

ON THE PHYLOGENETIC VALIDITY OF THE FLAGELLAR APPARATUS IN GREEN ALGAE AND OTHER CHLOROPHYLL A AND B CONTAINING PLANTS

ØJVIND MOESTRUP

Institut for Sporeplanter, Ø. Farimagsgade 2 D, DK-1353 Copenhagen, K. Denmark

The chlorophyll *a* and *b* containing eucaryotic plants (euglenoid flagellates, chlorophycean and charophycean green algae, prasinophytes, bryophytes and higher plants) have been studied fine structurally more than most other larger groups of organisms. Of the large number of articles published, over 100 contain some information on flagellar fine structure, of a similar number of species. Based on these data, it seems clear that the fine structure of the flagellar apparatus is an important phylogenetic indicator, not only on class level (or even higher), but also frequently on family and generic level. The few studies on species within a single genus hardly permit any conclusions.

The external appendages on the flagella are reasonably well known and include the unique covering of hairs in euglenoids, unmineralized scales and hairs (hair-scales?) in many prasinophytes and charophytes and very fine hairs in *Pedinomonas* (Loxophyceae *sensu* Christensen): 2 rows in *P. minor*, in *P. tuberculata* an apparently more even covering. Hair-shaped structures have also been found in *Chlamydomonas*, and in a few other members of the Volvocales (*Chlorogonium*, *Haematococcus*) the flagellar surface is covered by a close tomentum. Internally all the groups except the euglenoids contain a characteristic stellate pattern in the flagellar transition region, and an apparently coiled structure has been found in a prasinophyte, just above the stellate pattern.

The internal parts of the flagellar system, termed flagellar roots, are of 3 basic types. In prasino- and chlorophytes there are typically 4 cruciately arranged microtubular roots, in which the number of microtubules corresponds to X–2–X–2, X = 3 to approx. 8 (this includes *Chlamydomonas*). In the charophytes, bryophytes, and those higher plants which possess flagellated stages, there is a single unilateral root, in which the number of microtubules varies from 10–15 (many bryophytes) to over 10 000 (the cycad *Zamia*). In most cases this root contains a so-called multilayered structure (MLS), Dreiergruppe or Vierergruppe according to the number of layers in the MLS. The few euglenoids examined appear to possess 3 microtubular roots only, and in *Eutreptiella* one of these very surprisingly contains a MLS. It is less certain whether types intermediate between the 2 systems occur, but interesting deviations are known which possess only 2 roots, and in a few cases these both contain a MLS-like structure.

Cross-banded root components occur commonly in the primitive groups: prasino- (loxo-) and chlorophytes. They are also present in the euglenoid *Eutreptiella*, but have not been reported from charophytes, bryophytes, and higher plants.

Based on present information it is suggested that the primitive chlorophyll *a* and *b* containing eucaryotic plants (algae) were probably scale-covered, had some type of cross-banded flagellar root component, and in one line a X–2–X–2 microtubular root system. In the euglenoid line, the root system was probably different. Later, in the line leading towards the higher plants, scales were generally retained in charophytes, but lost in bryophytes, and higher plants. Also in this line, cross-banded components were generally lost and the microtubular root system transformed. In the euglenoid and chlorophycean lines of evolution, scales were lost except in very few cases, of which only three are known at present: *Ulvopsis* (*Monostroma*) *grevillei*, *Trichosarcina polymorpha* and *Pseudodoclonium basiliense* which form body scales.

1. Introduction

In both the animal and the plant kingdoms, the structure of flagellated cells, such as zoospores or sperms, is considered to be conservative and thus to have retained a number of characters relatively unchanged

during evolution. The structure of the flagellar apparatus in particular is considered conservative. Among the algae there are at present only 3 known examples of deviations from the ubiquitous 9 + 2 flagellar structure, the spermatozoids of the 2 examined centric diatoms (9 + 0, Manton and von Stosch, 1966) and of the freshwater plankton alga

Golenkinia (9 + 1, Moestrup, 1972), though non-motile variations are known to occur ("pseudocilia"), in which the central pair is absent (e.g., Lembi and Walne, 1971). Other flagellar characters, such as the presence of hairs and other appendages on the flagella, are among the basic differences between many algal classes.

Compared to the flagellar appendages, the internal parts of the flagellar apparatus are very rarely used in phylogeny, and in several classes have not been studied in detail.

In some of her first papers on algal fine structure Manton showed that the flagella were attached to the cell by "fibres" which she termed flagellar roots (Manton, 1952, Manton et al., 1955). She examined a large number of flagellated cells, brown and green algae in particular, and in her review of flagellar structure in plants (Manton, 1965) she pointed out that the subject of flagellar roots deserved further attention before the exact phylogenetic meaning could be interpreted.

Moestrup (1972) in a paper on the spermatozooids of the green alga *Golenkinia* included a list of the flagellar root systems known at that time in green algae and their allies and suggested that the structure of the flagellar root system is of genuine phylogenetic importance in these groups, this may also be the case in many other, so far less well-studied groups.

Since the beginning of the '70s a very great interest in green algae has considerably increased the available data on the flagellar apparatus in chlorophyll *a* and *b* containing plants. The published data and a number of new observations will be discussed in the present paper.

This review deals with approx. 100 papers and attempts to be complete, though some papers may have been overlooked, particularly on the bryophytes and higher plants. Certain flagellar characters will not be discussed, but are almost certainly phylogenetically important. The presence of a stellate pattern (star-shaped body) in the transition region between flagella and flagellar

bases is a characteristic of all the chlorophyll *a* and *b* eucaryotes except euglenoids (see Manton, 1965) and *Chlorachnion*, a green amoeba of unknown affinities (Hibberd, pers. comm.). The finding of a coiled structure just above that region in the flagellate *Pyramimonas orientalis* is as yet of unknown significance (Moestrup and Thomsen, 1974). The various fibrous structures which interconnect the flagellar bases will not be discussed here.

In number of species the chlorophyll *a* and *b* containing organisms have been very successful indeed. In addition to the bryophytes and higher plants 4 classes will be recognized here. The *euglenoid flagellates* have approx. 800 species listed by Huber-Pestalozzi (1955), though according to Leedale (1967) this number is probably somewhat too high. The class *Prasinophyceae* (probably a few hundred species) was recognized by Chadefaud (1941, 1947, 1960) under various names and given their nomenclaturally correct name by Christensen (1962). In this class a number of characters occur which are found in euglenoids, cryptomonads and dinoflagellates and therefore considered to be primitive: an anterior flagellar pit, muciferous bodies and trichocysts. The *Chlorophyceae* and *Charophyceae* (see the discussion regarding the difference between these 2 classes) are the well-known green algae which contain probably over 10 000 species (Bourrelly (1966) mentions 7800 in freshwater alone). The bryophytes and higher plants need no presentation.

In all the chlorophyll *a* and *b* containing plants the flagella are attached to the cell by flagellar roots. In most cases there are 4 roots arranged in a cruciate pattern (see e.g., Fig. 9), in others there are 3, 2 or 1. One group contains a high number of roots. Cross-banded structures (striated roots) often associate with the microtubular roots and the periodicity of the cross-banding may be phylogenetically significant.

The conclusion and discussion below is based on Tables 1—4 and on the comments to the Tables.

TABLE 1

Flagellar root systems in Chlorophyceae

	Microtubular root	Striated component, periodicity in nm	No. of flagella	Reference
<i>Golenkinia minutissima</i>	3—1—3—1	Not obs.**	2	Moestrup, 1972
<i>Chaetomorpha melagonium</i>	3—2—3—2	Not obs.	2	Manton et al., 1955
<i>Dichotomosiphon tuberosus</i>	3—2—3—2	Not obs.	2	Moestrup et al., 1975
<i>Chlamydomonas reinhardtii</i>	4—2—4—2	Not obs.	2	present paper
<i>Chlamydomonas</i> spp. (asymm. cell)	4—2—4—2	?	2	Birkbeck, 1976
<i>Chlamydomonas moewusii</i>	4—2—4—2*	Not obs.	2	Triemer et al., 1974
				Triemer, 1975
<i>Volvox carteri</i> , veg. cell	4—2—4—2*	?	2	Olson et al., 1970
<i>Volvox tertius</i> , spermatozoid	4—2—4—2*	Not obs.	2	Pickett-Heaps, 1970
<i>Tetraspora lubrica</i> , pseudocilia	4—2—4—2*	Not obs.	2	Lembi et al., 1969, 1971
<i>Dunaliella primolecta</i>	4—2—4—2	4, per. ** 25—30	2	Hyams et al., 1974
<i>Ulvopsis grevillei</i>	4—2—4—2	At least one, per. approx. 150	2	Chesnoy et al., 1973
				Jónsson et al., 1974
<i>Stigeoclonium</i> sp.	5—2—5—2	2, assoc. with 2-str., per. approx. 33	4	present paper
<i>Draparnaldia</i> sp.	5—2—5—2	Not obs.	4	Manton et al., 1955
<i>Fritschiella tuberosa</i>	5—2—5—2*	2, assoc. with 2-str., per. prob. like <i>Stig.</i>	4	Melkonian, 1975
<i>Ulothrix</i> sp.	5—2—5—2	Not obs.	4	Manton, 1965
<i>Chlamydomonas</i> (Ettl) No. 30)	5—2—5—2	?	2	Birkbeck, 1976
<i>Microthamnion kuetzingianum</i>	6—2—6—2	A very spec. type	2	Watson et al., 1973
<i>Tetraspora</i> sp. (LB 234) (flagella)	4—4—4—4 (?)	Nqt obs.	2	Watson, 1975
<i>Carteria radiosa</i>	4—4—4—4 (?)	At least 2, per. 85—120	4	Pickett-Heaps, 1973
<i>Carteria</i> sp. (LB 762)	4—4—4—4 (?)	Not obs.	4	Pickett-Heaps, 1975b
<i>Schizomeris leibleinii</i>	approx. 5—5—5—5	Not obs.	4	Lembi, 1975a
<i>Hydrodictyon reticulatum</i> (zoospore)	4 × 5—8	Not obs.	2	Birkbeck et al., 1974
				Marchant et al., 1972a
<i>(gamete)</i>		Not obs.	2	Marchant et al., 1972b
<i>Urospora penicilliformis</i>	9—9—9—9	4, per. approx. 160	2	Kristiansen, 1974
<i>Cylindrocapsa geminella</i>	4 × max 14	“layer 3”;	4	Hoffman, 1976
	(2 types?)	per. approx. 13	4	
<i>Schizochlamys</i> sp.	cruciate, prob. not 2-str.	Not obs.	4	Lembi et al., 1969
<i>Sorastrum</i> sp.	cruciate 6—2—6—2 (?)	Not obs.	2	Marchant, 1974a

Table 1 (continued)

	Microtubular root	Striated component, periodicity in nm	No. of flagella	Reference
<i>Ulva lactuca</i>	cruciate. One set 2-str., the other perhaps 3-str.	2 systems assoc. with 2-str. roots: I: per approx. 28 II: per. approx. 150	?	Micalef et al., 1972
<i>Enteromorpha intestinalis</i>	cruciate	At least 1, per. approx. 150	4	Evans et al., 1970
<i>Sphaeroplea annulina</i>	cruciate. One set 2–3, the other approx. 8-str.	Not obs.	2	Moestrup, 1975
<i>Aphanochaete</i> <i>Trichosarcina polymorpha</i>	cruciate, 2 root types	?		
<i>Pseudendocionium basiliense</i>	cruciate	Not obs.	4	Watson et al., 1973
<i>Pediastrum boryanum</i>	cruciate, one perh. 4-str. (loc. cit. Fig. 6A)	Not obs.	4	Mattox et al., 1973
<i>Chaetopeltis</i> sp., pseudocilia	cruciate, one set apparently 2-str.	Not obs.	4?	Mattox et al., 1973
<i>Asteromonas gracilis</i>	An apparently 4-str. root illustr.	Not obs.	2	Peterfi et al., 1968
<i>Eudorina illinoiensis</i>	4–5 str. root illustr.	?	2	Hobbs, 1971
<i>Tetraedron bitridens</i>	approx. 3-str. root in autospore forming cell	Not obs.	0	Pickett-Heaps, 1975a,b
<i>Acetabularia mediterranea</i>	prob. cruciate*	?	2	Woodcock et al., 1973
<i>Pleurosturm obovatum</i> (= <i>Leptosira obovata</i>)	2 roots, perh. 9-str. illustr.	?	2	Pickett-Heaps, 1975a
<i>Trebouxia</i>	2 roots	?	2	Stewart (pers. comm.)
<i>Polytomella agilis</i>	2 × 4–2–4–2	30	4	Brown et al., 1976
<i>Carteria crucifera</i>	3 complexes found: A: two 2-str roots	Not obs.	4	Moore et al., 1970
<i>Carteria eugametos</i>	B: two 4-str. roots and two (three) sigmoid shaped rods (?) C:			Lembi, 1975a
<i>Carteria olivieri</i>	incompletely studied			
<i>Gloeomonas simulans</i>	4 roots ass. with dense bands	approx. 5	2	Schnepf et al., 1976

<i>Oedogonium cardiacum</i> (spermatozoid)	3—3—3—	Many, per. 30—32	approx. 30
<i>Oedocladium sp.</i> (zoospore)	3—3—3—	Many, per. 30—32	approx. 120
<i>Bulbochaete hiloensis</i> (zoospore)	3—3—3—	Many	?
<i>Bulbochaete hiloensis</i> (spermatozoid)	3—3—3—	Many	approx. 40
<i>Trentepohlia aurea</i>	Possibly 2 roots only, with MLS-like structure, 6—8 microtubules in each root	Not. obs.	2
<i>Cephaleuros virescens</i>	Apparently as above	Not obs.	4
<i>Phycopeltis</i>	Probably 1 or 2 roots, with MLS-like structure as above	Not obs.	2—4

* Indicates that the number of microtubules either was not commented in the reference cited, or that I have interpreted the published micrographs with a conclusion which is different from the author's conclusion.

