Secondly, “halving” and “doubling” of cells also appears to be easily accomplished ontogenetically, at least in certain scaly green flagellates. *Micromonas squamata*, typically an asymmetrical scaly green flagellate with a single flagellum, sometimes has 2, 3 or 4 flagella (Manton and Parke, 1960). Manton

and Parke say these represent growth stages or giant cells representing "double divisions." It is pertinent to the present discussion that the quadriflagellate forms would be more nearly symmetrical than the typical uniflagellate form. Cells of *Pyramimonas* can "halve" in much the same way as *Micromonas* can double. During periods of rapid growth, the typically quadriflagellate and symmetrical *Pyramimonas* can lose its pyramidal shape, and the number of flagella can be reduced to 2 or even 1 (Manton, Oates and Parke, 1963), leading these authors to comment that in the uniflagellate condition the cells of *Pyramimonas* could be mistaken for *Micromonas* with the light microscope if the cells' origin were not known. It is thus clear that the difference between distinct asymmetry and near symmetry can be bridged in a culture of a single organism. It is also probable that "halving" and "doubling" of these cells is a common occurrence. In this context, we wish to point out that the shape of the asymmetrical zoospores of *Klebsormidium* and *Coleochaete* (Fig. 1B), with their near apical insertion of flagella, looks more nearly like a half-cell of *Pyramimonas* without a pit (Fig. 1A) than like such asymmetrical biflagellate genera as *Heteromastix* (Mattox and Stewart, 1977) and *Mesostigma* (Manton and Ettl, 1963), which have a more deeply lateral insertion.

A similar evolutionary sequence involving the loss of a pit and the transformation of rhizoplasts to microtubular rootlets could explain how a phycoplast-utilizing scaly unicell like the quadriflagellate *Platymonas* (Manton and Parke, 1965) could also have given rise to the zoospores of Chlorophyceae and advanced unicellular forms like *Chlamydomonas* (Fig. 2A). This can easily be envisaged for the zoospores of the filamentous *Pleurastrum* (Fig. 2B) because they are unusual in having only 2 broad microtubular rootlets instead of 4 smaller ones (Pickett-Heaps, 1975, Chap. 8; Conte, Stewart and Mattox, unpubl.), matching the number of rhizoplasts in *Platymonas*. A

doubling of rootlet or rhizoplast number could have given rise to the more common cruciate rootlet system (Fig. 2D). The past occurrence of these events is made more plausible by the fact that some of the events of cell division in *Pleurastrum* suggest that it is related to *Platymonas* (Molnar, Stewart and Mattox, 1975). Furthermore, a striated fiber similar to a rhizoplast often lies close to and parallels microtubular rootlets in the Chlorophyceae (Manton, 1964; Micallef and Gayral, 1972). There are some facts, however, that are difficult to explain. The most prominent is that chlorophycean flagellated cells often have rhizoplasts extending toward the nucleus (Watson and Arnott, 1973; Lembi, 1975) as well as a typical cruciate rootlet system. Since striated fibers parallel the rootlets in some chlorophycean flagellated cells and since some have typical rhizoplasts extending from the basal bodies toward the nucleus, we must suppose the system of striated fibers was not completely converted to microtubular rootlets. However, the zoospores and flagellated gametes of charophycean algae do not have a rhizoplast or striated fiber of any kind, leading us to believe that an MLS is the result of a complete conversion of the rhizoplast system. Moestrup's report, at this meeting, of an MLS in the euglenoid *Eutreptiella* demonstrates that the MLS is not restricted to green algae. We would interpret this as a parallel conversion of a rhizoplast to a microtubular rootlet rather than an indication of a relationship between green algae and euglenoids.

In summary, we see no evidence that there is a fundamental difference in overall symmetry between the monadal ancestors of the Charophyceae and Chlorophyceae. Indeed, there is some reason to suspect that the most immediate unicellular ancestors of both were quadriflagellate cells with anterior isokont flagella. It is also not possible to explain why the "half-cell" condition should have persisted in the charophyte line all the way to the multiflagellated gametes of land plants, where proliferation of the flagellar apparatus appar-

ently occurred by elaboration of the "half-cell" condition rather than by a doubling or multiplication of units as in some chlorophycean flagellated cells (see Birkbeck et al., 1974). The 2 MLS's in the symmetrical zoospores of *Trentepohlia* (Graham and McBride, 1975) demonstrated, however, that the "half-cell" arrangement is not universal in the Charophyceae.

