THE BOTANICAL REVIEW

VOL. 41 JANUARY-MARCH, 1975 NO. 1

COMPARATIVE CYTOLOGY, EVOLUTION AND CLASSIFICATION OF THE GREEN ALGAE WITH SOME CONSIDERATION OF THE ORIGIN OF OTHER ORGANISMS WITH CHLOROPHYLLS A AND B

Kenneth D. Stewart and Karl R. Mattox
Department of Botany
Miami University
Oxford, Ohio 45056

I. Introductory Review of Some Recent Comparative Cytological Studies in the Green Algae 105
II. A Classification of the Green Algae Based on Cytological Characteristics 113
III. Problems and Implications of a Cytological Classification 119
IV. The Origin of Land Plants 126
V. Summary 128
VI. Zusammenfassung 129
VII. References Cited 131

I. Introductory Review of Some Recent Comparative Cytological Studies in the Green Algae

It has become increasingly clear that the establishment of natural classes and orders of green algae is dependent upon the accumulation of detailed knowledge concerning their cytological characteristics, both structural and biochemical. We have presented evidence that in the past the necessary dependence on overall growth habit in the classification of many filamentous forms has led to the establishment of unnatural orders, families and genera (Floyd et al., 1972b; Mattox et al., 1974; Mattox and Stewart, 1974; Stewart et al., 1973). In addition, characteristics visible with the light microscope and perhaps even electron microscopic examination of interphase cells are insufficient for a proper evaluation of the taxonomic position of unicellular green algae (cf. Maiwald, 1971; Peterfi and Manton, 1968; Stewart et al., 1974).

Our primary purposes here are (*1*) to review the present state of progress in comparative cytological studies of the green algae, (*2*) to discuss reasons for considering certain fine structural characteristics to be important in the interpretation of phylogenetic affinities, (*3*) to offer our current views concerning the manner in which the classification of the green algae will be changed, and (*4*) to discuss briefly the implications that phycological studies may have in regard to the evolution of other organisms with chlorophylls a and b, particularly the origin of land plants.

Studies of mitosis and cytokinesis (Floyd et al., 1972a, 1972b; Pickett-Heaps, 1969, 1972b, 1972e; Stewart et al., 1973) provided the earliest evidence that a major revision of the overall classification of the green algae may be necessary, and such studies are still the ones that have been the most extensively pursued. Before our previous attempt to classify "ulotrichalean" algae on the basis of the events of cell division (Stewart et al., 1973), the detailed, pioneering work from Pickett-Heaps' laboratory on genera of the Charales, Oedogoniales, Zygnematales and Chlorococcales (cf. Pickett-Heaps, 1969, 1972e) had revealed that the green algae possess a fascinating diversity in the structural details of cell division. For example, mitosis and cytokinesis in *Chara* Vaillant proved to be essentially like that in vascular plants; that is, the mitotic spindle is open, and, cytokinesis is effected by a phragmoplast that proliferates from a persistent interzonal spindle (Pickett-Heaps, 1967). On the other hand, the spindle is closed in the Oedogoniales (Pickett-Heaps and Fowke, 1969, 1970a), and the nuclear envelope has only polar interruptions at metaphase in the Chlorococcales (cf. Pickett-Heaps, 1972c). The interzonal spindle disperses at telophase in the Oedogoniales and Chlorococcales, and a system of microtubules develops between the nuclei. However, these microtubules are transverse; that is, at right angles to the axis of the recent spindle and to the microtubules of a phragmoplast. Such a transverse system of microtubules has been called a phycoplast (Pickett-Heaps, 1972e). The phycoplast apparently facilitates the development of the new septum, which can develop as a cell plate (e.g., *Oedogonium* Wittrock) or as a furrow (e.g., *Tetraedron* Kützing, Pickett-Heaps, 1972c). In the algae with a phycoplast, the daughter nuclei usually lie close to the developing septum in contrast to the case in algae with phragmoplasts. In the Zygnematales (Fowke and Pickett-Heaps, 1969a,b; Pickett-Heaps, 1972a; Pickett-Heaps and Fowke, 1970b) the spindle tends to be open, and, for the most part, a septum develops as a furrow which is not associated with microtubules. However, Fowke and Pickett-Heaps (1969b) showed that in *Spirogyra* Link, a phragmoplast-like proliferation of microtubules and vesicles occurs late in the development of the furrow when its inward growth encounters the longitudinally oriented microtubules of a persistent interzonal spindle. Fowke and Pickett-Heaps then speculated that a phragmoplast such as occurs in *Chara* and vascular plants evolved in green algae with cell division similar to that in *Spirogyra.* Pickett-Heaps (1969, 1972e) later developed this idea further, and suggested that green algae with a phycoplast are not on a line of evolution leading to land plants. Later research in his and other laboratories, as discussed in the following paragraphs, has provided convincing support for Pickett-Heaps' idea.

When Pickett-Heaps first proposed that green algal ancestors of land plants were to be sought among forms with a persistent interzonal spindle at telophase, the only algae known to possess this characteristic were members of the Zygnematales and Charales. That distribution was rather perplexing at the time. The Zygnematales must be considered an evolutionary side branch characterized by loss of flagellated cells and the development of a peculiar mode of sexual reproduction. Although *Chara* is a very complex alga and has sometimes been considered to be related to an evolutionary line leading to land plants (e.g., Frye and Clark, 1937), its life cycle and the structural peculiarities of its reproductive organs led many to doubt a close relationship to land plant ancestors. However, the "ulotrichalean" algae, relatively unspecialized filamentous forms, had rarely been the subjects of fine structural investigations. In a comparative study of the vegetative interphase cells and cell division in *Ulothrix fimbriata* Bold, *Stigeoclonium helveticum* Vischer, and *Klebsormidium flaccidum* Silva, Mattox and Blackwell (Floyd et al., 1972a,b), we were rather surprised to find that *U. fimbriata* and *S. helveticum* are nearly indistinguishable in important cytological details and that these two species are vastly different from *K. flaccidum.* Cytokinesis in *S. helveticum* and *U. fimbriata* involves a phycoplast/cell plate, and the daughter nuclei lie close together during the development of the septum. The metaphase spindle is closed in *S. helveticum* and surrounded by a vesiculate nuclear envelope in *U. fimbriata.* Plasmodesmata occur in the transverse walls. Microbodies are small, numerous and scattered in the cytoplasm. In contrast, the spindle appears to be fully open in *K. flaccidum*. Plasmodesmata do not occur in the end walls. Each cell contains a single large microbody which lies between the nucleus and chloroplast and which divides during cell division and the division of the chloroplast. There is much spindle elongation at anaphase during which the distance from each chromosome plate to the corresponding spindle pole remains virtually the same as at metaphase. The daughter nuclei remain far apart during cytokinesis, which is accomplished by a furrow. Microtubules are not involved in the development of the furrow.

The results of the work with *S. helveticum*, *U. fimbriata*, and *K. flaccidum* were not anticipated for a number of reasons. The close similarity of *U. fimbriata* and *S. helveticum* does not agree with the past practice of placing branching filamentous green algae in a separate family or even order from those with simple filaments. On that basis *S. helveticum* was a member of the Chaetophoraceae or Chaetophorales, and *U. fimbriata* a member of the Ulotrichaceae or Ulotrichales. Much more surprising was the great cytological dissimilarity between *U. fimbriata* and *K. flaccidum*. *Ulothrix* Kützing and *Klebsormidium* Silva, Mattox and Blackwell (formerly *Hormidium* Klebs, see Silva et al., 1972) have always been considered to be very closely related genera and have even been difficult to distinguish with the light microscope (Floyd et al., 1972b). For example, *K. subtillissimum* Silva, Mattox and Blackwell was formerly classified as a species of *Ulothrix* (cf. Mattox and Bold, 1962). It seemed, therefore, that the differences between *K. flaccidum* and *U. fimbriata* might have more general importance than the mere establishment of clear differences between *Klebsormidium* and *Ulothrix* (Floyd et al., 1972b). Cell division in *K. subtillissimum* has since been studied by Pickett-Heaps (1972b) who found that it is essentially identical to *K. flaccidum*. In addition, however, he emphasized the presence of persistent interzonal spindle microtubules and persistent chromosomal microtubules, both of which he compared to features observed in the Zygnematales. On that basis then, Pickett-Heaps suggested that *Klebsormidium* may lie near a line of evolution leading to land plants.

Meanwhile, our interest had been drawn to the conspicuous and unusual microbody in *Klebsormidium*. We were aware that certain previous attempts to demonstrate the presence of catalase in algal microbodies by cytochemical means had yielded negative results (*Chlamydomonas* Ehrenberg, Giraud and Czaniski, 1971; *Polytomella* Aragao and *Chlorogonium* Ehrenberg, Gerhardt and Berger, 1971), but we employed the DAB technique (Novikoff and Goldfischer, 1969) in an effort to partially determine the nature of the microbody in *Klebsormidium*. The technique resulted in a clear localization of peroxidatic activity in the single large microbody (Stewart et al., 1972).

The staining of the microbody of *Klebsormidium* by the DAB technique appeared to have some significance in regard to work in Tolbert's laboratory on glycolate metabolism in green algae. The unicellular algae that had been studied possess a glycolate dehydrogenase rather than a glycolate oxidase as in vascular plants (Nelson and Tolbert, 1970; Tolbert, 1971). The fact that the action of glycolate dehydrogenase does not produce peroxide seemed, at that time, to correlate with the DAB-negative results with the microbodies of such algae as *Chlamydomonas* (Giraud and Czaniski, 1971). On that basis we pointed out that glycolate oxidase might be present in *Klebsormidium* as is the case in vascular plants (Stewart et al., 1972) and suggested that the kinds of enzymes present in green algal microbodies might be taxonomically important (Stewart et al., 1973). At about the same time, Tourte (1972) demonstrated that the microbodies of the zygnematalean alga, *Micrasterias* Agardh, are DAB-positive. In view of Pickett-Heaps' comparison of *Klebsormidium* with zygnematalean algae (see above), Tourte's results provided further support for the view that microbody enzymes might be of taxonomic value. Shortly thereafter, Frederick, Gruber and Tolbert (1973) published the results of a survey of green algae and lower land plants for the presence of glycolate oxidase and glycolate dehydrogenase. Their results seem to be very significant. Apparently, the photosynthetic tissues of all land plants, both bryophytes and tracheophytes, contain glycolate oxidase. In contrast, many of the green algae, both unicellular and filamentous, contain glycolate dehydrogenase, and only a few were found to possess glycolate oxidase. At the present the correlation of Frederick, Gruber and Tolbert's results with the fine structural characteristics appears to be perfect; that is, glycolate oxidase has been found in only those green algae with persistent interzonal microtubules at telophase. Although the presence of glycolate oxidase appears to be a very useful taxonomic character, it is now highly doubtful, however, that a positive reaction with the DAB/H_2O_2 technique indicates that an alga possesses glycolate oxidase. Catalase appears to be generally present in autotrophically grown green algae (Frederick et al., 1973), and Silverberg (1974) has very recently demonstrated a positive DAB/H_2O_2 reaction in the microbodies of green algae of diverse taxonomic positions. Further comparative studies of microbody metabolism in green algae would be most helpful.