* * obs, observed; per, periodicity.

TABLE 2

Flagellar root systems in Prasinophyceae (including Loxophyceae) and Euglenophyceae (for further explanation see Table 1)

	Microtubular root	Striated component, periodicity in nm	No. of flagella	Reference
Prasinophyceae				
<i>Pedinomonas tuberculata</i>	cruciate 3-2-3-2	per. approx. 20 per. approx. 20, this root attached to one 3-str.	1 (2)	Manton et al., 1960 Etli et al., 1964, Pickett-Heaps et al., 1974
<i>Pedinomonas minor</i>		per. approx. 50, but further divided ?	1(2)	Manton, 1967
<i>Monomastix minuta</i>	2 x 2 0-2-0-2? 4-2-0-0 4-2-4-2	per. indistinct per. approx. 290 per. approx. 250 per. 260-330 (500?)	4 4 4 4	Barlow, pers. comm. Moestrup et al., 1974 in prep.
<i>Mantoniella squamata</i>	cruciate 2, 3, 4 seen 2, 3, 4, 5 seen	per. approx. 250 per. approx. 30 (?)	4 4	Manton, 1968 Manton, 1966 Norris et al., 1975
<i>Pyramimonas orientalis</i>	4 groups, "at least 3 in each," ?	per. approx. 270 per. approx. 500 (2 roots)	8 4	in prep. Manton et al., 1965
<i>Pyramimonas tertrahynchus</i>	?	per. approx. 430 (2 roots)	4	McLachlan et al., 1967
<i>Pyramimonas amyliifera</i>	?	per. 160-200 (2? roots)	4	Parke et al., 1967
<i>Pyramimonas parkeae</i>	4 groups, "at least 3 in each," ?	per. 400-600 (2 roots)	4	Stewart et al., 1974
<i>Pyramimonas octopus</i>	?	per. 250-300 (2 roots)	4	Parke et al., 1965
<i>Platymonas tetrathele</i>	?	per. unknown	2	Manton, Rayns et al., 1965
<i>Platymonas impellucida</i>	?	per. approx. 25 (75?)	4	Manton et al., 1963
<i>Platymonas convoluta</i>	?			
<i>Platymonas subcordiformis</i>	?			
<i>Prasinocladus marinus</i>	2 types; 2-str. and 7-str. ?			
<i>Heteromastix rotunda</i>				
<i>Halosphaera minor</i>				
Euglenophyceae				
<i>Menoidium bibacillatum</i>	5-8-5	Not obs.	1(2)	Leedale et al., 1974
<i>Rhabdomonas costata</i>	6-8-2	Not obs.	1(2)	Leedale et al., 1974
<i>Eutreptiella cf. braarudii</i>	c.6-c.9-4	One, per. 45	2	in prep. and present paper.

TABLE 3

Flagellar root systems in Charophyceae, Bryophyta, Pteridophyta and Spermatophyta (for further explanation see Table 1)

	Microtubular root	Striated component periodicity in nm	No. of flagella	Reference
Charophyceae				
<i>Klebsormidium flaccidum</i>	20—36	Not obs.	2	Marchant et al., 1973
<i>Chaetosphaeridium globosum</i>	approx. 60	Not obs.	2	Moestrup, 1974
<i>Coleochaete scutata</i>	?	Not obs.	2	McBride, 1971
				Pickett-Heaps et al., 1972
<i>Chara corallina</i>	approx. 30	Not obs.	2	Moestrup, 1970
<i>Chara fibrosa</i>	approx. 30	Not obs.	2	Pickett-Heaps, 1968
<i>Nitella missouriensis</i>	approx. 20	Not obs.	2	Turner, 1968
Bryophyta				
<i>Pellia epiphylla</i>	15	Not obs.	2	Suire, 1970
<i>Blasia pusilla</i>	20	Not obs.	2	Carothers, 1973
<i>Sphaerocarpus donnellii</i>	12—26 (30)	Not obs.	2	Diers, 1967
				Heitz, 1961
<i>Marchantia polymorpha</i>	approx. 16	Not obs.	2	Carothers et al., 1967
<i>Anthoceros punctatus</i>	approx. 13	Not obs.	2	Kreitner et al., 1976
<i>Polytrichum juniperinum</i>	10	Not obs.	2	unpub. observations
<i>Polytrichum commune</i>	12	Not obs.	2	Paolillo et al., 1968
<i>Physcomitrium coorgense</i>	20—25	Not obs.	2	Lal et al., 1975
<i>Splachnum rubrum</i>	approx. 11	Not obs.	2	Fig. 6 in Heitz, 1961
<i>Eryum capillare</i>	approx. 15	Not. obs.	2	Bonnot, 1967
Pteridophyta				
<i>Marsilea vestita</i>	20—25	Not obs.	more than 100	Rice et al., 1967
				Myles, 1975
<i>Pteridium aquilinum</i>	approx. 150	Not obs.	approx. 40	Duckett, 1975
<i>Equisetum hyemale</i>	approx. 300	Not obs.	approx. 120	Duckett, 1973
<i>Lycopodium complanatum</i>	approx. 50 (?)	Not obs.	2	Carothers et al., 1975
<i>Selaginella kraussiana</i>	approx. 17	Not obs.	2	Robert, 1974
Spermatophyta				
<i>Zamia integrifolia</i>	approx. 60 000	Not obs.	10—12 000	Norstog, 1974, 1975

* obs. observed; per. periodicity.

2. Materials and methods

Fertile thalli of *Ulvopsis* (*Monostroma*; *grevillei*) were collected at Hvidøre, north of Copenhagen, on 22 April 1975 and kept moist but not wet in a plastic bag for a few hours (in a cooled ice-box). Following transfer to seawater, gametes were very soon released in large numbers. They were fixed overnight in 2% glutaraldehyde in 0.1 M cacodylate buffer at pH 7 with 0.2 M sucrose added.

Chlamydomonas reinhardtii, Cambridge 11/32a, was maintained at Institut for Sporeplanter, where it was grown at 15°C, on a 16 : 8 light : darkness regime in Bristol's soil water extract. It was fixed for 3 h in 1% glutaraldehyde in the medium.

Chlamydomonas reinhardtii, 21gr, was obtained from Dr. Ruth Sager, New York, and subsequently grown under the same conditions as 11/32a. It was fixed for 1 h in 2% glutaraldehyde in 0.05 M collidine buffer at pH 7.5.

All fixed material was rinsed in buffer for 1.5 h (3 changes, for *Ulvopsis* with decreasing sucrose concentration), and postosmicated for 5 h to overnight in 2% osmic acid in the cacodylate buffer, or (11/32a) in the medium. After a brief rinse in buffer they were dehydrated in an alcohol series and embedded in Spurr's resin mixture via propylene oxide. Thin sections were cut with glass or diamond knives on a Reichert OmU2 ultramicrotome. They were stained in 2% aq. uranyl acetate (30–90 min) followed by Reynold's lead citrate (10–30 min), or in the latter only. The sections were examined in a JEM-T8 electron microscope.

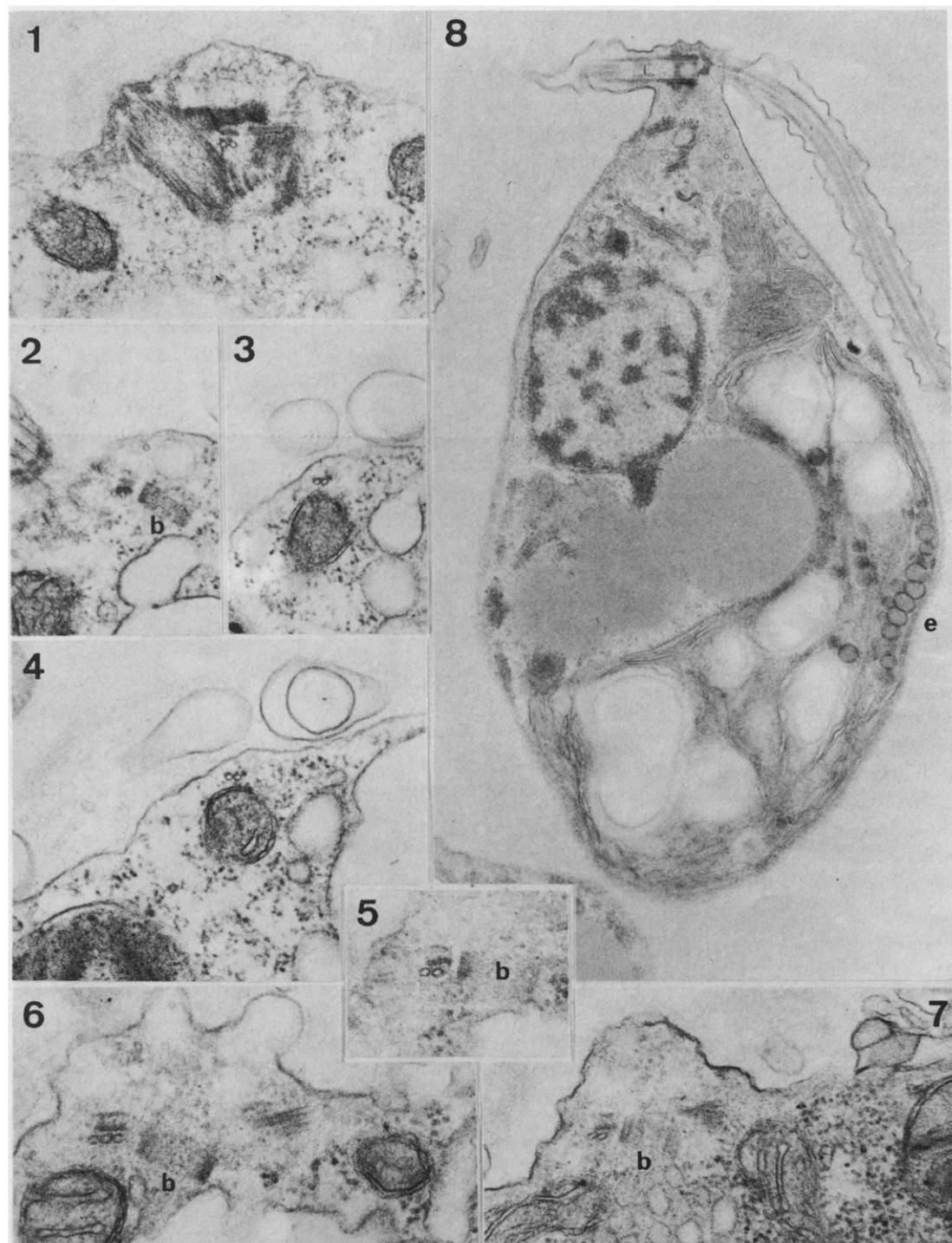
3. Comments to Tables 1–3, including new observations on *Chlamydomonas reinhardtii* and *Ulvopsis grevillei*

3.1. *Chlamydomonas reinhardtii*

In his excellent paper Ringo (1967) has described the flagellar apparatus of *C. reinhardtii*, including the roots, which he claimed to consist of 4 equal bands, each made of 4 microtubules. This claim was rather surprising, considering that all other green algae examined up to that time had been shown to possess 2 types of roots, differing in their number of microtubules. The only exceptions from this were the multiflagellate zoospores and spermatozoids of *Oedogonium*, in which the numerous roots are all identical.

