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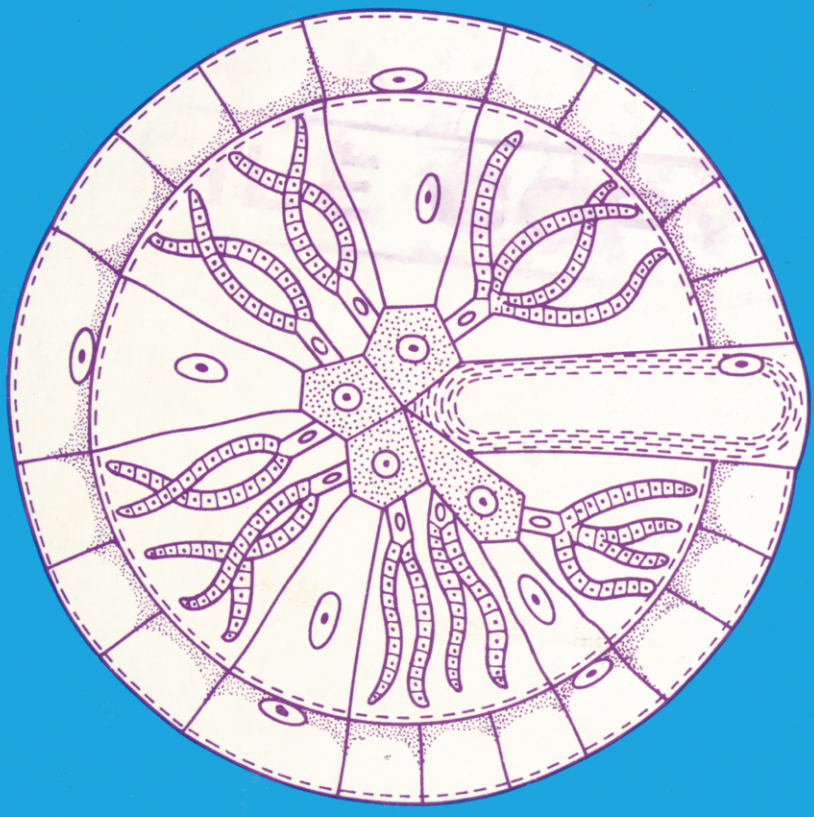
A Textbook on Algae

H D Kumar and H N Singh



A TEXTBOOK ON ALGAE

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Contents

Foreword

The study of algae is a rapidly expanding branch of botanical science. Some of these simple plants are being used as experimental material for advanced research in biochemistry, genetics and molecular biology; others are of great economic importance both as nuisances and as contributors to the productivity of soils and fisheries. Beyond this, most algae are extremely beautiful objects in themselves and perhaps in the ultimate analysis this is the main source of their attraction for scientists. Nevertheless, however good the reasons for studying the algae, they may be nullified if the student is faced with inadequate, uninspiring or intimidating textbooks. I am therefore very glad that two such active and distinguished Indian workers on algae as Professor H. D. Kumar and Dr. H. N. Singh have found time to write this introductory text. By providing a clear and sound background of the morphology which every student of the algae requires but at the same time indicating the other aspects and showing how one may begin practical work on the algae oneself, I believe that they have produced a book which will contribute greatly to the future development of the subject.

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The Marine Science Laboratories (Menai Bridge)
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Preface

The need for a suitable textbook on algae for undergraduates has been felt in many universities in recent years. Our main objective in writing this book is to fulfil this need and to present an updated account of morphology and reproduction of the more important genera, supplemented with brief descriptions of recent contributions on their physiology, biochemistry, cytology and genetics.

Any attempt at improving the standard of teaching of algae must begin in practical classes and field studies. One complete chapter is therefore exclusively devoted to field and laboratory techniques. It includes some of the simpler methods for culturing, microchemical study, separation of pigments by chromatographic techniques, preparation of herbarium sheets and permanent slides, estimation of growth and primary productivity, and ecological study of soil and freshwater forms.

The remaining chapters incorporate brief and simple accounts of some important and representative types. The discussion also covers topics of current interest such as the concept of Procaryota and Eucaryota; the ultrastructure of algal cells and their organelles; the developmental cycle and genetic recombination in certain blue-green algae; the physiology and biochemistry of nitrogen fixation and heterocyst differentiation; the nature of sexual substances (gamones) produced by algae; genetics in *Chlamydomonas*; and the physiology of growth and differentiation in synchronous cultures of *Chlorella*. The genera described in Part II are those usually prescribed for undergraduates. Minute variations in structure, reproduction and life history have been avoided so as to give proportionately greater coverage to micromorphology and modern experimental studies. It is hoped that the general comparative topics dealt with in Part I will be equally useful for advanced study.

Test questions and selected references for further reading are incorporated at appropriate places. Exhaustive lists of references have been avoided but care has been taken to include the latest references as far as possible, since the reader can always find most of the earlier works of individual authors in the citations given.

We are greatly indebted to Professor G. E. Fogg for many valuable suggestions and for critically reviewing the manuscript. We thank Professors R. N. Singh, T. V. Desikachary, H. A. Von Stotsch, M. B. E. Godward, B. M. Johri, Drs. V. P. Singh and F. E. Round for their helpful comments. We are grateful to Dr. Norma J. Lang of the University of California, Davis, for providing the electron micrograph of *Anabaenopsis circularis* (frontispiece).

Varanasi

H. D. Kumar
H. N. Singh

PART I

COMPARATIVE ACCOUNT OF ALGAE

1. (54-22/1972)

1
Introduction

DEFINITION

The algae comprise a large and heterogeneous assemblage of relatively simple plants which have little in common except their characteristic oxygen evolving type of photosynthesis. They exhibit great diversity in size and appearance and are found in freshwater of all kinds, barks, soils, rocks and marine habitats. Many unicellular forms, such as desmids and diatoms, are extremely beautiful microscopic objects. Some of the larger forms, especially the red seaweeds, are highly attractive and when mounted and dried on herbarium sheets appear like paintings.

Algae differ from fungi in possessing chlorophyll and characteristically **holophytic** (plant-like), photosynthetic mode of nutrition; parasitic, **heterotrophic** and even **holozoic** (animal-like) modes are, nevertheless, known in certain forms. They differ from bryophytes in lacking a jacket of sterile cells around their reproductive organs which are mostly unicellular. In brown algae (Phaeophyceae) multicellular reproductive organs are produced but all the cells of such a multicellular (plurilocular) sporangium or gametangium are fertile. The Charophyceae (green algae) constitute another exception since their reproductive structures are apparently multicellular and the **antheridium** is surrounded by an envelope of sterile cells. The female reproductive organ of algae is not organized into an **archegonium** (egg-producing multicellular organ) as is characteristic of bryophytes and pteridophytes.

With the exception of blue-green algae, most others can be sharply distinguished from bacteria. The main difference between blue-green algae and bacteria on the one hand, and the remaining algae, fungi and higher plants on the other, is the procaryotic nature of cells in the former group as contrasted with the eucaryotic nature in the latter. **Procaryota** lack organized nuclei, **chromatophores** and **mitochondria**. Their cell walls contain certain characteristic **mucopeptides** (or muropeptides), and **muramic acid**, not found in those of **Eucaryota**. They are susceptible to infection and lysis by viruses of similar morphology. However, blue-green algae resemble other algae and green plants in their oxygen evolving type of photosynthesis whereas in photosynthetic bacteria no oxygen is evolved.

VALIDITY OF DIVISION THALLOPHYTA

Algae, fungi and lichens have long been grouped in Thallophyta on account of the following characters which they share; (1) the zygote does not develop

into a multicellular embryo while still contained inside the oogonium, and (2) the reproductive organs, whether spore-producing or **gamete**-producing, are not enveloped by a wall of sterile cells.

Smith (1955) believes that the common features of algae and fungi are due to parallel evolution and not because of phylogenetic relationship. On this basis, therefore, algae and fungi should not be classified together in the Division Thallophyta. This idea is also shared by some mycologists such as C. J. Alexopoulos, G. C. Ainsworth and A. S. Sussman.

Researches carried out during the past decade (see Klein and Cronquist 1967) have, however, provided some indication of a phylogenetic connection between algae and fungi. Total absence of photosynthetic apparatus in fungi rules out the possibility that algae might have evolved from fungi. That fungi might have evolved from algae by the loss of chlorophyll is supported by: (1) similarity in certain structural and functional characteristics between the lower fungi (Phycomycetes, especially Oomycetes and Chytridiales) and some algae belonging to the Xanthophyta, Euglenophyta and Pyrrophyta; (2) similar flagellation, i.e., two unequal **flagella**—one whiplash type and the other pinnate type in both Oomycetes and Xanthophyta; (3) chemical resemblance of the cell wall polysaccharides of Vaucheriaceae (Xanthophyta) and Saprolegniaceae (Oomycetes); and (4) the presence in Euglenophyta and Pyrrophyta, like Chytridiales, of one whiplash flagellum and a nucleus in which the nuclear membrane persists throughout the division cycle.

The common features between Oomycetes and Xanthophyta, and among Chytridiales, Euglenophyta and Pyrrophyta, indicate that fungi might have evolved from algae and that their evolution was probably diphyletic: the Xanthophytan line giving rise to Oomycetes and the Euglenophytan and Pyrrophytan lines leading to Chytridiomycetes. Therefore, the classification of both algae and fungi in the same group Thallophyta does not seem unjustified. Nevertheless, a much better and rational way of classifying the lower plants is to group blue-green algae and bacteria as Procaryota, and other algae, fungi and higher forms in Eucaryota.

CLASSIFICATION

The algae have been classified mainly on the basis of the photosynthetic pigments (Table I), storage products of vegetative cells and the form of motile reproductive cells. Unlike bryophytes, pteridophytes and higher plants, the morphology of the mature vegetative stages does not afford a reliable criterion for distinguishing different classes or phyla of algae. Consequently, several examples are known where morphologically similar algae come to be placed in different classes, because they differ in their physiological and biochemical characters. Such a classification of morphologically similar but physiologically dissimilar forms in different classes is based on the view that there has been a parallelism in their evolution.

The main criteria (see Tables II and III) on the basis of which the algae

Table I. Distribution of Photosynthetic Pigments in Algae

Phylum	*Chlorophylls*					*Carotenes*				*Xanthophylls*																	*Phycobiliproteins*	
	a	b	c	d	e	α	β	γ	e	Lycopene	Flavacine	Echinenone	Lutein	Zeaxanthin	Violaxanthin	Neoxanthin	Antheraxanthin	Fucoxanthin	Diatoxanthin	Diadinoxanthin	Dinoxanthin	Peridinin	Myxoxanthophyll	Myxoxanthin	Siphonaxanthin	Astaxanthin	Oscillaxanthin	*Phycocyacnin*	*Phycoerythrin*
Cyanophyta	+	—	—	—	—	—	+	—	+	—	+	+	?	+	—	—	—	—	—	—	—	—	+	+	—	—	+	+	+
Chlorophyta	+																												
Chlorophyceae	+	+	—	—	—	+	+	+	—	—	—	—	+	—	+	+	—	—	—	—	—	—	—	—	+a	+	—	—	—
Charophyceae	+	+	—	—	—	—	+	+	—	+	—	—	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—
Xanthophyta	+	—	—	—	+	—	+	—	—	—	—	—	—	—	+?	+?	—	—	—	—	—	—	—	—	—	—	—	—	—
Chrysophyta	+	—	—	—	—	—	+	—	—	—	—	—	—	—	—	—	—	+	+	+	—	—	—	—	—	—	—	—	—
Bacillariophyta	+	—	+	—	—	—	+	—	+	—	—	—	—	—	—	—	—	+	+	+	—	—	—	—	—	—	—	—	—
Pyrrophyta	+	—	+	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	—	—	—	—	—	—	—
Cryptophyta	+	—	+	—	—	+	—	—	—	—	—	—	—	+?	—	—	—	—	+	—	—	—	—	—	—	—	—	+	+
Euglenophyta	+	+	—	—	—	—	+	—	—	—	—	+	—	+	—	+	+	—	—	—	—	—	—	—	—	+	—	—	—
Phaeophyta	+	—	+	—	—	—	+	—	—	—	—	—	+	—	+	—	—	+	+	—	—	—	—	—	—	—	—	—	—
Rhodophyta	+	—	—	+	—	+	+	—	—	—	—	—	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	+	+

? — Present in traces only

a — Siphonales

Table II. Diagnostic Features of Various Algal Phyla

Phylum	*Flagella*	*Genetic apparatus*	*Cell wall*	*Food reserves*
Cyanophyta	absent	nucleus absent; naked DNA fibrils (i.e., not associated with histones) present in nucleoplasm	mucopolymeric	proteinaceous cyanophycin and cyanophycean starch
Chlorophyta	1, 2 or 4 (or more), commonly equal, anterior, acronematic, smooth	nucleus with nucleolus; spindle and centromeric chromosomes made up of protein and DNA fibrils associated with histones	cellulosic	true starch
Xanthophyta	2 (rarely more), unequal, anterior, 1 acronematic and 1 pantonematic, 1 with stiff hairs and 1 smooth	as in Chlorophyta	when present either pectinaceous or cellulosic (Heterosiphonales)	oil and leucosin
Chrysophyta	2, anterior, equal or unequal, both acronematic or 1 acronematic and 1 pantonematic, 1 with stiff hairs and 1 smooth	as in Chlorophyta	organisms periplastic or with silicified scales, or calcified plates (coccoliths); non-cellulosic	leucosin and chrysolaminarin
Bacillariophyta	generally 1, anterior, pantonematic with stiff hairs	as in Chlorophyta	silicified, in 2 pieces, each with complex perforations	leucosin or chrysolaminarin

Pyrrophyta	2, terminal, inserted in a girdle in some forms, 1 acronematic and 1 pantonematic (1 spiral with fine hairs)	nucleus with nucleolus, nuclear membrane persistent; chromosomes mainly made up of DNA fibrils; no evidence of spindle	cells periplastic or with cellulosic wall divided into a number of plates or 2 halves	true starch or oil or both
Cryptophyta	2, terminal, sometimes lateral, either both pantonematic or 1 pantonematic and 1 acronematic, when 2, both have stiff hairs	nucleus with characteristic chromosomes	periplastic	as in Pyrrophyta
Euglenophyta	generally 1, anterior, pantonematic, sometimes 2, equal or unequal, having fine hairs	nucleus with one or more endosomes and many acentromeric chromosomes; spindle absent; nuclear membrane persistent	periplastic	paramylum
Phaeophyta	2, lateral, unequal, 1 acronematic and 1 pantonematic, 1 with stiff hairs and 1 smooth	as in Chlorophyta	cellulosic with alginic and fucinic acids as characteristic components	laminarins, leucosin and mannitol
Rhodophyta	absent	as in Chlorophyta	non-cellulosic polysaccharides and polyuronic acids; or with xylan, polyxylan, galactose and xylose; occasionally cellulosic	floridean starch, galactan sulphate polymers and floridioside

Table III. Diagnostic Characteristics of Various Algal Phyla

Phylum	*Photosynthetic apparatus*	*Eye spot*
Cyanophyta	chromatophores absent, thylakoids occur singly; pyrenoids absent	absent
Chlorophyta	chromatophores (chloroplasts) with 2–6 thylakoids grouped in a band; pyrenoids with starch sheath	part of chloroplast but distinct from flagella
Xanthophyta	chromatophores with 3 thylakoid bands; pyrenoids naked	part of chloroplast but situated adjacent to flagella
Chrysophyta	as in Xanthophyta	as in Xanthophyta
Bacillariophyta	as in Xanthophyta	absent
Pyrrophyta	as in Xanthophyta	variable
Cryptophyta	chromatophores with bands of 2 thylakoids each; pyrenoids naked	either as in Chlorophyta, or found internally in spur of chloroplast, at some distance from flagella
Euglenophyta	chromatophores with 2–6 thylakoids grouped into a band; pyrenoid projecting and with an apical sheath of paramylum	not part of chloroplast but associated with flagella
Phaeophyta	as in Xanthophyta; pyrenoids projecting and naked	as in Xanthophyta
Rhodophyta	chromatophores with single and widely separated thylakoids; pyrenoids naked	absent

have been classified are: (1) the chemical composition and relative amounts of the photosynthetic pigments; (2) the chemical nature of the food reserves; (3) the chemical and physical nature of the cell wall (precise details of the physical features can be revealed only under the electron microscope; and (4) the number, morphology and orientation of flagella, especially those of motile reproductive bodies, **zoospores** or gametes.

These principles are generally accepted as broad guidelines for the primary classification of algae. However, the systems of classification differ widely as regards the rank and status assigned to various taxa. For instance, Fritsch (1935, 1944) divides the algae into 11 classes whereas others classify them into 7–10 phyla. The number of orders within a class also varies considerably in different systems of classification.

The broad system of classification followed in this book is derived from the ideas of Stanier and van Niel (1962) and of Round (1965) except that we propose a total of 10 phyla whereas Round recognizes only eight. Our scheme also indicates the comparative position of viruses, bacteria and fungi.

The Subkingdom Eucaryota includes bryophytes, pteridophytes, higher plants and animals as well.

LIVING ORGANISMS

Kingdom 1 ACELLULAR ORGANISMS (Viruses)
Kingdom 2 CELLULAR ORGANISMS
- Subkingdom PROCARYOTA
  - Phylum SCHIZOMYCOPHYTA (Bacteria)
  - Phylum CYANOPHYTA (Blue-green algae)
- Subkingdom EUCARYOTA
  - Phylum CHLOROPHYTA
  - Phylum XANTHOPHYTA
  - Phylum CHRYSOPHYTA
  - Phylum BACILLARIOPHYTA
  - Phylum PYRROPHYTA
  - Phylum CRYPTOPHYTA
  - Phylum EUGLENOPHYTA
  - Phylum PHAEOPHYTA
  - Phylum RHODOPHYTA
  - Phylum MYXOMYCOPHYTA (Myxomycetes, slime-molds)
  - Phylum EUMYCOPHYTA (Fungi)

Each of the 10 algal phyla contains a single Class (-phyceae) except for Chlorophyta which includes two (Chlorophyceae and Charophyceae) and Chrysophyta which is divided into Haptophyceae (with 2 equal flagella) and Euchrysophyceae.

Some of the algal phyla show certain resemblances and interrelationships with other algal phyla. Thus, the Rhodophyta and Cyanophyta are characterized by the absence of flagellated bodies, and presence of biliprotein pigments and protoplasmic connections between adjacent vegetative cells. Despite these similarities, the red and blue-green algae do not seem phylogenetically related in view of the marked differences in their cell structure. Some relationship is possible between Chlorophyta and Euglenophyta since both contain chlorophyll-b, β-carotene and another pigment lutein, and also because some members of the Chlorophyta (Volvocales) possess an **eye spot** of the type found in Euglenophyta. The presence of chlorophyll-c is a feature which seems to link together the members of Pyrrophyta, Bacillariophyta, Cryptophyta and Phaeophyta. Similarly, the presence of the **periplastic** envelope, the **cytopharyngeal** apparatus associated with the gullet, and the **contractile vacuoles** seem to bring together the members of Cryptophyta, Pyrrophyta and Euglenophyta. Some phylogenetic connec-

tion may exist between Pyrrophyta and Cryptophyta since in both the nuclear membrane persists through the division cycle.

The recurrence of certain characters in different algal phyla, however, does not necessarily mean that these are phylogenetically related. It might rather be due to parallel evolution of these features in different phyla. Algae in general seem to have evolved from some photosynthetic procaryotic ancestors closely related to, but not identical with, the present day blue-green algae. This view is based on the hypothesis that chloroplasts of algae and higher plants are **cellular** symbionts which existed as photosynthetic procaryotes in the remote past (see Raven 1970).

TEST QUESTIONS

1. How would you distinguish cellular from acellular organisms ?
2. In spite of the functional equivalence between procaryotic and eucaryotic cells, the former are considered more primitive than the latter. Why ?
3. How would you justify the inclusion of Cyanophyta and Charophyceae among algae ?
4. What objections would you have if algal flagellates were to be included in the Phylum Protozoa ?
5. Why is a system of classification based on a single feature rather than a combination of characters considered invalid and unreliable ?
6. Zoologists generally group all unicellular animals in the single Phylum Protozoa but botanists do not put all unicellular plants in one major taxon. How can you account for this disparity ?

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2

Comparative Morphology

CELL STRUCTURE

In procaryotic algae (Cyanophyta) the photosynthetic, genetic and respiratory apparatuses are present in the cell but are not separated from each other by a bounding membrane of their own. Consequently, the cells lack

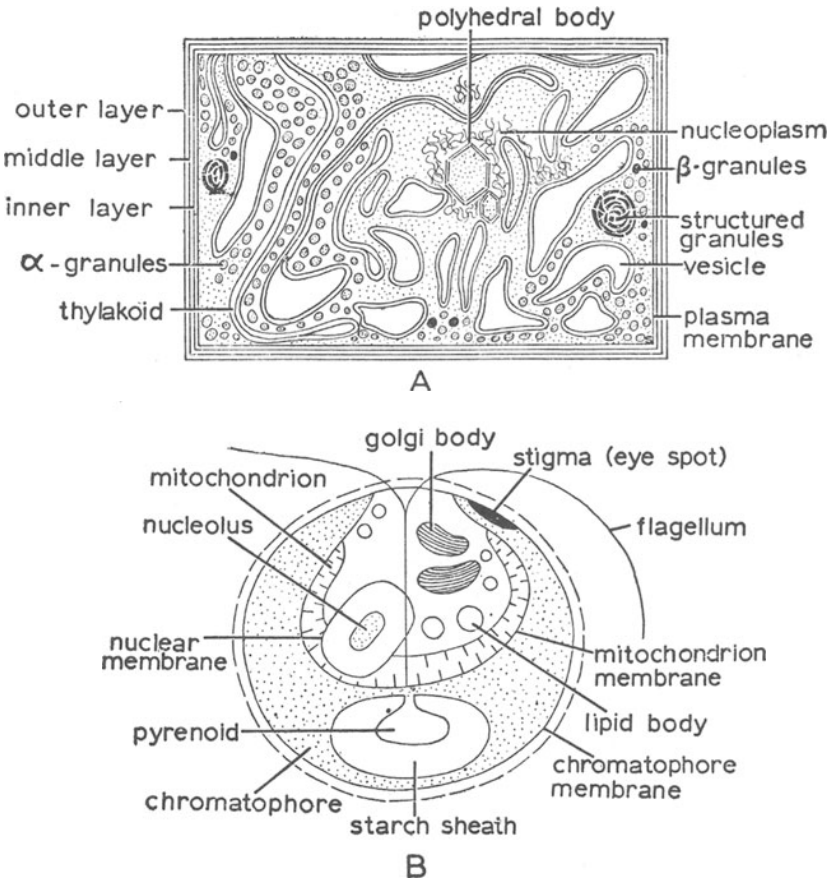

Fig. 2-1. Two basic types of cellular organization in algae. A, diagrammatic plan of a procaryotic cell of blue-green algae. B, *Heteromastix rotunda* showing eucaryotic organization (after Manton *et al.*, 1965).

organized nuclei, chromatophores and mitochondria (Fig. 2-1 A). In eucaryotic algae, on the other hand, the photosynthetic, genetic and

respiratory apparatuses are membrane-bounded and separated from each other (Fig. 2-1 B).