Recent observations have also established the importance of the fine structure of motile cells in the phylogenetic interpretation and classification of filamentous green algae. In a discussion of the phylogeny of green algae, Pickett-Heaps and Marchant (1972) pointed out that since *Klebsormidium* has a persistent interzonal spindle during cytokinesis, the zoospores of *Klebsormidium* might resemble the motile cells of *Coleochaete* Brébisson, the Charales, and lower land plants. Since the motile cells of the very complex genera, *Coleochaete* and *Chara*, resemble those of archegoniate plants

(Pickett-Heaps, 1968; Pickett-Heaps and Marchant, 1972) and are of a type not previously known among the relatively simple filamentous green algae, it was clear that a test of Pickett-Heaps and Marchant's prediction would have important implications concerning the value of mitotic and cytokinetic events in the establishment of phylogenetic trends in the green algae. Shortly thereafter, Marchant, Pickett-Heaps and Jacobs (1973) obtained zoospores of *Klebsormidium flaccidum* for electron microscopic examination and found that they were, indeed, much like those of *Coleochaete* and the Charales and distinctly different from other examined motile cells of filamentous green algae.

The differences between the two distinct types of motile cells in filamentous green algae have been discussed before (Pickett-Heaps and Marchant, 1972; Marchant et al., 1973; Birkbeck et al., 1974; Stewart et al., 1974). The flagellated cells of *Coleochaete* (Pickett-Heaps and Marchant, 1972; McBride, 1968), *Chara* (Pickett-Heaps, 1968), *Nitella* Agardh (Turner, 1968) and *Klebsormidium* (Marchant et al., 1973) have a single, broad band of closely adjacent microtubules with which the flagellar basal bodies are associated. In addition, the flagellar insertion is slightly, but very distinctly, lateral. The flagellated cells of all the other filamentous algae that have been carefully studied have four or more smaller microtubular rootlets associated with the basal bodies (cf. Birkbeck et al., 1974). In these the flagellar insertion is anterior. The cytoskeleton and the flagellar insertion in *Coleochaete*, *Chara*, *Nitella* and *Klebsormidium* are, then, asymmetrical, a fact which strongly supports Manton's statement of nearly a decade ago (Manton, 1965) that the asymmetry of the spermatozoids of land plants indicates that their monadal ancestors were asymmetrical and unlike *Chlamydomonas*.

The matters discussed above provide reason to view many of the green algae as belonging to two distinct groups. Furthermore, we believe that these algae could be assigned to two classes on that basis. In short, one group is characterized by having a persistent interzonal spindle during cytokinesis, glycolate oxidase, and motile cells (if produced) in which laterally attached flagella are associated with a single broad band of closely adjacent microtubules. The other group is characterized by collapse of the interzonal spindle at telophase, glycolate dehydrogenase, and motile cells (if produced) in which anteriorly attached flagella are associated with four, cruciately arranged, relatively narrow microtubular roots (more than four in Oedogoniales). Why should the characteristics mentioned be considered phylogenetically important? Among other things to be considered is the fact that unless one views these two groups as representing two independent evolutionary lines, it would be exceedingly difficult to explain why the three characteristics described for each group should occur together. The mode of mitosis and cytokinesis, the details of glycolate metabolism, and the structure of motile reproductive cells are not functionally related characteristics,

TABLE I

A Hypothetical Classification of Some Green Algae Based on Comparative Cytology

I. CHAROPHYCEAE

Persistent interzonal spindle during cytokinesis; glycolate oxidase present; motile cells, if produced, with laterally inserted flagella; flagellar basal bodies associated with a single broad band of microtubules; motile cells with two flagella; unicellular, filamentous or parenchymatous.

A. KLEBSORMIDIALES — Characteristics in addition to those of Charophyceae: Cytokinesis by centripetal growth of furrow which finally impinges upon microtubules of the persistent interzonal spindle; little decrease in spindle pole to chromosome distance during anaphase; chromosomal microtubules persistent at telophase; plasmodesmata absent; zoospores astigmatic; sexual reproduction unknown; unbranched filaments without holdfasts.

1) KLEBSORMIDIACEAE* — Characteristics as in the order. *Klebsormidium* is the only genus known.

B. ZYGNEMATALES — Characteristics in addition to those of Charophyceae: Cytokinesis by centripetal growth of a furrow which finally impinges upon the microtubules of the persistent interzonal spindle; little decrease in spindle pole to chromosome distance during anaphase; chromosomal microtubules persistent at telophase; plasmodesmata absent; flagellated cells not produced; sexual reproduction by conjugation; unicellular or unbranched filaments without holdfasts. Families and genera as given in current classifications.

C. COLEOCHAETALES — Characteristics in addition to those of Charophyceae: Cytokinesis by means of a phragmoplast/cell plate, the microtubules of which proliferate from the persistent interzonal spindle; little decrease in spindle pole to chromosome distance during anaphase; plasmodesmata present; some cells bearing sheathed setae; zoospores astigmatic; sexual reproduction oogamous with oogonia protected by sterile cells; plant body filamentous, branched or sometimes discoid.

1) COLEOCHAETACEAE — Characteristics as in the order. *Coleochaete* is the only genus studied with the electron microscope.

D. CHARALES — Characteristics in addition to those of Charophyceae: Cytokinesis by means of a phragmoplast/cell plate, the microtubules of which proliferate from the persistent interzonal spindle; plasmodesmata present; zoospores not produced; sexual reproduction oogamous with antheridia and oogonia protected by sterile cells; plant body very complex with apical growth and differentiation into nodes and internodes. Family and genera as in current classifications.

II. CHLOROPHYCEAE

Interzonal spindle not persistent until completion of cytokinesis; glycolate oxidase absent; motile cells, if produced, with anteriorly inserted flagella; flagellar basal bodies associated with four or more relatively narrow bands of microtubules; motile cells with 2, 3, 4, or more flagella; unicellular, colonial, filamentous or parenchymatous.

A. VOLVOCALES — Characteristics in addition to those of Chlorophyceae: Basal bodies or centrioles associated with but distinctly lateral to both spindle poles; phycoplast microtubules radiate from region of basal bodies or centrioles. Order restricted here to motile colonies.

   1) VOLVOCACEAE — Characteristics as given for the order. *Volvox aureus* is the only species in which cell division has been studied.

B. CHLOROCOCCALES — Characteristics in addition to those of Chlorophyceae: Cytokinesis effected by phycoplast/cleavage; microtubules of phycoplast usually, but not always, radiate from the centrioles; multiple mitoses before completion of cytokinesis; nuclei surrounded by perinuclear envelope of endoplasmic reticulum at prophase; non-motile unicells or non-motile colonies. Families as in current classifications. Investigated genera are *Kirchneriella* Schmidle, *Tetraedron*, *Scenedesmus* Meyen, *Pediastrum* Meyen and *Hydrodictyon* Roth.

C. MICROSPORALES — Characteristics in addition to those of Chlorophyceae: Cytokinesis effected by phycoplast/cleavage; microtubules of the phycoplast radiate from the region of centrioles between the daughter nuclei; plasmodesmata absent; motile cells with two or four flagella; filaments.

   1) MICROSPORACEAE — Characteristics in addition to those of the order: Cell walls composed of distinctive overlapping H-pieces; pyrenoid absent; simple filaments. The only genus studied with the electron microscope is *Microspora*.

D. ULVALES — Characteristics in addition to those of Chlorophyceae: Cytokinesis in vegetative cell division effected by a precocious cleavage furrow which is not associated with a phycoplast; plasmodesmata absent; centrioles associated with but distinctly lateral to the spindle poles; branched filaments and pseudoparenchymatous forms.

   1) ULVACEAE — Characteristics in addition to those of the order: Sexual reproduction isogamous or anisogamous. Genera to be included at present are *Ulva, Enteromorpha, Percursaria, Pseudendoclomium* and *Trichosarcina*.

   2) CYLINDROCAPSACEAE — Characteristics in addition to those of the order: Sexual reproduction oogamous. The only genus known is *Cylindrocapsa*.

E. CHAETOPHORALES — Characteristics in addition to those of Chlorophyceae: Cytokinesis in vegetative cell division effected by a phycoplast/cell plate; phycoplast does not radiate from the region of the centrioles, which remain on the side of the nuclei opposite the plane of cytokinesis; plasmodesmata present; motile cells with two to several flagella; unbranched filaments, branched filaments, and parenchymatous forms.

   1) CHAETOPHORACEAE — Characteristics in addition to those of the order: Motile cells with two or four flagella; system of four, cruciately arranged microtubular roots in the zoospores consists of two-microtubule roots alternating with five-microtubule roots; pyrenoids not traversed by thylakoids but bounded by thylakoids; hairs, when present, are multicellular; branched or unbranched filaments. Known genera or species are *Ulothrix fimbriata, Uronema, Stigeoclonium, Chaetophora, Draparnaldia* and *Fritschiella*.

2) APHANOCHAETACEAE — Characteristics in addition to those of the order: Motile cells with two or four flagella; motile cells with four, cruciately arranged microtubular roots, but number of microtubules is unknown; pyrenoids not bounded by thylakoids but penetrated shallowly by a few thylakoids; pyrenoids occur at edge of chloroplasts and cytoplasm often projects into the region on the chloroplast containing the pyrenoid; hairs, when present, are usually unicellular. The only genus studied with the electron microscope is *Aphanochaete*.