Ringo's claim, however, can no longer be accepted without reservation. Three different strains of *C. reinhardtii* have now been examined, of which two are illustrated here. In all 3 cases the root system has been found to be cruciate, but it is a 4–2–4–2 system rather than the symmetrical system suggested. Ringo illustrated roots with 2, 3 and 4 microtubules and interpreted these (serial sections were apparently not available) as belonging to 4-stranded roots, in which the microtubules terminated subsequently one just after the other near the flagellar bases. Fig. 1 in the present paper shows a section almost identical to Fig. 23 in Ringo (1967). With Figs. 2–4 it belongs to a series of sections through one root and, as the illustrations show, it remains 2-stranded throughout. Identical series have been obtained of strain 21gr, the strain used by Ringo. The same fixation procedure was applied (Fig. 5 shows a 2-stranded root from

Figs. 1–7. *Chlamydomonas reinhardtii*. Figs. 1–4 (strain 11/32a) are from a series of sections illustrating a 2-stranded root from near its point of origin at the flagellar bases (Fig. 1). In Fig. 2 an extra basal body is present (b). Figs 1 and 4, $\times 45\,000$; Figs. 2–3, $\times 40\,000$. In Figs. 5–7 (strain 21 gr) Fig. 5 shows a 2-stranded root at a higher magnification at some distance from the flagellar bases (an extra base is present on the right); Fig. 6 is from a series of sections through a 3-stranded root. Compare with Fig. 7 which is a very similar section taken from a series through the more commonly occurring cell type, which has 2-stranded roots. Figs. 5–6, $\times 60\,000$; Fig. 7, $\times 48\,000$. Fig. 8. Longitudinal section through a gamete of *Ulvopsis grevillei*, showing most of the organelles, including the 2 flagella, the nucleus, and the chloroplast, which contains an eyespot (e), approximately opposite the nucleus. $\times 15\,000$.



this strain, at some distance from the flagella). Gould (1975) used *C. reinhardtii* strain cw15 to isolate the flagellar apparatus for biochemical purposes and did not comment on the microtubule numbers. His elegant mounts show, however, the 2- and 4-stranded roots beautifully.

In *C. reinhardtii* the situation is complicated by the occurrence in some cells of 3-stranded rather than 2-stranded roots. Fig. 6 is from a series of 14 sections through a 3-stranded root. Compare with Fig. 7, which is from a series through another cell, showing a comparable 2-stranded root. In some sections of the 3-stranded series the adjacent root could be seen more clearly than in Fig. 7 to consist of at least 3 microtubules, thus further ensuring that the illustrated 3-stranded root is a variation of a 2-stranded root rather than a 4-stranded (see further below).

3.2. *Chlamydomonas moewusii*

According to Triemer and Brown (1974) "4 groups of 4 microtubules in a 3 over 1 arrangement as described by Ringo in *C. reinhardtii* associate with the basal body apparatus". This was, however, not examined in detail. Triemer and Brown's Fig. 4 shows a 4-stranded root, but in their Fig. 5 the root visible in a position comparable to that of Fig. 7 in the present paper appears 2-stranded. Triemer's (1975) Fig. 5 clearly shows a root with 2 microtubules. Considering that related species often have very similar root systems, it is reasonable to assume, until otherwise shown, that *C. moewusii* does also have a 4-2-4-2 system.

3.3 *Volvox carteri* and *V. tertius*

In *Volvox carteri* Olson and Kochert

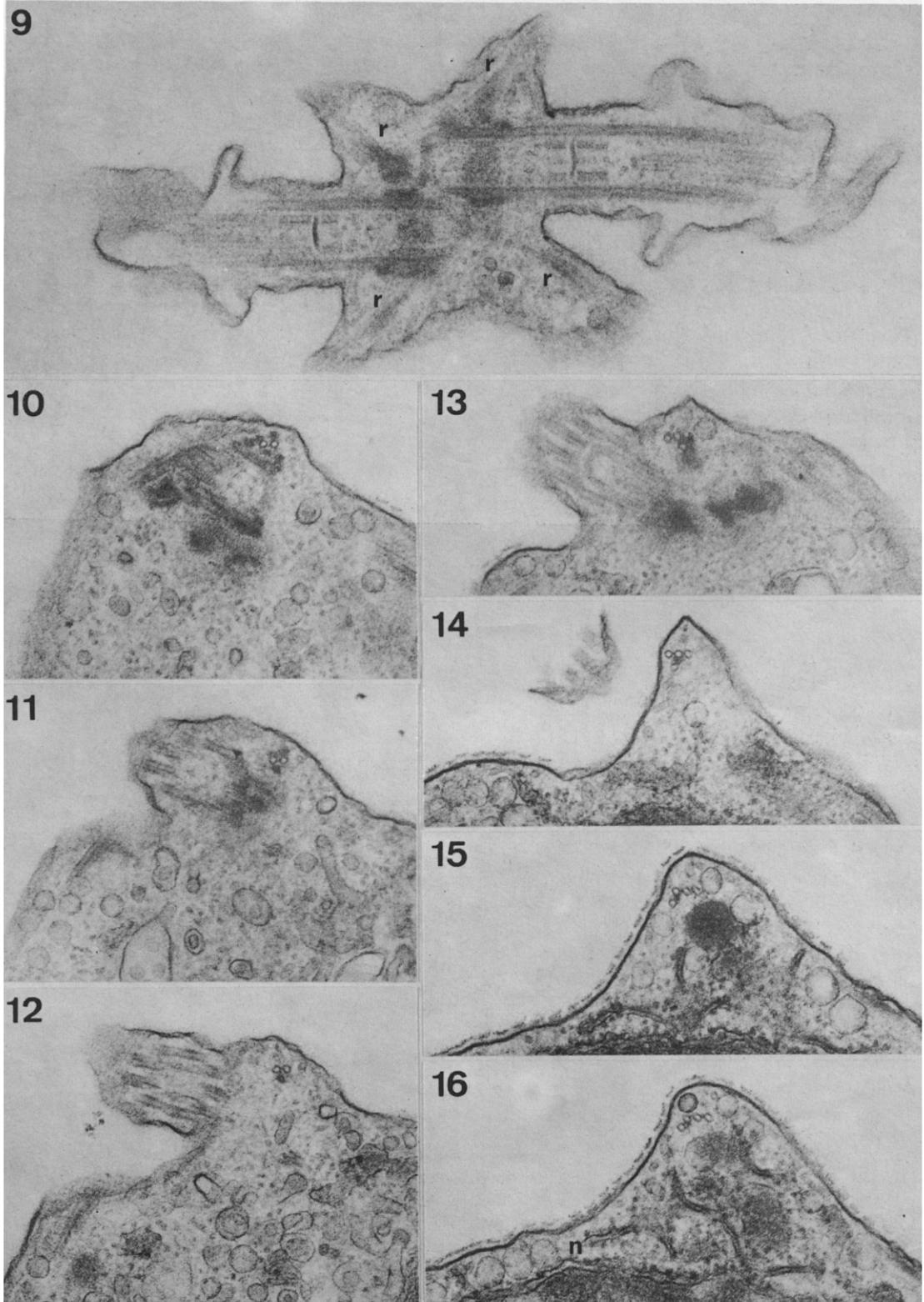
(1970) found that "4 groups of microtubules radiate from the kinetosome complex" and that "each set usually contains 3 or 4 microtubules". In a previous account of flagellar roots Moestrup (1972) referred to the root system in *Volvox* as being a 4-4-4-4, following the authors' remark that the similarity between *Volvox* and *Chlamydomonas* "is very impressive". Olson and Kochert's Figs. 1 and 2 very clearly show roots with 2 and 4 microtubules (vegetative cells), and in the transverse sections of *Volvox tertius* spermatozoids published by Pickett-Heaps (1970: Fig. 14, 1975b: Plate 2) 4 groups of 4, 2, 4, 2 microtubules may readily be distinguished. There can be little doubt, therefore, that the root system is a 4-2-4-2, and Olson and Kochert's remark upon the similarity between *Chlamydomonas* and *Volvox* is still valid.

It should also be mentioned that in Fig. 15 of Olson and Kochert a striated structure is illustrated, which resembles "système I" associated with the 2-stranded roots in *Ulva* (Micalef and Gayral, 1972). The periodicity appears however to be different.

3.4. *Tetraspora lubrica*

According to Lembi and Walne (1971), in each of the 4 roots of *T. lubrica* there are 4 microtubules "similar to that reported in *Chlamydomonas*". Their Fig. 2 shows 4 microtubules, but Fig. 2 (inset) and Figs. 3-5 show 2 microtubules, which the authors interpret in the same way as Ringo (1967) did with *Chlamydomonas*. I strongly suspect that these last 4 figures illustrate 2-stranded roots, rather than 4-stranded roots, in which 2 microtubules have terminated. The roots illustrated are identical to that shown by Ringo (his Fig. 23) and demonstrated above

Figs. 9-16. Gametes of *Ulvopsis grevillei*, all $\times 48\,000$. Fig. 9. Longitudinal section through the proximal parts of the 2 flagella and the 4 cruciate arranged flagellar roots (r), each of which passes into one of the 4 corners of the front end. Figs. 10-12. Three sections from a series through a 2-stranded root, in Fig. 10 near its point of origin at the flagellar bases. Figs. 13-16. A similar series through a 4-stranded root. The 4 microtubules are near the flagella arranged in a 3 over 1 pattern which gradually changes to 4 microtubules in a row. Eventually this root passes along the plasmalemma on the surface of the nucleus, which is just visible in the lower part of Figs. 15 and 16 (n).



to be 2-stranded (Figs. 1–4). In all the cases these roots are joined by a layer of dense material, located at some distance above the microtubules (i.e., on the side away from the basal body), and the innermost microtubule is in contact with the basal body. It is uncertain whether similar structures occur associated with 4-stranded roots.

3.5 *Dunaliella primolecta*

In this organism Hyams and Chasey (1974) have illustrated bifurcation of 4-stranded roots, using negative staining of isolated flagellar complexes. At some distance below the flagellar bases a branch leaves the root and takes a different path in the cell. When analysed, this branch was found to consist of a single microtubule.

3.6. *Ulvopsis (Monostroma) grevillei*

Gametes of this marine alga were examined a few years ago and the cell body shown to be covered by tiny diamond shaped scales (Chesnoy and Jónsson, 1973; Jónsson and Chesnoy, 1974). For reasons discussed below, following this finding, a study of the flagellar apparatus became very much needed, and Figs. 8–16 and 19–21 in the present paper illustrate some of the details. The gametes illustrated here are almost certainly female gametes, but a comparative study of the flagellar systems of male and female gametes has not been made.

The biflagellate gamete possesses a typical cruciate root system (Fig. 9). Two 4-stranded roots (starting near the flagellar bases as 3 over 1) pass from between the flagellar bases to the plasmalemma and continue along 2

opposite sides of the cell. For some distance one is situated on the nuclear surface (not illustrated, but compare with Figs. 13–16), the other on the eyespot (Figs. 19–21) which is located opposite the nucleus (Fig. 8). The other 2 roots are 2-stranded and extend from near the sides of the flagellar bases (Figs. 10–12). For as long as the roots have been followed in transverse sections of the cell, they were very exactly evenly spaced (illustrations not included). The presence of at least 1 striated root was confirmed (illustrated by Jónsson and Chesnoy, 1974). It resembles that of *Ulva* and *Enteromorpha* (Micalef and Gayral, 1972 and Evans and Christie, 1970, respectively).