CELL WALL

The zoospores, gametes, and mature cells of most of the algal flagellates are devoid of a typical cell wall and are merely bounded by the cytoplasmic membrane. In the majority of algae the cells possess a wall of non-living material outside the cytoplasmic membrane. The algal cell wall is composed of pure or mixed carbohydrates or may be entirely silicified. Sometimes it may contain proteins and may also be impregnated with calcium carbonates, iron, or chitin. It is usually made up of two layers, an outer amorphous layer which is mucilaginous or pectic and dissolves readily in boiling water, and an inner crystalline layer which is firm and relatively insoluble in boiling water and contains microfibrils. According to their composition, three categories of cell wall can be recognized in algae—cellulosic, silicified, and mucopolymeric. The cellulosic walls, characteristic of green algae, are fibrillar and the microfibrils may be composed of only glucose molecules, or of glucose plus other related sugars. The actual composition of the microfibrils varies so enormously that no generalization is possible. The microfibrils are embedded in the amorphous layer of the cell wall. In Xanthophyta the cell wall is mostly pectic and in Phaeophyta it is characterized by the presence of additional compounds such as hemicellulose, alginic acid, fucoidin and fucin. The members of the Rhodophyta and Chlorophyta have cell walls made of cellulose and pectin. In diatoms (Bacillariophyta) the cell wall is **valved** and composed of α-quartz embedded in a pectin-like matrix. Their silicified walls lack cellulose, hemicellulose or polymers of other sugars. The mucopolymeric cell wall is characteristic of blue-green algae and its principal components are **glucosamine**, amino acids, **diaminopimelic acid** and muramic acid with some amounts of sugar polymers.

In some algae the abutting walls of adjacent cells have pit connections and are traversed by fine protoplasmic strands known as **plasmodesmata** (cytoplasmic threads) which interconnect the protoplasts.

FLAGELLA

Most algal phyla, except Rhodophyta and Cyanophyta, include species which are either motile during their vegetative phase or reproduce by means of motile **asexual** or sexual cells, the motility being due to flagella they possess. Morphologically, there are two types of flagella in algae: (1) the whiplash or **acronematic** type (Fig. 2-2 A) which are smooth, and (2) the tinsel or **pantonematic** type (Fig. 2-2 B) which are covered with small filamentous appendages. A flagellum grows from a basal body, the **blepharoplast**, located in the peripheral region of the cytoplasm. Under the electron microscope it is seen to consist of a peripheral cylinder of 9 doublet fibrils surrounding a central pair of singlet fibrils (Fig. 2-2 C). Such an arrangement is known as the 9 + 2 arrangement and is characteristic of all flagellated

cells except bacteria. The entire group of fibrils is enclosed in a double sheath and the two central fibrils have a subsidiary (single) sheath of their own.

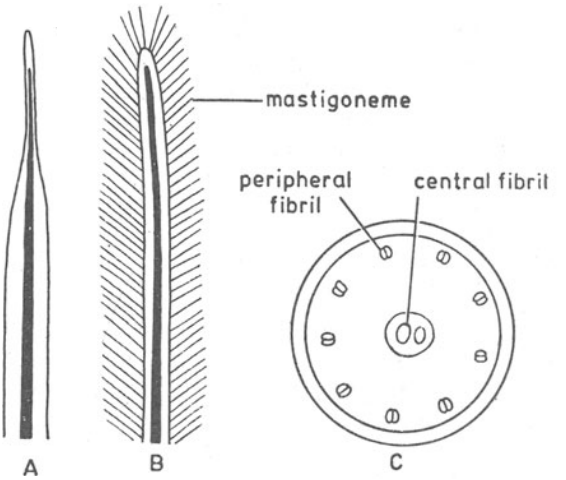

Fig. 2-2. A, upper portion of an acronematic flagellum; B, upper portion of a pantonematic flagellum (after Manton, 1966); C, cross section of a flagellum as diagrammatized from an electron micrograph.

The motile stages of the Chlorophyta possess two or four anteriorly inserted whiplash flagella of equal length. The Phaeophyta and Xanthophyta have one whiplash and one tinsel flagellum of unequal length, the flagellar attachment being lateral in the brown algae and anterior in the Xanthophyta. The Euglenophyta generally have two flagella, one of which emerges from the anterior invagination of the cell and has mastigonemata whereas the other is non-emergent, stops short within the reservoir and lacks mastigonemata.

PLASTIDS AND CHROMATOPHORES

The plastids are double membrane organelles of two types, the colourless or leucoplasts and the coloured or chromatophores. The usual practice is to designate the chlorophyll-a and chlorophyll-b containing plastids as chloroplasts; those which lack chlorophyll-b are termed chromatophores. Typical chromatophores or chloroplasts are found in all algae except the Cyanophyta in which the photosynthetic pigments are concentrated in the peripheral zone of the protoplasm.

According to its location in a cell the chromatophore may be parietal or axile. In the majority of algae only a single chromatophore is present in a cell but the members of Conjugales, Siphonales, Charales and Xanthophyceae generally contain more than one chromatophore per cell. The morphology of the chromatophore varies considerably in different algae (Fig. 2-3 A–F) but the following main types can be recognized: (1) cup-shaped, as in *Chlamydomonas*; (2) girdle-shaped, as in *Ulothrix*; (3) discoid,

as in *Vaucheria* and *Chara*; (4) reticulate, as in *Oedogonium*; (5) spiral, as in *Spirogyra*; and (6) stellate, as in *Zygnema*.

In some diatoms and the dinoflagellate *Pyrocystis lunula*, chloroplasts can change their shape and location within the cell in response to light. When exposed to light of either too high or too low intensity, the chloroplasts in *Surirella gemma* shrink and become rounded off, but when grown in moderate-intensity light, they expand into normal shape and size. Light-induced rotation of chloroplast, mediated by phytochrome, has been reported in the desmid *Mesotaenium*.

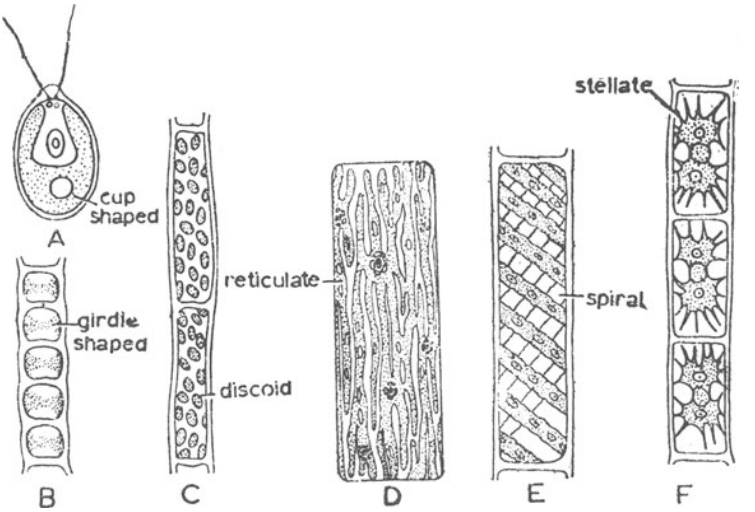

Fig. 2-3. Shapes of chromatophore. A, *Chlamydomonas*; B, *Ulothrix*; C, *Tribonema*; D, *Oedogonium*; E, *Spirogyra*; F, *Zygnema*. (After Fritsch, 1935.)

The electron microscopy of the algal chromatophore has revealed that it is a double membrane structure composed of a photosynthetic lamellar system traversing the colourless granular matrix, the stroma. The structural unit of the lamellar system is a closed double membrane disc termed **thylakoid**. A thylakoid membrane consists of a central lipid layer covered on both sides with protein particles, the lipid layer contains the fat-soluble photosynthetic pigments. The water-soluble pigments (phycoerythrin and phycocyanin) of Rhodophyta, Cyanophyta and Cryptophyta are located in characteristic particles, termed **phycobilisomes**, which are found linearly arranged along the surface of each thylakoid.

In Rhodophyta the thylakoids are widely separated and occur singly like cyanophycean thylakoids. In all other phyla they are stacked together into bands. The Phaeophyta generally contain a band of three thylakoids. In Chlorophyta and Euglenophyta a band of 4–6 thylakoids predominates.

Except for Chlorophyta and Rhodophyta, the chromatophores of most algae are enclosed in a characteristic sheath of endoplasmic reticulum lying external to the double-membraned envelope of the plastid.

The chloroplasts of most algae have electron-translucent areas contain-

ing DNA fibrils. In algae with a girdle band, e.g., *Ochromonas*, the plastid DNA is localized in a peripheral circular nucleoid inside the chromatophore but in algae without girdle bands, in general, the DNA areas are randomly scattered in the chromatophore matrix. Another characteristic feature of the chloroplast which is of some taxonomic and phylogenetic significance concerns the site of location and storage of starch grains. In the green algae starch grains are stored within the chloroplast; in dinoflagellates and red algae, in the cytoplasm outside the chromatophores; but in the Cryptophyta they are stored between the wall of the chloroplast and the sac of endoplasmic reticulum present around the plastid.

In all algae studied so far, plastid DNA has been found to have a lower guanine + cytosine content, a lower melting point, and a lower buoyant density than nuclear DNA. This is in sharp contrast to the situation in higher plants in which the chloroplast DNA has higher values of these parameters than nuclear DNA.

Chloroplasts or chromatophores are genetically semi-autonomous systems containing histone-free DNA (deoxyribonucleic acid), messenger RNA (ribonucleic acid), transfer RNA and ribosomes of bacterial type. Besides, new chloroplasts are always observed to arise from preexisting ones. These features have made it highly probable that chloroplasts are subcellular symbionts which had their origin in free living photosynthetic procaryotes (see Raven 1970).

PYRENOIDS

These are organelles composed of densely packed proteinaceous fibrils and are found lying within or on the surface of the chromatophore. Their function is to accumulate starch in green algae, or to concentrate some similar food reserve in other algae.

A chromatophore may contain a single pyrenoid as in *Chlamydomonas* or many as in *Oedogonium*. The pyrenoids have been demonstrated in almost all algal phyla except Cyanophyta. They may arise *de novo* or by the division of preexisting pyrenoids. In Chlorophyta the pyrenoid is located as a dense mass in the chloroplast, and a layer of starch plates encircles its proteinaceous core; in most other algae the pyrenoids do not seem to be associated with reserve food. Except in Phaeophyta, pyrenoids are invariably traversed by a variable number of chromatophore lamellae (Fig. 2-4).

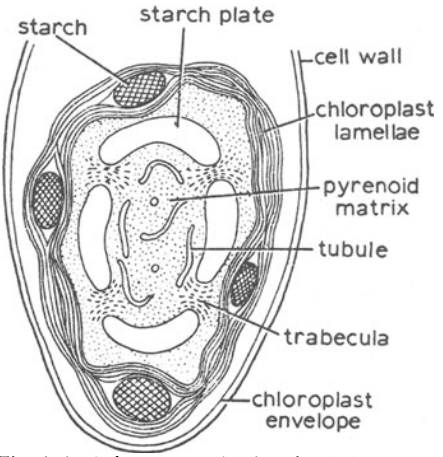

Fig. 2-4. Diagrammatic sketch of electron micrograph of pyrenoid of *Chlamydomonas*.

In many algae the pyrenoid is a transient structure which may be formed at certain stages but may disappear at other stages—the presence or absence possibly depending on photosynthesis and the status of reserve foods in a cell.

NUCLEI

A membrane-bounded genetic apparatus consisting of typical chromosomes and nucleoli is known as a nucleus and in this respect all algae except Cyanophyta and dinoflagellates are similar. In Cyanophyta the chromatin material consists of DNA fibrils which are not associated with basic proteins (histones) so that no organized chromosomes are found. The nuclear membrane is also lacking in the blue-green algae. In dinoflagellates there is a nuclear membrane but there are no organized chromosomes.

The nucleus is bound by two unit membranes of which the outer is continuous with the membranes of the endoplasmic reticulum. The nuclear envelope is traversed by numerous pores. In Xanthophyta, Chrysophyta, Bacillariophyta and Phaeophyta the nuclear envelope is intimately associated with a sac of the endoplasmic reticulum found around the chromatophores but no such plastid endoplasmic reticulum is met with in Chlorophyta and Rhodophyta (Leedale, 1970). The interphase nuclei of most eucaryotic algae contain uncoiled and expanded chromosomes but in Euglenophyta and Dinophyta the chromosomes, which lack centromeres, remain condensed. According to Leedale (1970), persistence of nuclear wall during mitosis, attachment of chromosomes to nuclear wall, lack of centromeres, and absence of a compact spindle are primitive features.

The cells of most algae are uninucleate but those of Cladophorales, Siphonales, Heterosiphonales and Charales are multinucleate or **coenocytic**. The position, size and shape of the nuclei is quite variable.

The nucleoli and chromosomes remain suspended in the granular matrix of the nucleus. In general, there is a single nucleolus per nucleus but in Conjugales there may be several nucleoli in a nucleus.

In Euglenophyta the nuclear membrane and nucleolus persist throughout the division cycle whereas in other algae the nucleoli break up during division and are reorganized after the completion of the nuclear division by a **nucleolar organizing chromosome**. In some species of *Spirogyra* and *Zygnema* the nucleolar organizing chromosomes are associated with **satellite chromosomes**. The nuclear division may or may not be accompanied by wall formation. In Conjugales the chromosomes have **diffused centromeres**.

Recently, the characteristic structures called **synaptonemal complexes** have been observed in the meiotic nuclei of certain red and brown algae. Under the electron microscope, a synaptonemal complex appears to consist of two lateral longitudinal rods which give rise transversely to minute fibrils extending inward. Each such complex is thought to correspond to a bivalent. The existence of synaptonemal complexes is the most reliable evidence for meiosis not only in algae but also in higher plants.

The nuclei of some euglenoids harbour certain bacteria but the biological nature of such an association is not known.

GOLGI APPARATUS

Structurally, the Golgi apparatus is an association of stacks of smooth lamellae or cisternae in which each stack is referred to as a **dictyosome** and consists of 2–20 cisternae. In cells having a single dictyosome, the Golgi apparatus is equivalent to one dictyosome. Excepting Cyanophyta dictyosomes occur in most other algae. They may be located near the chromatophore, the flagellar base or the nucleus.

In other organisms the Golgi bodies have been implicated in excretory functions but their precise role in algae has not been conclusively established. Dictyosomes are involved in the formation of the scales in some green algae and Chrysophyceae (including Haptophyceae and Coccolithophorids) and also in the formation of the cellulosic wall component in Chrysophyceae.

SEPTA

The cross walls between adjacent cells of a **filamentous** or **parenchymatous** thallus are known as septa, and each consists of a central core, the middle lamella and a peripheral portion—the primary wall. Cell division usually takes place by transverse furrowing of the cytoplasm midway between the two extremities of a cell. Within the furrow a transverse wall of pectic substance known as the middle lamella is first laid down, over which the subsequent apposition layers (primary wall) consisting chiefly of cellulose, are deposited.

The septa may be complete or incomplete. The former are mainly of five types: (1) **plane**, (2) **replicate**, (3) **semireplicate**, (4) **colligate**, and (5) **unduliseptate** (Fig. 2-5 A–E). The plane type has a homogeneous contour as in *Ulothrix*. In some species of *Spirogyra* the middle lamella shows circular infoldings. If each of the adjoining cells has two infolds in opposite directions then the septum is called replicate, and if there is only a single infold in alternate positions, it is known as semireplicate. The colligate septum is an H-shaped cross wall as seen in *Microspora*. In the unduliseptate type, the middle lamella is much expanded and has a wavy, undulating margin, as in *Spirogyra undulisepta*.

The incomplete septum as found in most Rhodophyta and some Cyanophyta has a central opening known as a pit through which the cytoplasm of adjacent cells remains connected (Fig. 2-5 F–H). In *Sphaeroplea* (Chlorophyta) the septa appear to be composed of radiating ingrowths of side walls which fail to meet in the centre and form a central opening (Fig. 2-5 G). During cell division in *Oedogonium*, a septum is laid down across the middle of the cell and remains unconnected with the side walls (i.e., free-floating) for some time.

Except for the plane septa, the other types are thought to assist in the

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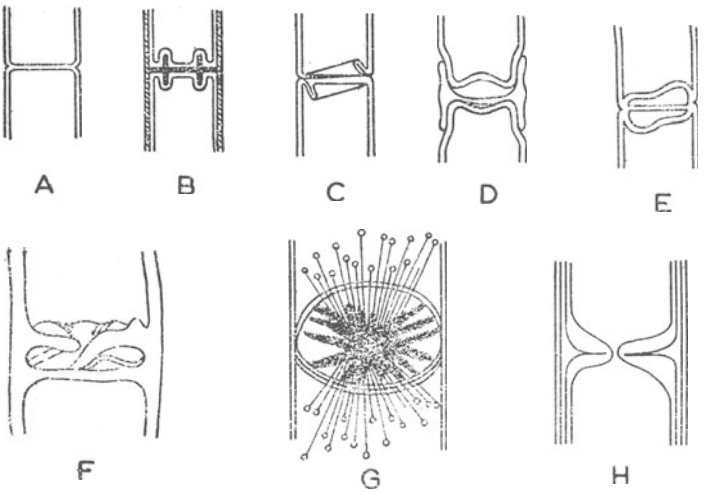

Fig. 2-5. Types of septa. A, plane; B, replicate; C, semireplicate; D, colligate; E, unduliseptate; F, *Sphaeroplea*, thick septum showing finger-shaped processes; G, *Sphaeroplea*, septum in surface view showing central opening; H, a pitted septum. (Figs. A–E after Randhawa, 1959; F–H after Fritsch, 1935.)

fragmentation of the filaments. An alteration in the turgor pressure inside the cell leads to the development of a shearing strain in the cross wall which finally ruptures, resulting in fragmentation of the filament.

MISCELLANEOUS STRUCTURES AND CELLULAR ORGANELLES

Stigma

The motile, flagellated cells of algae have a pigmented organelle known as the **stigma** or eye spot, which has been accepted as a light-sensitive photoreceptor organ. However, certain mutant strains of *Chlamydomonas* lacking eye spot still respond to light, this has cast some doubt on its photosensitive nature. The stigma appears as a reddish streak or dot in the anterior, median or posterior region of the cytoplasm. It is found in the coloured (photosynthetic) as well as colourless motile cells. It is a collection of separately membraned lipid droplets arranged randomly in *Euglena*, or in rows of two in *Chlamydomonas*, with the space in between containing a pair of thylakoids (Fig. 2-6 A). In the pigmented species it is often located near the edge of the chloroplast or the base of the flagellum. In the spermatozoid of *Fucus* it lies outside the chromatophore and is unique in having a pigment layer composed of a large number of chambers. The orange-red pigments of the eye spot are present in the lipid droplets.

Comparative studies have demonstrated that in *Chlamydomonas* and other green algae the eye spot is located on the chloroplast whereas in euglenoids it is found in the cytoplasm outside the chloroplast.

During cell division the eye spot may divide into two, each daughter cell receiving an eye spot. Or, the eye spot of the parent cell may go to one of the daughter cells whereas in the other it arises *de novo*.

Vacuole

Except in Cyanophyta, the mature cells of algae characteristically possess one or more **vacuoles** bound by distinct membranes. Such vacuoles play an important role in osmotic relations and absorption or regulation of solutes and water.

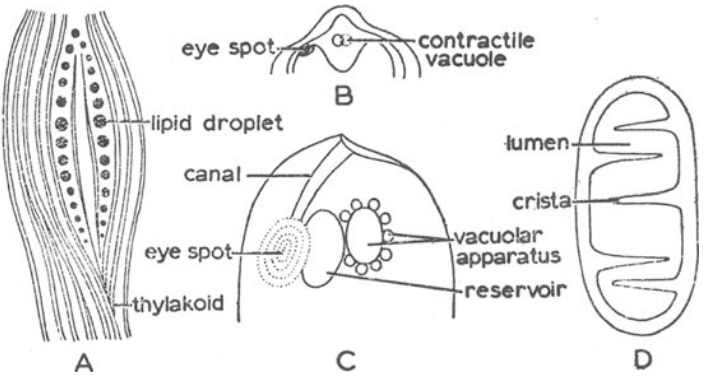

Fig. 2-6. A, *Chlamydomonas*, stigma with two rows of lipid droplets and a pair of thylakoids in between; B, *Chlamydomonas*, upper part of cell with contractile vacuoles and stigma; C, upper part of a euglenoid cell showing a canal (cytopharynx), reservoir, vacuolar apparatus and stigma (after Fritsch, 1935); D, mitochondrion with the inner membrane infolded into cristae which project into the lumen.

In motile algae, two types of **vacuolar apparatus** are recognized: (1) the simple vacuoles, known as contractile vacuoles (Fig. 2-6 B), which contract periodically and expel their contents to the exterior as in Chlorophyta; and (2) the complex vacuolar apparatus which consists of a cytopharynx (canal), a reservoir, and a group of vacuoles of varying size (Fig. 2-6 C) as is characteristic of the Euglenophyta, Dinophyceae and some members of the Chrysophyta. All these parts work in close coordination: the smaller vacuoles feed their contents into larger one which then empties and releases the contents into the reservoir. Periodical contraction of the reservoir then expels the contents to the outside through cytopharynx.