3) SCHIZOMERIDACEAE — Characteristics in addition to those of the order: Microtubular roots of zoospores all with equal number of microtubules; zoospores with two, three, four, five, six, or more flagella; pyrenoids traversed by several undulating thylakoids; chloroplasts perforate; mature thallus unbranched and parenchymatous. *Schizomeris* is the only known genus.

F. OEDOGONIALES — Characteristics in addition to those of Chlorophyceae: Motile cells with a ring of numerous flagella; peculiar type of cytokinesis and cell elongation involving migration of a phycoplast/cell plate and extension of "wall rings"; plasmodesmata present; plasmodesmata of unique type having a "barrel shape" in longitudinal section and peripheral subunits; oogamous; reticulate chloroplasts; largest chromosomes known in green algae; unbranched or branched filaments. Family and genera as in current classification.

---

*The names Klebsormidiales and Klebsormidiaceae are here formally based on Klebsormidium Silva, Mattox and Blackwell, Taxon 21: 645. 1972 the only genus of the family and order.

and there appears to be no explanation for their correlation other than that they reflect attributes of ancestral organisms. In addition, the structural features of algal motile cells have long been used as taxonomic criteria at the highest levels of classification. In particular the mode of insertion of flagella and the structural features related to flagella and motility have been considered important and relatively conservative features (e.g., see Manton, 1965). Pickett-Heaps (1972b) has discussed reasons for considering the structural features of mitosis and cytokinesis conservative. In support of his claim is the fact that cell division is relatively uniform within a number of groups. For example, features of mitosis and cytokinesis are relatively uniform within the metazoa and within higher plants. In addition, cell division appears to be fairly uniform within some of the fungal groups. Finally, it should be mentioned that with relatively simple plants such as these algae, the use of biochemical characteristics along with the fine structural features of vegetative interphase cells, mitosis, cytokinesis, and the motile reproductive cells is a step toward a "whole-plant taxonomy." Very significantly, it is the ulotrichalean, ulvalean and chaetophoralean algae that recent studies have shown to contain unnatural groupings. Those algae had been classified mainly on the basis of one character, growth habit, because no cytological characteristics such as are used to partially characterize the Zygnematales and Oedogoniales were previously available.

II. A Classification of the Green Algae Based on Cytological Characteristics

A partial classification of the green algae based both on recently observed cytological features and on a number of classical characteristics is shown in Table I. We are not proposing that such a classification be put to general use at the present time because information is lacking for several important filamentous genera and for numerous important unicellular and colonial forms. As in a previous attempt to classify certain ulotrichalean, ulvalean and chaetophoralean algae on the basis of mitotic and cytokinetic events (Stewart et al., 1973), the taxa listed in Table I are intended merely to be reasonable hypotheses based on presently available information. We have prepared Table I and the discussion in this section and Section III in an effort to both clearly show the present state of progress in the comparative cytological study of green algae and to emphasize that a great number of gaps still exist in the information necessary to make such a classification more complete, accurate and useful. Nevertheless, for reasons discussed in the previous section, we believe that a natural classification of the green algae must eventually incorporate data on mitosis, cytokinesis, the fine structure of motile cells, and biochemical characteristics as does the classification of Table I.

A classification based primarily on cytological characteristics that are largely undetectable with the light microscope might draw criticism from some as being impractical. It cannot be denied that such a classification may be difficult to establish. However, apart from the argument that a natural classification is preferable in any case, it seems to us that with the biology of today the only "practical" classification may be a natural one. A case in point is Frederick, Gruber and Tolbert's survey of green plants for the presence of glycolate oxidase (1973). Their findings suggest that glycolate oxidase is universal in bryophytes and vascular plants. In the green algae, however, it is present in the Zygnematales, *Coleochaete*, *Nitella* and *Klebsormidium*, but is absent in *Chlorella* Beijerinck, *Stigeoclonium helveticum*, *Microspora* Thuret, *Dunaliella* Teodoresco and others. Their results would have been very perplexing, indeed, if it had been necessary to base interpretation on the present classification of green algae. However, their results were seen to agree with recent observations on mitosis and cytokinesis (Frederick et al., 1973). Frederick, Gruber and Tolbert's selection of algae to study was, of course, prompted by those recent fine structural studies, a fact which illustrates another important point concerning the "practicality" that a natural system would have. The more natural a classification, the better it would serve a researcher wishing to select green algae for comparative biochemical studies. In addition, if biochemical studies of green algae are to be interpreted with respect to the biochemistry of land plants, the most valuable classification is one that reflects phylogeny as nearly as possible.

Matters discussed in Section I provide evidence that the algae included in Table I should be divided into two classes, which we have called Charophyceae and Chlorophyceae. When more information is accumulated, we believe that the orders and families included in the Charophyceae and Chlorophyceae can, in part, be defined by the same kind of characteristics used to define the classes; that is, characteristics of mitosis, cytokinesis, fine structure of motile cells, and biochemistry. Unfortunately, fine structural information on motile reproductive cells and data from comparative biochemical studies are scarce. Observations on critical details of fine structure are also very unevenly distributed among the different kinds of green algae. For that reason some of the features of the classification shown on Table I are supported by a number of facts whereas others are based on a minimum of data and are, therefore, more speculative and more likely to be modified by future investigations. In the discussion below, we try to make clear how much or how little evidence supports the establishment of each group. Many green algae, particularly coenocytic and unicellular forms, have been omitted from Table I because there is such a paucity of information that even their speculative inclusion would be difficult. Such problems are discussed further in Section III.

As reflected in the classification of Table I, it is the Ulotrichales, Ulvales and Chaetophorales that appear to be the most unnatural among the orders of advanced green algae in current classifications. Those orders that have long been based on cytological and reproductive peculiarities such as the Oedogoniales and the Zygnematales still appear to be natural. The phylogenetic affinities of the Zygnematales and Oedogoniales, however, have only recently begun to be understood as a result of comparative fine structural studies (Birkbeck et al., 1974; Pickett-Heaps, 1972b).

Charophyceae and Chlorophyceae

Since the reasons for the establishment of these two classes have been discussed in Section I, the consideration here can be brief. The apparent affinity of *Klebsormidium* and the Zygnematales with a line of evolution leading to *Chara* and *Coleochaete* and the fundamental differences between *Klebsormidium* and many other of the "ulotrichalean" algae have been very surprising but could not have become clear with the light microscope alone.

In the descriptions of the two classes we chose to contrast the character of "glycolate oxidase present" with "glycolate oxidase absent," rather than to characterize the Chlorophyceae as having glycolate dehydrogenase, for certain reasons. In a recent report, Roth-Bejerano and Lips (1973) claim that glycolate dehydrogenase is present in vascular plants when nitrate and active nitrate reductase are present. If that is the case, it is conceivable that charophytes may be able to make glycolate dehydrogenase under certain conditions.

Klebsormidiales

The cytological classification requires the removal of *Klebsormidium* from the Ulotrichaceae to a separate class from several of the other organisms that have been included in that family. Since *Klebsormidium* differs in reproductive characteristics from the Zygnematales and is a much simpler organism that those in the Coleochaetales and Charales, it must also be placed in a new order. Similarities in cell division and in the degree of complexity of the plant body, however, indicate that an organism such as *Klebsormidium* could have been ancestral to the Zygnematales (see Pickett-Heaps, 1972b, and descriptions of the two orders in Table I). At present, no other genus is certainly known to belong in the Klebsormidiales. Pickett-Heaps has shown that *Stichococcus* Naegeli and *Raphidonema* Lagerheim (personal communication) have cell division similar to that of *Klebsormidium. Stichoccus* and *Raphidonema* should, therefore, be placed in the Charophyceae, but since they are not known to produce motile cells or to reproduce sexually, it is presently difficult to assess whether they are more closely related to the Klebsormidiales or to the Mesotaeniaceae of the Zygnematales. The name Klebsormidiaceae (Table I) has not been used previously, but since *Klebsormidium* is presently the only genus included, the Latin description of the genus (Silva, Mattox and Blackwell, 1972) can serve for the family.

The characterization of the Klebsormidiales in Table I includes the fact that there is virtually no decrease in chromosome to spindle pole distance during anaphase. This characteristic is also listed for the Zygnematales and Coleochaetales but may not be exclusive to orders of the Charophyceae. It has also been noted that there is relatively little decrease in chromosome to spindle pole distance in ulvalean algae (Løvlie and Bråten, 1970; Stewart et al., 1973; Mattox and Stewart, 1974) as the spindle elongates. Ris and Kubai (1974) have recently given their views concerning the sequence of events that may have occurred in the evolution of the spindle and suggest that a primitive characteristic could be that chromosomal microtubules do not shorten during chromosome segregation. If so, this phenomenon might be considered a primitive characteristic in both the Charophycean and Chlorophycean lines. Maintenance of chromosome to pole distance during anaphase may have been a feature of very early green algae, and the monadal ancestors of both the Charophyceae and Chlorophyceae may have possessed this characteristic.

Zygnematales

Cell division in the Zygnematales has been well studied (Fowke and Pickett-Heaps, 1969a,b; Pickett-Heaps, 1972a; Pickett-Heaps and Fowke, 1970b). Frederick et al., (1973) have shown that *Netrium* Itzigsohn et Rothe and *Spirogyra* contain glycolate oxidase. Recent studies have supported the concept of the Zygnematales as a natural group and have also clearly shown that they are to be assigned to the Charophyceae of the present classification.

Coleochaetales

Cell division in *Coleochaete* (Marchant and Pickett-Heaps, 1973) and the fine structure of its motile cells have been examined (Pickett-Heaps and Marchant, 1972). The presence of glycolate oxidase has been demonstrated (Frederick et al., 1973). In contrast to the relative simplicity of *Klebsormidium*, *Coleochaete* is a highly complex alga. The main features of its advancement are the protection of oogonia by sterile cells and the cytokinesis by means of a phragmoplast/cell plate with the concomitant formation of plasmodesmata. Some other genera with sheathed setae may belong to this order, but their inclusion must wait until they are carefully examined with the electron microscope.

Charales

Cell division and the structure of the spermatozoid in *Chara* have been studied by Pickett-Heaps (1967, 1968). Glycolate oxidase has been found in the Charalean genus *Nitella* (Frederick et al., 1973).