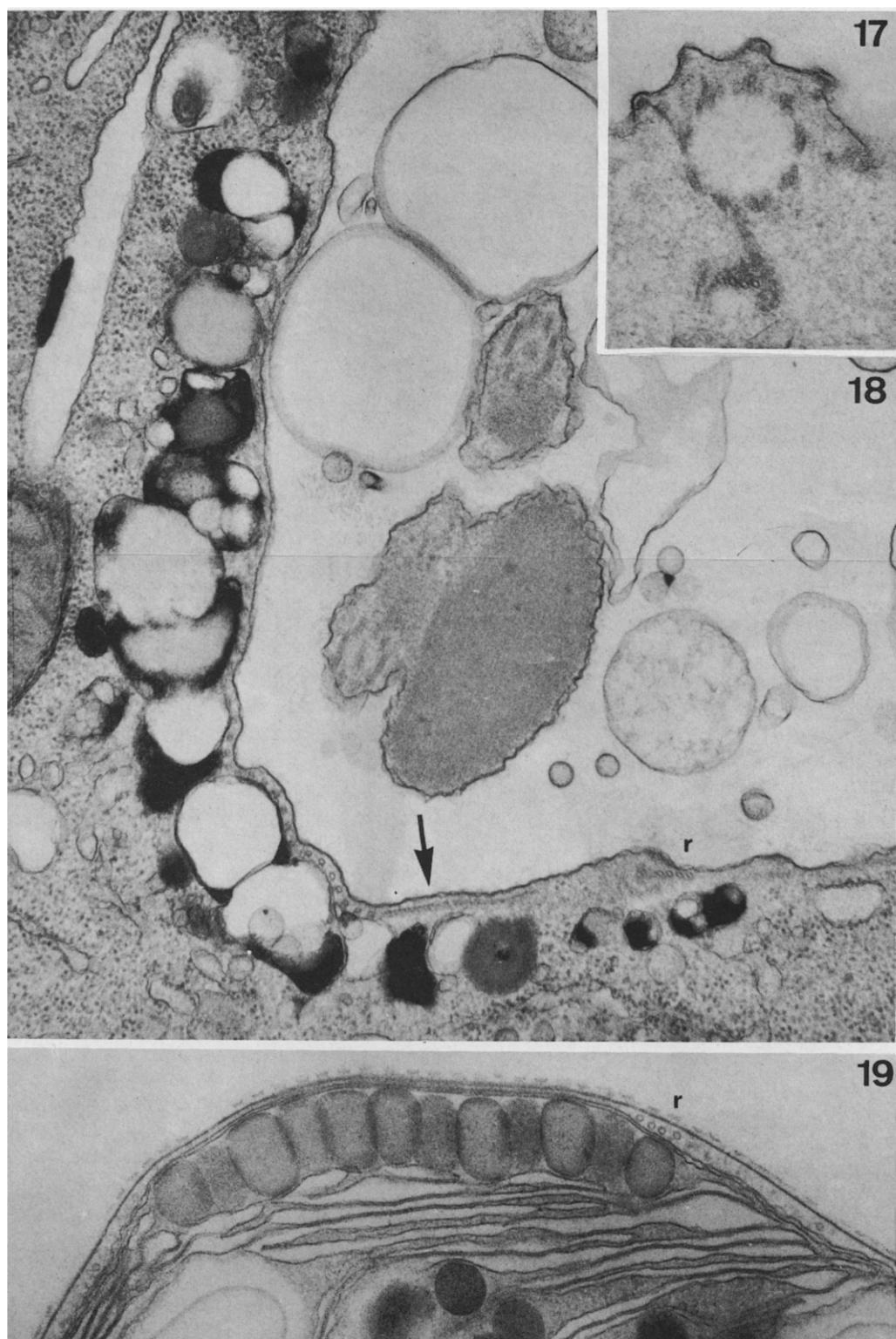
3.7. *Fritschia tuberosa*

In his excellent paper Melkonian (1975) has described a cruciate root system made of 2 and 7–8 microtubules, respectively. In my opinion the published micrographs of the broad root can also be interpreted as showing 5 microtubules, the extra being cytoplasmic microtubules which pass near the flagellar apparatus. Melkonian has commented on the striking similarity between the zoospore of *Stigeoclonium* and *Fritschia*. This is even further enhanced if the interpretation above is correct, in which case they both have a 5–2–5–2 system, and the 2-stranded roots associated with a striated component of apparently similar periodicity.

3.8. *Carteria radiososa* and sp.

Lembi (1975a,b) reports that all cross sections indicate the 4 roots to be composed of 4 microtubules. One is illustrated in her Fig. 5.

Figs. 17–18. *Euteptiella* cf. *braarudii*. Fig. 17. Transverse section through a flagellar base and an attached 4-stranded flagellar root. $\times 48\ 000$. Fig. 18. The eyespot consists of a group of osmophilic globules arranged near the plasmalemma. In between there is a considerable number of evenly spaced microtubules. The 4-stranded flagellar root (r) passes in a similar position near the periphery of the eyespot. Part of the space between the 2 microtubular systems is occupied by a thin plate, which in Fig. 18 appears as a thin line (arrow). One of the flagella in the eyespot region possesses a large flagellar swelling. $\times 39\ 000$. Fig. 19. In *Ulvopsis grevillei* a 4-stranded root (r) occupies the narrow space between the periphery of the eyespot and the plasmalemma. The gamete is scale-covered, also on the surface of the eyespot region. $\times 48\ 000$.



3.9. *Schizochlamys* sp.

Fig. 5 in a study by Lembi and Walne (1969) indicates that the number of microtubules in the 4 roots is higher than 2.

3.10. *Sorastrum* sp.

Marchant's (1974a) Fig. 19 shows a 6-stranded root (5 over 1).

3.11. *Sphaeroplea annulina*

The spermatozoids were examined by Moestrup (1975) in a preliminary way, and exact data are not available. The number of microtubules per root is approx. 2 and 8.

3.12. *Chaetopeltis* sp.

The published micrographs show roots with 2 and 3 microtubules (Wujek and Chelune, 1975). Whether this represents a 3-2-3-2 system or whether the 3-stranded root represents a variation of a 2-stranded (the other (broad?) root in that case not illustrated) is not known. In the published micrographs the very similar position of the root profiles suggests the latter explanation.

3.13. *Eudorina illinoiensis*

Hobbs (1971) mentions that "root microtubules are striated with a periodicity of 13.5 nm", but this is not illustrated or further discussed. The periodicity is similar to that of the striated structure in *Volvox* (see above).

3.14. *Acetabularia mediterranea*

Woodcock and Miller (1973) found "a band of 15 to 20 microtubules encircling the cell in a longitudinal direction", but it is not known whether this is part of the root system. The few micrographs published (in particular Fig. 16 in Woodcock and Miller, 1973) indicate a cruciate root system, which may be a X-2-X-2 system, though this must be further examined.

3.15. *Gloeomonas simulans*

The periodicity of the striated band which interconnects the flagellar base is difficult to measure (Fig. 4 in Schnepf et al., 1976) and should be reinvestigated.

3.16. *Pedinomonas minor*

The 4 roots (3-2-3-2) soon after having left the flagellar bases become arranged in 2 opposite pairs. One pair is on the nuclear surface, in the narrow space between the nucleus and the plasmalemma. The 3-stranded root is attached to the nucleus by the striated component. The other pair (or at least one root) is located in the narrow space between the chloroplast and the plasmalemma. This pair was also illustrated by Pickett-Heaps and Ott (1974), and in their paper the 3-stranded root is shown on the edge of the eyespot. In Fig. 6 they show a 5-stranded root in the same position, but it is not known whether this is an abnormal cell in which the 2 roots have fused, or whether it shows a variation in the number of microtubules of the "broad" root. Other cases in which the 4 roots arrange themselves in 2 groups are *Microthamnion* (Watson and Arnott, 1973) and *Dichotomosiphon* (Moestrup and Hoffman, 1975).

3.17. *Platymonas convolutae*

Measurements of the periodicity of the striated root may be obtained from Fig. 45 in Parke and Manton (1967). The difference between this and other species of *Platymonas* cannot at present be explained.

3.18. *Chara* and *Nitella*

In *Chara corallina* (Moestrup, 1970) a ring of approx. 30 microtubules surrounds the row of mitochondria in the anterior (flagellar) region of the spermatozoid. In one half of the ring the microtubules are interconnected and this group is restricted to the mitochondrial region. The other half extends almost throughout the length of the cell, and only this group is present in the one examined species of *Nitella* (Turner, 1968). Whether the micro-

tubules are all part of a single root is uncertain, but in Pickett-Heaps' (1968) investigation on spermatogenesis in *Chara fibrosa* 1 row of 27 microtubules was found to be formed near the flagellar bases, and they therefore presumably belong to one root. Information on any interconnections is not given.

Pickett-Heaps (1968) also comments on a group of 3–4 microtubules which was found near the group of 27, but their origin and fate during spermatogenesis could not be established (and extra root?). In *Nitella*, too, a group of 2 or 3 microtubules can be distinguished, not directly aligned with the root (Turner, 1968: Figs. 28e, 28f; 21, also 22?).

Intraspecific variation in the root systems

Variations in the number of microtubules per root are reported very rarely. Above I have mentioned the occurrence in *Chlamydomonas reinhardtii* of the 2-stranded root in the 4–2–4–2 system being replaced by a 3-stranded, a variation which is important if it is accepted that the primitive root type is X–2–X–2 (see below). A variation in the broad root has also been reported, in *Dichotomosiphon* (Moestrup and Hoffman, 1975), the 3-stranded root in the 3–2–3–2 system in some cells was replaced by a 4-stranded.

In a few cases the different motile stages have been investigated of those organisms which produce more than one flagellated stage during the life cycle. In *Oedogonium cardiacum* the number of flagella in the spermatozoids is approx. 30 as opposed to the much larger zoospores with approx. 120 flagella. In addition to the reduced number of flagella in the spermatozoids, other details are also different, e.g., the amount of dense material which supports the flagellar bases and the number of flagellar roots. The same was found in the related genus *Bulbochaete* (Retallack and Butler, 1972, 1973). Each individual root, however, still contains 3 microtubules, and in fact the 3-stranded type is the only one known in the 3 genera of the

Oedogoniales, from the 3–5 μm long spermatozoids of *Bulbochaete hiloensis* with its 6–9 flagella and to the 10 times longer zoospores of *Oedogonium cardiacum* with 15–20 times more flagella.

In *Volvox* the published papers on vegetative cell structure and spermatozoids also indicate that, in spite of a number of other differences, the microtubular root systems are the same (see above).

In *Hydrodictyon reticulatum* Marchant and Pickett-Heaps (1972a,b) mention that both gametes and the somewhat larger zoospores have cruciate root systems, but a detailed study was not made.

5. Interspecific variation in the root system

As mentioned above all examined species of the *Oedogoniales* (all 3 genera) possess 3-stranded roots, and it will appear from the Tables that within a genus there is generally very little variation in the microtubular root system, at least as far as can be concluded at the present time.

In an abstract Birkbeck (1976) mentions that *Chlamydomonas sp.* (termed Ettl No. 30) has a 5–2–5–2 system rather than the usual 4–2–4–2 and, when more species have been examined, it may be expected that a greater degree of variation is discovered. The 2 types which Lembi (1975a) has described within the genus *Carteria* are, however, unusually different. One group (*Carteria radiosa* and *C. sp.*) contains the "normal" cruciate root system, the other (*C. crucifera*, *C. eugametos* and *C. olivieri*) an unusual type which differs from any other known alga, although 2 and 4 membered roots are present. The difference between the 2 groups may eventually justify separation into different genera.

In the genera *Pyramimonas* and *Platymonas* a number of species have been examined, and generally the periodicity of the striated roots (rhizoplasts) is constant within the genus, allowing for a certain variation in the accuracy of the given magnifications. In *Pyramimonas*

TABLE 4

Chlorophyll *a* and *b* containing organisms, in which an association between a flagellar root and the eyespot has been demonstrated. "E" indicates the eyespot root (for further explanation see Table 1).

	E	
Euglenophyceae		
<i>Eutreptiella cf. braarudii</i>	c.6—c.9—4	present paper
Prasinophyceae	—	
<i>Pedinomonas minor?</i>	—	Ettl et al., 1964 Pickett-Heaps et al., 1974
Chlorophyceae		
<i>Stigeoclonium sp.</i> (zoospore)	5—2—5—2	Manton, 1964
<i>Microthamnion kuetzingianum</i> (zoospore)	6—2—6—2	Watson, 1975
<i>Ulvopsis grevillei</i> (gamete)	4—2—4—2	present paper
<i>Fritschella tuberosa</i> (zoospore)	5—2—5—2	Melkonian, 1975
<i>Aphanochaete</i>	—	Watson, 1975
<i>Volvox tertius</i>	4—2—4—2	Pickett-Heaps, 1975a,b
<i>Eudorina illinoiensis</i>	—	Hobbs, 1971

the periodicity in 4 of 5 examined species is 250–300 nm. The fifth is *P. parkeae*, in which the periodicity of approx. 30 nm indicates affinity with *Halosphaera* (*Pyramimonas* stage of *Halosphaera*?).

In *Platymonas* the periodicity is 400–600 nm, the only exception being *P. convolutae*, in which species only the proximal part of the root was illustrated.

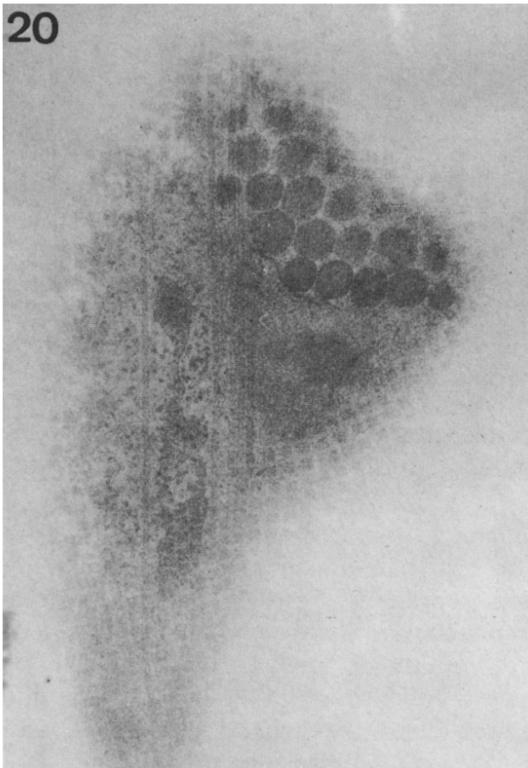
Among the possible descendants from the green algae, in only the bryophyte *Polytrichum* more than 1 species appears to have been examined. In *P. juniperinum* the spermatozoids are reported to have 10 microtubules in the multilayered structure, in *P. commune* 12 (Paolillo et al., 1968).