The vacuolar system is generally considered as an organ of osmoregulation involved in pumping liquids out of the cytoplasm against an osmotic gradient. In some holozoic algae the cytopharynx functions as a gullet (for ingesting food particles) besides being an emptying canal for the vacuoles. Sometimes food reserves such as laminarin and chrysolaminarin are also stored in the vacuole.

Mitochondrion

Except in Cyanophyta, the algal cells invariably contain mitochondria, the

organelles of respiratory metabolism. Under the electron microscope they appear to be bound by a two-layered membrane, the inner being infolded into finger-like processes which project into the cavity (Fig. 2-6 D). The size and number of mitochondria per cell vary widely and except for a very few species (e.g., Fig. 2-1 B) most algae have more than one mitochondrion per cell. Mitochondria are independently multiplying cytoplasmic organelles having DNA, RNA and ribosomes of bacterial type. Like chloroplasts, they are also considered to have originated from free living heterotrophic procaryotes (see Raven 1970).

Endoplasmic Reticulum

A system of tubules and vesicles traversing the cytoplasm and termed as the **endoplasmic reticulum** is known to exist in algal cells except in the blue-green algae. These tubules appear to have some particles (believed to be ribosomes) disposed along their surface. The function of these particles is to synthesize proteins or enzymes. Two different kinds of ribosomes, differing in their physical properties and location, have been demonstrated in *Chlamydomonas*. The 70S ribosomes, characteristic of procaryotic cells, are found in the chloroplast whereas the 80S ribosomes, typical of eucaryotic cells, occur in the cytoplasm. The existence of the procaryotic type of ribosomes in chloroplasts of *Chlamydomonas* indicates that the plant chloroplast might be of the nature of an intracellular symbiont derived from ancestral unicellular photosynthetic procaryotes (see Raven 1970).

Granules

Diverse kinds of granules such as **polyhedral bodies**, structured granules, **cyanophycin granules**, **α-granules** and **β-granules** are found within the blue-green algal cell. The exact function of most of these granular inclusions is not clearly understood.

Cuticle

Studies on the organization and chemistry of the cuticle of algae belonging to Phaeophyta, Rhodophyta and Chlorophyta have shown that it contains much protein and consists of several alternate microfibrillar and amorphous layers. In this respect the algal cuticle differs from the cuticle of higher plants which lacks the protein components (Haine and Craigie, 1969).

RANGE OF VEGETATIVE STRUCTURE AND HABIT

The organization of thallus in algae is basically of two types, the unicellular or the multicellular. The enormous range of forms that algae exhibit arises from a modification or elaboration of these types. The cells of a multicellular thallus may be loosely held together by a mucilaginous matrix or cemented together through middle lamellae. During its growth cycle an alga passes through two phases, the **vegetative** or **developmental**, and **reproductive** or **multiplicative**. In unicellular algae the growth in size constitutes

the vegetative phase whereas increase in cell numbers by cell division constitutes the reproductive phase. In multicellular thalli, both the vegetative and the reproductive phases involve cell division and cell growth.

UNICELLULAR HABIT

All phyla except Phaeophyta include unicellular forms which may be motile or non-motile. The motile type is of two kinds, the flagellated kind moving by means of flagella (found in all phyla except Cyanophyta and Rhodophyta), and the **rhizopodial** kind having the fine protoplasmic projections (the **rhizopodia**) and showing an amoeboid movement (Xanthophyta and certain other algae; Fig. 2-7 A). The cell envelope of the rhizopodial forms is characteristically periplastic and rather soft, thus permitting extensive changes in shape and size of the thallus. The flagellated forms may be periplastic, e.g., *Euglena* (Fig. 2-7 B), or may be provided with a definite

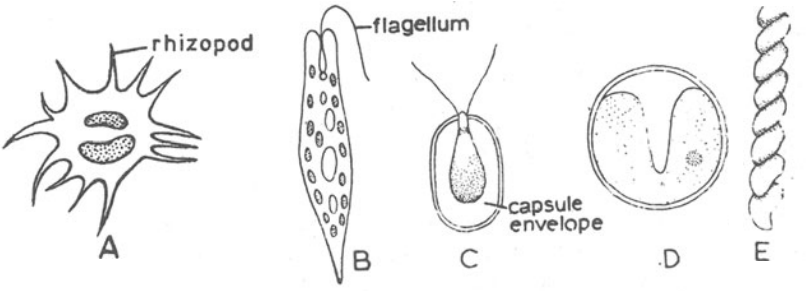

Fig. 2-7. A, *Chrysamoeba*, motile rhizopodial unicell; B, *Euglena*, motile flagellated unicell; C, *Phacotus*, motile encapsuled unicell; D, *Chlorella*, non-motile, coccoid unicell; E, *Spirulina*, spiral filamentous unicell. (Figs. A, C and E after Fritsch, 1935.)

cell wall, e.g., *Chlamydomonas*. External to periplast, some flagellates have a special envelope which is provided with pores for the protrusion of the flagella. The calcareous envelope is separated from the cell proper by a space (Fig. 2-7 C). Such forms are known as the **encapsulated** forms. In some the capsule may become calcareous.

The non-motile or **coccoid** type includes forms of diverse shape and size. They are provided with a rigid cell wall and are non-flagellated. Some typical examples are *Chlorella* (Chlorophyta; Fig. 2-7 D) and *Synechocystis* (Cyanophyta). In *Spirulina* (Cyanophyta) the cell is elongated into a helical filament (Fig. 2-7 E).

MULTICELLULAR HABIT

Depending on the manner in which cells are produced and arranged during the vegetative phase, three principal types of habit are recognized—**colonial,** filamentous and **siphoneous.**

Colonial Habit

A colony is a group of separate cells generally similar in structure and function and aggregated together by a mucilaginous envelope. On the basis of morphology there are four main types of colonial organization—**coenobial, palmelloid, dendroid** and rhizopodial. A **coenobium** has a definite number of cells arranged in a particular manner which is determined at the juvenile stage and does not increase during its subsequent growth even though the cells enlarge. The coenobium may be motile or non-motile. In the motile kind the cells are flagellated, e.g., *Volvox* (Fig. 2-8 A). In the non-motile type the cells are coccoid and more or less fused together, e.g., *Hydrodictyon* (Fig. 2-8 B).

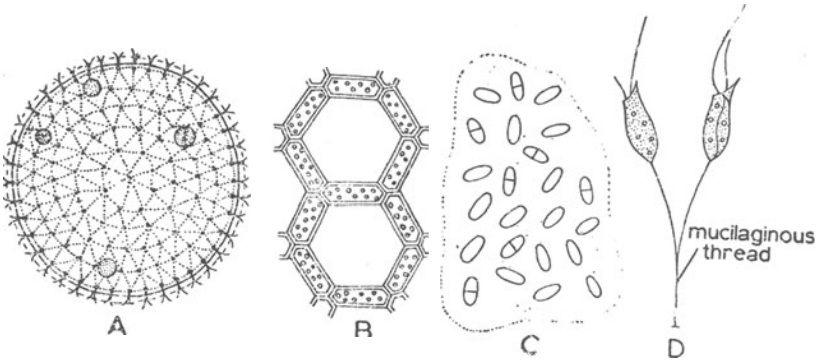

Fig. 2-8. A, *Volvox*, showing a flagellated motile coenobial colony (after Smith, 1955); B, *Hydrodictyon*, non-motile colony (coenobium); C, *Aphanothece*, palmelloid colony; D, *Chrysodendron*, dendroid colony (after Fritsch, 1935).

Unlike the coenobium, the palmelloid, dendroid and rhizopodial types of colony are not of constant shape and size and their cells divide leading to increase in the size of the colony. In palmelloid colonies, the cells remain embedded in a mucilaginous matrix of irregular shape and size (Fig. 2-8 C). The matrix is formed from the walls of individual daughter cells which gelatinize and mix together with the gelatinized walls of daughter cells embedded in the common matrix. Some examples of palmelloid habit are met with in *Aphanothece* (Cyanophyta) and *Tetraspora* (Chlorophyta).

In the dendroid colonies the cells are united in a branching manner by localized production of mucilage at the base of each cell (Fig. 2-8 D). The whole colony looks like a tree in habit. Such an organization is found in *Ecballocystis* (Chlorophyta) and *Chrysodendron* (Chrysophyta).

The cells of rhizopodial colonies are united through rhizopodia, as in *Chrysidiastrum* (Chrysophyta).

FILAMENTOUS HABIT

A uniseriate row of cells joined end to end in a transverse plane through middle lamellae constitutes a filament. Such a habit arises as a result of vegetative divisions being restricted to the transverse plane. In Cyanophyta

a filament includes both the trichome (i.e., the uniseriate row of cells) and its sheath. The filaments may be unbranched or branched. The unbranched filaments of *Ulothrix* (Chlorophyta) and *Anabaena* (Cyanophyta) do not show any polarity but those of the Rivulariaceae (Cyanophyta) exhibit well-marked polarity (Fig. 2-9 A, B).

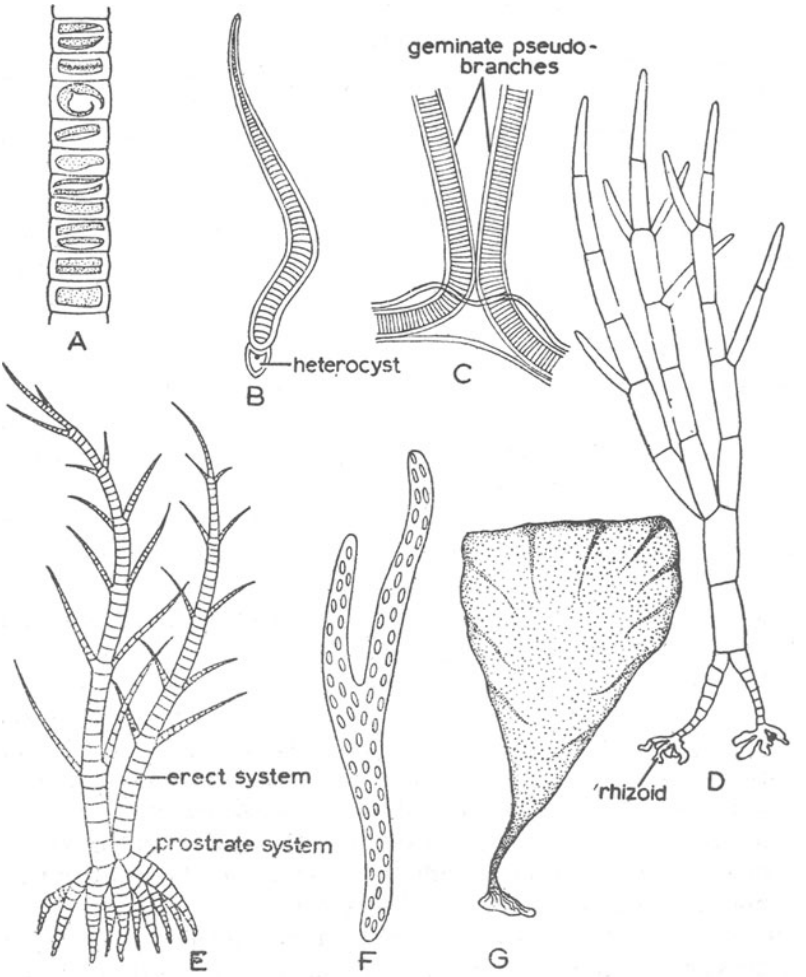

Fig. 2-9. A, *Ulothrix*, simple filamentous habit; B, *Calothrix*, polar filamentous habit; C, *Scytonema*, pseudobranching; D, *Cladophora*, true branching; E, *Stigeoclonium*, heterotrichous, branched filament; F, *Vaucheria*, branched siphoneous habit; G, *Ulva*, parenchymatous habit. (Figs. B and D after Fritsch, 1935.)

The branching of the filaments is of two kinds—false and true. In **false branching**, which occurs in the Scytonemataceae (Cyanophyta), the trichome generally fragments due to the degeneration of an intercalary cell (or by the formation of biconcave separation discs) after which one or both of its ends adjacent to the dead cell grow out of the parent sheath, giving the semblance of branching (Fig. 2-9 C). Sometimes false branching may arise through

loop-formation. In this method, a group of intercalary cells of the trichome becomes meristematic and for a time continues to divide at two localized points, leading to the formation of a loop. The top (middle) cell of the loop finally degenerates setting free the two arms of the loop as a pair of pseudobranches.

True branching results from repeated transverse divisions of the lateral outgrowths produced by a few or many scattered cells of the main filament. The truly branched thalli are of four types: (1) simple branched filament, e.g., *Cladophora* (Chlorophyta; Fig. 2-9 D); (2) **heterotrichous** in which the thallus is differentiated into an erect and a prostrate system of branched filaments (Fig. 2-9 E); (3) parenchymatous; and (4) **pseudoparenchymatous** in which the thalli show **uniaxial** or **multiaxial** construction.

The heterotrichous habit is the most highly evolved type of filamentous construction in algae. During its development a system of branched creeping filaments is produced first and functions as the holdfast. These creeping filaments give rise to a system of erect branched filaments. More or less equally well-developed prostrate and erect systems are found in *Stigeoclonium* (Chlorophyta) and *Ectocarpus* (Phaeophyta). The more advanced heterotrichous thalli show a progressive reduction or elimination of either the prostrate system (e.g., *Draparnaldiopsis*) or the erect system (e.g., *Coleochaete scutata*).

The parenchymatous habit is a further variant of the filamentous construction and results from vegetative divisions taking place in more than one plane followed by the failure of the division products to separate. Examples are met with in *Ulva* (Fig. 2-9 G) and *Prasiola* (Chlorophyta) and *Porphyra* (Rhodophyta).

The pseudoparenchymatous habit results from a close juxtaposition of the branched filaments of a single or many axial filaments. If branches from a single filament are involved, as in *Batrachospermum*, the thallus construction is spoken of as uniaxial. If branches of many axial filaments aggregate together, the thallus is multiaxial, e.g., *Nemalion.*

The vegetative growth by cell division of filamentous and parenchymatous thalli occurs in four ways—**diffuse, intercalary, trichothallic** and apical. In simple filamentous forms like *Ulothrix* and *Nostoc*, the growth of the thallus is diffuse because each vegetative cell is potentially capable of growth and division. A good example of intercalary growth is furnished by the Laminariales (Phaeophyta) in which the growth of the thallus is brought about by the activity of a meristem located at the junction of the **stipe** and the blade. The trichothallic growth is also intercalary but the meristem is located at the base of a terminal hair, e.g., in Rivulariaceae, and Cutleriales. The thalli of the Charophyceae, Dictyotales and certain other algae grow by the activity of a single or a group of apical cells.

SIPHONEOUS HABIT

A siphoneous thallus is multinucleate and lacks septation except during the

formation of reproductive organs. The simplest form of such an organization is found in *Protosiphon* (Chlorophyta) and *Botrydium* (Xanthophyta), both having a vesicular, unicellular thallus attached to the substratum by a simple or branched rhizoid. In *Vaucheria* (Fig. 2-9 F), the siphoneous filament is branched, advanced members of the Siphonales (Chlorophyta) show uniaxial and multiaxial forms.

TEST QUESTIONS

1. Would you regard the cell wall as an essential part of an algal cell? Explain.

2. Which of the following parts of an algal cell is alive: cell wall, protoplasm, vacuole?

3. Differentiate between a chloroplast and a chromatophore and justify the suggestion that variation in the external morphology of chromatophores is of little significance in the phyletic classification of algae.

4. Discuss the statement that electron microscopy has in no way altered the primary basis of classification of algae as previously emphasized by light microscopy.

5. What arguments can you offer in favour of the view that unicellular flagellates are the most primitive forms of green algae?

6. Why is the motile colonial habit considered to have ended with a *Volvox*-like form?

7. On what grounds is the heterotrichous habit considered to represent the most highly evolved type of filamentous organization in algae? What light does this habit throw on the origin of land plants?

8. Even though the basic structural unit of photosynthesis is the same in both algae and higher plants, yet the chromatophore organization in the former is considered more primitive than in the latter. Why?

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3

Reproduction and Perennation

REPRODUCTION

It is a process by which a living organism propagates and multiplies, and increases the number of its individuals. Being devoid of the capacity for active locomotion, most plants depend on dispersal of reproductive bodies for movement from one favourable habitat to another. The algae generally reproduce in three ways—vegetative, asexual and sexual.

Vegetative propagation does not involve rejuvenation of the protoplasts, and the cell walls of its reproductive units are either entirely parental, or partly parental and partly new. Asexual reproduction, however, involves rejuvenation of the protoplasts, and the reproductive bodies are either naked or provided with a newly synthesized wall of their own. Further, asexual reproduction is accomplished by using specially differentiated cells that are capable of directly multiplying the alga. Both the vegetative and asexual reproduction are accessory means of propagation and are not concerned with bringing together in one cell lineage the genes from different cell lineages.

In contrast to vegetative propagation and asexual reproduction, the sexual reproduction involves alternation of syngamy (fusion of male and female gametes) and karyogamy (fusion of gametic nuclei) on the one hand, and meiosis on the other. This leads to the creation of new combinations of genes by pooling together in one line of descent the genes derived from two different parents, thus resulting in a reshuffling of the gene material. The **recombinants** so produced can then be subjected to natural selection. In procaryotic organisms recombinants are formed by such processes as **conjugation, transformation** and **transduction,** none of which involves true karyogamy and meiosis. This genetic recombination in bacteria and blue-green algae accordingly falls short of the true and complete definition of sexuality in the sense adopted here, even though it fulfils the same function as true sexuality.

VEGETATIVE PROPAGATION

Vegetative propagation is very common in algae and takes place in a variety of ways. In unicellular algae, flagellates, desmids and diatoms, a cell may divide into two halves to produce new individuals. In multicellular forms the thallus often breaks into small fragments each of which then grows to become a new plant. *Dictyosphaerium* and *Aphanothece* multiply by splitting

of their colonies. Proliferation of filamentous thalli involves two methods—fragmentation, and **hormogone** formation. The fragmentation of filaments may be either due to mechanical pressure exerted on them, or by dissolution of the cross walls because of the development of shearing strains by differences in turgor pressure between adjoining cells. In such algae as *Stichococcus*, the tendency to fragment is so pronounced that filaments longer than a few cells each are seldom found.

Filamentous blue-green algae propagate vegetatively through a specialized process known as hormogone formation. The hormogones, which are short fragments of a trichome, are produced either as a result of fragmentation, or by the formation of biconcave, intercalary separation discs spaced quite a few cells apart from each other. The vegetative cells lying between two separation discs constitute a hormogone. Devoid of a sheath and endowed with the power of movement, the hormogones constitute an efficient and quick method of vegetative propagation and dispersal of the species during environmentally favourable growth periods.

Some algae reproduce by means of adventitious thalli as in *Dictyota*, or by the formation of a few-celled branches, known as **propagules**, which become detached from the parent and subsequently develop into new plants. In *Protosiphon*, vegetative propagation takes place by budding of the vesicle. In species of *Trentepohlia* cells of the prostrate system form a powdery mass which is dispersed by wind and can give rise to new plants.

ASEXUAL REPRODUCTION

Asexual reproduction involves the formation of one or more of the following types of spores: zoospores, **aplanospores, autospores, hypnospores, carpospores, tetraspores, endospores** and **exospores**. These may be formed in any vegetative cell (*Ulothrix*), or in specially differentiated cells called sporangia (*Trentepohlia, Ectocarpus* and *Dictyota*).

The zoospore which probably constitutes the most important kind of asexual spore, is a motile body equipped with one or more flagella and commonly contains a chromatophore, a nucleus and an eye spot. Aplanospores are non-flagellated, non-motile and have a definite cell wall distinct from the parent wall. If the aplanospore, while still within the parent wall, appears like the parent cell in all respects except size, it is spoken of as an autospore. The endospores and exospores of Cyanophyta are analogous to the aplanospores of Chlorophyta but not homologous because the term aplanospore indicates a derivation from the motile zoospores which are not met with in blue-green algae.

The zoospores may be biflagellate (e.g., *Ectocarpus*), quadriflagellate (e.g., macrozoospores of *Ulothrix*), or multiflagellate (e.g., *Oedogonium, Vaucheria*). During zoospore formation in *Oedogonium*, the entire protoplast assumes a pear shape and escapes from the cell and develops a ring of flagella around a colourless circular area towards its anterior end. In other algae, e.g., *Cladophora*, the protoplast of a cell may give rise to 8, 16, 32,

or more zoospores by undergoing repeated divisions either after the completion of all nuclear divisions (**simultaneous division**) or after each nuclear division (**successive division**).

Aplanospores are produced in *Ulothrix*, *Microspora* and certain other algae, and may be formed either from the entire protoplast of a cell, or a single cell may give rise to many aplanospores. Autospore formation occurs in many members of the Chlorococcales, e.g., *Chlorella*.

Endospores are produced by the division of the protoplast in three planes, e.g., *Pleurocapsa* and *Dermocarpa*. In exospore formation, which occurs in *Chamaesiphon*, the cell wall ruptures apically and the protoplast thus exposed successively abstricts spherical spores.

The asexual spores produced by meiotic division in the unilocular sporangium of *Ectocarpus*, in the tetrasporangium of *Polysiphonia*, and during germination of some zygotes are known as **meiospores** or **gonospores.** Such spores are unlike those produced by mitotic division (**mitospores**) in that they are haploid and are concerned with the perpetuation of only the haploid thalli. The mitospores may be either haploid or diploid and accordingly perpetuate the haploid or the diploid generation.

SEXUAL REPRODUCTION

Typical or conventional sexuality consisting of karyogamy and meiosis is brought about by a variety of methods invariably involving the union of the male and the female sexes. The reproductive units participating in the sexual fusion are variously named according to the morphology, behaviour, and the nature of the organs in which they are produced. The dictionary meaning of the term gamete is "a germ cell carrying with it the implication of either sex", and for sexual reproduction to occur, the gametes of opposite sexes must be present.