The complexities of the plant body and the reproductive organs in the Charales show significant advances over *Coleochaete*. Nevertheless, those evolutionary advances are not so fundamental as those shown by the Coleochaetales over the Klebsormidiales. Since there are clear similarities in cell division in *Coleochaete* and *Chara* and clear similarities in the flagellated cells of *Klebsormidium*, *Coleochaete* and the Charales, the Charales cannot be isolated in a separate class or division as is often done. The added complexities and specializations of the Charales certainly call for ordinal rank, but a higher rank would serve only to obscure relationships.

Volvocales

We restrict the order to include only motile colonial forms because it appears to be too early to attempt to assign motile unicellular forms to orders. There is so little information on this group that its complete omission was considered. The only study of cell division is that of Deason and Darden (1971) on *Volvox aureus* Ehrenberg. Their description of cell division and conclusion that cytokinesis there is like that in *Chlamydomonas* as described by Johnson and Porter (1968) prompted our inclusion of the order. A few other genera and species must be studied, however, before it is certain that all motile colonial forms fall into the same order.

Chlorococcales

The characteristics given for this order are based on the extensive investigations in Pickett-Heaps' laboratory. That work is partially summarized in his paper on *Tetraedron* (Pickett-Heaps, 1972c). The organisms studied by Pickett-Heaps appear to form a natural assemblage. There is, however, very little information on the structure of motile cells in this large group. There is certainly the possibility that some of the many forms which are currently assigned to this order may prove to be unlike those that have been investigated with the electron microscope.

Microsporales

More characteristics are needed to establish the validity of this order, which we have based almost entirely upon features of cell division, particularly cytokinesis. Cell division has been studied in *Microspora* (Pickett-Heaps, 1973b; Stewart et al., 1973). Information on the fine structure of zoospores is not available.

Ulvales

The algae of this order have been relatively well studied, but further examination is necessary. Cell division has been followed in *Ulva* L. (J. Agardh) (Løvlie and Bråten, 1970), *Pseudendoclonium* Wille, *Trichosarcina* Nichols and Bold (Mattox and Stewart, 1974) and *Cylindrocapsa* Reinsch (Pickett-Heaps, personal communication). Certain observations have been made on the zoospores of *Ulva* (Bråten and Løvlie, 1968; Micalef and Gayral, 1972), *Enteromorpha* Link (Evans and Christie, 1970), *Pseudendoclonium* and *Trichosarcina* (Mattox and Stewart, 1973). Assays for glycolate oxidase and glycolate dehydrogenase have not been made for any member of this order.

Were it not for the fact that a phycoplast is absent in the vegetative cell division of the Ulvales, the Chlorophyceae could be characterized as having a phycoplast in addition to having a non-persistent spindle at telophase. In this order a precocious furrow grows between the daughter nuclei immediately upon their separation (Mattox and Stewart, 1974). A few longitudinally oriented microtubules are often seen near the region where the developing furrow occurs between the daughter nuclei (Mattox and Stewart, 1974, Fig. 14), and these may represent the remnants of an interzonal spindle. However, the daughter nuclei appear to be held apart by the precocious cleavage furrow rather than by interzonal microtubules as in the Charophyceae. Although the situation here is somewhat different from the other orders of the Chlorophyceae, it appears that the interzonal spindle of the Ulvales is essentially non-persistent. It will be necessary to determine whether or not a phycoplast exists during the cleavages that occur during zoosporogenesis or gametogenesis.

In the past the Ulvales contained those green algae which are basically filamentous and whose mature thalli are more or less unbranched and pluriseriate or parenchymatous. In the classification here, the order includes filamentous forms whose mode of cytokinesis in vegetative cell division is distinct from other filamentous orders of the Chlorophyceae (see Mattox and Stewart, 1974). Although the criteria here are different, almost all of the studied genera that have been included in the past remain in the Ulvales. *Schizomeris* Kützing, however, must not be included in the order (Mattox et al., 1974; Birkbeck et al., 1974). The greatest change in the Ulvales that would result from adoption of the description in Table I would be an expansion of the order by the inclusion of a number of filamentous genera of diverse growth habit. Our work (Mattox and Stewart, 1973, 1974) has shown that *Pseudendoclonium basiliense* Vischer, which has been assigned to the Chaetophorales in the past, is very similar to *Ulva* in cell division and other cytological details. It seems very likely that further observations will result in the inclusion of genera with uniseriate, unbranched filaments.

Pickett-Heaps' work with *Cylindrocapsa involuta* Reinsch (personal communication) has shown that cell division there is like that in *Pseudendoclonium* and *Trichosarcina.* We have, therefore, included *Cylindrocapsa* in the Ulvales but have assigned it to a separate family. The separation of the Cylindrocapsaceae is done almost solely on the basis of the occurrence of oogamy in *Cylindrocapsa.* Since there are anisogamous forms in the Ulvaceae, the oogamy in *Cylindrocapsa* may not justify a separate family. The motile cells of *Cylindrocapsa* should be examined to determine their degree of similarity to those of *Ulva* and *Enteromorpha.* Hoffman (personal communication) has observed a well developed phycoplast in a preliminary study of cell division in a collected *Cylindrocapsa* with stellate chloroplasts. Since *Cylindrocapsa involuta,* the type species and the one studied by Pickett-Heaps, lacks a phycoplast and has parietal chloroplasts, it is very likely that the genus *Cylindrocapsa* presently includes two very distinct kinds of algae and that those with stellate chloroplasts will eventually have to be removed from the genus.

Chaetophorales

A number of these organisms have been examined with the electron microscope. Observations on cell division have been published on *Ulothrix fimbriata, Stigeoclonium helveticum* and *Schizomeris* (Floyd et al., 1972a; Mattox et al., 1974; Stewart et al., 1973). Our unpublished observations on *Uronema* Lagerheim, *Chaetophora* Schrank, *Draparnaldia* Bory, *Fritschiella* Iyengar, and *Aphanochaete* A. Braun indicate that cell division in those genera is essentially like that of *U. fimbriata, S. helveticum,* and *Schizomeris.* In addition McBride's (1970) figures show daughter nuclei lying close to a cell plate in *Fritschiella* as they do in other genera of this order. Some information is available on the zoospores of *Stigeoclonium* Kützing (Manton, 1964), *Draparnaldia* (Manton et al., 1955), *Ulothrix* (Manton, 1965), and *Schizomeris* (Birkbeck et al., 1974).

Features of cytokinesis (see Table I) and the related formation of plasmodesmata (see Stewart et al., 1973) provide the main basis of the distinction of the Chaetophorales from the Microsporales and Ulvales. Many of the genera of this order also exhibit a greater degree of complexity of the thallus than is seen in the Microsporales and Ulvales. The position of the Chaetophorales in the Chlorophyceae parallels that of the Coleochaetales in the Charophyceae; it is here that a greater degree of specialization of cell types and a greater degree of differentiation of the thallus corresponds with the presence of plasmodesmata (see discussion of plasmodesmata in Stewart et al., 1973).

Most of the genera included in the Chaetophorales are placed in the Chaetophoraceae. The organisms that we have included in that family are cytologically homogeneous to the degree that the vegetative interphase cells of any one are virtually indistinguishable from those of the others. In addition, a 5-2-5-2 system of microtubular roots occurs in all of the zoospores of the Chaetophoraceae that have been examined, *Ulothrix*, *Draparnaldia* and *Stigeoclonium.*

The Schizomeridaceae is separated because of distinctive features of the vegetative interphase cells, differences in the number of microtubules in the flagellar roots, and the variability in flagellar number (see Mattox et al., 1974; Birkbeck et al., 1974). The Aphanochaetaceae is separated because of differences in pyrenoid structure (Stewart et al., 1973, and unpublished observation) and the usual presence of unicellular hairs. The zoospores of *Aphanochaete* have been reported to have a cruciate root system (Watson and Arnott, 1973) and should be examined further to determine whether they possess any differences from those of the Chaetophoraceae and Schizomeridaceae.

Oedogoniales

The fine structural characteristics of this distinctive order have been well documented (Fraser and Gunning, 1969; Hill and Machlis, 1968; Hoffman and Manton, 1962; Pickett-Heaps and Fowke, 1969, 1970a; Pickett-Heaps, 1971, 1972d). The phycoplast/cell plate indicates that the Oedogoniales should be included in the Chlorophyceae. Similarities between the organization of the flagellar apparatus in *Oedogonium* and that of the quadriflagellate zoospores of *Schizomeris*, whose zoospores often possess more than four flagella, may also show an affinity between the Chaetophorales and Oedogoniales (Birkbeck et al., 1974).

III. Problems and Implications of a Cytological Classification

The distinction between the Charophyceae and Chlorophyceae is one of the most clearly established features of the cytological classification. However, the descriptions of the two classes will probably have to be altered when motile unicellular forms are incorporated into the classification. For example, some apparently primitive green monads (see discussion below) have two microtubular roots instead of one (Charophyceae) or four or more (Chlorophyceae). Further study on advanced genera in both classes may also result in the discovery of motile cells which do not fit the rather simple descriptions given in Table I. Since some primitive unicellular algae have two microtubular roots, this characteristic could conceivably occur in some advanced forms. Indeed, Graham and McBride's (1974) very recent abstract on the zoospores of *Trentepohlia* Martius indicates that the microtubular system there might be somewhat different from that described in other filamentous green algae.

Green Monads

Extensive comparative cytological studies of the unicellular green algae could well have a profound effect upon the concepts of phylogeny in the green algae and their overall classification. At this time, however, motile unicellular green algae and those that are often placed in the Tetrasporales must be omitted from the preliminary classification because of the scarcity of information. Cell division has been studied in *Chlamydomonas reinhardii* Dangeard (Johnson and Porter, 1968), *Platymonas subcordiformis* (Wille) Hazen (Stewart et al., 1974), *Pyramimonas* Schmarda (Norris and Pearson, 1973), and *Tetraspora* Link (Pickett-Heaps, 1973a).

The first electron microscopic study of cell division in a green monad was that of *C. reinhardii*. The fact that cytokinesis involves a phycoplast indicates that *C. reinhardii* should be included in the Chlorophyceae of the present classification. While it seems likely that *Chlamydomonas* and many other of the genera of green monads will have the characteristics given here for the Chlorophyceae, it is equally likely that at least a few will not. Before an attempt is made to assign green monads to classes and orders, much more must be known concerning their range of variation in mitosis, cytokinesis, the structure of the flagellar apparatus and other characteristics.