6. Connections between flagellar roots and eyespots in chlorophyll *a* and *b* containing organisms

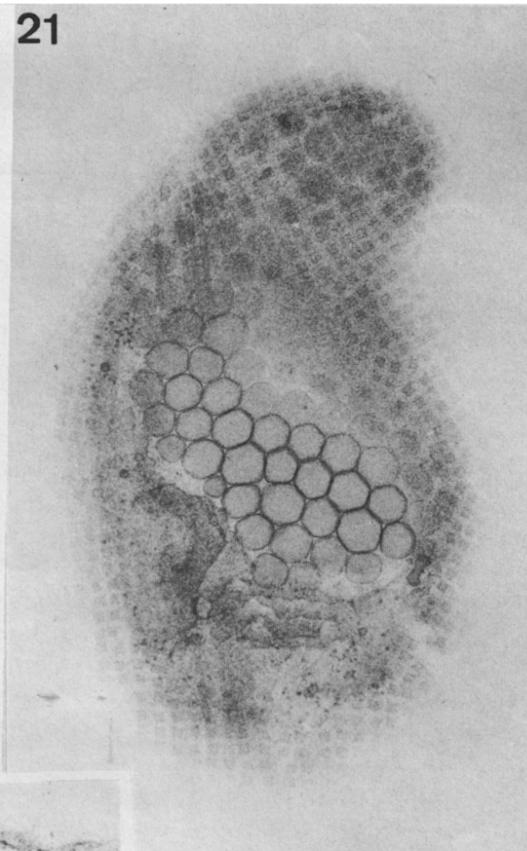
The few examples known to me of structural connections between the flagellar apparatus and the eyespot are listed in Table 4. Since the connection was first observed by Manton (1964) in *Stigeoclonium*, a number of other examples have been found in green algae and it may be significant that the eyespot root is never a 2-stranded, always a "broad" root. This root passes from the flagellar apparatus along the plasmalemma to the eyespot region, but it never passes over the middle of the eyespot. This part is

Figs. 20–21. Grazing section through the eyespot region of the scale-covered gamete of *Ulvopsis grevillei*, showing 4 microtubules on the surface of the eyespot (a 4-stranded flagellar root). An extra microtubule passes at some distance from the eyespot (compare with Fig. 19). The root microtubules pass along the outermost rows of eyespot globules. Fig. 20, $\times 35\,000$; Fig. 21, $\times 39\,000$. Figs. 22–25. *Eutreptiella cf. braarudii*. In *Eutreptiella* both a striated component (Fig. 23) and a multilayered structure (MLS) are part of the flagellar root system. The MLS (Figs. 22, 24 and 25) contains at least 3 layers: an uppermost microtubular layer, a layer consisting of thin parallel plates, spaced approx. 14 nm apart, and an underlayer (Fig. 22) which is probably made of very short plates arranged at an angle to the layer above. Fig. 22, $\times 60\,000$; Figs. 23–25, $\times 48\,000$.

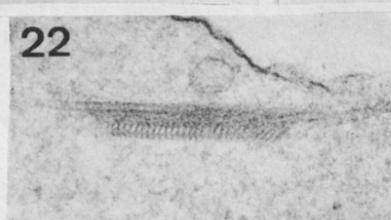
20



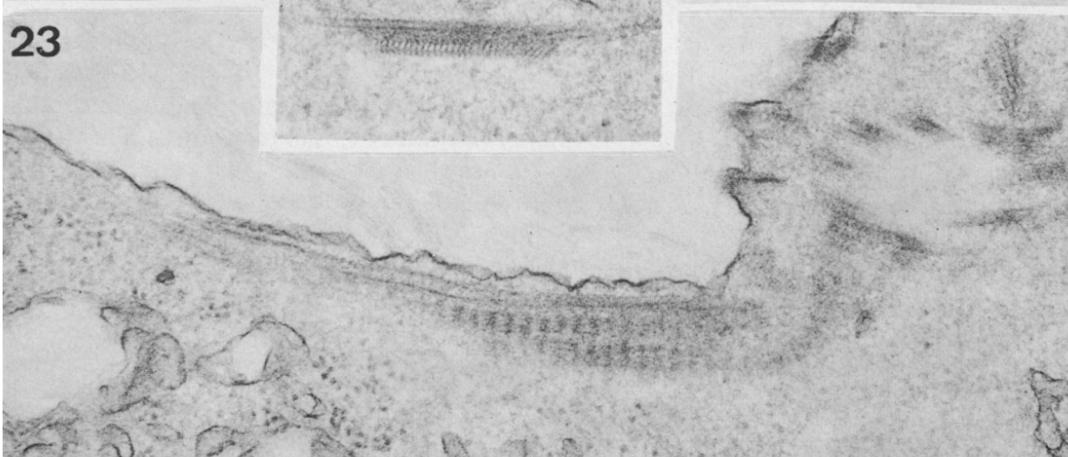
21



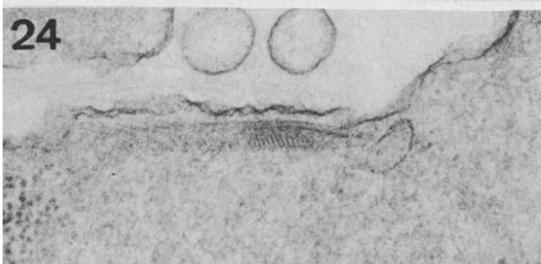
22



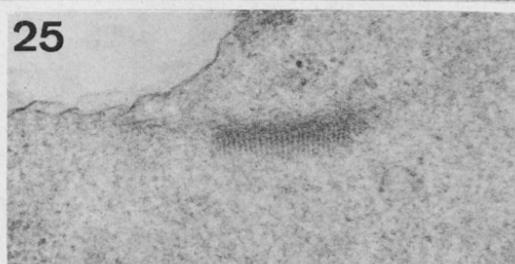
23



24



25



generally closely appressed to the plasmalemma (see also Figs. 19–21), leaving no space for the microtubules, which are arranged in a short row over the peripheral part of the eyespot (the first 1–4 rows of eyespot globules) where this curves away from the plasmalemma. In *Fritschia* Melkonian (1975) observed the root to become disorganized distally from the eyespot, finally into single microtubules.

In *Pedinomonas minor* (Ettl and Manton, 1964, Pickett-Heaps and Ott, 1974) the 4 roots at some distance below the flagella are arranged in 2 pairs on opposite sides of the cell. The eyespot is located in the rather narrow area between the roots of 1 pair, but whether this represents a situation comparable to that of the other organisms in Table 4 is not certain.

In euglenoids it is well known that the large eyespot is covered with a layer of evenly spaced microtubules (see also Fig. 18). In *Eutreptiella* (and probably also in other euglenoids) these microtubules are not in contact with the flagella, they terminate at some distance above the flagellar bases. Very interestingly, however, there is in *Eutreptiella* a situation comparable to that described above. One of the 3 roots (a 4-stranded: Fig. 17) passes over the periphery of the eyespot at some distance from the other microtubules (Fig. 18). The 2 groups of microtubules are kept apart by a thin plate-like structure. Further studies on flagellar roots in other euglenoids are needed to establish whether this situation is of more general occurrence.

The finding of this characteristic association also in euglenoids adds to the idea of a functional relationship between eyespot and flagella. This could be a structural connection, or it could be a physiological connection in which impulses are transferred between the flagella and the eyespot. A similar function has been suggested for the plasmamembrane which covers both the flagella and the eyespot (see further in Walne and Arnott, 1967). Also the initial assemblage of globules into an eyespot has been suggested to be mediated by

microtubules (Bouck, 1970). Very interestingly, Watson (1975) found in a few zoospores of *Microthamnion* 2 eyespots, and these were both in contact with the root, one above the other.

7. Conclusion and discussion

From the Tables it appears that deviations from the cruciate root type are rare among the chlorophyll *a* and *b* containing algae, with the exception of the euglenoids. In the euglenoids the few observations (showing only 3 (different) flagellar roots) should be extended to include more genera before conclusions can be made. The 3 genera studied represent, however, both primitive (*Eutreptiella*) and advanced types (*Menodium*, *Rhabdomonas*), cf. Table B in Leedale (1967). In the other chlorophyll *a* and *b* containing organisms there are 2 basic root types, and these are best discussed separately.

7.1. The cruciate type

Approximately 40 of the examined green algae possess 4 roots. In the majority of species the roots are of 2 types, and one usually contains 2 microtubules. This is so conspicuous that I suggest the primitive condition of the cruciate type was what may be termed an X–2–X–2 system. *Golenkinia* has a 1-stranded rather than a 2-stranded root, and in *Chlamydomonas reinhardtii* a variation has been mentioned above in which the 2-stranded became replaced by a 3-stranded root. The X root contains 3 to approx. 8 microtubules. Genera which for other reasons are considered related have very similar root systems: in *Draparnaldia*, *Stigeoclonium*, and *Fritschia* the root system appears to be a 5–2–5–2, with probably identical striated components (at least in the latter 2, *Draparnaldia* has been examined in a preliminary way only). In *Microthamnion*, which is usually also included in the family Chaetophoraceae, though in an isolated position, the system is

6—2—6—2, and the striated component very different (in fact with no equivalent known so far). *Chlamydomonas*, *Volvox* and *Tetraspora* also appear to have similar root systems (4—2—4—2); striated components of the *Stigeoclonium* type have not been found. Very interestingly, however, the colourless flagellate *Polytomella*, usually considered a relative of *Chlamydomonas*, when examined recently was found to possess 8 roots, so far the only case known outside the Oedogoniales with more than 4 roots. Except in number the microtubular parts of the roots appear identical to *Chlamydomonas reinhardtii*, as would be expected in a supposedly related organism, but, very puzzling, the striated part associated with the 2-stranded root is the type present in the *Stigeoclonium* group mentioned above and in the Oedogoniales.

Other deviations from the cruciate type are the few organisms found so far to possess 2 roots only (regarding the *Trentepohlia* group which contains MLS-like structures, see below): in *Monomastix minuta* (Prasinophyceae) Manton (1967) found 2 2-stranded roots, and recently *Trebouxia* (Stewart, pers. comm.) and *Pleurastrum obovatum* (Pickett-Heaps, 1975a) have been reported to possess 2 broad roots. These cases may represent organisms in which the 2 roots of a X—2—X—2 system have been lost completely, or they may represent special types of root systems. In contrast to these 3 genera (in which the 2 roots are opposite and identical) is *Mantoniella squamata*, a scale-covered prasinophycean flagellate, which possesses one normal (9 + 2) flagellum associated with a 4-stranded and a 2-stranded root, and 1 very short flagellum (9 + 0) with no trace of roots (Barlow, pers. comm.). *Pedinomonas*, a somewhat similar flagellate sometimes considered a relative of *Mantoniella*, also has one 9 + 2 flagellum only (an extra basal body is, however, present), but the root system is the usual cruciate type. *Mantoniella* and *Pedinomonas* probably represent a reduced biflagellate type (both basal bodies replicate during cell division: Barlow, 1977, Pickett-Heaps and Ott, 1974),

in which the 2 roots associated with the non-functional flagellar base have been lost completely in *Mantoniella*, but retained in *Pedinomonas*.

The order Oedogoniales with their symmetrical flagellar root system (all roots identical, the flagellar apparatus ring-shaped) has been repeatedly discussed in the literature (e.g., Ringo, 1967) in the hope of finding the ancestors of this rather isolated group. From the Tables it appears that only a few genera are reported to have all roots identical. In *Tetraspora* sp. (LB 234) and in 2 species of *Carteria* a 4—4—4—4 system has been suggested, but this must be accepted with some reservation until they have been examined by serial sectioning. *Tetraspora* sp., in particular, is not expected to have a system which differs from *Tetraspora lubrica* (probably 4—2—4—2). In the 2 species of *Carteria*, however, the 4 basal bodies are arranged in a ring (or in the 4 corners of a square) - adjacent basal bodies being interconnected by fibres (Lembi, 1975a), distal fibres in Ringo's terminology (Ringo, 1967). Lembi states that these species "more likely represent a specialized offshoot of *Chlamydomonas* or some other monad", and although this may be true their position as possible ancestors of the Oedogoniales cannot at present be wholly excluded. Also the genera *Schizomeris*, *Hydrodictyon*, *Urospora* and *Cylindrocapsa* are reported to have identical roots. *Hydrodictyon* was, however, not examined in detail, and in *Cylindrocapsa* Hoffman (1976) indicates that 2 different root types may be present. The 2 remaining genera in which the evidence indicates a symmetrical root system (*Schizomeris* and *Urospora*) both occupy (as does *Cylindrocapsa*) rather isolated systematic positions and have few characters in common with the Oedogoniales. In *Schizomeris* Birkbeck et al. (1974) claim that the 4 basal bodies of the quadriflagellate zoospores are arranged in a ring, adjacent basal bodies connected by striated distal fibres, but both *Urospora* and *Schizomeris* should be further examined for possible similarities with the

Oedogoniales before any phylogenetic conclusions are made.