If the male and female gametes are flagellated and motile, they are termed **zoogametes**. **Aplanogametes** are non-flagellated and may show amoeboid movement. The antheridia and oogonia are the well differentiated gametangia producing, respectively, the male and female gametes which differ in morphology and behaviour. The gametes produced in antheridia are the flagellated, motile **antherozoids** (or spermatozoids), whereas the gamete produced in the oogonium is the non-motile, non-flagellated egg or **ovum.** In Rhodophyta the oogonium having a swollen base (containing the egg) and a slender projecting neck, the trichogyne, is called a **carpogonium**, and the non-motile, non-flagellate male gamete is termed **spermatium**, the structure producing it being known as a spermatangium.

True sexuality has been established for most members of the Chlorophyta, Phaeophyta and Rhodophyta and for some members of the Xanthophyta, Bacillariophyta, Pyrrophyta and Chrysophyta. In the Cyanophyta, true sexuality in the sense of karyogamy and meiosis is lacking but a **parasexual** phenomenon involving recombination of genes in a single line of descent from two genetically distinct parents has been reported in three

different genera—*Anacystis, Cylindrospermum* and *Anabaena.*

In unicellular forms, sexual fusion may take place between male and female (or minus and plus) gametes produced either by division of protoplasts of the parent cells, or by fusion of the mature individuals themselves. The latter phenomenon, known as **hologamy**, occurs in a few species of *Chlamydomonas.* Besides, three other modes of sexual reproduction based on the morphology and behaviour of the mating gametes are known. These are **isogamy, anisogamy** and **oogamy.** In isogamy, the gametes of a fusing pair are morphologically as well as physiologically alike and are distinguishable genetically as plus or minus mating types. In anisogamy the gametes differ in size and motility, the smaller, more active gametes being male and the larger, less active being female. In oogamy, the gametes of the two sexes are sharply differentiated, and with a few exceptions are produced in specialized organs known as the antheridium and the oogonium. Both in isogamy and anisogamy, the gametes of opposite sexes mate outside the parent cells except in the Conjugales in which mating is facilitated by the formation of a conjugation tube and may occur either within the conjugation canal itself, e.g., *Zygnema,* or in the cell containing the female (plus) gamete, in which case the male gamete moves to the female through the conjugation tube, e.g., *Spirogyra.* In the majority of oogamous green algae the egg is not liberated from the oogonium and fertilization takes place inside the oogonium.

Isogamy, anisogamy and oogamy are stages of a progressive series in the differentiation of gametes and this series seems to have evolved independently in different phyla. Thus, the Chlorophyta, Phaeophyta, Xanthophyta and Bacillariophyta include forms ranging from isogamy through anisogamy to oogamy. However, in diatoms isogamy is secondary and oogamy is primitive. The members of Rhodophyta are characteristically oogamous. In the Bacillariophyta, only those forms as show a progressive decrease in cell size during vegetative multiplication undergo sexual reproduction; and the formation of **auxospores** from the zygote serves as a device to offset reduction in cell size resulting from continued vegetative propagation.

Sexually reproducing thalli may be **homothallic (monoecious)** showing fusion between gametes derived from a single parent individual, or **heterothallic (dioecious)** permitting fusion between gametes of different parentage.

ORIGIN OF SEX

The origin of sex is inherently related to the origin of gametes which are thought to have been derived from asexual zoospores that had become too small or too weak to produce healthy new plants by themselves (Fig. 3-1). In forms such as *Chlamydomonas* and *Ulothrix* with isogamous sexual reproduction, the gametes are very much like the zoospores except for the difference in size. In the isogamous algae, both plus and minus gametes resemble the zoospores whereas in the anisogamous or oogamous species, only the motile gametes (antherozoids) show this resemblance. The zoo-

spores and gametes differ fundamentally in the way they give rise to new plants: the former germinate directly into new plants whereas the latter

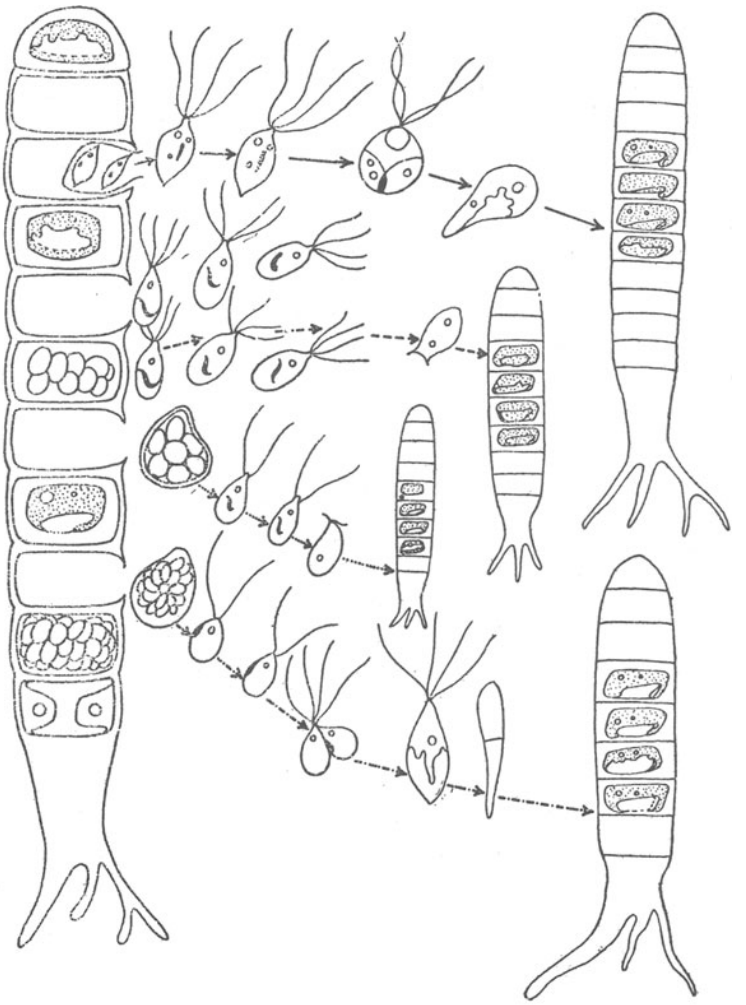

Fig. 3-1. Hypothetical diagram illustrating how sex might have originated in such forms as *Ulothrix*. Note the four types of swarmers (indicated by different kinds of arrows) and their fate, and also the relative sizes of the germlings produced.

copulate in pairs to form the zygote whose division products ultimately develop into new plants. How a mere variation in size can account for such a fundamental difference in their behaviour is the basic question. The larger zoospores contain the minimum level of factors that help them grow individually into new plants. Comparatively, the smaller zoospores are not self-sufficient and therefore fail to grow. The chance fusion of such

zoospores in pairs is considered to restore the factors to optimum level for subsequent development. The precise nature of the factors is not known but it is likely that chemical, hormonal or electrical components may be involved.

The difference in size of the larger and weaker zoospores which can potentially act as gametes, results from the number of divisions the parent protoplast undergoes. If this number is small, the products are large and behave as zoospores, and if the number is large, the resulting cells are small and behave as gametes. This view is supported by the observation that under certain conditions if the gametes fail to copulate they can act like zoospores. All these facts indicate that the gametes are derived from zoospores and that sex in algae has originated as a result of accidental fusions between small-sized, weaker zoospores.

The origin of gametes (and hence the sex) from zoospores is a hypothesis developed mostly from observations made on materials collected from nature and it has yet to receive conclusive experimental support. To our knowledge, neither genetical nor physiological factors that might be involved in regulating the zoospore-gamete balance sheet have been experimentally verified.

The mechanism of sexuality has been investigated in detail mostly in the genus *Chlamydomonas*, especially in heterothallic and isogamous species in which sex is determined genotypically. In these species, gametogenesis is induced by a variety of environmental factors such as light, temperature, nitrogen content, and pH. The mating reaction between gametes proceeds through a number of distinct phases: (1) morphological and physiological differentiation leading to gamete formation, (2) clumping, (3) pairing, (4) cell fusion, and (5) nuclear fusion. Recent work on mutants of *Chlamydomonas* lacking flagella has indicated that the mating type or sexual substances are located on the flagellar tips of the isogametes. The mating reaction between such gametes is initiated by the agglutination of their flagellar tips and then gradually proceeds downward till the bodies of the two gametes finally fuse.

LIFE CYCLES AND ALTERNATION OF GENERATIONS

The zygote is a diploid cell resulting from gametic fusion and is provided with a thin or thick wall. Thin-walled zygotes often germinate directly, without undergoing any resting period, as in most marine algae. Thick-walled zygotes generally have their walls differentiated into three layers and such zygotes (zygospores or oospores) normally remain dormant for a while before germination. Both karyogamy and meiosis may take place in the zygote which on germination produces a haploid plant. If these processes become separated in time and space, the zygote merely remains a place for karyogamy and its germination gives rise to a diploid plant. It is during sporogenesis by the diploid plant that meiosis occurs. The interpolation of a diploid somatic phase between karyogamy and meiosis probably makes

the individuals more stable, genetically and physiologically, with the consequent attainment of greater adaptability and survival.

The growth and development of an alga proceeds through a number of distinct morphological and cytological stages. The sequence of these orderly changes is termed life cycle or life history. Accordingly, a life cycle has two aspects, the somatic or morphological, and the cytological or chromosomal. A generation is regarded as a somatic phase; the alternation of generations refers to a situation in which a plant has two somatic phases: (1) the haploid or gametophytic, and (2) the diploid or sporophytic, which regularly alternate during the life cycle. Both morphological and cytological considerations have led to the recognition of four main types of algal life cycle: haplontic, diplontic, **isomorphic** and **heteromorphic.** The haplontic type includes algae in which the somatic phase only is haploid and the diploid stage is restricted to the zygote whereas in the diplontic type the vegetative phase only is diploid and the haploid phase is restricted to the gametes. In between these two types are those life cycles in which a regular alternation of gametophytic and sporophytic generations occurs. If the two alternating somatic phases of the life cycle are morphologically alike, the life cycle is termed isomorphic and if the two phases differ morphologically, it is termed heteromorphic.

The haplobiontic haplontic life cycle is found in such algae as *Chlamydomonas*, *Ulothrix*, *Oedogonium* (Fig. 3-2) and *Chara*. The diplontic type is characteristic of *Chlorochytrium*, most Siphonales, Fucales (Fig. 3-3) and Bacillariophyta. Most Nemalionales (Fig. 3-3) have a haplobiontic life cycle in which the haploid gameophytic phase alternates with a haploid asexual carposporophytic phase; the life cycle in this case is biphasic with two morphologically dissimilar, but cytologically similar, generations and the only diploid stage in the life cycle is constituted by the zygote itself. The isomorphic life cycle occurs in Ulvaceae, *Stigeoclonium*, *Draparnaldiopsis* and *Ectocarpus* (Fig. 3-4) and the heteromorphic type is characteristic of the Laminariales (Fig. 3-4). The life cycle of the Florideae is complicated by the interpolation of a parasitic **carposporophyte** between the gametophytic and sporophytic generations. The carposporophyte is a carpospore-producing sporophyte consisting of a cluster of sterile cells and carposporangia which are borne terminally on special filaments called **gonimoblasts.** In more advanced members of the Florideae, e.g., *Polysiphonia* (Fig. 3-5), the life cycle is triphasic, involving a succession of three generations: gametophytic, carposporophytic, and tetrasporophytic. The carposporophyte and the tetrasporophyte are diploid and the latter is morphologically similar to the gametophytic plant.

According to Von Stotsch (personal communication), most probably there are no haplobiontic Nemalionales at all and even *Batrachospermum* seems to lack zygotic meiosis.

Drew (1955), and Chapman and Chapman (1961) have reviewed the algal life cycles, introducing complex terminologies. According to the nuclear cycle a **haplont** is one in which the zygote is diploid and meiosis

takes place during the germination of the zygote; a **diplont** is one in which the plant is diploid and the gametes alone are haploid. A haplobiont possesses only one kind of plant (a haploid or a diploid) whereas a diplo-

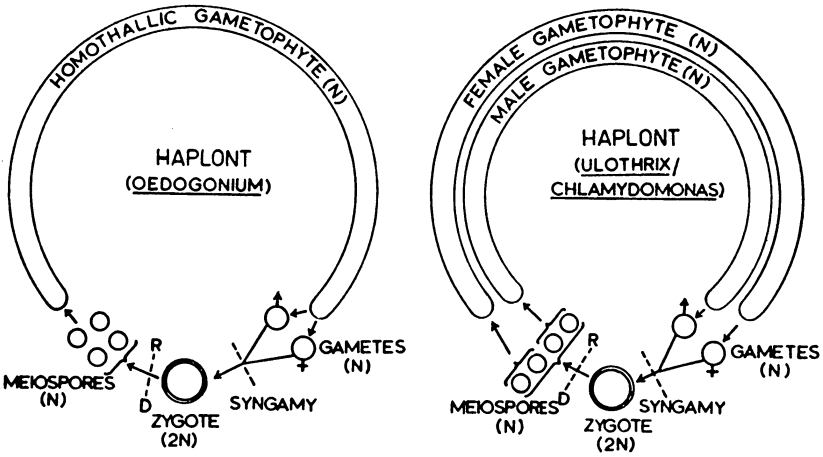

Fig. 3-2. Haplobiontic life cycles.

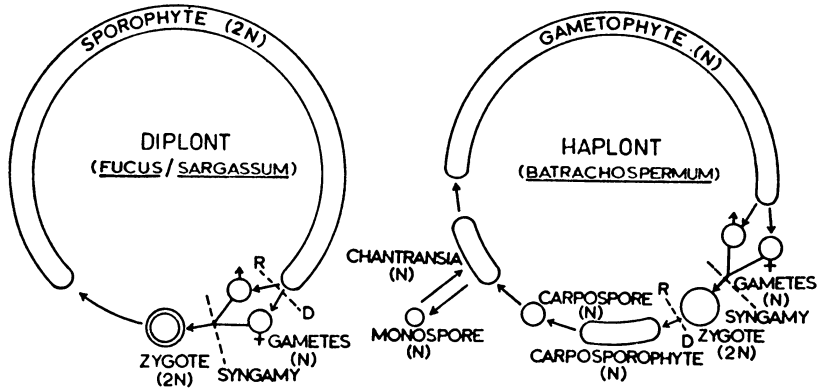

Fig. 3-3. Types of life cycle.

biont possesses both the haploid and the diploid plants in its life cycle. These workers favour the idea that an algal life cycle should be qualified both cytologically and morphologically. Based on morphology, the life cycle can be of three types: (1) the **monomorphic**, in which the gametophytic and sporophytic generations are morphologically alike, (2) the **dimorphic**, in which the two generations are morphologically different, and (3) the trimorphic, in which a succession of three morphological types occurs. In Drew's terminology, the life cycle of *Ectocarpus* and *Cladophora*

is monomorphic, **diplohaplont** and that of the Nemalionales like *Batrachospermum* trimorphic, haplont.

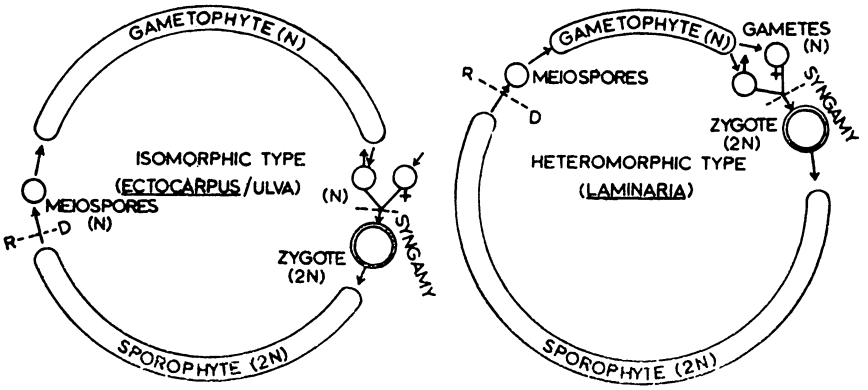

Fig. 3-4. Isomorphic and heteromorphic alternation of generations.

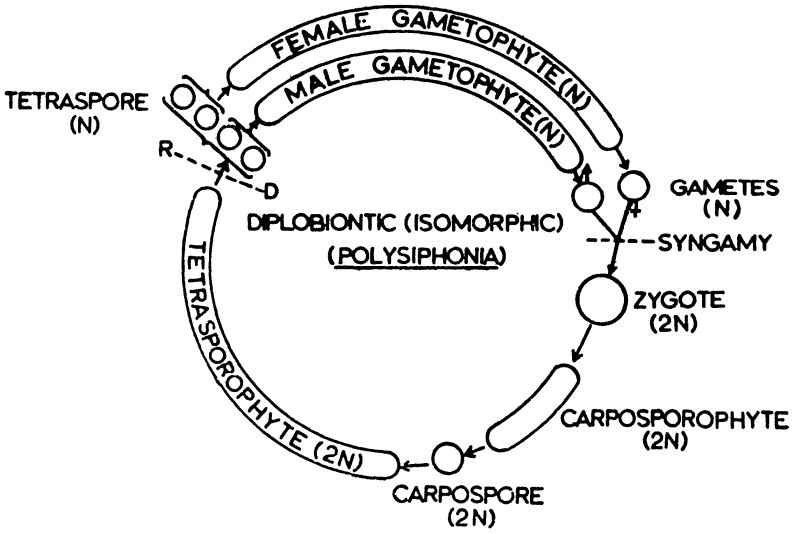

Fig. 3-5. Isomorphic alternation of generations.

PERENNATION

When the environmental conditions become unfavourable, many algae form resting stages for perennation, i.e., for tiding through adverse periods. In many Chlorophyceae, the only perennating stage in the life cycle is the thick-walled zygospore or oospore that commonly has a highly viscous protoplast containing an orange or red oily pigment known as **haematochrome.** They can tolerate unfavourable environmental conditions without any adverse effects.

Many terrestrial or **subaerial** blue-green algae can perennate as such, i.e., without any apparent modification of the vegetative cell or filament. The thick, stratified or gelatinous sheaths around their cells or trichomes, the gel-like consistency and viscosity of their protoplast, and the virtual universal absence of vacuoles in their cells, are probably some features that enable

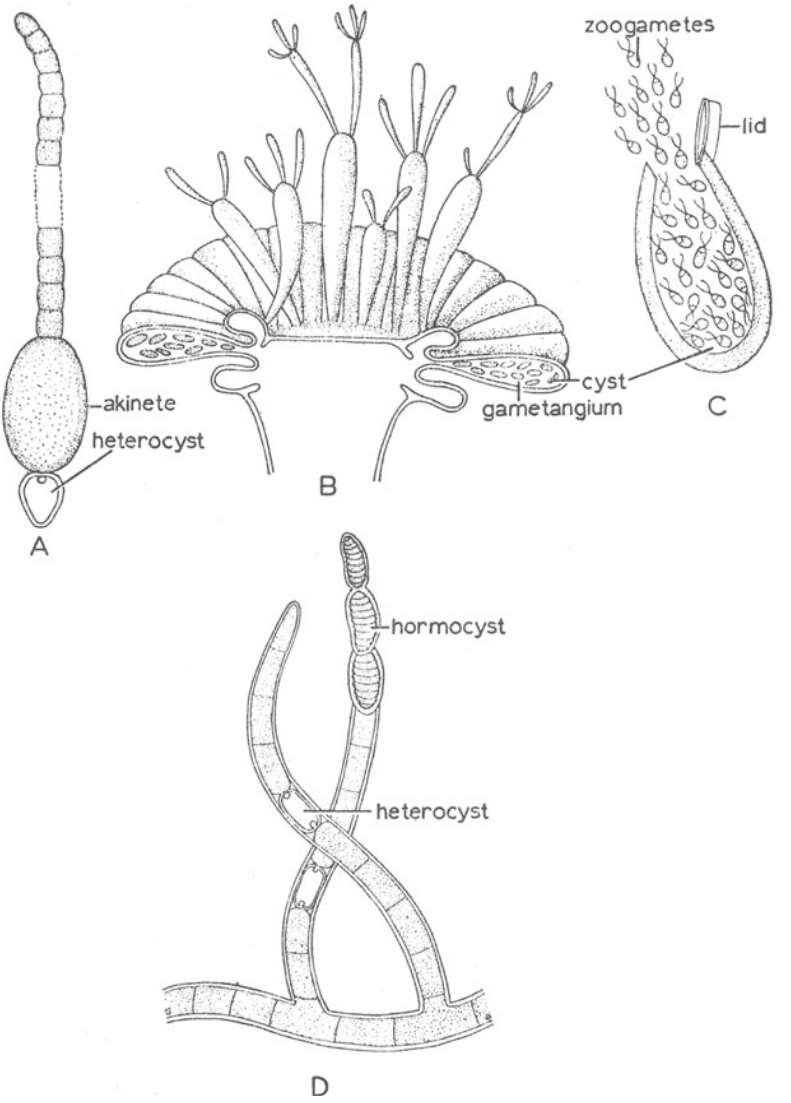

Fig. 3-6. A, *Cylindrospermum*, akinete and heterocyst; B, *Acetabularia*, vertical section through fertile disc showing gametangial rays and cysts (after Bold, 1967); C, same showing germination of cyst; D, *Westiella*, hormocysts and heterocysts.

them to endure and survive unfavourable conditions without much structural modification.

Perennation in other algae involves the formation of specially differen-

tiated resting cells. Some common examples are:

1. **Akinetes or "Spores".** These are enlarged vegetative cells which become thick-walled and store abundant reserve food material (Fig. 3-6 A). With the advent of unfavourable conditions the ordinary vegetative cells die whereas the akinetes successfully endure the harsh climate. When favourable conditions recommence during the next spring or rainy season, the akinete germinates and produces a vegetative filament, e.g., *Pithophora*, *Gloeotrichia* and *Cylindrospermum* (Fig. 3-6 A). Akinete formation is widespread in the nitrogen-fixing blue-green alga, *Aulosira fertilissima*, which occurs abundantly in tropical rice fields. Here, the vegetative filaments become transformed into chains of akinetes (spores) when the stagnant water in the fields begins to dry up, and it is in this sporulating condition that the alga tides over dry and hot periods.