Variations in the structural features of cell division in filamentous green algae suggest that a similar diversity may occur in green monads; that is, the differences in cell division between the filamentous Chlorophyceae and Charophyceae may reflect differences between their respective monadal ancestors. Pickett-Heaps (1972e) has suggested that since the persistent spindle is widespread in organisms (e.g., found in both higher plants and animals), it is likely to be the primitive condition, whereas the collapsing spindle is a derived condition associated with the evolution of the phycoplast. Pickett-Heaps' view is quite logical and attractive. Its acceptance, of course, leads to the assumption that the early ancestors of both the Chlorophyceae and Charophyceae possessed a persistent interzonal spindle at telophase and that a search for the most primitive green monads should be among those that might prove to have that characteristic.

There is also reason to suspect that the most primitive green algae are among those naked or scaly green monads that have occasionally been placed in a separate class, Prasinophyceae. Manton (1965) has suggested before that the asymmetry of "prasinophytes" and certain other of their characteristics indicate that they are primitive. As with the persistent spindle, asymmetry in motile cells is the rule in organisms (protozoa, chrysophytes, xanthophytes, water molds, etc.) and radial symmetry the exception. Such considerations led us to study cell division in *Platymonas subcordiformis*, a green monad with scaly flagella (Stewart et al., 1974). Somewhat to our surprise we found that the interzonal spindle in *Platymonas* collapses at telophase and that cytokinesis involves a phycoplast similarly as in *Chlamydomonas reinhardii.* Since cytokinesis in *Platymonas* G. S. West could not be considered primitive or of a type ancestral to the Charophyceae, we suspected that "prasinophytes" might be diverse in their mitotic and cytokinetic mechanisms (see discussion in Stewart et al., 1974). There were other reasons to suppose that scaly green monads might not form a natural group and that they may include types ancestral to both chlorophytes and charophytes (see Birkbeck et al., 1974). Scales have been observed on the zoospores of the chlorophycean algae, *Pseudendoclonium basiliense* and *Trichosarcina polymorpha* (Mattox and Stewart, 1973), as well as on the spermatozoids of the charophycean genera, *Nitella* and *Chara* (Turner, 1968; Pickett-Heaps, 1968). Scales are also present in chrysophytes, protostelids (Furtado and Olive, 1971) and certain water molds (Darley et al., 1973). We were led to suggest that scales were characteristic of very early phytoflagellates and that the presence of scales in "prasinophytes" indicates a degree of primitiveness but does not necessarily mean that scaly green monads can be assigned to a single natural group clearly distinct from other groups of green algae (Stewart et al., 1974). While it is quite possible, or even likely, that scaly green monads have a common ancestor, their divergence could be very ancient. Pickett-Heaps (personal communication) has recently demonstrated that cytokinesis is, indeed, not uniform in these organisms by finding that *Pedinomonas minor* Korchikoff has a persistent interzonal spindle.

The matters discussed above suggest that "prasinophytes" will eventually be separated and assigned to different classes. Research on scaly green monads has provided information of great value and has established the importance of these organisms to any consideration of the overall classification of green algae. The concept of the Prasinophyceae, however, has never been clearly drawn. Parke and Manton (1965) have discussed some of the problems. Peterfi and Manton (1968) could not determine whether *Asteromonas gracilis* Artari should be included in the Prasinophyceae, and Maiwald (1971) could not determine whether *Pyramimonas montana* Geitler is actually a member of that genus or a volvocalean monad.

Some of the characteristics that have been listed for the Prasinophyceae (see Peterfi and Manton, 1968; Round, 1971) are (*1*) cells naked or scaly, (*2*) flagella arising from a pit or groove rather than from a papilla, (*3*) the presence of a cross-striated "root" connecting the flagellar basal bodies and the nucleus, and (*4*) cytoplasmic extensions penetrating the pyrenoid. An examination of the literature reveals that those characteristics are not adequate to define a division or class distinct from other green algae. The cells of *Dunaliella*, a volvocalean monad, are naked as are the zoospores of many filamentous and colonial green algae, and scales are present on the male gametes of the Charales (Pickett-Heaps, 1968; Turner, 1968) and the zoospores of *Pseudendoclonium* (Mattox and Stewart, 1973). Maiwald (1971) has shown that *Pyramimonas montana* has the intriguing characteristic of a "papilla in a pit." Some of the "prasinophytes" do not have a cross-striated root connecting the basal bodies and nucleus (*Mesostigma* Lauterborn and *Spermatozopsis* Korchikoff, see Peterfi and Manton) while the male gametes of the filamentous or thalloid *Prasiola* Agardh do possess such a structure (Manton and Friedmann, 1959) as do the zoospores of the filamentous genus, *Microthamnion* Naegeli (Watson and Arnott, 1973). Not all "prasinophytes" possess cytoplasmic extensions into the pyrenoid while such extensions do occur in *Oedogonium* (Hoffman, 1968). The literature suggests that at least a majority of "prasinophytes" have two microtubular roots rather than four as in the Chlorophyceae or one as in the Charophyceae, but it is not certain that all possess that characteristic or even that some other monads or zoospores of chlorophytes or charophytes will not prove to have two roots.

It is possible, then, that a future major division among green monads may be based on the persistence of the interzonal spindle as with the forms included in the classification here. It is difficult to judge, however, which additional characteristics will prove to be important in devising a detailed classification. One characteristic which may prove to be valuable is the manner in which the flagella are connected; Patenaude and Brown (1973) have reported that opposite basal bodies are connected by a striated fiber in the quadriflagellate *Polytomella*, but the arrangement must be somewhat different in such a genus as *Polyblepharides* Dangeard which can have more than four flagella. It must also be determined whether certain details of spindle formation are significant. For example, the spindle in *Tetraspora* appears to be unicentric (Pickett-Heaps, 1973a) while that in *Platymonas* is acentric (Stewart et al, 1974). In *Volvox aureus*, whose cells have a chlamydomonad type of organization, centrioles are associated with, but are lateral to, both spindle poles (Deason and Darden, 1971). The great number and diversity of species of green monads indicates that much work will be necessary to classify them properly.

Cytokinesis

The details of cytokinesis are considered to be important in the cytological classification (Table I). Cytokinetic characteristics distinguish among the first three filamentous orders of the Chlorophyceae and, to a large extent, between the Klebsormidiales and Coleochaetales of the Charophyceae. In the Charophyceae, it appears that the phragmoplast/cell plate evolved from such systems as those in *Klebsormidium* and *Spirogyra* where a furrow impinges upon a persistent interzonal spindle (Fowke and Pickett-Heaps, 1969b; Pickett-Heaps, 1972d; also see discussion in Section I). In the Chlorophyceae, the phylogenetic relationships of the different types of cytokinesis are not so obvious.

In the Microsporales, phycoplast microtubules appear to radiate from a region near the centrioles which lie in the plane of cytokinesis (Pickett-Heaps, 1973b; Stewart et al., 1973). This type of cytokinesis is very similar to that in certain unicellular green algae, *Chlamydomonas* (Johnson and Porter, 1968), *Tetraspora* sp. (Pickett-Heaps, 1973a), and *Platymonas* (Stewart et al., 1974). Its presence in the filamentous Microsporales could well indicate that that order evolved from such green monads with little alteration in the cytokinetic process.

The Ulvales do not possess a phycoplast during vegetative cell division (Mattox and Stewart, 1974). It could be that the spindle has evolved the tendency to collapse and that the function of holding daughter nuclei apart has been transferred from persistent interzonal spindle microtubules to the precocious cleavage furrow characteristic of the order (see Mattox and Stewart, 1974). On the other hand, the possibility must be considered that ulvalean algae have replaced a phycoplast with the precocious furrow. In *Microspora* (Pickett-Heaps, 1973b; Stewart et al., 1973) the centrioles are at the spindle poles at metaphase and must migrate to the plane of cytokinesis before the phycoplast can form. It is possible that in ulvalean algae the precocious furrow replaced a phycoplast in the course of evolution as an alternative to the migration of centrioles or their associated microtubule organizing centers. Further research is necessary before any hypothesis can be favored.

Cytokinesis in the Chaetophorales also involves peculiarities rather difficult to interpret phylogenetically. The presence of a cell plate and the related formation of plasmodesmata make cytokinesis here and in the Oedogoniales the most advanced in the Chlorophyceae. The development of the cell plate involves transverse microtubules as does the furrow in the Microsporales, but the development of the phycoplast in the Chaetophorales is not associated with the centrioles, which remain on the side of the daughter nuclei opposite the plane of cytokinesis (Floyd et al., 1972a; Stewart et al., 1973). A granular material devoid of ribosomes appears between the daughter nuclei immediately upon their separation. Phycoplast microtubules seem to be synthesized at the granular region and later radiate from the area between the nuclei (Stewart et al., 1973). It is necessary to try to provide an explanation for the fact that the centrioles in the Chaetophorales are not associated with the phycoplast as they are in possibly ancestral monads with collapsing spindles. One possibility lies with the fact that in *Tetraspora* sp. (Pickett-Heaps, 1973a) and *Chlamydomonas reinhardii* (Johnson and Porter, 1968) the development of the phycoplast not only involves microtubules which radiate from the basal bodies but also a second system of microtubules which appears to arise and radiate from a region between the daughter nuclei similarly to the phycoplast in the Chaetophorales. In the ancestors of the Chaetophorales a close association of the centrioles with the spindle poles could have resulted in the function of the phycoplast being taken over entirely by the second system of microtubules. Although *Tetraspora* sp. and *Chlamydomonas reinhardii* have both systems of cytokinetic microtubules, the Chaetophorales, *Platymonas* (Stewart et al., 1974), *Pleurastrum terrestre* Fritsch and John (Molnar, unpublished results), and *Gloeotilopsis sterilis* Deason (Kao, unpublished results) have only one or the other. Since the differences in the development of transverse cytokinetic microtubules in various chlorophycean algae could be the result of evolutionary divergence and therefore of taxonomic value, we believe that the terminology should be altered so that a more precise distinction can be made. The term phycoplast should be retained, as it has been used in the past, as a general term for all transverse cytokinetic microtubules in the green algae. In addition we propose that a phycoplast that develops and radiates from a region near the centrioles be referred to also by a more specific term "trochoplast" (prefix chosen in reference to the centriolar "cartwheel"). Similarly we propose that phycoplast microtubules that develop among the remnants of the collapsed interzonal spindle be referred to also by a more specific term "mesoplast" (in reference to its development between the daughter nuclei). With that usage the Chlorococcales and Microsporales could be described as having a trochoplast, and the Chaetophorales and Oedogoniales as having a mesoplast. It should be pointed out, however, that a trochoplast can occur in an alga without centrioles. For example, *Gloeotilopsis sterilis* (Kao, unpublished results) lacks centrioles in the vegetative cells and has apparently lost the ability to make zoospores. Nevertheless, the transverse cytokinetic microtubules in *G. sterilis* clearly develop from a region which would be occupied by centrioles if they were present.