**3. Scaly green flagellates and the origin of advanced green algae**

While the immediate ancestors of advanced green algae might have been nearly symmet-

rical, it is still quite possible that the ultimate ancestors of green algae were asymmetrical. The predominance of asymmetry in the flagellated cells of other algae, protozoans and water molds suggests that the rare radial symmetry is derived. Among algae, the Chlorophyta are nearly unique in that regard. Although some haptophytes may appear to have an external symmetry, the cytoskeleton does not reflect it. In extant scaly green flagellates, it is not at all clear whether symmetry or asymmetry is the more primitive. The apparent extreme reduction in size of the tiny asymmetrical, uniflagellate *Micromonas* makes it appear possible that it is derived from a nearly symmetrical *Pyramimonas*-like cell by "halving" (see also ideas

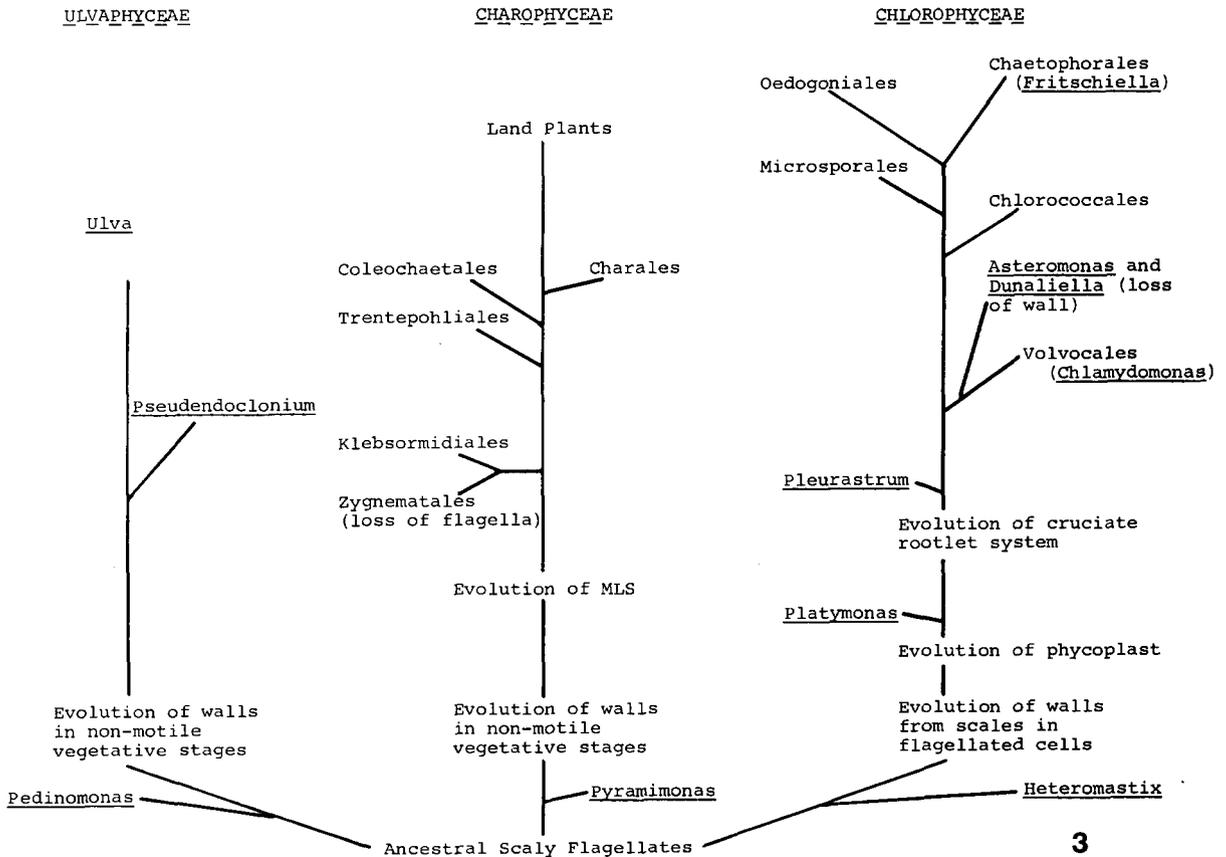


Fig. 3

expressed by Chadeffaud, 1977). On the other hand, the asymmetry of the anisokont *Heteromastix* and the near symmetry of its more advanced isokont relative *Platymonas* (Mattox and Stewart, 1977) imply the reverse.