2. **Hypnospores or Cysts.** Like akinetes, these are also thick-walled but, as a rule, are formed by repeated divisions of the protoplast rather than by the modification of a cell into a single akinete as in *Acetabularia* (Fig. 3-6 B, C). *Protosiphon botryoides* and *Sphaerella lacustris* also furnish good examples of cyst formation. During conditions of drought or light of high intensity, their cytoplasm breaks up into a number of thick-walled hypnospores which in *Sphaerella* may remain embedded in a common gelatinous matrix.

In *Fritschiella tuberosa* and *Botrydium* cysts are formed in the subterranean parts. When favourable conditions recur, the cysts either grow direct into a new cell or give rise to zoospores.

3. **Hormocysts.** These are hormogonia which, instead of being liberated from the parent filament, remain *in situ* and become thick-walled. They may also be regarded as many-celled akinetes. Typical hormocysts are met with in the blue-green alga *Westiella* (Fig. 3-6 D).

TEST QUESTIONS

1. Discuss the advantage of sexual reproduction when an alga can effectively multiply by asexual or vegetative means.

2. Why do algae produce a variety of spores, e.g., zoospores, aplanospores, and hypnospores, when the function of asexual reproduction can be effectively discharged by zoospores alone ?

3. Distinguish between: (a) akinete and hypnospore; (b) autospore and endospore; (c) aplanospore and autospore; and (d) vegetative cell division and binary fission.

4. How does the sexuality in eucaryotic algae differ from that in bacteria ?

5. Why are isogamy, anisogamy and oogamy regarded as progressive stages in the evolution of gametes in algae ?

6. What is the evolutionary significance of the algae with heteromorphic life cycle ?

7. Occasionally, when gametes fail to fuse, they may directly give rise to new plants. What is its significance in relation to the origin of sex in algae ?

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4

Economic Importance

With the phenomenal increase in urban population and industrial growth during the past few decades, some of the major problems of a basic nature confronting mankind are those of quantity and quality of food, feed, drinking water, disposal of sewage and industrial wastes, and of proper conservation of soils and waters. Suitably controlled algal growth can provide positive help in tackling these problems.

POSITIVE IMPORTANCE

EDIBLE FORMS

People of coastal countries, e.g., China and Japan, have long been using seaweeds and certain other algae as a source of food. Some of the more commonly used are *Porphyra, Ulva, Alaria, Chlorella, Chondrus* (Fig. 4-1 A–E), *Rhodymenia* and *Nostoc*. These not only form an important ingredient of soups but are also used for flavouring meat. Sometimes the blades and stipes of seaweeds are eaten after frying, with or without salt.

Besides being rich in organic iodine, which serves as a precursor of thyroxine, seaweeds contain significant amounts of other mineral elements considered indispensable for a well-balanced diet. Vitamins B, C, folic acid, and niacin are also found in them. They are, however, poor in protein content.

Among all the edible seaweeds the most important is the red alga *Porphyra tenera* which is cultivated in shallow sheltered seacoasts on a commercial scale in Japan. Chemical analyses of the processed alga by several Japanese scientists established that it is very rich in proteins (30–35%) and carbohydrates (40–45%). Fairly high concentrations of vitamins A, B, C and niacin were also detected.

In the farming of *P. tenera* a large number of bamboo networks, each about 40 metres long and 1.25 metres wide, are placed horizontally so as to be near the surface of water in the shallow sea. These frameworks are held in place with the help of bamboos fixed vertically in the seabed. Such bamboo frames provide a favourable habitat for the germination of monospores into young thalli. After about a month the entire bamboo networks together with the young thalli are transferred to river estuaries along the sea where the concentration of such nutrients as nitrogen, phosphorus and others is quite high. These substances stimulate the growth of the transplants. A number of *Porphyra* crops can be harvested successively between

November and April. Harvesting is carried out by plucking mature thalli by hand, washing and cutting them into pieces which are then resuspended in water. The algal suspension is filtered through sieves made of bamboo

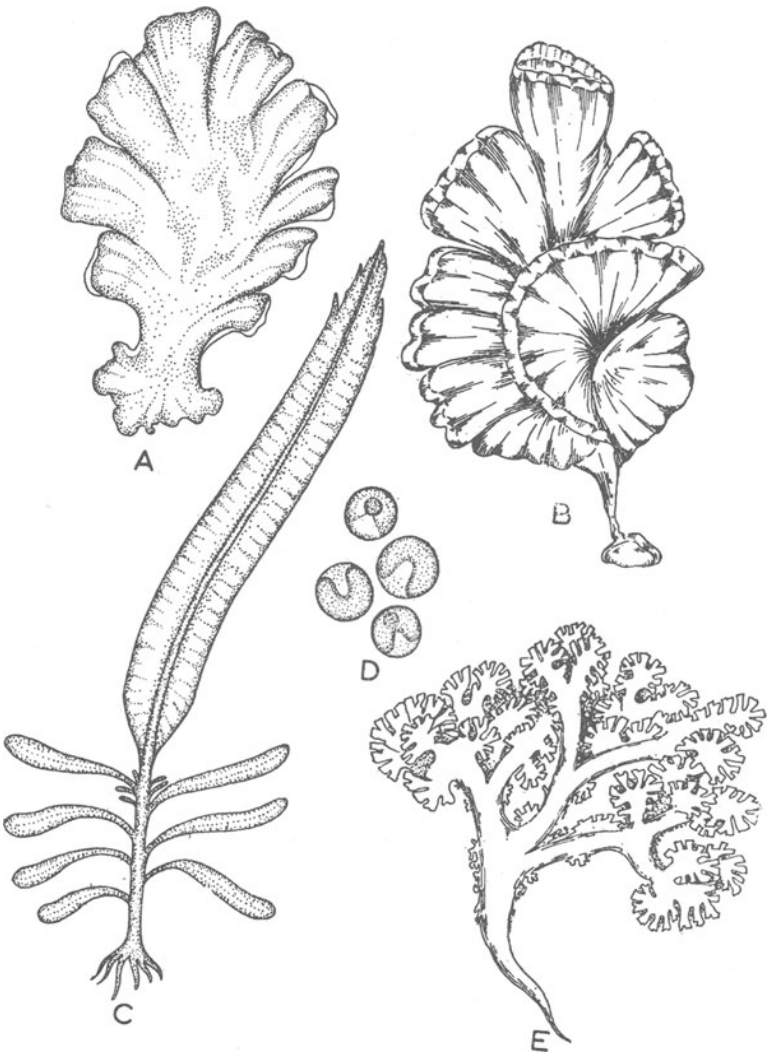

Fig. 4-1. Some common edible algae. A, *Porphyra*; B, *Ulva*; C, *Alaria*; D, *Chlorella*; E, *Chondrus*.

frames, dried and then pressed into sheets for sale. These *Porphyra* sheets are eaten with rice. *Porphyra* soup is highly prized in Europe and elsewhere.

The late Professor K. M. Drew-Baker of the University of Manchester, on the basis of her researches in the fifties, conclusively established that the alga then known as *Conchocelis*, found growing on oyster shells, was actually a juvenile stage in the life cycle of *Porphyra*, the important "Nori" of the Japanese seaweed industry. Her recognition of the *Conchocelis* stage in shells has enabled the Japanese to devise an artificial method of spore cultivation. The significance of this discovery was so great that it saved the Japanese *Porphyra* industry from near collapse.

Many developing countries are facing grave food shortage. In order to meet this challenge, suitable substitutes for conventional foods have to be evolved. Added to this is the alarming rate of population increase for which additional accommodation has to be provided at the cost of agricultural land. These facts necessitate a search for alternative crops that could be grown a number of times in a year within minimum space at a cost well within the reach of the common man, and with a nutritional value comparable to that of the conventional foods. Researches carried out in Germany, Japan and the United States on the large scale production, chemical composition and nutritional value of algae suggest that they are not only suitable substitutes for conventional crops but also yield greater amount of food per unit land area (Dawson, 1965). Moreover, for their growth the algae require simple inorganic nutrients, light and carbon dioxide (CO_2) which they can easily obtain when grown in sewage **oxidation ponds**. Even otherwise their growth requirements can easily be met without much economic burden.

Although *Porphyra* has been in use as a supplementary diet in Japan for a long time, its restriction to marine habitat, slow growth rate, and relatively poor vitamin and protein contents make it a less favourable algal food than *Chlorella*. The latter grows very fast under different conditions and therefore a much greater number of crops can be harvested. It is also richer in protein and lipid content than *Porphyra* and contains a wider variety as well as higher concentrations of vitamins. The nutritional value of *Chlorella* is comparable to that of a mixture of soya beans and spinach. Simple growth requirements, production of little waste material and high growth rate are the features which have encouraged the mass cultivation of *Chlorella* in Germany, Japan and the United States.

Chlorella grows actively in culture when provided with sunlight (or artificial light), CO_2 and mineral nutrients. A provision has also to be made for frequent or continuous agitation of the culture suspension so as to prevent the cells from settling down, and to ensure a proper distribution of nutrients, CO_2 and light. The open circulation system of mass culture devised by Tamiya (1960) in Japan consists of an open, shallow culture pond containing rotating pipes with numerous fine jets. These jets agitate the culture suspension and keep it enriched with CO_2. At frequent intervals aliquots of culture suspension are pumped out and the algal cells harvested by centrifugation. The harvested cells are washed and dried in vacuum or extracted with methanol to remove the pigments. Finally the dried algal mass is ground in a mill and stored in powdered form. On an average an annual

yield of nearly 13 metric tons per acre can be expected if the cultivation continues throughout the year.

Cultivation, harvesting and drying are the three major processes for turning *Chlorella* into foodstuffs. Out of these, the harvesting technique commonly employed, is still the most inefficient and expensive so that the greatest hurdle in the large scale exploitation of *Chlorella* as food is its high cost of production. Further, the indigestibility of the algal cell wall prevents its direct use as food on an industrial scale.

Researches are also being conducted in the United States on *Chlorella* and *Synechococcus* regarding their possible use in space ships and nuclear submarines as oxygen regenerating and food and water recycling organisms.

In addition to their direct use as food, many algae provide meal to human beings indirectly by virtue of their strategic position in the food webs in natural habitats. In both fresh and marine waters, algae are ingested by lower animals (protozoa, insect larvae, copepods and rotifers) which in turn are eaten by higher animals such as fish. Fish constitutes an important source of protein.

MINERALS AND ELEMENTS

The brown seaweeds popularly known as kelps are a rich source of soda, potash, iodine and alginic acid. Besides, they can also yield a good amount of ammonia and tar or charcoal when carefully processed.

The weed is collected, air dried and then burnt in cylindrical kilns. The resulting ash is used to recover soda and potash which are used in the manufacture of soap, glassware and alum.

In Japan, the extraction of iodine from kelps represents 5–7% of the world production. The genera commonly used for iodine production are *Laminaria, Fucus, Ecklonia* and *Eisenia.* It is the most important component of thyroid hormones and therefore has got a large application in chemotherapy of thyroids. The high iodine yielding Phaeophyta are used for the treatment of goitre as well.

Seaweeds are also rich in copper, iron, zinc, cobalt, vanadium, molybdenum, manganese, boron and chromium. This has led to their use as supplements to fodders and fertilizers.

ALGINATES

Alginates are the salts of alginic acid found in the cell wall of Phaeophyta. The genera most commonly used for alginate extraction are *Fucus, Laminaria, Macrocystis, Cystoseira, Lessonia* and *Ecklonia.* Flame-proof fabrics as well as plastic articles are prepared from alginates. Plastic materials of alginates find many applications in medicine, e.g., in the preparation of dental impressions. Alginic acid can effectively stop bleeding and is employed as a highly efficient gauze in internal operations. Because of their non-toxicity and colloidal properties, alginic acid derivatives have a number

of applications in preparation of commercial products for public consumption, e.g., in the preparation of soups, sauces and creams of various kinds and of antibiotic (aureomycin) capsules. Alginates are extensively used as thickeners in the cosmetic, textile and pharmaceutical industries, and as emulsifying agents in the preparation of polishes and paints.

AGAR-AGAR

A jelly-like substance, agar-agar (often called simply agar), is a complex polysaccharide of great economic value. It is extracted in water from certain species of red algae belonging to *Gelidium*, *Gracilaria* and other genera which produce and store it along with cellulose in their cell walls.

The most extensive use of agar is as a base for culture media for algae, fungi, bacteria and tissues. It is also used as a stabilizer or emulsifier in food, cosmetics, leather and pharmaceutical industries. Agar also finds good application in the canning of fishes, sizing of fabrics and in paper industry. It is often given as a laxative and is sometimes prescribed for treating a prolapsed stomach.

CARRAGEENIN

Chemically, carrageenin is very much like agar and occurs as a cell wall polysaccharide esterified with sulphate. It is usually extracted from *Chondrus crispus* (popularly known as 'Irish moss'), though sometimes species of *Gigartina* are also employed. Carrageenin is often used as a remedy for cough and as a pharmaceutical emulsifier. It is also utilized in the textile, leather, cosmetics and brewing industries.

FODDER

The brown algae *Ascophyllum*, *Laminaria* and *Fucus* are used as stock feed for sheep and cattle in maritime districts. In Ireland and Scotland there are established industries for processing them into a commercial feed. The seaweed meal is very nutritious because of its high vitamin and mineral content. The milk produced by cows that feed on such meals is richer in fat content than by those fed on conventional fodder. Likewise, hens fed with seaweed meal produce eggs rich in iodine.

The primary producers in aquatic habitats, including the sea, are mainly algae and therefore algal metabolism and productivity has a great bearing on the natural flora and fauna of the aquatic ecosystem. Nitrogen-fixing blue-green algae maintain and regulate the nitrogen budget of aquatic habitats, and by excretion of fixed nitrogen into the surrounding water, aid the growth of other microorganisms in places where nitrogen is a limiting factor (Stewart, 1967). Planktonic algae form the major food of protozoans, crustaceans and fishes (Fig. 4-2). Marine flagellates and other microalgae are cultivated for rearing the larvae of marine fishes, especially shell fish.

Practical methods for increasing algal feed are devised by placing bunches of bamboo in fish ponds; this provides an enormous surface for the growth of algal epiphytes which serve as food for fish. Vitamin-rich fish oils are in fact derived from diatomaceous feed. Efficient pisciculture involves the joint cultivation of fishes and algae, the former feeding on algae and the

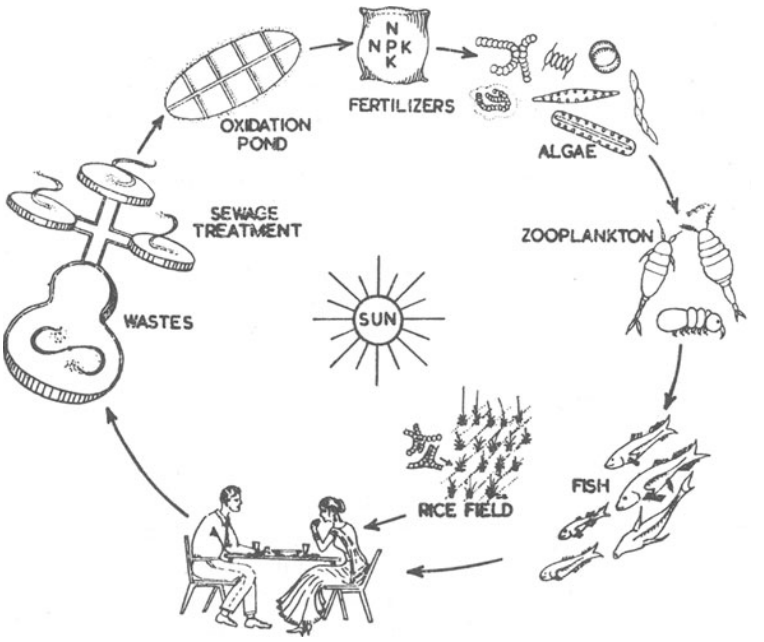

Fig. 4-2. Role and significance of algae in biological food webs, and in converting waste materials into foodstuffs.

latter using the waste products of fishes and other organisms for their own nutrition and growth.

FERTILIZER

Seaweeds, being rich in potassium, phosphorus, trace elements and growth substances, are extensively utilized as manure by people of coastal districts. The weeds are either allowed to rot in the field or composted with other organic materials. The resulting manure, when added to agricultural fields, not only enriches them in mineral nutrients but also helps in soil binding, in breaking down clays and in promoting good crumb formation. Such coralline or lime-depositing algae as *Lithothamnion* and *Lithophyllum* are used for liming the crop fields.

The Irish collect and use *Fucus* as manure on a large scale. In India, a comparative study of the yield of 'bhindi' (*Abelmoschus esculentus*) grown separately on a mixture of cowdung and wood ash manure, and on seaweed manure, indicated the superiority of the latter over the former (Thivy,

1960). The seaweed manure, however, is poorer in nitrogen and phosphorus than the farmyard manure. The blue-green algae, which are rich in nitrogen and phosphorus, are better fertilizers than seaweeds. In tropical countries, bottom mud of dried up ponds is regularly used as manure in crop fields; the manurial value is mostly due to the high content of blue-green algae. A suitably blended mixture of seaweed and cyanophycean manures (e.g., bloom of *Microcystis*) may serve as an ideal fertilizer and this can relieve the acute shortage of fertilizers in developing countries.

The fertility of natural habitats benefits from algal growth in many other ways as well. Many algae found in such habitats are believed to produce considerable amounts of extracellular substances, including organic acids, amino acids and polypeptides which chelate organic and inorganic ions and maintain them in a state readily available to plants. The extracellular products also serve as sources of carbon and nitrogen for other micro-organisms that regulate the overall balance of soil nitrogen, sulphur, phosphorus and carbon.

In respect of nitrogen status of natural habitats, the nitrogen-fixing blue-green algae deserve special mention. These algae grow luxuriantly in tropical habitats, e.g., rice fields. Experiments conducted with *Tolypothrix tenuis* in Japan and with *Aulosira fertilissima* in India have shown that the yield of paddy is substantially increased following the inoculation of fields with these algae. Algologists at the Central Rice Research Institute, Cuttack (India) inoculated rice fields with four species of nitrogen-fixing blue-green algae; the grain yield increased by nearly 30 per cent.

In the Sambhar Salt Lake in Rajasthan (India), cartloads of algal mats consisting of *Anabaenopsis* and *Spirulina* are produced annually, from September to December, and are employed by local farmers as manure.

SOIL RECLAMATION

Singh (1961) has demonstrated that blue-green algae can be used to help reclaim saline and alkaline wastelands in various parts of India. These algae in general prefer alkaline pH, waterlogging and high humidity coupled with high temperature for their growth, and can successfully grow in alkaline and saline wastelands during summer monsoons when all these requirements are available. Reclamation experiments conducted on wasteland enclosed by a 0.5 metre earth embankment so as to encourage waterlogging during the rains, resulted in decrease in pH and increase in nitrogen, phosphorus and organic matter content of the field thus converting it, in due course, into a fertile cultivable land.

ANTIBIOTICS

Some algae produce antibacterial substances effective against a number of pathogenic bacteria. Chlorellin, obtained from *Chlorella*, was the first such substance. Extracts of *Cladophora*, *Lyngbya* and certain other algae

kill strains of *Pseudomonas* and *Mycobacterium* and exhibit antiviral activity. However, the antibacterial or antiviral substances concerned have not yet been isolated in a pure form.

Certain Cyanophyta and Characeae have been claimed to possess larvicidal properties since very few mosquito larvae occur in waters supporting the growth of such algae.

DIATOMITE

It constitutes the cell wall material of diatoms. Large deposits of diatomaceous earth have been discovered in marine and freshwater basins. Diatomite is highly porous and insoluble and therefore is ideally suited as a filter for oils and for clearing solvents. Most industrial filtration devices are made of diatomite. It is also employed in wine and paper industries. An effective insulator, it is light, and fireproof and finds application in the preparation of high temperature furnaces.

SEWAGE DISPOSAL

Sewage consists of water-borne domestic and industrial waste which is fairly rich in dissolved or suspended organic and inorganic constituents, but very poor in oxygen. It harbours a number of faecal and other anaerobic bacteria, some of which may be pathogenic. The sewage has to be treated before its disposal for two main reasons. Firstly, the anaerobic digestion of sewage produces stinking and offensive odours. Secondly, the contamination of potable water with sewage infested raw material is potentially a rich source for inorganic nutrients such as nitrogen, phosphorus, potassium and sulphur. This aspect of sewage assumes considerable importance in view of the fact that many developing countries are facing an acute shortage of inorganic fertilizers on the one hand and an explosive population increase on the other.

Sewage oxidation ponds have been created for bringing about its complete oxidation into mineral components. Such ponds support luxuriant growth of unicellular algae, e.g., *Chlorella, Chlamydomonas, Scenedesmus* and *Euglena.* These help in the bacterial decomposition of sewage by providing oxygen; in addition, they recover the mineral nutrients from sewage which would otherwise have been lost in the effluent.

Sewage algae have also been used as animal feed, as manure, and for ingestion by fish fingerlings in fish ponds. The main limitation preventing a wider exploitation of sewage algae is the lack of a cheap and effective harvesting technique.

NEGATIVE IMPORTANCE

TOXICITY AND PARASITISM

Prymnesium parvum, Gymnodinium veneficum and species of *Microcystis* cause mortality in fish, and in domestic animals that drink water infested with

these algae. Various species of *Gonyaulax* produce endotoxins which accumulate in the digestive glands of shellfishes feeding on them. If such shellfishes are consumed by other animals, including man, paralysis and even death may result.

Bloom-forming blue-green algae such as *Microcystis aeruginosa*, *Anabaena flos-aquae* and *Aphanizomenon flos-aquae* have been implicated in animal poisoning in temperate countries. Depending on the time interval that elapses between injection of the toxic algae into mice and their resulting death, these toxins have been classified into three categories: Very Fast Death Factor (VFDF), Fast Death Factor (FDF) and Slow Death Factor (SDF), killing mice within 10 min, 2 hr and 48 hr respectively.