Organization in Filamentous Forms

As pointed out previously (Stewart et al., 1973), only those filamentous green algae that have plasmodesmata possess a significant degree of differentiation of the thallus. Plasmodesmata, specialized regions of the thallus, non-pigmented hairs or rhizoids, and distinctly localized growth are found only in the Chaetophorales, Oedogoniales, Coleochaetales, and Charales among the filamentous orders included in the present classification. Since groups both with and without plasmodesmata occur in both the Charophyceae and Chlorophyceae, it is concluded that plasmodesmata evolved independently in the two classes.

The evolution of truly multicellular plants appears to coincide with the evolution of plasmodesmata or other cytoplasmic continuity. In a discussion of evolution in animals, Jägersten (1972) states that one of the most important and mysterious or little understood events in the evolution of animals is the transition from unicellular to multicellular organisms. It would appear that at least some of the structural innovations associated with such a transition in plants are somewhat more clear.

A long standing controversy in considerations of algal evolution is the mode of origin of the filamentous habit. Filaments have evolved in other algal groups, and it seems probable that filaments evolved independently at least twice and possibly more times in the green algae. Some have considered it likely that filaments evolved by a transformation of colonies of non-motile cells such as occur in the Tetrasporales of present classifications (cf. Smith, 1950, p. 61). Fritsch (1935, p. 198), however, championed the idea that filaments evolved more directly from motile unicells. He believed that the germination of zoospores of filamentous algae reflects the sequence in the evolution of filaments.

Pickett-Heaps (1973b) has discussed one theory of the evolution of filaments in which "H-shaped" wall pieces such as occur in *Microspora* would represent a primitive condition. A point worth consideration is that the zoospores of most, if not all, filamentous algae are naked or scaly. That fact could possibly mean that the filamentous habit evolved in some cases before cell walls and that some early filaments were scaly.

Euglenoids

In the preceding pages we have tried to show how a more natural classification of the green algae could result from the systematic accumulation of knowledge concerning the structure of motile cells, mitotic phenomena, and biochemistry. If such characteristics are important, it might also be expected that they would eventually be useful in an overall definition of the green algae. At the present time green algae are united almost solely on the basis of the occurrence of chlorophyll b. Even that sparse characterization might be more satisfying were it not for the existence of the photosynthetic euglenoids. Even though euglenoids have long been recognized to have some features distinct from other organisms with chlorophylls a and b, they are still often regarded as having some sort of affinity with green algae. In the most recent consideration of the overall classification of the green algae, Round (1971) treated euglenoids as a group of green algae, however distinctive, and in 1964 Ettl and Manton suggested that certain "prasinophytes" might be as distinct from other green algae as are euglenoids.

If euglenoids have any sort of direct affinity with green algae, the characteristics that we have considered important in the classification of green algae take on a diminished significance. Nuclear division in euglenoids (see Leedale, 1970), their cytoskeletal structure, and biochemical characteristics other than those of the chloroplasts are fundamentally different from those of green algae. The special pathway of lysine synthesis that euglenoids share with many fungi (see review by Bartnicki-Garcia, 1970), the similarity of euglenoid *arom* aggregates to those of certain fungi (Berlyn et al., 1970), the extraplastidic eyespot (Walne and Arnott, 1967), and the extraplastidic storage of a peculiar carbohydrate, paramylon, suggest that the relatives of euglenoids are to be found among organisms that are non-photosynthetic or at least lack chlorophyll b. On the other hand few, if any, biologists would wish to postulate an independent origin of chlorophyll b in euglenoids. There are, of course, other cases in which two groups of organisms with similar pigments are very different in other respects. The best known example involves dinoflagellates and chrysophytes.

The origin of chlorophyll b in euglenoids is, therefore, a most intriguing mystery. We think it highly significant that recent work on dinoflagellates, mentioned above, seems to point the way toward an eventual solution to this problem. Dodge (1971) reported that the dinoflagellate *Glenodinium foliaceum* Stein possesses two nuclei, one a mesocaryotic nucleus characteristic of dinoflagellates and the other a eucaryotic nucleus. Investigating this phenomenon further in another such dinoflagellate, *Peridinium balticum* (Levander) Lemmermann, Tomas and Cox (1973) were able to show that the eucaryotic nucleus belongs to an endosymbiont which is separated from the cytoplasm of the host cell by its own plasmalemma. They also showed that the chloroplasts of *P. balticum* are part of the endosymbiont rather than the host cell. The endosymbiont's chloroplasts are limited by an envelope of two membranes and are also surrounded by "chloroplast ER" (term proposed by Bouck, 1965) for a total of four membranes. Tomas and Cox suggest that the chloroplasts present in uninucleate dinoflagellates evolved by the reduction of a eucaryotic endosymbiont such as occurs in *P. balticum.* Noting that uninucleate photosynthetic dinoflagellates have chloroplasts surrounded by three membranes, Tomas and Cox further suggest that the triple membrane could have evolved by a fusion of two of the four membranes mentioned above, an hypothesis with some similarity to one advanced earlier by Dodge (1968).

It might be highly significant, therefore, that the chloroplasts of euglenoids are also surrounded by three membranes (cf. Leedale, 1967, and Gibbs, 1970). It is conceivable that euglenoids have obtained their chloroplasts by the evolutionary reduction of a eucaryotic green alga. If such an origin should prove to be the case, it would no longer be necessary to postulate an independent origin for chlorophyll b or, as Raven (1970) has, the former existence of procaryotes with chlorophylls a and b.

IV. The Origin of Land Plants

Comparative cytological studies of green algae have provided convincing support for the viewpoint that the Charophyceae (Table I) are related to the ancestry of archegoniates and that the Chlorophyceae are remote from this line because their divergence from charophytes apparently began with the primitive monadal ancestors of chlorophytes. There can be little doubt that the phragmoplast is a universal feature of land plants. Among green algae, however, the phragmoplast is rare and known only in the Coleochaetales and Charales (see Pickett-Heaps and Marchant, 1972, and Section I of this paper). Likewise, glycolate oxidase appears to be universal in land plants (Frederick et al., 1973) but is known only in the Charophyceae of the classification given here. Finally, the microtubular cytoskeleton of the spermatozoids of archegoniate plants (Carothers and Kreitner, 1968; Duckett, 1973; Duckett and Bell, 1969; Marchant et al., 1973) possesses striking similarities to the cytoskeleton of the motile cells of the algae we have included in the charophyceae but bears little resemblance to that of the motile cells of the Chlorophyceae. It seems possible to conclude that the origin of the land flora is essentially monophyletic, at least to the extent that only advanced charophycean algae could have been ancestral.

The position of the Coleochaetales and Charales as the most advanced algae related to a line of evolution leading to archegoniate plants may have important implications concerning the nature of the early terrestrial ancestors of the land flora. The life cycle of *Coleochaete* and charalean algae (oogamous and haplobiontic - H, h) provides support for the idea that a multicellular dependent sporophyte was "interpolated" in the life cycle upon the invasion of land. The zygote of *Coleochaete* remains within its protective layer of sterile cells and, at the end of the winter rest period, undergoes meiosis and sometimes further mitotic divisions to produce 8 to 32 zoospores (see Smith, 1950). Such a system appears to be much more likely to be ancestral to an "embryophyte" than an alga with an alternation of free-living multicellular generations in which the zygote is not retained in a structure. It is very interesting that past proponents of the "antithetic" theory (e.g., Bower, 1908; Campbell, 1918; Frye and Clark, 1937) have often cited *Coleochaete* and *Chara* as possible ancestors of land plants and that they did so in the absence of good evidence that *Coleochaete* and *Chara* are related organisms. *Chara* was cited because of its conspicuously high level of vegetative organization and both *Chara* and *Coleochaete* were favored because of the protection of oogonia by sterile cells. It is often stated that the protection of gametangia by the enveloping growth of vegetative cells, as occurs in *Coleochaete* and the Charales, is fundamentally different from the development of gametangia in archegoniates (e.g., see Schuster, 1966, pp. 137-138, 170-271). However, such opinions appear to be based less on development than on the conviction that the gametangia of archegoniates evolved by the sterilization of outer layers of plurilocular gametangia such as are found in the Phaeophyta but which are absent in the Charophyceae. Since nothing is known about the evolutionary origin of the antheridia and archegonia of land plants, it cannot be assumed that the case in the Coleochaetales and Charales is fundamentally different.

The "antithetic" theory of the origin of the sporophyte of land plants was once in general favor because it was supported by Bower (1908) and Campbell (1918). Pringsheim's enunciation (1877, 1878) of the homologous theory was not popular because, for a long time, no green alga was known to possess an alternation of multicellular generations. The later demonstration of a homologous alternation in *Ulva* and other green algae, however, caused many to lean toward the hypothesis that the algal ancestors of archegoniate plants possessed a homologous alternation. Fritsch (1935, 1945), in particular, developed the concept that the algal ancestors of the land flora had such a life cycle and were heterotrichous and isogamous. The filamentous terrestrial green alga *Fritschiella* was named after Fritsch because it appeared to possess all of the characteristics that Fritsch had hypothesized for an ancestor of land plants. Not too long ago, the reported presence of a cell plate during cytokinesis in *Fritschiella* (McBride, 1970) was thought to support the idea that *Fritschiella* may be related to the ancestry of land plants (McBride, 1970; Floyd et al., 1971). However, much important information on the fine structure of green algae has accumulated since then, and it is now very clear that *Fritschiella* is a chlorophycean alga (see Table I and text in Section I and II). It is now inconceivable that *Fritschiella* could be related to the ancestry of land plants.