In any case, it is now reasonably clear that scaly flagellates gave rise to all other green algae, including advanced unicellular forms like *Chlamydomonas*. In fact, the range of diversity in the Chlorophyceae suggests that, in that line, scaly flagellates gave rise to walled flagellates which in turn gave rise to the colonial, filamentous and parenchymatous forms (see Mattox and Stewart, 1977). It is also becoming more clear that the groups of advanced green algae differ from each other because of early evolutionary divergences among their scaly flagellated ancestors. We believe that the information at hand suggests the pattern of divergence in Fig. 3. All of the rationale behind this scheme cannot be given here; in addition to the matters discussed in this paper, see Mattox and Stewart (1977). It now appears that *Ulva* and its relatives might represent a third line, distinct from the Chlorophyceae and Charophyceae, because these algae have neither an MLS or a phycoplast (Mattox and Stewart, 1977). The placement of *Pedinomonas* at the base of the line leading to *Ulva* is the most tentative aspect of Fig. 3, and is based on the fact that *Pedinomonas* has body scales only, as do the zoospores of some ulvacean algae, and on the fact that *Pedinomonas* possesses some features of mitosis (Pickett-Heaps and Ott, 1974) different from those of scaly flagellates that can be associated with the chlorophycean and charophycean lines.

## References

- Birkbeck, T.E., K.D. Stewart and K.R. Mattox, 1974, The cytology and classification of *Schizomeris leibleinii*. II. The structure of quadriflagellate zoospores, *Phycologia* 13, 71–79.
- Cachon, J., M. Cachon, L.G. Tilney and M.S. Tilney, 1977, Movement generated by interactions between the dense material and the ends of microtubules and monactin-containing microfilaments in *Sticholonche zanzlea*. *J. Cell Biol.* 72, 314–338.
- Chadeffaud, M., 1977, Les Prasinophycées: Remarques historiques, critiques et phylogénétiques, *Bull. Soc. Phycol. Fr.* 22, 1–18.
- Graham, L.E. and G.E. McBride, 1975, The ultrastructure of multilayered structures associated with flagellar bases in motile cells of *Trentepohlia aurea*, *J. Phycol.* 11, 86–96.
- Lembi, C.A. 1975. A rhizoplast in *Carteria radiosa* (Chlorophyceae), *J. Phycol.* 11, 219–221.
- Manton, I., 1964, Observations on the fine structure of the zoospore and young germling of *Stigeoclonium*, *J. Exp. Bot.* 15, 399–411.
- Manton, I., 1965, Some phyletic implications of flagellar structure in plants. in: *Advances in Botanical Research* R.D. Preston, (ed.), Vol. 2. (Academic Press, London and New York) pp. 1–34.
- Manton, I., B. Clarke and A.D. Greenwood, 1955, Observations with the electron microscope on biciliate and quadriciliate zoospores in green algae, *J. Exp. Bot.* 6, 126–128.
- Manton, I. and H. Ettl, 1963, Observations on the fine structure of *Mesostigma viride* Lauterborn, *J. Linn. Soc.* 59, 175–182.
- Manton, I., K. Oates and M. Parke, 1963, Observations on the fine structure of the *Pyramimonas* stage of *Halosphaera* and preliminary observations on three species of *Pyramimonas*, *J. mar. biol. Assoc. U.K.* 43, 225–238.
- Manton, I. and M. Parke, 1960, Further observations on small green flagellates with special reference to possible relatives of *Chromulina pusilla* Butcher, *J. mar. biol. Assoc. U.K.* 39, 275–278.
- Manton, I. and M. Parke. 1965. Observations on the fine structure of two species of *Platymonas* with special reference to flagellar scales and the mode of origin of the theca, *J. mar. biol. Assoc. U.K.* 45, 743–754.
- Manton, I., D.G. Rayns, H. Ettl and M. Parke. 1965. Further observations on green flagellates with scaly flagella: the genus *Heteromastix* Korschikoff, *J. mar. biol. Assoc. U.K.* 45, 241–255.
- Marchant, H., J.D. Pickett-Heaps and K. Jacobs, 1973, An ultrastructural study of zoosporogenesis and the mature zoospore of *Klebsormidium flaccidum*, *Cytobios* 8, 95–107.
- Mattox, K.R. and K.D. Stewart, 1973, Observations on the zoospores of *Pseudendoclonium basilense* and *Trichosarcina polymorpha*, *Can. J. Bot.* 51, 1425–1430.
- Mattox, K.R. and K.D. Stewart, 1977, Cell division in the scaly green flagellate *Heteromastix angulata* and its bearing on the origin of the Chlorophyceae, *Am. J. Bot.* 64, 931–945.
- McBride, G.E., 1971, The flagellar base in *Coleochaete*