If some of the toxic planktonic algae happen to be ingested either along with drinking water or during swimming, they may cause various disease syndromes. For example, *Anabaena* and *Microcystis* cause gastric trouble; *Gymnodinium brevis* produces respiratory disorders, and *Lyngbya* and *Chlorella* are responsible for certain skin infections.

Species of the parasitic green alga *Cephaleuros* (Chlorophyceae) cause 'red rust of tea' and inflict heavy economic losses by seriously affecting the yield of tea.

EFFECTS ON AQUATIC ANIMALS

The excessive growth of certain algae (*Microcystis aeruginosa*) in a body of water often results in severe depletion of oxygen in the habitat. This leads to mass mortality of fish due to suffocation. Sometimes, high temperature and bright sunlight result in massive disintegration of algal blooms, releasing into the medium their noxious components. Choking of the mouth or gills of fish by these algae is also partly responsible for their death.

FOULING OF MARINE VESSELS

Some seaweeds may grow on the metal hulls and woodwork of ships and boats producing a fouling, corroding and destructive effect. A thick growth of weeds sometimes results in considerable increase in friction between hull and water thereby accentuating wear and tear and shortening the life of the vessel.

IMPORTANCE IN MUNICIPAL WATER SUPPLIES

The problems associated directly or indirectly with algal growths in water reservoirs and water supplies are: (1) loss of recreational (swimming and aesthetic) and fishing values of pools, ponds and lakes due to excessive growth of *Microcystis*, *Spirogyra*, *Cladophora* and *Pithophora*; (2) imparting of abnormal tastes and odours by the metabolic or decomposition products of nuisance organisms such as *Synura*, *Synedra*, *Asterionella*, *Anabaena*, *Microcystis* and *Dinobryon*; (3) clogging of water filters by *Oscillatoria*,

Spirogyra and certain diatoms, thereby shortening the filter runs leading to serious economic losses; (4) colouration of raw and finished waters due to the presence of planktonic algae, e.g., *Chlorella*, *Chlamydomonas*, *Euglena* and *Oscillatoria*; (5) spoilage of the quality of commercial products of food, pharmaceutical and pulp industries due to the use of waters contaminated with algal slimes; (6) production of toxic substances; (7) corrosion of concrete and metallic walls of pipes and boilers by carbonic, oxalic and silicic acids excreted by certain algae, e.g., *Anacystis* and *Chaetophora*; (8) interference with purification of water; and (9) changes in pH, CO_2, bicarbonate and oxygen contents of water.

In water treatment plants, the raw water is chlorinated in order to kill algae and other microorganisms. However, even this water is sometimes not completely free from algae since some of them are chlorine-resistant and their presence may alter its quality and potability.

The only useful purposes the algae serve in water supplies are: (1) maintenance of aerobic conditions by checking putrefaction of organic substances, and (2) reduction of total hardness of water by consuming bicarbonates and the nearly insoluble carbonates.

CONTROL OF UNDESIRABLE SPECIES

BIOLOGICAL CONTROL

The virus, **cyanophage** LPP-1, and several other phycoviruses (e.g., LPP-2, SM-1, N-1 and AS-1) infect various blue-green algae and could conceivably be employed to control their growth in surface water since they cause lysis of the sensitive forms. Recently, the eucaryotic algae *Chlorella* and *Sirodotia* have also been reported to be attacked by phycoviruses. Addition of virus particles to cultures of susceptible algae results in considerable increase in virus titres and rapid fall in algal cell numbers (see Safferman 1973).

A better method for algal control consists in introducing suitable crustaceans or fish fingerlings into the water body. These animals feed on algae either directly or indirectly, and may be harvested for food at maturity.

CHEMICAL CONTROL

The growth of algae in reservoirs and lakes is controlled by appropriate applications of **algicide** copper sulphate which selectively kills the algae within a dose range of 0.25–9.5 ppm. The effective dose varies with the chemical character of water as well as the concentration of algae. Chlorophenyl dimethyl urea, a photosynthetic inhibitor, has also been found effective in checking the growth of planktonic algae. Besides, there are other promising algicides such as antibiotics, quinones, substituted hydrocarbons and phenols. But they are not in common use because of their higher cost.

The quinone 2,3-dichloro-1,4-naphthoquinone is selectively toxic to

many bloom forming cyanophytes as compared to other algae. This can be effectively used to control water blooms caused by blue-green algae.

A particularly suitable algicide in widespread use is Dichlorophen ("Panacide" of British Drug Houses Ltd.). It is inexpensive, potent and non-toxic to animals and human beings, and can be used for preventing algal growth in open air swimming pools.

The Metropolitan Water Board of London has found that artificial circulation of water in reservoirs is an effective method of reducing growth of planktonic algae. The basis underlying this method is not clear but the breakdown of chemical stratification in water appears to be significant (G. E. Fogg, personal communication).

TEST QUESTIONS

1. Why is *Chlorella* preferred as a suitable substitute for conventional foods?
2. What purpose do the bamboo net beds serve in *Porphyra* farming?
3. The utilization of agar, alginate and carrageenin as food is restricted. Why?
4. How do nitrogen-fixing blue-green algae serve to reclaim 'usar' lands?
5. What factors determine a good manure? Suggest reasons for the superiority of algal manure over farmyard manure.
6. Do you think that growth of algae in water reservoirs, and water treatment and distribution systems, is to some extent helpful in improving the potability of water? If so, give reasons.
7. Enumerate the preventive measures that can be adopted to control the growth of algae in water reservoirs and water supply systems.
8. What role can algae play in a food production unit for space travel so that most of the food used by the space travellers is produced in the space vehicle itself rather than being carried from earth?

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PART II

STRUCTURE AND REPRODUCTION OF SELECTED ALGAE

4. (54-22/1972)

5

Cyanophyta

The Phylum Cyanophyta (Myxophyceae, blue-green algae) differs from other algae in having a procaryotic cell organization, i.e., it lacks organized double membraned nuclei, chromatophores and mitochondria, and possesses characteristic photosynthetic pigments including biliproteins, myxoxanthin and myxoxanthophyll in addition to chlorophyll-a and β-carotene. No flagellated cells are formed and movement of motile stages is brought about by a characteristic gliding action. However, certain specialized cells known as **heterocysts** are produced in many species. True sexuality, defined as alternating karyogamy and meiosis, does not occur but genetic recombination which fulfils the function of sex is known in some members.

OCCURRENCE AND ECOLOGICAL AMPLITUDE

The blue-green algae are perhaps the most widely distributed algal organisms, especially in tropical countries. They are ubiquitous and cosmopolitan, found in clean and polluted waters of lakes, ponds and reservoirs, in fresh or salt waters, and in stagnant or flowing waters. They occur in the spray zone of seacoasts as well as in surface water of oceans, and are widespread on or in tropical soils of diverse kinds. One of the most characteristic and favourite habitats is the tropical paddy field. Other conspicuous places include tree barks, rocks and stones, footpaths and plant pots.

The blue-green algae form a significant part of the free-floating population of microscopic organisms which constitute the plankton of **eutrophic** (organically rich) lakes and oceans. They are also found in the **benthos** of lakes and ponds and are largely responsible for the high fertilizing qualities of bottom muds of dried ponds and tanks. In tropical countries the ponds that are heavily polluted and exposed to high temperatures and intense sunlight, provide optimum conditions for the growth of blue-green algae. Species of *Microcystis* and certain other planktonic blue-greens multiply so prolifically in such conditions that they impart bluish-green colour to the pond water which appears as "pea soup". Such **water blooms** in tropical ponds are more or less perennial but similar blooms, though ephemeral, are also formed in temperate climates either sporadically or annually.

Except for certain bacteria, the blue-green algae are the only organisms which grow in adverse and harsh environments. While most of them thrive in biologically normal habitats, some can successfully endure extremes of temperature, light intensity, desiccation, and high or low concentrations of dissolved nutrients. Species of *Mastigocladus* and *Phormidium* are impor-

tant members of the flora of hot water springs and can tolerate temperatures as high as 75°C. On the other hand, species of *Phormidium* are found in the frigid arctic lakes and certain others, collected from the Antarctic, have experimentally been shown to survive freezing. Tolerance to extremes of light intensity is illustrated by species found in caves which are virtually dark, on bare rocks exposed to intense sunlight, or those growing in tropical deserts. Certain species of *Nostoc* and *Chlorogloea* have remained viable after prolonged storage in a calcium chloride desiccator. Likewise, some blue-greens thrive in waters of highly polluted ponds or of non-polluted mountain streams.

The Cyanophyceae are pioneer colonizers of bare rocks or virgin lands and grow luxuriantly on alkaline or saline soils. Several species grow in or on calcareous strata. Some have been found in oil field sump ponds and in samples of floating tarry scums. A few species of *Nostoc* and *Anabaena* grow as **endophytes** in the roots of cycads, in *Azolla* and in *Anthoceros*. A number of blue-greens are the algal constituents of various lichens. Others live symbiotically in diverse unicellular animals. In nature, they interact with and affect the behaviour of a wide variety of diverse procaryotic and eucaryotic organisms.

THALLUS ORGANIZATION AND RANGE OF FORMS

Unicellular, colonial (palmelloid), filamentous and heterotrichous forms are common but more elaborate macroscopic thalli are rare. The products of cell division, instead of being liberated as distinct individuals, often remain united in an assemblage of cells (a colony). Such colonies may be definite (e.g., *Merismopedia*) or irregular and indefinite (e.g., *Aphanothece*). The actual shape of a colony is largely determined by the number of planes in which the cells divide.

A filamentous blue-green alga consists of a number of cells arranged end to end. Simple, unbranched, uniseriate filaments are characteristic of *Lyngbya*, *Anabaena* and many other genera of the Nostocales. Here, the term filament includes the row of cells as well as the surrounding gelatinous sheath; if it consists of cells without a distinct sheath, it is designated trichome. The mucilage sheath is also found around unicellular and colonial forms. Most filaments have a single trichome each, but in some more than one trichome are so disposed within the common sheath that the filament appears to be branched. This phenomenon is known as pseudo or false branching and is characteristic of such genera as *Scytonema* and *Tolypothrix*. It is caused either by the growth of free ends of some trichomes through the sheath of the parent filament, or by the degeneration of the top cell in a loop, leading to the division of an originally single and continuous trichome into two independent pseudobranches. In some genera, true (lateral) branching occurs.

CELL STRUCTURE

The most striking difference in the cell structure of blue-green and other algae is the lack of differentiation of parts in the former. There are no organized nuclei, chromatophores, pyrenoids, mitochondria, or true vacuoles. The only prominent structures seen within the blue-green algal cell under the light microscope are the different kinds of granules whose number and size vary in different genera and depend on physiological and nutritional factors. These are proteinaceous and are called cyanophycin granules. Reserve food is also stored in the form of a carbohydrate known as cyanophycean starch (=glycogen). Other storage products are trehalose or sulpholipid.

In addition to the cyanophycin granules, the healthy cells of many planktonic blue-green algae contain numerous small bodies of irregular shapes, known as **pseudovacuoles** or **gas vacuoles.** These appear black under low power and reddish under high power of a microscope. Under an electron microscope the gas vacuoles appear to consist of packed arrays of cylindrical vesicles, each with conical ends and bound by a single membrane. Fogg's (1970) study on the biology of gas vacuoles proves that they contain metabolic gases and therefore serve as a mechanism for buoyancy regulation. The gas vacuoles are produced in low light intensity and disappear in high light intensity. In bright light there is greater production of sugars through photosynthesis resulting in an increase in osmotic pressure of the cells which causes the collapse of gas vacuoles. The ecological significance of this finding is that the blue-green algae growing below the water surface receive less light and form gas vacuoles, thus increasing their buoyancy. Such cells then rise to the surface where they receive more light, develop higher cellular osmotic pressure which leads to the collapse of gas vacuoles. This results in reduction of buoyancy and consequently the cells sink to deeper layers of water.

The core or central region of a blue-green algal cell appears somewhat transparent and contains most of the genetic material. There are no nuclei or nucleoli. Since DNA is not associated with protein material (histones or protamines), no organized or firm chromosomes, characteristic of higher plants and animals, are found in blue-green algae. Besides DNA, RNA is present.

The cells possess a wall which may be differentiated into an inner and an outer layer. Outside the cell wall there is a gelatinous sheath which is either homogeneous and colourless or thick, stratified and pigmented. Such a sheath is characteristic of those blue-green algae which grow in dry or xerophytic habitats. A firm, pigmented sheath is indeed an asset to the terrestrial or subaerial forms because it enables them to perennate. This is due to the water-absorbing and water-retaining capacity of the sheath. The cell wall contains muramic acid and other components not found in other algae.

MICROMORPHOLOGY

Since the cells or filaments are spherical or cylindrical rather than flat, the structure of deep-seated inner regions constituting the core of the sphere cannot usually be observed by merely mounting the alga on a slide and examining it under the microscope. Only the outer or peripheral parts can be observed in this way. For examining the inner or central regions, sections must be cut. Ultrathin sections of a typical cyanophycean cell studied under an electron microscope at magnifications of about 25000 times reveal many important details (Pankratz and Bowen, 1963) which are diagrammatically represented in Fig. 2-1 A (see also the Frontispiece), and are described here.

SHEATH AND CELL WALL

The gelatinous sheath consists of three layers of microfibrils disposed reticulately within an amorphous matrix. The chemical nature of these microfibrils is not known but recent studies indicate that cellulose is absent. Inside the sheath is a double-layered cell wall whose inner layer contains mucopeptide and muramic acid.

The cytoplasmic membrane is found inside the cell wall and generally consists of two electron opaque layers separated by a translucent layer.

LAMELLAE

The peripheral regions of the cell are traversed by a large number of photosynthetic lamellae or thylakoids which may be arranged regularly in two or more parallel stacks, or disposed irregularly. A lamella appears as an elongated, flattened sac and consists of two unit membranes, each of about 75 Å thickness.

GRANULES

The various kinds of granules observed in different cyanophycean cells include the cyanophycin granules, polyhedral bodies, ribosomes, α-granules and β-granules. Their actual significance and function are not clearly understood but some of them, e.g., ribosomes, are concerned with protein synthesis, whereas others, e.g., cyanophycin granules, are thought to be of the nature of reserve food. In planktonic forms pseudovacuoles are found; their ultrastructure has been described earlier.

CENTRAL REGION OF THE CELL

The genetic material, consisting of DNA fibrils, is concentrated in the centre of the cell and this region is also traversed by a few thylakoids.

HETEROCYSTS

These are specialized cells which differ from ordinary vegetative cells in having a thickened wall and one or two pores. Usually they are slightly larger than the vegetative cells, and under the light microscope appear as empty, homogeneous, double-walled and markedly distinctive cells. A recent study of heterocyst development with the electron microscope has shown that the general sequence of events during the differentiation of a vegetative cell into heterocyst includes cell enlargement, synthesis of a many-layered, non-cellulosic polysaccharide wall, gradual loss of photosynthetic pigments, decrease in granular inclusions, and reorientation of the photosynthetic lamellae into a complex reticulate pattern (see Lang 1968). The heterocyst lacks the normal lipid components of the photosynthetic lamellae present in the vegetative cells or chloroplasts of algae and higher plants, but contains two novel types of lipids—**glycolipid** and **acyl lipid**—not found in vegetative cells. Besides, the mature heterocysts contain chlorophyll-a but lack the accessory pigment, phycocyanin, which is normally required for the operation of the Photosystem-II of photosynthesis or in Hill reaction.

Heterocysts are found in many but not all filamentous blue-green algae belonging to the orders Nostocales and Stigonematales. They may occur either terminally or in an intercalary position in the trichome.

Many views have been advanced about the function of the heterocyst. An earlier belief that heterocysts are moribund or dead cells can be safely dismissed because in exceptional cases they have germinated to produce new filaments or endospores indicating their spore-like nature. The changes in structure of the cell wall and formation of endospores within some heterocysts, however, do not seem to support this belief. Their potentiality to germinate under certain conditions, led some workers to regard them as archaic reproductive bodies which have now become almost functionless, though sometimes they revert to their original reproductive function. Unusually frequent germination of heterocysts in certain strains of *Gloeotrichia ghosei* has been reported by Singh and Tiwari (1970).

The heterocyst has also been assigned a role in sporulation (Wolk, 1966) and nitrogen fixation (Fay *et al.*, 1968). Evidences suggesting its role in the sporulation of *Anabaena cylindrica* are: (1) the vegetative cells adjacent or proximal to a heterocyst sporulate earlier than those distal to it and (2) the removal of the heterocysts from the filaments prevents sporulation. According to Kulasooriya *et al.* (1972), the carbon: nitrogen balance is the major factor which induces and controls the differentiation of a vegetative cell into a heterocyst.

Researches during the past three years have conclusively established that the heterocyst is the site of nitrogen fixation in most blue-green algae. Evidences in support of this conclusion are that: (1) virtually all the reported nitrogen-fixing blue-green algae are heterocystous forms; (2) combined inorganic nitrogen inhibits both heterocyst differentiation and formation of the enzyme **nitrogenase**; (3) the nitrogenase requires an anaerobic atmos-

phere for its nitrogen-fixing activity and the heterocyst has just such an atmosphere; the lack of phycocyanin, the inability to photoassimilate CO_2, and the high respiratory activity of the heterocysts measured in terms of oxygen uptake and the T.T.C. (Triphenyl Tetrazolium Chloride) reaction, indicate that they have a highly reducing atmosphere; and (4) the localization of active nitrogenase within the heterocysts is indicated by the fact that isolated heterocysts (free of vegetative cells) can reduce acetylene to ethylene when supplied with a reducing agent (sodium dithionite) and an energy source, ATP (adenosine triphosphate).

In vitro studies of Smith and Evans (1970) on *Anabaena cylindrica* indicate that nitrogenase is also present in vegetative cells. The report that *Gloeocapsa*, a colonial, non-heterocystous blue-green alga also fixes nitrogen seems to raise the possibility of nitrogenase activity in vegetative cells of non-heterocystous families as well (see Wyatt and Silvey 1969).

Two other hypotheses concerning the functions of heterocyst are that: (1) they help in splitting a trichome into hormogonia; and (2) they are enzyme receptacles.

The varied types of functions attributed to heterocysts suggest that probably they function differently under diverse conditions. It is also likely that their function involves interaction with adjacent cells. With the electron microscope connections between heterocysts and vegetative cells, and between pairs of vegetative cells, have been demonstrated in some blue-green algae.

COMPARATIVE ORGANIZATION OF VEGETATIVE CELLS, HETEROCYSTS AND SPORES

Recent cytological and biochemical studies reveal that these cell types differ in structure, composition and function (Lang, 1968). Both heterocysts and spores develop from vegetative cells. As compared to vegetative cells, the wall of heterocyst includes an additional layer lacking true cellulose, whereas that of spore consists of several additional sheath layers with the outermost 1 or 2 layers often being variously sculptured. The vegetative cells and spores have nearly the same type of thylakoid arrangement. The heterocyst development, however, is accompanied by extensive reorganization of thylakoids in a reticular fashion at its two poles. In addition, heterocysts contain osmiophilic **polar nodules**. The number of various granules is larger in spores and smaller in heterocysts than in vegetative cells. **Polyphosphate granules**, which function both as an energy source and inorganic phosphate reserves, are found in vegetative cells as well as spores but not in heterocysts. The lipid composition of the three cell types differs (see under Heterocysts). The vegetative cells contain a variety of photosynthetic pigments, e.g., chlorophyll-a, phycocyanin, β-carotene, myxoxanthophyll and other carotenoids. Heterocysts have only chlorophyll-a and β-carotene. In spores no other pigments except carotenoids are found. The distribution of pigments in these cell types is highly significant in their metabolic

activities.

The vegetative cells carry out the aerobic type of photosynthesis characteristic of other algae and generate both the reducing power, NADPH (reduced Nicotinamide Adenine Dinucleotide Phosphate) and the energy source (ATP). The heterocysts include only Photosystem-I and therefore can only have the anaerobic type of photosynthesis as found in photosynthetic bacteria. The spores are photosynthetically defunct. The nitrogenase activity is confined to heterocysts and not to spores.

PIGMENTS AND CHROMATIC ADAPTATION

The chief pigments are chlorophyll-a, β-carotene, myxoxanthophyll, myxoxanthin (echinenone) and c-phycocyanin. These are not located in any chromatophore but are found in photosynthetic lamellae (thylakoids). Smaller amounts of c-phycoerythrin, oscilloxanthin, zeaxanthin and lutein may also be found. However, their proportion, especially of phycocyanin (blue) and phycoerythrin (red), varies greatly under different habitat conditions. Forms which are exposed to bright sunlight are rich in phycocyanin and appear deep bluish-green; those growing in low light intensity generally have more of phycoerythrin so that they appear reddish. The filamentous alga *Trichodesmium erythraeum* which grows in the plankton of the Red Sea is an extreme example where the phycoerythrin content is so high, and phycocyanin so low, that it appears distinctly reddish even though it is actually a blue-green.

In contrast to other pigments which are fat-soluble, the phycocyanin and phycoerythrin, collectively known as biliproteins or phycobilins, can be extracted in water after cell disruption and are proteinaceous.

In a few species of Cyanophyta, the pigmentation seems to be determined by the quality of light in which they are grown. For instance, some species of *Oscillatoria* can assume a green colour in red light, reddish in green light, and bluish-green in yellow light. Such a capacity to change colour complementary to that of the light is known as **chromatic adaptation** or **Gaidukov phenomenon.** This adaptation is of definite advantage since it enables the alga to maximally absorb the available light for photosynthesis.

Scheibe (1972) has isolated a new phytochrome-like photoreversible pigment from *Tolypothrix tenuis.* The absorption maxima for the two photoreversible forms of this pigment lie in the green and red portions of the spectrum instead of in red and far-red (red and far-red are characteristic of the higher plant phytochrome). This new pigment may possibly control in cyanophytes several morphogenetic phenomena, including chromatic adaptation.

MOVEMENTS

Although motile flagellate cells are absent, mature individuals of many Oscillatoriaceae and hormogonia of most filamentous forms are capable of

movement. A pendulum-like, oscillatory, swinging movement of the trichomes can be readily observed when living material of *Oscillatoria* is examined under a microscope. In different genera, the movement may be a backward and a forward gliding, a slow waving of one end of the trichome, or a spiral progression or retrogression. Some trichomes rotate around their axes, simultaneously gliding forward or backward. The gliding movement differs from swimming in that it occurs in contact with some solid or semi-solid substratum, but without any visible organ or a visible change in the shape of the alga.