From the data assembled here the only other groups in which the flagellar apparatus shows some similarity with the Oedogoniales are (a) the flagellate *Polytomella* and (b) the filamentous *Stigeoclonium-Fritschia* group. In both these groups the striated roots are of the same type as in *Oedogonium*, *Oedocladium*, and *Bulbochaete* (apparently same repeating pattern, same periodicity). The presence of these rather conspicuous structures may be phylogenetically more important than generally realized. The roots should be isolated and studied in further detail, applying the technique Hoffman (1970) used with *Oedogonium*.

Of the green algae examined so far, no taxa except those of the Oedogoniales have more than 4 flagella and it may be interesting to study whether the rare cases of cells with more than 4 flagella possess root types intermediate between the cruciate and the Oedogoniales type. For example, in some members of the Chaetophoraceae the number of flagella is rather variable (e.g., *Schizomeris* as suggested by Birkbeck et al., (1974)), and flagellates with more than 4 flagella also exist. From the Tables it appears that in the Oedogoniales the number of flagella may be as low as 6–9, one example being the spermatozoids of *Bulbochaete hiloensis*.

Two genera in which the flagellar root system at first appears to be basically different from the cruciate type is *Carteria*, type II, and *Gloeomonas*. In the latter Schnepf et al., (1976) consider the structure of the flagellar root system to be a better generic characteristic than for example the position of the contractile vacuoles, which is often used. The root system is indeed striking (for details see Schnepf et al., 1976), but the presence of 4 microtubular roots only, 2 associated with each flagellum, indicates that it may be a modification of the cruciate system. This modification is probably associated with the unusual feature that in non-dividing cells the 2 flagella are separated from each other by a

distance of 3–5 μm . The 8 dense structures which are present and some of which interconnect the flagella may have a function in for example coordinating the flagellar beating. In *Carteria* type II the situation is also complicated, but again may be associated with the fact that the 4 flagellar bases (in contrast to *Carteria* type I) are not in direct contact, but arranged in 4 corners of a square, approx. 0.75 of a micron apart. Both 2-stranded and 4-stranded roots are present.

The details of the flagellar system of *Ulva* and *Enteromorpha* are only partly known, but the published data indicate a close relationship between these 2 genera and *Ulvopsis*, e.g., a striated root type with a periodicity of approx. 150 nm. This is a very important discovery, because in *Ulvopsis* the gametes are covered on the body by tiny scales of a shape found only in a very small number of algae with cruciate roots and chlorophyll *a* and *b*: a number of prasinophytes, and in *Trichosarcina* and *Pseudendoclonium* (Mattox and Stewart, 1973), usually included in the Chaetophoraceae. Details of the flagellar apparatus of these latter 2 are not known. As mentioned in the Introduction the Prasinophyceae are probably among the most primitive organisms with chlorophyll *a* and *b*, and Moestrup (1973, 1974) has previously suggested an evolutionary scheme in which the present day Prasinophyceae and the green algae with cruciate roots evolved from a common ancestor. The available evidence indicates that this ancestor had an X–2–X–2 system and was probably scale covered. Scales are now being found in so many groups that their presence may be considered a primitive character (at present observed in at least 5 of Christensen's 12 eucaryotic algal classes (Christensen, 1962)). Apparently scales were retained in most of the prasinophytes, though occasionally one or more layers were lost: in *Pyramimonas orientalis* and its allies the layer of diamond shaped scales is absent except in the flagellar pit (Moestrup and Thomsen, 1974). The presence of scales in *Ulvopsis* but not in the related genera *Ulva* and *Enter-*

morpha indicates that the opposites has happened in the cruciate Chlorophyceae, the scales were lost in almost all cases.

In *Trichosarcina polymorpha* scales were seen only during zoosporogenesis, whilst in *Pseudoendoclonium basiliense* body scales were present on the mature zoospore (Mattox and Stewart, 1973). The Chaetophoraceae (to which the 2 genera are generally referred) and the Ulvaceae are often included in the same order, Ulotrichales. Further information on the flagellar apparatus of the 2 genera is needed to be compared with (1) the *Ulvopsis* type, and (2) the *Stigeoclonium* type.

7.2. The unilateral type

In a review by Moestrup (1972) only 3 cases of unilateral roots were included: *Coleochaete*, *Chara*, and *Nitella*. In *Coleochaete* McBride (1971) reported, in addition to the microtubular root, a multilayered structure, though unfortunately the micrographs were never published and the details therefore remained unknown for some time. In the 3 genera the motile cells were also found to be covered with scales. The flagella are covered with tiny diamond shaped scales whereas the body in *Chara* and *Nitella* possess somewhat similar scales and in *Coleochaete* the body is covered with pyramidal scales. These were suspected to be a modification of the diamond shaped scale. Figs. 26 and 29, which have very kindly been placed at my disposal by Dr H. Marchant, show that this is indeed the case, the base of each scale is diamond shaped. *Coleochaete* also has hairs (probably hair scales) on its flagella as do many prasinophytes.

Without knowing of each others' work Pickett-Heaps and Marchant in Colorado and myself in Copenhagen continued work on these organisms. Pickett-Heaps and Marchant arrived at an evolutionary scheme based mainly upon their studies of mitosis and cytokinesis (Pickett-Heaps and Marchant, 1972); the scheme proposed by me was based exclusively on motile cell structure (Moestrup,

1973, 1974). Almost incredibly, however, the 2 evolutionary schemes were practically identical and both suggested that the higher plants and bryophytes have evolved, not from a *Fritschia* type as has previously been suggested, but from an evolutionary line which gave rise also to the *Coleochaete* and the *Chara* group. Marchant and Pickett-Heaps included in their scheme the Zygnematales, in which flagellated cells are unknown, and *Klebsormidium*, which they later showed to have a unilateral root but no scales (Marchant et al., 1973). I found *Chaetosphaeridium* to be a close relative of *Coleochaete*, although the scales covering both the flagella and the cell body were of the diamond shaped type (the flagella also had hairs). A similar type has however now been found on the zoospores of another species of *Coleochaete*, *C. nitellarum* (Dr. H. Marchant, unpublished, illustrated in Figs. 27–28).

Stewart and Mattox (1975) suggested the erection of a new class, Charophyceae, to comprise the above mentioned algae, an idea which I fully support. The lack of flagellated cells in the Zygnematales makes the inclusion of this group in the Charophyceae slightly less convincing, but it is clear that the class should contain at least the genera listed in Table 3. I suspect that the apparently rare *Chlorokybus* should also be included in the Charophyceae, but this must await EM examination of the zoospores, which are difficult to obtain. Based on light microscopy of the zoospores an affinity to *Klebsormidium/Coleochaete/Chaetosphaeridium* is suggested (Rieth, 1972; Moestrup unpubl. obs.) Pickett-Heaps (1974) has shown that *Stichococcus* and *Raphidonema*, which as the Zygnematales do not form any (known) flagellated stages, should probably also be included in the class.

A basic character of the 5 genera listed in Table 3 (Charophyceae) is the unilateral root, which extends from 2 subapical flagella. This root in *Klebsormidium*, *Coleochaete*, and *Chaetosphaeridium* contains a multilayered structure (MLS), a very complex structure characteristic of all examined spermatozoids

of bryophytes and higher plants, though in many cases in a reduced form in the mature spermatozoids. The structure consists of 3 (Dreiergruppe) or 4 layers (Vierergruppe), 1 of which is microtubular. In the 3 examined species of the Charales only 1 layer has been found (the microtubular layer). There is no obvious sign of extra layers, not even during spermatogenesis. None of the chlorophyll *a* and *b* containing plants with a unilateral root possess a striated component in the root, whereas a glance at Table 2 shows this to be very commonly present in these more primitive organisms, suggesting that the presence of a striated component is a primitive character. Similar structures are present in many other flagellates, and they have now been found also in euglenoids (Moestrup in prep., and Fig. 23).

An eyespot is also lacking in all the unilateral types. Several functions have been suggested for the flagellar roots in addition to their being attaching structures for the flagella and a means by which the stress created during flagellar action is absorbed. As discussed above, the fact that in a number of algae with cruciate roots one of the broad roots is attached to the eyespot, suggests a functional relationship. If this is true, one may speculate that during the evolution of the unilateral types the eyespot root disappeared together with the eyespot.

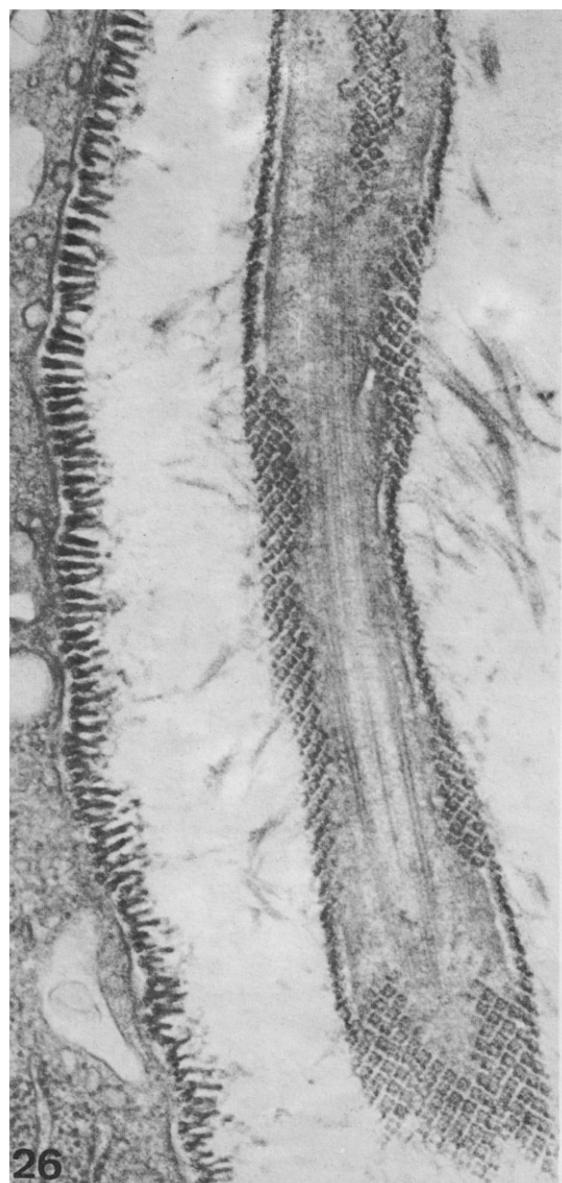
The number of microtubules in the unilateral roots varies from approx. 15 in bryophytes and *Selaginella* to the incredible number of approx. 60 000 in the very large spermatozoid of *Zamia*. Whether the number is related directly to cell size is not clear, however. In *Chaetosphaeridium* the zoospores may be twice the size of the zoospores in *Klebsormidium*, and they possess twice the

number of microtubules. Duckett (1973) has however suggested that in some cases the number of microtubules is related to the shape of the nucleus.

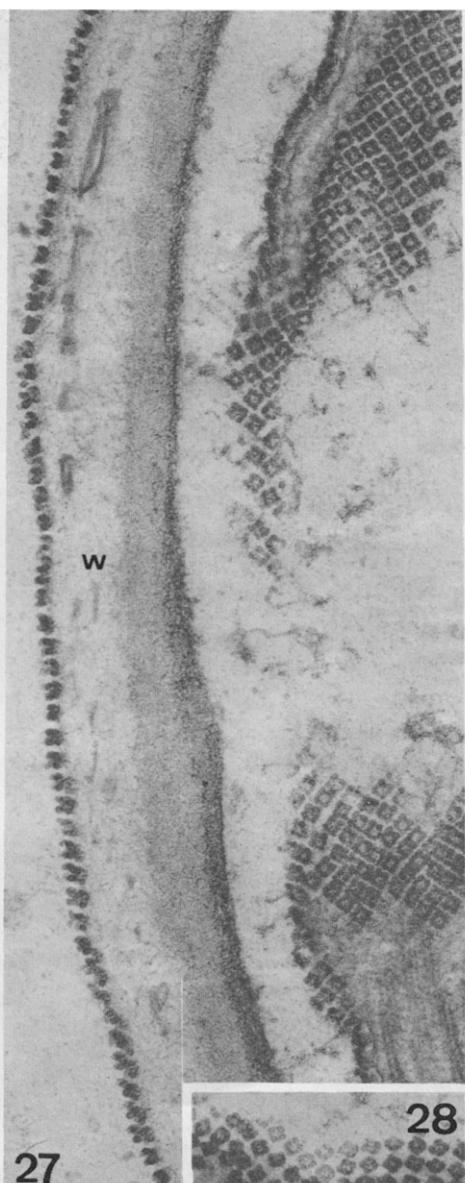
If the evolutionary schemes proposed by Pickett-Heaps and Marchant (1972) and Moestrup (1974) are valid, it suggests that the unilateral root type evolved from a cruciate type, or perhaps from a root system containing 2 roots only. It is therefore likely that intermediate types may be found. At present 2 organisms may be considered to show intermediate types. In *Chara* and *Nitella* the few extra microtubules present (see above) may represent remains of a second root, and in the zoospores of *Trentepohlia* Graham and McBride (1975) found 2 opposite roots only, both associated with a structure reminiscent of a MLS. The layers of this structure are not clearly visible, however. At present the phylogenetic position of *Trentepohlia* (and its relatives *Cephaeluros* and *Phycopeltis*) is very uncertain, and a comparison between the *Trentepohlia* group and the 3 genera which contain only 2 (identical) roots (*Monomastix*, *Trebouxia*, *Pleurastrum*) is greatly needed.