There is, of course, no good reason to suppose that some charophycean algae could not have evolved a homologous alternation such as occurs in *Ulva*. There is some very recent evidence that *Trentepohlia*, which has been reported to have an alternation of multicellular generations but has not been recently studied, is a charophycean alga (Graham and McBride, 1974). *Trentepohlia* might, therefore, replace *Fritschiella* as the example to point to for those who favor the homologous theory. It is our present opinion that the Coleochaetales and Charales lie closer to the main line of evolutionary advance. We base our opinion on (*1*) the greater elaboration of the thallus in the Coleochaetales and Charales, (*2*) the protection of gametangia by sterile cells in the Coleochaetales and Charales, and (*3*) the fact that the early information on the zoospores of *Trentepohlia* (Graham and McBride, 1974) indicate that their structure is less like the flagellated cells of archegoniate plants than are those of the Coleochaetales and Charales.

Summary

Recent comparative studies of the green algae have shown that the advanced genera belong to two lines of evolution very distinct from each other. The divergence of the two lines probably began with primitive green unicells. One line is characterized by having a persistent interzonal spindle at telophase, motile cells in which the flagella are associated with a single broad band of microtubules, and glycolate oxidase. The other line has a collapsing interzonal spindle at

telophase, motile cells in which the flagellar apparatus includes four or more relatively narrow microtubular roots, and lacks glycolate oxidase. The genera within each line appear to fall into groups on the basis of other cytological characteristics.

A partial classification of green algae based on recent comparative studies (Table I) is constructed to show how the classification of green algae might eventually be changed by the accumulation of information on mitosis, cytokinesis, the structure of motile cells and biochemistry. The classification is not proposed for general use but is meant to be a tabular presentation of the current state of progress in comparative cytological studies of the green algae.

Many green algae, particularly coenocytic and motile unicellular forms, have had to be omitted from the preliminary classification because of the paucity of information. Some of the characteristics that might prove to be of value in the classification of motile unicellular forms are discussed. It is probable that the Prasinophyceae is an undefinable assemblage.

Consideration is given to the evolutionary origin of other plants with chlorophylls a and b. The Charales and Coleochaetales are the most highly advanced algae cytologically similar to archegoniate plants. The nature of those two orders (haplobiontic and oogamous) supports the idea that the sporophyte was interpolated in the life cycle of the early terrestrial ancestors of the land flora. In regard to euglenoids it is suggested that certain recent observations and future research might eventually lead to the conclusion that photosynthetic euglenoids have obtained their chloroplasts by the reduction of green eucaryotic endosymbionts.

Zusammenfassung

Kürzliche komparative Studien auf dem Gebiet grüner Algen haben gezeigt, dass die fortgeschrittenen Gattungen zweiet Evolutionsrichtungen, angehören die untereinander sehr unterschiendlich sind. Die Divergenz beider Richtungen begann wahrscheinlich mit primitiven grünen Einzelzellen. Die eine Richtung ist charakterisiert durch eine konstante, interzonale Spindel in der Telophase, durch bewegliche Zellen, in der die Geiseln ein einziges breites Band von Mikrotubuli darstellen und durch glycolate oxidase; die andere Richtung durch eine zusammengefallene zwischenzonale Spindel in der Talophase, durch bewegliche Zellen, in denen die Geiseln aus vier oder mehr relativ schmalen winzigen röhrenartigen Wurzeln bestehen, jedoch keine glycolatoxidase hat. Die Gattungen beider Richtungen scheinen in Gruppen bezüglich anderer zellen Charakteristika zu fallen.

Eine vorläufige und einseitige Klassifikation der Grünalgen, die auf einer kurzichen komparativen Studie beruht (s. Tabelle I) soll zeigen wie die Klassifikation der Grünalgen vielleicht durch die Anhäufung der Kenntnis bezüglich der Kernteilung, Zellteilung der Stuktur der beweglichen Zellen und der Biochemie eventuell verändert werden könnte. Die Klassifikation sollte nicht zum Allgemeingebrauch vorgeschlagen werden, sondern eher als Tabellenpräsentation des gegenwärtigen Standes des Fortschritts in komparativen Zellenstudien der Grünalgen.

Viele Grünalgen, besonders im Stadium der Kervermehrung und der beweglichen Einzelzellen, dürfen wegen mangelnder Information nicht zu der vorläufigen Klassifikation gezählt werden. Einige der Charakteristika, die in der Klassifikation der beweglichen Einzelzellen einen Nutzwert haben, werden besprochen. Es ist möglich, dass die Prasinophycea eine gezwungene Anhäufung ist.

Der evolutionäre Ursprung anderer Pflanzen wurde unter Chlorophylle a und b betrachtet. Die Charales und Coleochaetales sind die fortgeschrittensten Algen, die den archegoniate Pflanzen ähnlich sind. Die Art dieser beiden Ordnungen (zygotischer Kernphasenwechsel und oogamie) bestärkt die Idee dass der Sporophyt in Beziehung stand zu dem Lebenszyklus der frühen Erdflora. Bezüglich der Euglenales könnte die gegenwärtige und zukünftige Forschung vielleicht zu einer Schlussfolgerung führen, dass nämlich photosynthetische Euglenales ihre Chloroplasten durch die Verminderung grüner eucaryotischer endosymbionts erhalten haben.

References Cited

Bartnicki-Garcia, S. 1970. Cell wall composition and other biochemical markers in fungal phylogeny. *In*: Phytochemical phylogeny, ed. by J. B. Harborne. Academic Press, New York.

Berlyn, M. B., S. I. Ahmed, & N. H. Giles. 1970. Organization of polyaromatic biosynthetic enzymes in a variety of photosynthetic organisms. J. Bacteriol. **104**: 768-774.

Birkbeck, T. E., K. D. Stewart, & K. R. Mattox. 1974. The cytology and classification of *Schizomeris leibleinii*. II. The structure of quadriflagellate zoospores. Phycologia **13**: 71-79.

Bouck, G. B. 1965. Fine structure and organelle associations in the brown algae. J. Cell Biol. **26**: 523-537.

Bower, F. O. 1908. The origin of a land flora. London.

Bråten, T., & A. Løvlie. 1968. On the ultrastructure of vegetative and sporulating cells of the multicellular green alga *Ulva mutabilis* Føyn. Nytt Mag. Bot. **15**: 209-219.

Campbell, D. H. 1918. The structure and development of mosses and ferns. 3rd Ed. New York.

Carothers, Z. B., & G. L. Kreitner. 1968. Studies of spermatogenesis in the Hepaticae. II. Blepharoplast structure in the spermatid of *Marchantia*. J. Cell Biol. **36**: 603-616.

Darley, W. M., D. Porter, & M. S. Fuller. 1973. Cell wall composition and synthesis via golgi-directed scale formation in the marine eucaryote, *Schizochytrium aggregatum*, with a note on *Thraustochytrium* sp. Arch. Mikrobiol. **90**: 89-106.

Deason, T. D., & W. H. Darden, Jr. 1971. The male initial and mitosis in *Volvox*. *In*: Contributions in phycology, ed. by B. C. Parker & R. M. Brown, Jr. Allen Press, Lawrence, Kansas.

Dodge, J. D. 1968. The fine structure of chloroplasts and pyrenoids in some marine dinoflagellates. J. Cell Biol. **3**: 41-48.

————. 1971. A dinoflagellate with both a mesocaryotic and a eucaryotic nucleus. I. Fine structure of the nuclei. Protoplasma **73**: 145-157.

Duckett, J. G. 1973. An ultrastructural study of the differentiation of the spermatozoid of *Equisetum*. J. Cell Sci. **12**: 95-129.

————, & P. R. Bell. 1969. The occurrence of a multilayered structure in the sperm of a pteridophyte. Planta **89**: 203-211.

Ettl, H., & I. Manton. 1964. Die feinere Struktur von *Pedinomonas minor* Korschikoff. Nova Hedwigia **8**: 421-451.

Evans, L. V., & A. O. Christie. 1970. Studies on the ship-fouling alga *Enteromorpha*. I. Aspects of the fine structure and biochemistry of swimming and newly settled zoospores. Ann. Bot. **34**: 451-466.

Floyd, G. L., K. D. Stewart, & K. R. Mattox. 1971. Comparative ultrastructure of several ulotrichalean algae. J. Phycol. 7(suppl.): 9.

————, ————, & ————. 1972a. Comparative cytology of *Ulothrix* and *Stigeoclonium*. J. Phycol. **8**: 68-81.

————, ————, & ————. 1972b. Cellular organization, mitosis and cytokinesis in *Klebsormidium*. J. Phycol. **8**: 176-184.

Fowke, L. C., & J. D. Pickett-Heaps. 1969a. Cell division in *Spirogyra*. I. Mitosis. J. Phycol. **5**: 240-259.

————, & ————. 1969b. Cell division in *Spirogyra*. II. Cytokinesis. J. Phycol. **5**: 273-281.

Fraser, T. W., & B. E. S. Gunning. 1969. The ultrastructure of plasmodesmata in the filamentous green alga, *Bulbochaete hiloensis* (Nordst.) Tiffany. Planta **88**: 244-254.

Frederick, S. E., P. J. Gruber, & N. E. Tolbert. 1973. The occurrence of glycolate dehydrogenase and glycolate oxidase in green plants: An evolutionary survey. Plant Physiol. **52**: 318-323.

Fritsch, F. E. 1935. Structure and reproduction of the algae, volume 1. Cambridge University Press, London. 791 pp.

————. 1945. Studies in the comparative morphology of the algae. IV. Algae and archegoniate plants. Ann. Bot. N.S. **9**: 1-29.

Frye, T. C., & L. Clark. 1937-1947. Hepaticae of North America. University of Washington Press, Seattle. Parts I-V.

Furtado, J. S., & L. S. Olive. 1971. Ultrastructure of the protostelid *Ceratiomyxella tahitiensis* including scale formation. Nova Hedw. **21**: 537-576.

Gerhardt, B., & C. Berger. 1971. Microbodies und Diaminobenzidin-Reaktion in den Acetat-Flagellaten *Polytomella caeca* und *Chlorogonium elongatum*. Planta **100**: 155-166.

Gibbs, S. P. 1970. The comparative ultrastructure of the algal chloroplast. Ann. N.Y. Acad. Sci. **175**: 454-473.

Giraud, G., & Y. Czaniski. 1971. Localisation ultrastructurale d'activites oxydasiqués chez le *Chlamydomonas reinhardi*. Comptes Rendus Ser. D. **273**: 2500-2503.

Graham, L. E., & G. E. McBride. 1974. Multilayered structures in motile cells of *Trentepohlia aurea* and the evolutionary implications. J. Phycol. **10**(suppl.): 8.