- and its evolutionary significance. (Abstr.), *J. Phycol.* 7, Suppl. 13.
- Micalef, H. and P. Gayral, 1972, Quelques aspects de l'infrastructure des cellules vegetatives et des cellules reproductrices d'*Ulva lactuca* L. (Chlorophyceae). *J. de Microscopie* 13, 417-428.
- Moestrup, Ø. and H.A. Thomsen, 1974, An ultrastructural study of the flagellate *Pyramimonas orientalis* with particular emphasis on golgi apparatus activity and the flagellar apparatus, *Protoplasma* 81, 247-269.
- Molnar, K.E., K.D. Stewart and K.R. Mattox, 1975, Cell division in the filamentous alga *Pleurastrum* and its comparison with the unicellular *Platymonas* (Chlorophyceae), *J. Phycol.* 11, 287-296.
- Norris, R.E. and B.R. Pearson, 1975, Fine structure of *Pyramimonas parkeae*, sp. nov. (Chlorophyta, Prasinophyceae), *Arch. Protistenkd.* Bd. 117, 192-213.
- Pearson, B.R. and R.E. Norris, 1975, Fine structure of cell division in *Pyramimonas parkeae* Norris and Pearson (Chlorophyta, Prasinophyceae), *J. Phycol.* 11, 113-124.
- Pickett-Heaps, J.D., 1968, Ultrastructure and differentiation in *Chara (fibrosa)*. IV. Spermatogenesis, *Aust. J. Biol. Sci.* 21, 655-690.
- Pickett-Heaps, J.D., 1975, *Green Algae: Structure, Reproduction and Evolution in Selected Genera* (Sinauer Associates, Inc., Sunderland, Mass.).
- Pickett-Heaps, J.D. and H.J. Marchant, 1972, The phylogeny of the green algae: a new proposal, *Cytobios* 6, 255-264.
- Pickett-Heaps, J.D. and D.W. Ott, 1974, Ultrastructural morphology and cell division in *Pedinomonas*, *Cytobios* 11, 41-58.
- Ringo, D.L., 1967, Flagellar motion and fine structure of the flagellar apparatus in *Chlamydomonas*, *J. Cell Biol.* 33, 543-571.
- Stewart, K.D., K.R. Mattox and C.D. Chandler, 1974, Mitosis and cytokinesis in *Platymonas subcordiformis*, a scaly green monad, *J. Phycol.* 10, 65-79.
- Stewart, K.D. and K.R. Mattox, 1975a, Comparative cytology, evolution and classification of the green algae with some consideration of the origin of other organisms with chlorophylls *a* and *b*, *Bot. Rev.* 41, 104-135.
- Stewart, K.D. and K.R. Mattox, 1975b, Some aspects of mitosis in primitive green algae: phylogeny and function, *BioSystems* 7, 310-315.
- Watson, M.W. and H.J. Arnott, 1973, Ultrastructural morphology of *Microthamnion* zoospores, *J. Phycol.* 9, 15-29.