In 1962 Christensen proposed the division Chlorophyta to comprise all chlorophyll *a* and *b* containing algae, including the euglenoids, which several other authors prefer to keep separate. Some authors (e.g., Chadefaud, 1977) even suggest that chlorophyll *b* has evolved more than one time. It is quite clear that the flagellar apparatus of the euglenoids is very different from that found in other chlorophyll *a* and *b* containing organisms. There is a unique covering of hairs, a stellate pattern is absent in the flagellar transition region, the root system (as far as it is known) consists of 3 unequal roots. It was therefore very surprising indeed that during sectioning of

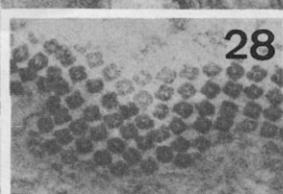
Figs. 26–29. *Coleochaete scutata* and *C. nitellarum*. In the zoospore of *Coleochaete nitellarum* both the flagella (Fig. 27, right) and the cell body (Fig. 28) are covered with small diamond shaped scales. The scales may be retained when the zoospores germinate and form a cell wall: in Fig. 27 scales are still present on the outside of the zoosporangial wall (w). In *C. scutata* the flagella bear similar scales (Fig. 26, right), but on the body each scale possesses a central pyramidal spine (Fig. 26, left). The base is, however, still diamond shaped (Fig. 29). All $\times 50\,000$. (The micrographs on this plate were all provided by Dr. H. Marchant, Canberra).



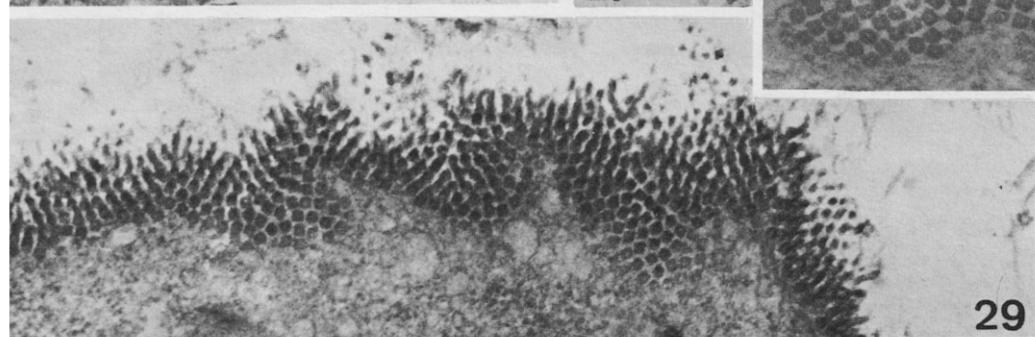
26



27



28



29

the euglenoid *Eutreptiella*, considered to be one of the most primitive in the class (cf. Leedale, 1967), a multilayered structure was detected (Figs. 22–25). Next to a microtubular layer there is a layer of thin plates spaced approx. 14 nm apart, and in suitable sections such as Fig. 22 a third layer is evident. The similarity between this and e.g., the MLS of *Chaetosphaeridium* (Moestrup, 1974, Fig. 4C) is unmistakable, and a survey of the literature on multilayered structures showed that the thin plates are always spaced 12–15 nm apart, thus further confirming the identity of the MLS in *Eutreptiella*. A similar structure was not observed in the colourless euglenoids *Rhabdomonas* and *Menodium* (Leedale and Hibberd, 1974).

The finding of a MLS in a euglenoid is very unexpected and further studies on the other algal classes (in particular those without chlorophyll b) are required before its significance can be assessed.

Note added in proof

Since this paper was prepared, 4 more articles have appeared which contain observations on flagellar roots: In *Chlorosarcinopsis minuta* and *C. sp.* M. Melkonian (1977) describes a 4–2–4–2 system (Plant Syst. Evol. 128, 79–88) and questions Ringo's findings of identical roots in *Chlamydomonas*. In *Bryopsis maxima* T. Hori (1977) observed a cruciate root system (2 pairs, one pair containing 3–5 microtubules (J. Phycol. 13, 238–243). Finally the flagellar system of the bryophyte *Phaeoceros laevis* has been described in detail by J.W. Moser, J.G. Duckett and Z.B. Carothers (1977) (Am. J. Bot. 64, 1097–1116) and J.G. Duckett and P.R. Bell (1977) in a paper on *Equisetum* included a review of the MLS (Phil. Trans. R. Soc. Ser. B 277, 131–158), which contains unpublished information.

Acknowledgements

I extend my best thanks to Dr. H. Marchant, Canberra, for supplying micrographs of unpublished data, and to Dr. R. Sager, New

York, for a culture of *Chlamydomonas reinhardtii*.

I also thank Prof. T. Christensen, Copenhagen, for many discussions on green algal phylogeny.

Finally I wish to thank the Danish Research Council, which covered travel expenses to Canada.

References

- Barlow, S., 1977, Fine structure and cell division of the scale-covered uniflagellate *Mantoniella squamata* (Chlorophyta, Prasinophyceae). J. Phycol. 13, Suppl., Abstr. 18.
- Birkbeck, T.E., 1976, Variation of the flagellar apparatus within the genus *Chlamydomonas*. J. Phycol. 12, Suppl., Abstr. 72.
- Birkbeck, T.E., K.D. Stewart and K.R. Mattox, 1974, The cytology and classification of *Schizomeris leibleinii* (Chlorophyceae). II. The structure of quadriflagellate zoospores. Phycologia 13, 71–79.
- Bonnot, E.-J., 1967, Le plan d'organisation fondamental de la spermatide de *Bryum capillare* (L.) Hedw. C. r. Acad. Sci., Paris, Sér. D 265, 958–961.
- Bouck, G.B., 1970, The development and post-fertilization fate of the eyespot and the apparent photoreceptor in *Fucus* sperm. Ann. N.Y. Acad. Sci. 175, 673–685.
- Bourrelly, P., 1966, Les algues d'eau douce. T. 1. Les algues vertes, (N. Boubée et Cie, Paris) pp. 1–511.
- Brown, D.L., A. Massalski and R. Patenaude, 1976, Organization of the flagellar apparatus and associated cytoplasmic microtubules in the quadriflagellate alga *Polytomella agilis*. J. Cell Biol. 69, 106–125.
- Carothers, Z.B., 1973, Studies of spermatogenesis in the Hepaticae. IV. On the blepharoplast of *Blasia*. Am. J. Bot. 60, 819–828.
- Carothers, Z.B. and G.L. Kreitner, 1967, Studies on spermatogenesis in the Hepaticae. I. Ultrastructure of the Vierergruppe in *Marchantia*. J. Cell Biol. 33, 43–51.
- Carothers, Z.B., R.R. Robbins and D.L. Haas, 1975, Some ultrastructural aspects of spermatogenesis in *Lycopodium complanatum*. Protoplasma 86, 339–350.
- Chadefaud, M., 1941, Sur l'organisation et la position systématique des Flagellés du g. *Pyramidomonas*. Revue Scient., Paris 79, 113–114.
- Chadefaud, M., 1947, Études sur l'organisation de deux Volvocales sédentaires marines: *Prasinocladus lubricus* et *Chlorodendron subsalsum*. Revue Scient., Paris 85, 862–865.

- Chadefaud, M., 1960, Les végétaux non vasculaires (Cryptogamie), in "Traité de Botanique, Systématique", T. 1, (Masson et Cie, Paris) pp. 1–1018.
- Chadefaud, M., 1977, Les Prasinophycées. Remarques historiques, critiques et phylogénétiques. Bull. Soc. Phyc. Fr. 22, 1–18.
- Chapman, R.L., 1977, Scanning and transmission electron microscopic observations on zoosporegenesis in *Cephaleuros virescens* (Chlorophyta; Chroolepidaceae). J. Phycol. 13, Suppl., Abstr. 57.
- Chapman, R.L. and B.H. Good, 1977, Some comparisons among *Cephaleuros*, *Phycopeltis*, *Trentepohlia* and other green algae. J. Phycol. 13, Suppl., Abstr. 58.
- Chapman, R.L. and S.W. Madison, 1976, Observations on the zoospores of *Cephaleuros virescens* (Chroolepidaceae). J. Phycol. 12, Suppl., Abstr. 70.
- Chesnoy, L. and S. Jónsson, 1973, Étude ultrastructurale du développement du zygote calcicole d'une Chlorophycée marine, le *Monostroma grevillei* (Thuret) Wittrock. C. r. Acad. Sci. Paris Sér. D. 276, 299–302.
- Christensen, T., 1962, Alger, in "Botanik", Bd. 2, "Systematisk Botanik", No. 2, T.W. Böcher, M. Lange and T. Sørensen (eds), (Munksgaard, Copenhagen) pp. 1–178.
- Coss, R.A. and J.D. Pickett-Heaps, 1974, Gametogenesis in the green alga *Oedogonium cardiacum*. II. Spermiogenesis. Protoplasma 81, 297–311.
- Diers, L., 1967, Der Feinbau des Spermatozoids von *Sphaerocarpus donnellii* Aust. (Hepaticae). Planta 72, 119–145.
- Duckett, J.G., 1973, An ultrastructural study of the differentiation of the spermatozoid of *Equisetum*. J. Cell Sci. 12, 95–129.
- Duckett, J.G., 1975, Spermatogenesis in pteridophytes, in "The Biology of the Male Gamete", J.G. Duckett and P.A. Racey (eds), Biol. J. Linn. Soc., 7, Suppl. 1, pp. 97–127.
- Ettl, H. and I. Manton, 1964, Die feinere Struktur von *Pedinomonas minor* Korschikoff. Nova Hedwigia 8, 421–451.
- Evans, L.V. and A.O. Christie, 1970, Studies on the shipfouling alga *Enteromorpha*. I. Aspects of the fine-structure and biochemistry of swimming and newly settled zoospores. Ann. Bot. 34, 451–466.
- Gould, R.R., 1975, The basal bodies of *Chlamydomonas reinhardtii*. Formation from probasal bodies, isolation, and partial characterization. J. Cell Biol. 65, 65–74.
- 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.
- Heath, I.B. and W.M. Darley, 1972, Observations on the ultrastructure of the male gametes of *Biddulphia levii* Ehr. J. Phycol. 8, 51–59.
- Heitz, E., 1960, Über die Geisselstruktur sowie die Dreiergruppe in den Spermatiden der Leber- und Laubmoose, in "Proc. Eur. reg. conf. electron microscopy, Delft, 1960", A.L. Houwink and B.J. Spit (eds), vol. 2, (Nederlandse Vereniging voor Elektronenmicroscopie, Delft) pp. 934–937.
- Hobbs, M.J., 1971, The fine structure of *Eudorina illinoiensis* (Kofoid) Pascher. Br. phycol. J. 6, 81–103.
- Hoffman, L.R., 1970, Observations on the fine structure of *Oedogonium*. VI. The striated component of the compound flagellar "roots" of *O. cardiacum*. Can. J. Bot. 48, 189–196.
- Hoffmann, L.R., 1976, Fine structure of *Cylindrocapsa* zoospores. Protoplasma 87, 191–219.
- Hoffmann, L.R. and I. Manton, 1962, Observations on the fine structure of the zoospore of *Oedogonium cardiacum* with special reference to the flagellar apparatus. J. exp. Bot. 13, 443–449.
- Hoffmann, L.R. and I. Manton, 1963, Observations on the fine structure of *Oedogonium*. II. The spermatozoids of *O. cardiacum*. Am. J. Bot. 50, 455–463.
- Huber-Pestalozzi, G., 1955, Das Phytoplankton des Süßwassers. 4. Euglenophyceen, (Schweizerbart, Stuttgart) pp. 1–606.
- Hyams, J. and D. Chasey, 1974, Aspects of the flagellar apparatus and associated microtubules in a marine alga. Exp. Cell Res. 84, 381–387.
- Jónsson, S. and L. Chesnoy, 1974, Étude ultrastructurale de l'incorporation des axonèmes flagellaires dans les zygotes du *Monostroma grevillei* (Thuret) Wittr., Chlorophycée marine. C. r. Acad. Sci., Paris, Sér. D. 278, 1557–1560.
- Kreitner, G.L. and Z.B. Carothers, 1976, Studies of spermatogenesis in the Hepaticae. V. Blepharoplast development in *Marchantia polymorpha*. Am. J. Bot. 63, 545–557.
- Kristiansen, J., 1974, The fine structure of the zoospores of *Urospora penicilliformis*, with special reference to the flagellar apparatus. Br. phycol. J. 9, 201–213.
- Lal, M. and P.R. Bell, 1975, Spermatogenesis in mosses, in "The Biology of the Male Gamete", J.G. Duckett and P.A. Racey (eds), Biol. J. Linn. Soc. 7, Suppl. 1, pp. 85–95.
- Leedale, G.F., 1967, Euglenoid flagellates, (Prentice-Hall Inc., New Jersey) pp. 1–242.
- Leedale, G.F. and D.J. Hibberd, 1974, Observations on the cytology and fine structure of the euglenoid genera *Menoidium* Perty and *Rhabdomonas* Fresenius. Arch. Protistenkd. 116, 319–345.
- Lembi, C.A., 1975a, The fine structure of the flagellar apparatus of *Carteria*. J. Phycol. 11, 1–9.
- Lembi, C.A., 1975b, A rhizoplast in *Carteria radiosa* (Chlorophyceae). J. Phycol. 11, 219–221.