Hill, G. J. C., & L. Machlis. 1968. An ultrastructural study of vegetative cell division in *Oedogonium borisianum*. J. Phycol. **4**: 261-271.

Hoffman, L. R. 1968. Observations on the fine structure of *Oedogonium*. V. Evidence for the de novo formation of pyrenoids in the zoospores of *Oe. cardiacum*. J. Phycol. **4**: 212-218.

————, & I. Manton. 1962. Observations on the fine structure of the zoospore of *Oedogonium cardiacum* with special reference to the flagellar apparatus. J. Exptl. Bot. **13**: 443-449.

Jagersten, G. 1972. Evolution of the metazoan life cycle; a comprehensive theory. Academic Press, New York. 282 pp.

Johnson, U. G., & K. R. Porter. 1968. Fine structure of cell division in *Chlamydomonas reinhardi*. J. Cell Biol. **38**: 403-425.

Leedale, G. F. 1967. Euglenoid flagellates. Prentice-Hall, Englewood Cliffs, New Jersey. 242 pp.

————. 1970. Phylogenetic aspects of nuclear cytology in the algae. Ann. New York Acad. Sci. **175**: 429-443.

Løvlie, A., & T. Bråten. 1970. On mitosis in the multicellular green alga *Ulva mutabilis* Føyn. J. Cell Sci. **6**: 109-129.

Maiwald, M. 1971. A comparative ultrastructural study of *Pyramimonas montana* Geitler and a *Pyramimonas spec.* Arch. Protistenk. **113**: 334-344.

Manton, I. 1964. Observations on the fine structure of the zoospore and young germling of *Stigeoclonium*. J. Exp. Bot. **15**: 399-411.

————. 1965. Some phyletic implications of flagellar structure in plants. *In*: Advances in botanical research, ed. by R. D. Preston. Academic Press, London and New York. **2**: 1-34.

————, B. Clarke, & A. D. Greenwood. 1955. Observations with the electron microscope on biciliate and quadriciliate zoospores in green algae. J. Exp. Bot. **6**: 127-128.

——————, & I. Friedmann. 1959. Gametes, fertilization and zygote development in *Prasiola stipitata* Suhr. II. Electron microscopy. Nova Hedwigia 1: 443-462.

Marchant, H. J., & J. D. Pickett-Heaps. 1973. Mitosis and cytokinesis in *Coleochaete scutata*. J. Phycol. 9: 461-471.

——————, ——————, & K. Jacobs. 1973. An ultrastructural study of zoosporogenesis and the mature zoospore of *Klebsormidium flaccidum*. Cytobios 8: 95-107.

Mattox, K. R., & H. C. Bold. 1962. Phycological studies. III. The taxonomy of certain ulotrichalean algae. Univ. Texas Publ. 6222.

——————, & K. D. Stewart. 1973. Observations on the zoospores of *Pseudendoclonium basiliense* and *Trichosarcina polymorpha*. Can. J. Bot. 51: 1425-1430.

——————, & ——————. 1974. A comparative study of cell division in *Pseudendoclonium basiliense* and *Trichosarcina polymorpha* (Chlorophyceae). J. Phycol. 10: 447-456.

——————, ——————, & G. L. Floyd. 1974. The cytology and classification of *Schizomeris leibleinii*. I. The vegetative thallus. Phycologia 13: 63-70.

McBride, G. E. 1968. Ultrastructure of the *Coleochaete scutata* zoospore. (Abstr.). J. Phycol. 4(suppl.): 6.

——————. 1970. Cytokinesis and ultrastructure in *Fritschiella tubersoa* Iyengar. Arch. Protistenk. 112: 365-375.

Micalef, H., & P. Gayral. 1972. Quelques aspects de l'infrastructure des cellules vegetatives et des cellules reproductrices d'*Ulva lactuca* L. (Chlorophyceés). J. de Microscopie 13: 417-428.

Nelson, E. B., & N. E. Tolbert. 1970. Glycolate dehydrogenase in green algae. Arch. Biochem. Biophys. 141: 102-110.

Norris, R. E., & B. R. Pearson. 1973. Ultrastructure of mitosis and cytokinesis in a marine species of *Pyramimonas*. J. Phycol. 9(suppl.): 16.

Novikoff, A., & S. Goldfischer. 1969. Visualization of peroxisomes (microbodies) and mitochondria with diaminobenzidine. J. Histochem. Cytochem. 17: 675-680.

Parke, M., & I. Manton. 1965. Preliminary observations on the fine structure of *Prasinocladus marinus*. J. Mar. Biol. Ass. U. K. 45: 525-536.

Patenaude, R. D., & D. L. Brown. 1973. Microtubule distribution in *Polytomella agilis*. J. Phycol. 9(suppl.): 7.

Peterfi, L. S., & I. Manton. 1968. Observations with the electron microscope on *Asteromonas gracilis* Artari emend (*Stephanoptera gracilis* (Artari) Wisl. with some comparative observations on *Dunaliella* sp. Brit. Phycol. Bull. 3: 423-440.

Pickett-Heaps, J. D. 1967. Ultrastructure and differentiation of *Chara* sp. II. Mitosis. Aust. J. Biol. Sci. 20: 883-894.

——————. 1968. Ultrastructure and differentiation in *Chara* (*fibrosa*). IV. Spermatogenesis. Aust. J. Biol. Sci. 21: 655-690.

——————. 1969. The evolution of the mitotic apparatus: An attempt at comparative ultrastructural cytology in dividing plant cells. Cytobios 3: 257-280.

——————. 1971. Reproduction by zoospores in *Oedogonium*. I. Zoosporogenesis. Protoplasma 72: 275-314.

——————. 1972a. Cell division in *Cosmarium botrytis*. J. Phycol. 8: 343-360.

——————. 1972b. Cell division in *Klebsormidium subtillissimum* (formerly *Ulothrix subtillissima*) and its possible phylogenetic significance. Cytobios 6: 167-183.

——————. 1972c. Cell division in *Tetraedron*. Ann. Bot. 36: 693-701.

——————. 1972d. Reproduction by zoospores in *Oedogonium*. II. Emergence of the zoospore and the motile phase. Protoplasma 74: 149-168.

——————. 1972e. Variation in mitosis and cytokinesis in plant cells: Its significance in the phylogeny and evolution of ultrastructural systems. Cytobios 5: 59-77.

——————. 1973a. Cell division in *Tetraspora*. Ann. Bot. 37: 1017-1025.

——————. 1973b. Cell division and wall structure in *Microspora*. New Phytol. 72: 347-355.

——————, & L. C. Fowke. 1969. Cell division in *Oedogonium*. I. Mitosis, cytokinesis and cell elongation. Aust. J. Biol. Sci. 22: 857-894.

——————, & ——————. 1970a. Cell division in *Oedogonium*. II. Nuclear division in *O. cardiacum*. Aust. J. Biol. Sci. 23: 71-92.

——————, & ——————. 1970b. Mitosis, cytokinesis and cell elongation in the desmid, *Closterium littorale*. J. Phycol. 6: 189-215.

——————, & H. Marchant. 1972. The phylogeny of the green algae: A new proposal. Cytobios 6: 255-264.

Pringsheim, N. 1877. Über den Generationswechsel der Thallophyten und seinen Anschluss an den Generationswechsel der Moose. Monatsbericht d. K. Akad. Wiss. (Berlin) 1876: 869-911.

——————. 1878. Über Sprossung der Moosfruchte und den Generationswechsel der Thallophyten. Jahrb. Wiss. Bot. 11: 1-46.

Raven, P. H. 1970. A multiple origin for plastids and mitochondria. Science 169: 641-646.

Ris, H., & D. F. Kubai. 1974. An unusual mitotic mechanism in the parasitic protozoan *Syndinium* sp. J. Cell Biol. 60: 702-720.

Roth-Bejerano, N., & S. H. Lips. 1973. Glycolate dehydrogenase activity in higher plants. Israel J. Bot. 22: 1-7.

Round, F. E. 1971. The taxonomy of the Chlorophyta. II. Br. Phycol. J. 6: 235-264.

Schuster, R. M. 1966. The Hepaticae and Anthocerotae of North America, volume I. Columbia University Press, New York.

Silva, P. C., K. R. Mattox, & W. H. Blackwell, Jr. 1972. The generic name *Hormidium* as applied to green algae. Taxon 21: 639-645.

Silverberg, B. 1974. Selected studies in the fine structure of Characean cells. Ph. D. Dissertation, University of Toronto, Toronto.

Smith, G. M. 1950. Fresh-water algae of the United States. 2nd Ed. McGraw-Hill, New York, 719 pp.

Stewart, K. D., G. L. Floyd, K. R. Mattox, & M. E. Davis. 1972. Cytochemical demonstration of a single peroxisome in a filamentous green alga. J. Cell Biol. 54: 431-434.

——————, K. R. Mattox, & G. L. Floyd. 1973. Mitosis, cytokinesis, and distribution of plasmodesmata and other cytological characteristics in the Ulotrichales, Uvales and Chaetophorales — phylogenetic and taxonomic considerations. J. Phycol. 9: 128-141.

——————, ——————, & C. D. Chandler. 1974. Mitosis and cytokinesis in *Platymonas subcordiformis*, a scaly green monad. J. Phycol. 10: 65-79.

Tolbert, N. E. 1971. Microbodies — peroxisomes and glyoxysomes. Ann. Rev. Plant Physiol. 22: 45-71.

Tomas, R. N., & E. R. Cox. 1973. Observations on the symbiosis of *Peridinium balticum* and its intracellular alga. I. Ultrastructure. J. Phycol. 9: 304-323.

Tourtè, M. 1972. Mise en evidence d'une activité catalasique dans les peroxysomes de *Micrasterias fimbriata* (Ralfs). Planta 105: 50-59.

Turner, F. R. 1968. An ultrastructural study of plant spermatogenesis. Spermatogenesis in *Nitella*. J. Cell Biol. 37: 370-393.

Walne, P. L., & H. J. Arnott. 1967. The comparative ultrastructure and possible function of eyespots: *Euglena granulata* and *Chlamydomonas eugametos*. Planta 77: 325-353.

Watson, M. W., & H. J. Arnott. 1973. Ultrastructural morphology of *Microthamnion zoospores*. J. Phycol. 9: 15-29.