The precise mechanism of the gliding movement is obscure. Many theories have been advanced but without conclusive experimental support. Most workers consider that movement is due to secretion of a mucilaginous material through minute pores in the cell wall. That the gliding movement is shown only by those species of *Oscillatoria* which produce mucilage, supports this viewpoint. Another hypothesis claims that the movement is due to rhythmic waves of alternate expansions and contractions along the length of the trichome. A third view has recently been proposed by Halfen and Castenholz (1970). According to them the gliding movement may be the result of waves travelling in one direction along the cell surface acting against some solid substrate or an associated elastic sheath. Since there are no flagella or muscular threads of any kind, the basic mechanism of how a filament glides or oscillates back and forth is unknown. It may possibly be associated, as in other movements, with phosphorylation and ATP.

NITROGEN FIXATION

Certain blue-green algae possess the capacity to convert (or fix) elementary nitrogen found in the air to useful nitrogenous compounds which can be assimilated. This property makes them truly **autotrophic** plants in the sense that they can fix not only atmospheric CO_2 but also gaseous nitrogen.

Like heterocysts, the nitrogen-fixing power is restricted to certain filamentous species such as *Aulosira*, *Tolypothrix*, *Anabaena*, *Cylindrospermum*, *Nostoc* and *Mastigocladus*. Such species contribute greatly to the fertility of rice fields in tropical countries. In temperate regions also nitrogen fixation seems to be mainly a cyanophycean process rather than bacterial. A number of blue-green algae are found in symbiotic association with other plants and the nitrogen fixed by them may be largely responsible for the success of the partnership.

In the past decade significant advances have been made in elucidating the biochemistry and mechanism of nitrogen fixation. By far the most important of these researches has been carried out in the laboratory of Professor R. H. Burris at the University of Wisconsin. Prior to 1960, studies on the mechanism of this process had practically come to a standstill because no methods were known for extracting nitrogen-fixing enzymes from the organisms. Its successful demonstration in 1960 in cell-free extracts from nitrogen-fixing blue-green algae (and also bacteria) facilitated the biochemical

study. A second interesting discovery by the Wisconsin group is that of acetylene reduction by nitrogen-fixing cell-free extracts. It was further established that the reduction of acetylene to ethylene is brought about by the enzyme nitrogenase whose primary function is to reduce nitrogen. The importance of this reduction system is so great that it is now being envisaged and claimed as an effective method of scanning blue-greens or other organisms for nitrogen fixing capacity.

The nitrogen fixed by blue-green algae can be assimilated by themselves as well as by other organisms. Substantial amounts of soluble nitrogenous compounds are liberated from healthy cells of nitrogen-fixing blue-green algae either into the culture medium or in their natural habitats. Besides, when the algae die, their nitrogenous materials decay and form ammonia which may then be transformed into nitrates by nitrifying bacteria thus enriching the fertility of the habitat.

NUTRITION

Cyanophytes have a greatly restricted range of their nutrition and in this respect they differ sharply from most other procaryotes. With the exception of some strains of *Nostoc*, *Tolypothrix* and *Chlorogloea*, most of them are obligate photoautotrophs and cannot grow in darkness even in the presence of organic substrates. The biochemical basis of their obligate phototrophy is still not clear. Two hypotheses have recently been put forward to explain this phototrophy. One suggests that since the key respiratory enzymes, α-ketoglutarate dehydrogenase and nicotinamide adenine nucleotide oxidase (NAD), are absent from blue-green algae, the incomplete functioning of the tricarboxylic acid cycle might be responsible for their phototrophic behaviour (Hoare *et al.*, 1970). The second hypothesis implicates the lack of enzyme repression control mechanism in the tricarboxylic acid cycle and amino acid metabolism as the principal causative feature for the obligate phototrophy (Carr, 1970). The enzyme repression control operates at the **transcription (cistron)** level, and acts by stopping synthesis of biosynthetic enzymes in response to the exogenous availability, or over production of their end products. The inhibition of enzyme synthesis is termed **repression** and its release **derepression.**

REPRODUCTION, GENETICS AND LIFE CYCLE

In many unicellular and colonial forms, the chief method of reproduction is by division of a parent cell into two daughter cells, or by fragmentation of a colony into two or more colonies. Sometimes, in addition to cell division or fission, non-motile spores (endospores or exospores) are produced; each of these gives rise to a new plant. The spores are commonly produced in ordinary vegetative cells rather than in differentiated sporangia. Unlike eucaryotic algae, mitosis and meiosis are not involved in cell division or sporogenesis.

Filamentous forms belonging to the Nostocales and Stigonematales regularly multiply by the breaking of their trichomes into hormogonia which are generally motile and later mature into filaments. Some forms produce spores or akinetes which function as reproductive or perennating cells.

True sexuality does not exist but a kind of parasexual phenomenon, known as genetic recombination, first reported in *Anacystis nidulans* by Kumar in 1962, has since been confirmed by other workers (Bazin, 1968). Genetic transformation (a mode of gene transfer leading to genetic recombination) for antibiotic resistance characters has been demonstrated in *A. Nidulans* (Shestakov and Khyen, 1970). Genetic recombination differs from true sexuality in that it is not attended by syngamy or meiosis, and yet the function of true sexuality (namely, to bring about gene recombination) is achieved. Besides *Anacystis nidulans*, genetic recombination has been reported in *Cylindrospermum majus* (Singh and Sinha, 1965) and *Anabaena doliolum.*

Lazaroff and Vishniac (1962) claim that under certain conditions different filaments of *Nostoc muscorum* can actually fuse in a manner reminiscent of **heterokaryotic** phenomenon in certain fungi. According to them the alga passes through a life cycle involving alternation between a heterocystous and a sporogenous phase (Fig. 5-1). The former occurs in the presence of light and the latter when the alga is grown heterotrophically in the dark. It appears that the fusion of short non-heterocystous filaments from the dark-grown cultures, which takes place after exposure to light, initiates the heterocystous phase of development.

Nothing is known about the genetic systems involved in recombination in the Cyanophyta. However, it is likely that recombination is brought about by conjugation between donor and recipient cells, as in bacteria. Recent studies demonstrating the susceptibility of some blue-green algae to a virus (known as cyanophage, phycovirus or blue-green algal virus) warrant the possibility that gene recombination may also be caused by transduction, a process in which the virus (as prophage) acts as a vector of certain genes, transferring them from donor to recipient cells.

The blue-green algal virus, cyanophage LPP-1, was discovered by Safferman and Morris in 1963. It was so named as to indicate its host-range (*Lyngbya*, *Plectonema* and *Phormidium*). Since then many more viruses attacking diverse blue-green algae have been reported (Safferman, 1973), and can now be broadly classified into the following categories: (1) LPP-1 viruses; (2) LPP-2 viruses, which are generally similar to LPP-1 viruses morphologically but different serologically; (3) SM-1 viruses (on hosts *Synechococcus* and *Microcystis*), which are polyhedral and have much shorter tails than those of LPP viruses; (4) N-1 virus (on host *Nostoc*), with general structure similar to LPP-1 but with contractile tail; (5) AS-1 (on hosts *Anacystis* and *Synechococcus*), largest phycovirus described so far and morphologically similar to LPP-1 but with particle size (head diameter 90 nm and tail length about 245 nm) much larger than LPP-1, and contractile tail like N-1; and (6) miscellaneous phycoviruses, infecting certain

species of *Cylindrospermum*, *Anabaenopsis*, *Raphidiopsis*, or *Anabaena*.

Although the nucleic acid and protein components of some of the above phycoviruses have not been analyzed in detail, available information (mostly on LPP-1) suggests that cyanophages contain double-stranded DNA.

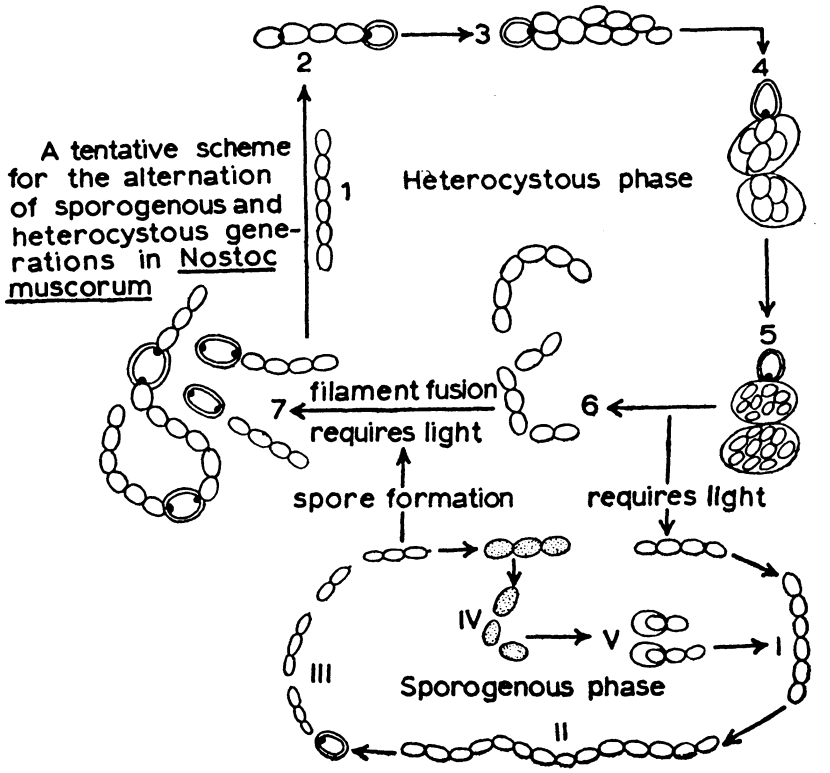

Fig. 5-1. *Nostoc muscorum*, life cycle and alternation of heterocystous and sporogenous generations. (After Lazaroff and Vishniac, 1962.)

A study of the replication cycle of LPP-1 in *Plectonema boryanum* revealed the following stages: (1) attachment of virus to host cell wall by means of the tail; (2) injection of viral DNA into the algal cell; (3) replication of viral DNA in the nucleoplasm of host cell and breakdown of host cell DNA; (4) migration of replicating viral DNA from nucleoplasm to photosynthetic thylakoids, leading to slight displacement of the latter; and (5) synthesis of protein coats and their enclosure around DNA particles, leading to virus assemblage.

Recent researches have also provided a strong indication of the existence of temperate cyanophages similar in properties to the well known bacteriophages. Cannon *et al.* (1971) have discovered a new strain of LPP-1 which successfully lysogenized the host alga, *P. boryanum*. In this system, the majority of the host cells were, of course, lysed but a few cells resisted lysis

and underwent lysogeny (i.e., they survived and divided in step with the cyanophage DNA harboured internally).

TAXONOMY AND CLASSIFICATION

The taxonomy of the blue-green algae has long been in a highly confused state. Enormous number of new species and genera have been created by different workers on trivial grounds, and borderline forms are extremely difficult to classify. Two biotic characteristics have contributed a great deal to the present state of confusion. One is the way in which various morphological features vary with differences in ecological conditions. The second is the variability of physiological and biochemical response observed under different conditions. This may merely be the physiological counterpart of the morphological variability or may represent genetically determined responses.

A few attempts have been made to produce a rational and orderly system of classification or taxonomic delimitation by reducing the number of genera and species but these have either been drastic or confined to a study of only one or two families rather than the entire phylum. A satisfactory solution can be found only after a combined approach involving a thorough study of the physiology, cytology and genetics of different taxa has been made. This seems a distant goal.

In this book the classification system of Fritsch (1945) has been followed. He divides the blue-green algae into five orders:

I. Chroococcales. Unicellular or colonial (palmelloid) forms; reproduction by cell division and by endospore formation.

II. Chamaesiphonales. Unicellular or colonial lithophytes or epiphytes exhibiting marked polarity; reproduction by endospores or exospores.

III. Pleurocapsales. Heterotrichous filamentous forms lacking heterocysts; reproduction by endospores; hormogonia absent.

IV. Nostocales. Non-heterotrichous filamentous forms often showing false branching; heterocysts commonly present; reproduction mostly by hormogonia and akinetes.

V. Stigonematales. Heterotrichous filamentous forms, mostly with heterocysts, showing true branching and usually pit connections between adjacent cells; multiplication mostly by hormogonia and rarely by hormocysts and akinetes.

PHYLOGENETIC RELATIONSHIPS

The blue-greens are the most primitive of algal organisms. In their general cytology, genetics and morphology as well as procaryotic nature they seem closely related to bacteria but differ from them in their photosynthetic metabolism and lack of flagellated cells. If the view that chloroplasts are cellular symbionts is accepted, the relationship to be considered is not that

between the procaryotic blue-green algae and the eucaryotic algae but between the former and the chloroplasts of the latter. Thus blue-green algae as a whole can be compared only with the chloroplasts of Rhodophyta or Cryptophyta. Some affinity with red algae is indicated by the presence of phycoerythrin and pit connections, and lack of flagellated structures. Further, the blue-green and red algae have a common pattern of fatty acid formation which differs from other plants in that the lipid content does not increase as the thallus grows. In these algae, nitrogen starvation is also not a factor in fatty acid accumulation (see Klein and Cronquist 1967). Phycobilins link Cryptophyta to blue-green algae.

The resemblance between blue-green algae and bacteria appears stronger and more fundamental than between blue-green and red algae. The chemical composition and ultrastructure of their cell wall and cytoplasm, the organization of photosynthetic apparatus, and aspects of hydrogen, nitrogen and sulphur metabolism link the blue-green algae with the rest of the Schizomycophyta via the photosynthetic bacteria. Clearly, the blue-green algae and the bacteria are two groups of the same Kingdom, Procaryota (Klein and Cronquist, 1967).

Order: CHROOCOCCALES
Family: Chroococcaceae
Genus: *Gloeocapsa*

This is a terrestrial or subaerial alga generally found on rocks, walls or soils. It is a palmelloid, colonial form. Each colony consists of a few cells (Fig. 5-2 A, B) which are more or less spherical and have conspicuous, coloured, stratified and concentrically lamellate gelatinous sheaths around them. The

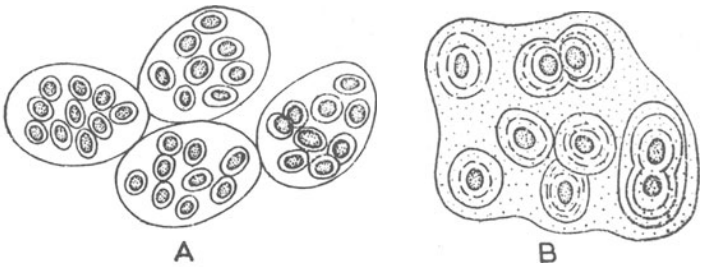

Fig. 5-2. A, *Gloeocapsa magma*; B, *G. janthina*.

sheaths not only surround individual cells but also the entire colony. In most species the photosynthetic thylakoids are arranged in rows at right angles to the plasma membrane, with the outer thylakoids being directly connected with plasma membrane; but in *G. alpicola* they lie parallel to longitudinal walls. Reproduction occurs by simple cell division, in three

planes, at right angles to one another, so that more or less spherical daughter cells are produced.

Order: NOSTOCALES
Family: Oscillatoriaceae
Genus: *Oscillatoria*

It is common and widespread in polluted waters, temporary rainwater pools, drains and streams. *O. rubescens* forms dense deep-water blooms in temperate lakes and ponds and its migration and colonization in deep water lakes is often associated with high level of eutrophication. Also characteristic of highly eutrophic waters are the populations of certain species having very narrow trichomes (less than 3 μm in diameter). Some species are marine. In the living state many species exhibit a characteristic swinging oscillatory movement of their trichomes. In streams, so long as the trichomes continue to move, they are not attacked by protozoan predators but once movement ceases, the trichomes are invaded and devoured by protozoa.

It is an unbranched trichomatous alga. The trichome consists of a number of simple, undifferentiated cells, commonly broader than long (Fig. 5-3 A, B). There are no constrictions between adjacent cells, no recognizable base or apex but the end cell in some species may by round, conical or dilated with its outer surface sometimes covered by a thickened hood known as calyptra. The end of the trichome is occasionally bent on one side. No heterocysts or spores are found in *Oscillatoria*.

The cells exhibit a typical cyanophycean structure, and prominent pseudovacuoles are formed in planktonic forms. Reports of a true nucleus or chromosomes in certain species require confirmation. Even if these are correct, it must be borne in mind that no one species of this genus possesses an entire nucleus and mitotic apparatus.

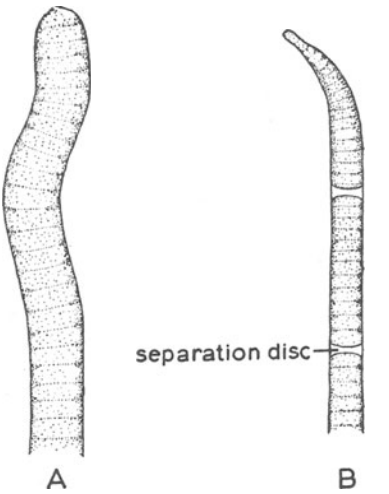

Fig. 5-3. A, *Oscillatoria curviceps*; B, *O. brevis*.

Whitton and Peat (1969) have studied the developmental variability of *O. redekei* grown in unialgal culture. They observed that gas vacuoles, trichome sheath and terminal cells having pointed ends were present at certain stages but absent at others. Their presence or absence depended upon physiological and environmental conditions. Furthermore, at certain stages of its development, *O. redekei* could not be distinguished from certain other species of *Oscillatoria*.

Reproduction is brought about by the breaking up of trichome into hormogonia as a result of the formation of biconcave separation discs (Fig. 5-3 B) at certain points along the trichome.

Family: Nostocaceae
Genus: *Nostoc*

It is a freshwater or terrestrial alga, abundantly found in paddy fields. Some species grow on moist soil intermingled with mosses and lichens whereas others may constitute the algal components of lichens or occur as endophytes in higher plants. *N. sphaericum* forms a close symbiotic association with the phycomycetous fungus *Geosiphon pyriforme*. The capacity for nitrogen fixation has been established in bacteria-free cultures of *Nostoc* sp. isolated from lichens and it has been shown that in the lichen *Peltigera aphthosa*, the nitrogen fixed by *Nostoc* is utilized by the fungal partner.

An interesting feature concerning the association of *Nostoc* with the angiosperm *Gunnera* is that the filaments are intracellular rather than intercellular, are devoid of sheath, but are enveloped by a layer of host cell cytoplasm.

It is a filamentous form and the filaments are often aggregated into ball-like gelatinous colonies which vary a great deal in size and shape, some being as large as a hen's egg. The filaments are usually contorted and twisted in various ways. Like *Oscillatoria* they are always unbranched but the cells have prominent constrictions between them. Consequently they seem to be arranged like beads in a string (Fig. 5-4 A, B). Such a habit is called

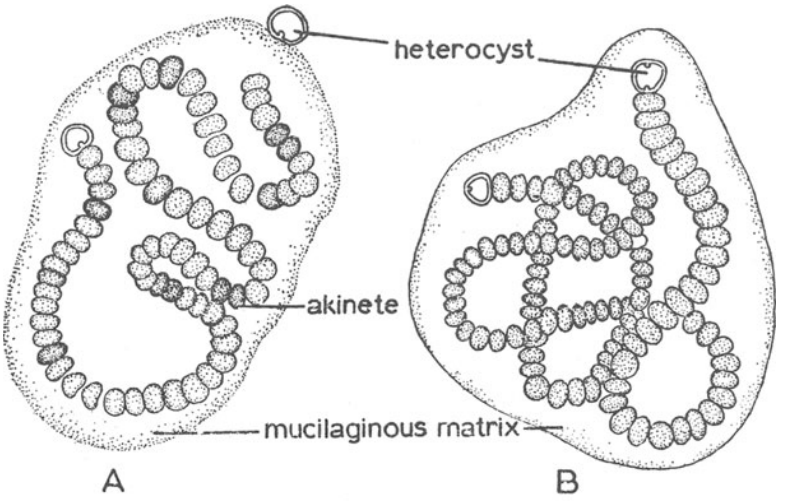

Fig. 5-4. A, *Nostoc commune*; B, *N. punctiforme*.

moniliform. Unlike *Oscillatoria*, the filaments regularly form heterocysts and occasionally also akinetes. The heterocysts are commonly two-pored and intercalary but rarely one-pored and terminal.

The sheaths of adjoining trichomes may coalesce to form a common gelatinous matrix of the colony. Cell structure is typically cyanophycean.

Reproduction takes place by hormogonia and akinetes. Lazaroff and Vishniac (1962) have also reported the occurrence of fusions and anastomoses between different filaments.

Based on developmental patterns, the various species of *Nostoc* may be broadly classified into three groups: (1) the *commune* type, in which the colonies have a firm pellicle; (2) the *piscinale* type, in which firm pellicle is absent; and (3) the *punctifanue* type, in which filamentous organization is lost and aseriate form results.

The essentiality of bacteria in the formation of normal colonies of *N. sphaericum* has been established by Schwabe and Mollenhauer (1967). When grown in the absence of bacteria, only minute colonies were formed instead of the normal, macroscopic colonies formed in bacterized cultures.

Family: Scytonemataceae
Genus: *Scytonema*

It occurs in fresh and saltwaters, terrestrial and subaerial habitats, grass lawns, on tree barks and on a wide variety of other substrata.