- Lembi, C.A. and P.L. Walne, 1969, Interconnections between cytoplasmic microtubules and basal bodies of tetrasporalean pseudocilia. *J. Phycol.* 5, 202–205.
- Lembi, C.A. and P.L. Walne, 1971, Ultrastructure of pseudocilia in *Tetraspora lubrica* (Roth) Ag. *J. Cell Sci.* 9, 569–579.
- Manton, I., 1952, The fine structure of plant cilia. *Symp. Soc. exp. Biol.* 6, 306–319.
- 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 structures in plants, in "Advances in Botanical Research" 2, R.D. Preston (ed.), (Academic Press, London and N.Y.) pp. 1–34.
- Manton, I., 1966, Observations on scale production in *Pyramimonas amyliifera* Conrad. *J. Cell Sci.* 1, 429–438.
- Manton, I., 1967, Electron microscopical observations on a clone of *Monomastix Scherffel* in culture. *Nova Hedwigia* 14, 1–11.
- Manton, I., 1968, Observations on the microanatomy of the type species of *Pyramimonas* (*P. tetrarhynchus* Schmarda). *Proc. Linn. Soc. Lond.* 179, 147–152.
- 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., 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, 275–298.
- 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* Korschikov. *J. mar. biol. Assoc. U.K.* 45, 241–255.
- Manton, I. and H.A. von Stosch, 1966, Observations on the fine structure of the male gamete of the marine centric diatom *Lithodesmium undulatum*. *J.R. microsc. Soc.* 85, 119–134.
- Marchant, H.J., 1974a, Mitosis, cytokinesis, and colony formation in the green alga *Sorastrum*. *J. Phycol.* 10, 107–120.
- Marchant, H.J., 1974b, Mitosis, cytokinesis, and colony formation in *Pediastrum boryanum*. *Ann. Bot.* 38, 883–888.
- Marchant, H.J. and J.D. Pickett-Heaps, 1972a, Ultrastructure and differentiation of *Hydrodictyon reticulatum*. III. Formation of the vegetative daughter net. *Aust. J. biol. Sci.* 25, 265–278.
- Marchant, H.J. and J.D. Pickett-Heaps, 1972b, Ultrastructure and differentiation of *Hydrodictyon reticulatum*. IV. Conjugation of gametes and the development of zygospores and azygospores. *Aust. J. biol. Sci.* 25, 279–291.
- Marchant, H.J., J.D. Pickett-Heaps and K. Jacobs, 1973, An ultrastructural study of zoosporogenesis and the mature zoospore of *Klebsormidium flaccidum*. *Cytobios* 8, 95–107.
- Markowitz, M.M., 1976, An ultrastructural investigation of the flagellar apparatus of *Oedocladium* zoospores. *J. Phycol.* 12, Suppl., Abstr. 71.
- Mattox, K.R. and K.D. Stewart, 1973, Observations on the zoospores of *Pseudodoclonium basiliense* and *Trichosarcina polymorpha* (Chlorophyceae). *Can. J. Bot.* 51, 1425–1430.
- McBride, G.E., 1971, The flagellar base in *Coleochaete* and its evolutionary significance. *J. Phycol.* 7, Suppl. 13.
- McLachlan, J. and M. Parke, 1967, *Platymonas pellucida* sp. nov. from Puerto Rico. *J. mar. biol. Assoc. U.K.* 47, 723–733.
- Melkonian, M., 1975, The fine structure of the zoospores of *Fritschella tuberosa* Iyeng. (Chaetophorineae, Chlorophyceae) with special reference to the flagellar apparatus. *Protoplasma* 86, 391–404.
- Micalef, H. and P. Gayral, 1972, Quelques aspects de l'infrastructure des cellules végétatives et des cellules reproductrices d'*Ulva lactuca* L. (Chlorophyceae). *J. Microsc. (Fr.)* 13, 417–428.
- Moestrup, Ø., 1970, The fine structure of mature spermatozoids of *Chara corallina*, with special reference to microtubules and scales. *Planta* 93, 295–308.
- Moestrup, Ø., 1972, Observations on the fine structure of spermatozoids and vegetative cells of the green alga *Golenkinia*. *Br. phycol. J.* 7, 169–183.
- Moestrup, Ø., 1973, New observations on scales in green algae. *Br. Phycol. J.* 8, 214 (Abstract).
- Moestrup, Ø., 1974, Ultrastructure of the scale-covered zoospores of the green alga *Chaetosphaeridium*, a possible ancestor of the higher plants and bryophytes. *Biol. J. Linn. Soc.* 6, 111–125.
- Moestrup, Ø., 1975, Some aspects of sexual reproduction in eucaryotic algae, in "The Biology of the Male Gamete", J.G. Duckett and P.A. Racey (eds.), *Biol. J. Linn. Soc.* 7, Suppl. 1, pp. 23–35.
- Moestrup, Ø. and L.R. Hoffman, 1975, A study of the spermatozoids of *Dichotomosiphon tuberosus* (Chlorophyceae). *J. Phycol.* 11, 225–235.
- 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.
- Moore, J., M.H. Cantor, P. Sheeler and W. Kahn, 1970, The ultrastructure of *Polytomella agilis*. *J. Protozool.* 17, 671–676.
- Myles, D.G., 1975, Structural changes in the sperm of *Marsilea vestita* before and after fertilization, in "The Biology of the Male Gamete", J.G. Duckett and P.A. Racey (eds.), *Biol. J. Linn. Soc.* 7, Suppl. 1, pp. 129–134.
- Norris, R.E. and B.R. Pearson, 1975, Fine structure of *Pyramimonas parkeae*, sp. nov. (Chlorophyta, Prasinophyceae). *Arch. Protistenkd.* 117, 192–213.
- Norstog, K., 1974, Fine structure of the spermatozoid of *Zamia*: the Vierergruppe. *Am. J. Bot.* 61, 449–456.
- Norstog, K., 1975, The motility of cycad spermatozoids in relation to structure and function, in "The Biology of the Male Gamete", J.G. Duckett and P.A. Racey (eds.), *Biol. J. Linn. Soc.* 7, Suppl. 1, pp. 135–142.
- Olson, L.W. and G. Kochert, 1970, Ultrastructure of *Volvox carteri*. II. The kinetosome. *Arch. Mikrobiol.* 74, 31–40.
- Paolillo, D.J., G.L. Kreitner and J.A. Reighard, 1968, Spermatogenesis in *Polytrichum juniperinum*. I. The origin of the apical body and the elongation of the nucleus. *Planta* 78, 226–247.
- Parke, M. and I. Manton, 1965, Preliminary observations on the fine structure of *Prasinocladus marinus*. *J. mar. biol. Assoc. U.K.* 45, 525–536.
- Parke, M. and I. Manton, 1967, The specific identity of the algal symbiont in *Convoluta roscoffensis*. *J. mar. biol. Assoc. U.K.* 47, 445–464.
- Peterfi, L.S. and I. Manton, 1968, Observations with the electron microscope on *Asteromonas gracilis* Artari emend. (*Stephanoptera gracilis* (Artari) Wisl.), with some comparative observations on *Dunaliella* sp. Br. *phycol. Bull.* 3, 423–440.
- Pickett-Heaps, J.D., 1968, Ultrastructure and differentiation in *Chara (fibrosa)*. IV. Spermatogenesis. *Aust. J. biol. Sci.* 21, 655–690.
- Pickett-Heaps, J.D., 1970, Some ultrastructural features of *Volvox*, with particular reference to the phenomenon of inversion. *Planta* 90, 174–190.
- Pickett-Heaps, J.D., 1971, Reproduction by zoospores in *Oedogonium*. I. Zoosporogenesis. *Protoplasma* 72, 275–314.
- Pickett-Heaps, J.D., 1972, Reproduction by zoospores in *Oedogonium*. II. Emergence of the zoospore and the motile phase. *Protoplasma* 74, 149–167.
- Pickett-Heaps, J.D., 1973, Cell division in *Tetraspora*. *Ann. Bot.* 37, 1017–1025.
- Pickett-Heaps, J.D., 1974, Cell division in *Stichococcus*. Br. *phycol. J.* 9, 63–73.
- Pickett-Heaps, J.D., 1975a, Green algae: structure, reproduction, and evolution in selected genera (Sinauer Associates, Inc., Sunderland, Mass.) pp. 1–606.
- Pickett-Heaps, J.D., 1975b, Structural and phylogenetic aspects of microtubular systems in gametes and zoospores of certain green algae, in "The Biology of the Male Gamete", J.G. Duckett and P.A. Racey (eds.), *Biol. J. Linn. Soc.* 7, Suppl. 1, pp. 37–44.
- 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.
- Retallack, B. and R.D. Butler, 1972, Reproduction in *Bulbochaete hiloensis* (Nordst.) Tiffany. I. Structure of the zoospore. *Arch. Mikrobiol.* 86, 265–280.
- Retallack, B. and R.D. Butler, 1973, Reproduction in *Bulbochaete hiloensis* (Nordst.) Tiffany. II. Sexual reproduction. *Arch. Mikrobiol.* 90, 343–364.
- Rice, H.V. and W.M. Laetsch, 1967, Observations on the morphology and physiology of *Marsilea* sperm. *Am. J. Bot.* 54, 856–866.
- Reith, A., 1972, Über *Chlorokybus atmophyticus* Geitler 1942. *Arch. Protistenkd.* 114, 330–342.
- Ringo, D.L., 1967, Flagellar motion and fine structure of the flagellar apparatus in *Chlamydomonas*. *J. Cell Biol.* 33, 543–571.
- Robert, D., 1974, Étude ultrastructurale de la spermiogenèse, notamment de la différenciation de l'appareil nucléaire, chez *Selaginella kraussiana* (Kunze) A. Br. *Ann. Sci. nat. (Bot.)*, Sér. 12, 15, 65–118.
- Schnepf, E., G. Deichgräber and H. Ettl, 1976, *Gloeomonas* oder *Chlamydomonas*? Elektronenmikroskopische Untersuchungen an *Gloeomonas simulans*. *Plant Syst. Evol.* 125, 109–121.
- 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, 1975, 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.
- Suire, C., 1970, Recherches cytologiques sur deux Hépatiques: *Pellia epiphylla* (L.) Chorda (Metzgeriale) et *Radula complanata* (L.) Dum. (Jungermanniale). Ergastome, sporogenèse et spermogenèse. *Botaniste*, Sér. 53, 125–392.
- Triemer, R.E., 1975, The ultrastructure of fertilization in *Chlamydomonas moewusii*. *Protoplasma* 84, 315–325.

- Triemer, R.E. and R.M. Brown, 1974, Cell division in *Chlamydomonas moewusii*. *J. Phycol.* 10, 419-433.
- Turner, F.R., 1968, An ultrastructural study of plant spermatogenesis. Spermatogenesis in *Nitella*. *J. Cell Biol.* 37, 370-393.
- Walne, P.L. and H.J. Arnott, 1967, The comparative ultrastructure and possible function of eyespots: *Euglena granulata* and *Chlamydomonas eugametos*. *Planta* 77, 325-353.
- Watson, M.W., 1975, Flagellar apparatus, eyespot and behavior of *Microthamnion kuetzingianum* (Chlorophyceae) zoospores. *J. Phycol.* 11, 439-448.
- Watson, M.W. and H.J. Arnott, 1973, Ultrastructural morphology of *Microthamnion* zoospores. *J. Phycol.* 9, 15-29.
- Woodcock, C.L.F. and G.J. Miller, 1973, Ultrastructural features of the life cycle of *Acetabularia mediterranea*. I. Gametogenesis. *Protoplasma* 77, 313-329.
- Wujek, D.E. and P. Chelune, 1975, The taxonomic position of *Chaetopeltis*. *Br. phycol. J.* 10, 265-268.