It differs from *Oscillatoria* and *Nostoc* in having branched filaments. The branching is false, and both single and double pseudobranches may be formed. Typically, however, two false branches are produced remote from two intercalary heterocysts (Fig. 5-5 A). A filament or its branches may have a few to many heterocysts though these are usually always two-pored and intercalary. The trichomes are not moniliform and the cells are more or less quadratic (as long as broad). Filaments have distinct mucilage

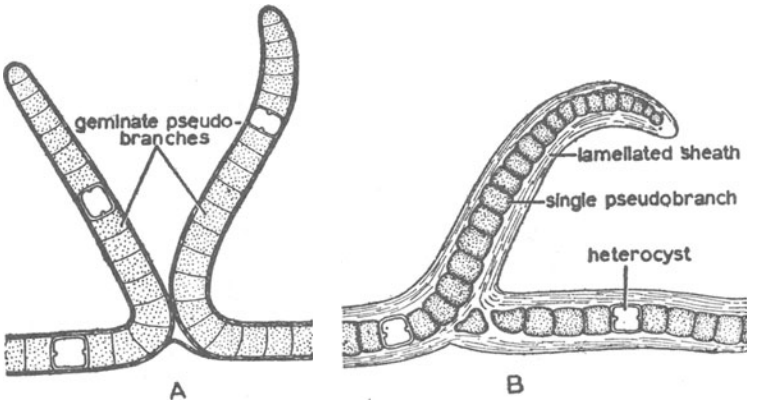

Fig. 5-5. A, *Scytonema ocellatum*; B, *S. insigne*.

sheaths (Fig. 5-5 B) which are thick, stratified and variously pigmented in terrestrial species.

False branches arise by the degeneration of a cell in the trichome follow-

ed by outgrowth of one or both ends of the interrupted trichome through the parent sheath as branches. Alternatively, they may arise by loop formation.

Reproduction occurs by hormogonia which are commonly liberated through the extremities of the filaments or branches.

TEST QUESTIONS

1. Mention the primitive characters of Cyanophyta which resemble bacteria. Are blue-greens more closely related to other algae, or to bacteria ?
2. In what respects does the ultrastructure of a cyanophycean cell differ from that of other algae ?
3. "Heterocyst is a botanical enigma", discuss this statement. Is it still an enigma ?
4. A pond develops a thick growth of algae such as *Plectonema*, *Phormidium* and *Lyngbya*. Can you suggest any quick method of destroying such a growth ?
5. Can the blue-green algae be employed to replace chemical fertilizers for increasing the fertility of soil ? Give reasons for your answer.
6. Discuss the significance of blue-green algae in relation to public health.
7. The blue-green algae have a wide ecological amplitude. What structural or physiological characteristics make this possible ?
8. Compare the genetic recombination found in Cyanophyta with the sexuality of other algae.
9. How would you distinguish between ordinary vacuoles, contractile vacuoles and gas vacuoles ?

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6

Chlorophyta

The Phylum Chlorophyta (green algae) includes two classes of eucaryotic algae, the Chlorophyceae and the Charophyceae. These have, as in higher plants, the same pigments (chlorophyll-a, chlorophyll-b, various carotenes and xanthophylls) and in the same proportion. The pigments are located in definite chloroplasts. The excess photosynthates are commonly stored in the form of starch. Inner layer of the cell wall is completely or partly cellulosic. The flagella, when present, are usually two or four in number, equal in length and of whiplash type.

OCCURRENCE AND ECOLOGICAL AMPLITUDE

The Chlorophyta comprise three main types of forms, namely, free living, attached and parasitic, and all or some of these types may be aquatic, amphibious, terrestrial or subaerial. Of the aquatic forms, the Ulvaceae and Siphonales are mostly marine whereas Conjugales and Oedogoniales are almost entirely freshwater.

Although the green algae are predominantly freshwater forms, quite a few species grow on or within the surface of moist soil, tree barks, moist and shaded rocks and on a variety of other terrestrial and subaerial habitats. Species of *Trentepohlia* constitute some of the best known examples of subaerial algae whereas those of *Cladophora* and some members of Chaetophorales grow attached on shells of molluscs in freshwater ponds and lakes. Some species of *Chlamydomonas* and *Characium* flourish on crustaceans, turtles or other aquatic animals.

Chlorochytrium lemnae is a space parasite (endophyte) in *Lemna* whereas *Cephaleuros* and *Rhodochytrium* are true parasites. Species of *Trebouxia*, *Chlorella* and *Trentepohlia* form symbiotic associations with fungi in various lichens. Some unicellular green algae are regularly found within the epidermal tissues of certain lower animals.

In contrast to Cyanophyta, Chlorophyta are mostly cold water forms although some strains of *Chlorella* are known to be thermophilic. *Chlamydomonas spp.* and various chlorococcaleans occur in such abundance on snow that they impart to it a reddish colour. This is due to the haematochrome (mostly secondary carotenoids dissolved in oil) which they possess.

RANGE OF VEGETATIVE STRUCTURE

The thallus exhibits a wide variation in its habit and morphology, ranging

from a motile or non-motile cell through colonial, filamentous, parenchymatous and siphoneous habits to the highly evolved heterotrichous filament. The highest type of specialization seems to have been attained in such genera as *Draparnaldiopsis* and *Chara* which have a highly differentiated, complicated and organized thallus. However, no elaborate and bulky parenchymatous or pseudoparenchymatous forms, as are met with in the Laminariales and Fucales of the Phaeophyta, are found in green algae. Simple, pseudoparenchymatous thalli are, however, known in the Siphonales.

CELL WALL

Except for a few primitive flagellate forms, the vast majority of green algae have a distinct, faitly rigid wall around their protoplast. The cell wall consists of two or three layers of which the inner may either be completely cellulosic (consisting of pure glucose residues), or a mixture of cellulosic and non-cellulosic chains. Outside the inner cellulosic layer is a layer of pectose or mucilaginous substance. The latter is water-soluble and hence is continuously regenerated in aquatic species. The gelatinous wall layer may be impregnated with lime in some members of the Siphonales. In *Cladophora* and *Oedogonium* a third chitinous layer may be laid down outside the pectose layer.

FLAGELLA

The motile vegetative cells and most zoospores and gametes generally have one, two, four, or rarely many, anteriorly inserted whiplash flagella of equal length. These are associated with a neuromotor apparatus which connects them with a **centrosome** lying within or just outside the nucleus.

PROTOPLAST AND CELL STRUCTURE

The green algae are typically eucaryotic, having definite nuclei, chloroplasts and mitochondria. A central vacuole is generally found in mature cells, with the cytoplasm confined to just inside the cell wall. In some species of *Spirogyra* the vacuolar sap contains purple pigments that mask the green colour of the chloroplasts.

Most green algae have uninucleate cells but those of Chlorococcales, Cladophorales, Siphonales and *Sphaeroplea* are multinucleate (coenocytic). The nucleus has a double-layered porous nuclear membrane, one or more nucleoli and centromeric chromosomes. Well-developed complex nucleoli occur in Conjugales and in *Acetabularia.*

There is a great diversity in the number, morphology and arrangement of their chloroplasts, and this has been interpreted to suggest the manifestation of evolutionary tendencies among different orders of the phylum.

A single, cup-shaped or bowl-shaped chloroplast per cell, with one or a

few pyrenoids, is characteristic of the Volvocales though rarely an axile stellate (*Chlamydomonas arachne*) or reticulate (*Sphaerella lacustris*) chloroplast may be found.

Single parietal chloroplast, usually having a single pyrenoid, is found in the Chlorococcales but *Trebouxia* has single, lobed, axile chloroplast with one pyrenoid whereas *Eremosphaera* has many, small, discoid chloroplasts devoid of pyrenoids.

A reticulate chloroplast with several pyrenoids is characteristic of *Hydrodictyon, Cladophora* and Oedogoniales, and a parietal ring-, collar- or girdle-shaped chloroplast containing a few pyrenoids is found in many Ulotrichaceae. A parietal chloroplast having a variable number of pyrenoids is also found in most of the Chaetophorales. In more advanced members of this order, e.g., *Draparnaldia* and *Draparnaldiopsis*, the main axes of central cells have a diminutive chloroplast restricted to the middle of the cell.

The Conjugales have three basic and common types of chloroplasts: (1) a flat axile plate with several pyrenoids (*Mesotaenium* and *Mougeotia*); (2) a pair of axile stellate chloroplasts, each with a single pyrenoid (*Zygnema*); and (3) one or a few spiral, ribbon-shaped chloroplasts, each with many pyrenoids (*Spirogyra*).

Many discoid chloroplasts with or without pyrenoids are found in Siphonales and in Charales.

The pyrenoid of green algae commonly consists of a proteinaceous core which is surrounded by minute plates of starch. It is often highly refractile.

The cells of the primitive, motile green algae commonly possess a pair of contractile vacuoles near the base of the flagella. The chloroplasts of motile cells generally also possess an eye spot near the anterior end.

The mitochondria, Golgi bodies and endoplasmic reticulum characteristic of eucaryotic cells are also found in the cells of Chlorophyta.

In Volvocales, Oedogoniales and Ulotrichales, the excess photosynthates are stored in the form of starch. Some Chlorococcales accumulate both starch and lipid, whereas most Siphonales and some Conjugales store fat.

CELL DIVISION

In the simple forms belonging to Volvocales and Chlorococcales, cell division is linked with reproduction but in most other green algae it may be both vegetative and reproductive.

During nuclear division all the four stages, i.e., prophase, metaphase, anaphase and telophase are evident. There are wide variations in the number and morphology of chromosomes but these seem to bear little, if any, taxonomic correlation or significance. In general, the nuclear cytology reveals two important features: (1) most green algae, except Conjugales, have centric chromosomes with localized centromeres in the median, sub-median or

terminal position; the Conjugales, on the other hand, have diffused centromeres; and (2) the wide range of variation in the chromosome number of different species is mostly due to **aneuploidy** although a few examples of **polyploidy** have also been reported.

Following nuclear division, the cell divides by a transverse furrowing of the cytoplasm, accompanied by the secretion of new cell wall material. Cytokinesis ranges from cleavage by invagination of the plasma membrane (as in *Chlamydomonas*) to the development of a distinct cell plate (as in *Fritschiella*), similar to that in higher plants.

REPRODUCTION

It may be brought about by any one or all of the three methods: (1) vegetative; (2) asexual; and (3) sexual.

VEGETATIVE

This takes place by splitting of colony or fragmentation of filament. The latter results from some external mechanical pressure, septum dissolution or from dying out or consumption during sexual or asexual reproduction of one or a few intercalary cells of the filament. In *Ulothrix*, *Oedogonium* or *Sphaeroplea*, the number of filaments increases by occasional fragmentation of one filament into two, each of which then grows to the adult size. The best known example is, however, constituted by *Stichococcus* in which the tendency to fragment is so strong and pronounced that as soon as the filament grows to the stage of four or six cells, fragmentation occurs so that filaments longer than 4–6 cells are seldom found.

ASEXUAL

The commonest method is by repeated division of the protoplasts of certain cells. The daughter protoplasts are either transformed into motile and flagellate, commonly naked zoospores, or they round off into non-motile aplanospores provided with true cell walls. The number of zoospores or aplanospores produced within a mother cell varies from one (*Oedogonium*), two, four or more (in many algae) to as many as several thousand in *Hydrodictyon*. These asexual cells may be produced either in ordinary, unmodified cells (*Ulothrix*) or in specially modified cells known as zoosporangia (*Trentepohlia*).

In many forms, zoospores are produced during night and liberated in the morning. Their formation is most active during favourable growth periods when the alga is young and healthy. Formation of zoospores may sometimes be artificially induced by modifications of environmental conditions such as pH, temperature, light, and transference from flowing to still water.

Zoospores are liberated either by gelatinization of the parent cell wall or, more commonly, through one or more pores formed in the lateral walls.

When liberated they are generally naked and swarm around in this state for a few hours or a few days. They then come to rest and attach themselves to some substratum by their anterior ends, lose flagella, secrete a wall, and become quiescent for some time before growing into new individuals.

SEXUAL

Most green algae, except some Chlorococcales, reproduce sexually. In the Conjugales the fusing gametes are amoeboid and non-flagellate whereas in other orders at least the male gamete is always flagellated.

The Chlorophyta exhibit wide variations in the kind and formation of their gametes. When the fusing gametes are morphologically identical and indistinguishable they are known as isogametes; such isogamous reproduction is noticed, among others, in species of *Chlamydomonas*, *Ulothrix* and *Zygnema*. When the fusing gametes are both motile and flagellate but unequal in size, they are termed anisogamous as found in *Chlamydomonas braunii*, *Aphanochaete*, and others. Oogamous reproduction involves fusion between a small flagellate and actively motile male gamete (known as the antherozoid) and a large, non-motile, non-flagellate and passive female gamete, the ovum or oosphere. The ovum is generally produced singly within a specially differentiated and enlarged cell called the oogonium, e.g., *Oedogonium* and *Coleochaete*. Antherozoids are, however, many and are produced within an antheridium; in *Oedogonium*, nevertheless, only two antherozoids are produced per antheridium.

In *Spirogyra*, the fusing gametes, which are non-flagellate, are morphologically isogamous but physiologically anisogamous since one of them behaves as male and the other as female. *Sirogonium* provides an isolated example of morphological anisogamy in the order Conjugales since it forms a small male and large female gametangium.

The advancement in sexual reproduction, from isogamy through anisogamy to oogamy, seems to have been accompanied by a reduction in number and increase in size of the female gametes. The evolution from isogamy to oogamy perhaps occurred independently in different orders of the green algae. Progressive evolutionary series illustrating the transition from isogamy to oogamy are met with in the coenobial Volvocales and in certain Chaetophoraceae, e.g., *Stigeoclonium*, *Aphanochaete*, *Chaetonema* and *Coleochaete*.

With the exception of three species (*Chlorogonium oogamum*, *Carteria iyengarii* and *Chaetonema irregulare*) in which the ovum is released from the oogonium prior to fertilization, in all others fertilization takes place while the ovum is still within the mother cell.

Generally the two members of a pair of fusing gametes are derived from two different individuals, such individuals being termed heterothallic. A number of green algae are however homothallic; in these fusion can take place between two gametes which have been derived from the same parent individual.

The zygote formed as a result of isogamous or anisogamous reproduction

or conjugation is called zygospore, whereas that produced as a result of oogamous fertilization is termed oospore. In most haploid Chlorophyta, the diploid zygospore or oospore secretes a thick wall and becomes transformed into a resting cell. In the next favourable season it germinates to produce one or more plants. On the other hand, in many marine Chlorophyta, some Cladophorales and certain diploid forms, the zygote germinates directly into a vegetative plant.

Occasionally, some gametes, without fusion, may directly give rise to new plants, or may form resting cells known as parthenospores which subsequently produce new plants. Such parthenogenesis occurs in *Ulothrix* and certain other algae.

PERENNATION

Certain green algae produce special resting stages that enable the alga to pass through adverse or unfavourable environmental conditions. Such resting cells may be vegetative (akinetes), asexual (hypnospores) or sexual (zygospores or oospores).

Akinetes are prominently differentiated and enlarged vegetative cells which become thick-walled and densely laden with food reserves to tide over adverse periods. In these the spore wall fuses with that of the parent cell and a few additional wall layers are deposited outside the whole structure. Characteristic intercalary and terminal akinetes are found in species of *Pithophora*. Hypnospores are modified aplanospores that secrete specially thickened cell walls around them, e.g., in *Sphaerella* and *Protosiphon*.

In many Chlorophyta the only mode of perennation is by the formation of resting zygospores or oospores.

The ordinary vegetative cells of *Pleurococcus*, *Trebouxia*, *Prasiola* and certain other terrestrial or subaerial algae can perennate as such, i.e., without the formation of any special stages.

LIFE CYCLE AND ALTERNATION OF GENERATIONS

Four main types of life cycle—haploid, diploid, isomorphic or homologous, and heteromorphic—are met with in the green algae.

Ulothrix, *Pandorina*, *Coleochaete* and *Oedogonium*, for example, are haploid, with the zygote representing the only diploid stage in the life cycle. In these, meiosis occurs during the first division of the zygote nucleus (Fig. 3-2).

In the diploid forms such as many Siphonales and some Chlorococcales, the mature vegetative plant is diploid and meiosis occurs during the formation of gametes which represent the only haploid stage. The zygote germinates direct into the diploid plant.

The isomorphic life cycle involves an alternation between a haploid gametophyte and a diploid sporophyte, both of which are morphologically quite indistinguishable from each other. The haploid phase produces

gametes that fuse to form the zygote which in turn germinates into a diploid sporophytic plant and produces zoospores without undergoing any meiotic division. Meiosis occurs during the formation of zoospores and hence they are haploid. These grow into haploid gametophytes (Fig. 3-4). This kind of alternation of generations is found in the Ulvaceae, many Cladophorales and certain Chaetophorales, e.g., *Draparnaldiopsis* and *Fritschiella.*

Urospora, in which the two free living alternating generations are morphologically dissimilar, provides an example of the heteromorphic life cycle.

CLASSIFICATION

The use of electron microscope has considerably added to our understanding of the micromorphology of green algae. This study in conjunction with their chemical and physiological features has led many phycologists to propose different systems of classification of Chlorophyta. These systems vary mainly in the degree of emphasis placed on a particular combination of phyletic characters.

Emphasizing the morphological and reproductive features, Fritsch (1935) proposed a widely accepted system of classification. He divided the green algae into nine orders (Volvocales, Chlorococcales, Ulotrichales, Cladophorales, Chaetophorales, Oedogoniales, Conjugales, Siphonales and Charales). In this book we have followed the same system of classification but have raised Charales to the rank of a Class (Charophyceae) coordinate with Chlorophyceae. Thus, the Chlorophyta includes two classes of which the Chlorophyceae has eight orders whereas the Charophyceae has single order, the Charales.

Two of the more widely followed systems of classification that may be mentioned here are those of Bold (1967) and Round (1971).

Bold, following some earlier workers, has raised Charales to the rank of a Phylum, the Charophyta. The Division Chlorophycophyta includes only one Class, the Chlorophyceae, having ten orders (Volvocales, Chlorosphaerales, Chlorococcales, Ulotrichales, Ulvales, Cladophorales, Siphonales, Dasycladales, Oedogoniales and Zygnematales).

Round (1971) has divided the green algae (Chlorophyta *sensu Fritsch*) into three phyla containing six classes and 37 orders as follows:

I. Phylum CHLOROPHYTA
   - Class Chlorophyceae—Orders Chlamydomonadales, Volvocales, Polyblepharidales, Tetrasporales, Chlorodendrales, Chlorosarcinales, Chlorococcales, Ulotrichales, Codiolales, Ulvales, Prasiolales, Cylindrocapsales, Microsporales, Chaetophorales, Trentepohliales, Pleurococcales, and Ulvellales.
   - Class Oedogoniophyceae—Order Oedogoniales.
   - Class Zygnemaphyceae—Orders Mesotaeniales, Zygnematales, Gonatozygales, and Desmidiales.
   - Class Bryopsidophyceae—Orders Cladophorales, Sphaeropleales,

Acrosiphonales, Dasycladales, Siphonocladales, Chlorochytriales, Derbesiales, Codiales, Caulerpales, Dichotomosiphonales, and Phyllosiphonales.

II. Phylum PRASINOPHYTA
Class Prasinophyceae—Orders Pyramimonadales, Prasinocladales, and Halosphaerales.

III. Phylum CHAROPHYTA
Class Charophyceae—Order Charales.

EVOLUTIONARY TRENDS WITHIN CHLOROPHYTA

While discussing the phyletic relationships of any taxon the terms "primitive" and "advanced" are frequently encountered. Their meaning is relative and can be understood with reference to two hypothetical taxa A and B; B can be considered advanced over A if its life cycle includes a stage that resembles A. Such a generalization holds true only for organisms that are phyletically related.

In tracing the phyletic trends within Chlorophyta, the use of three characters has been emphasized: (1) thallus organization, (2) sexual reproduction and (3) life cycle.

The motile or non-motile unicell, the motile or non-motile colony, and the colonial, the filamentous and the parenchymatous thalli constitute an ascending series in which the unicellular habit is considered the most primitive and the filamentous or parenchymatous the most advanced. Similarly, in the isogamous-anisogamous-oogamous series, isogamy is considered primitive whereas oogamy represents an advanced type of sexuality.

The unicellular motile forms such as *Chlamydomonas* are generally accepted as the most primitive among green algae from which evolution has progressed along three different lines—volvocine, tetrasporine and chlorococcine (coccoid). The volvocine line represents a series in which the products of the vegetative cell division are flagellated and are retained in a common mucilaginous envelope giving rise to a motile colony. This line ends abruptly in *Volvox*. The tetrasporine line parallels the volvocine in all aspects except that its vegetative phase is predominantly non-motile and the motile phase is mainly confined to the reproductive stages. The chlorococcine line differs from tetrasporine line in its inability to divide vegetatively.

According to Fritsch (1935) the filamentous and parenchymatous forms found in the Ulotrichales are derived from motile unicellular flagellates of the *Chlamydomonas* type; the cell division is in only one plane, i.e., transverse, followed by the holding together of its products through the formation of a common cross wall between the cells, thus leading to the development of the filamentous habit from the unicellular ancestors. Cell division of the unicellular ancestor in two or more planes seems to have given rise to the parenchymatous habit of the present day *Ulva* and other similar algae.

Some members of the Tetrasporales which show a filamentous tendency appear to have given rise to the Ulotrichales. The Chaetophorales probably

originated from the Ulotrichales. For the Charales, many authors have proposed a chaetophoralean ancestry. There seems to be a general unanimity on the derivation of the Siphonales from the Chlorococcales. The origin of Conjugales and Oedogoniales is more uncertain; however, Chapman (1962) favours a ulotrichalean ancestry for the Conjugales, and Klein and Cronquist (1967) likewise suggested a possible ulotrichalean ancestry for the Oedogoniales.

Class: CHLOROPHYCEAE
Order: VOLVOCALES

The unicellular and colonial green algae with either a permanently motile, or a sedentary vegetative phase that is readily revertible to motility, are all included in this order. On the basis of vegetative habit this order has been divided into three suborders: (1) the Chlamydomonadineae, comprising motile individuals, (2) the Tetrasporineae, consisting of palmelloid forms and (3) the Chlorodendrineae, including dendroid species. The suborders Tetrasporineae and Chlorodendrineae have many *Chlamydomonas*-like characters, such as eye spot, contractile vacuoles and chloroplast.

The Chlamydomonadineae includes four families distinguishable by cell wall morphology: the Chlamydomonadaceae has a cellulosic cell wall; the Sphaerellaceae has a thickened gelatinous wall provided with a number of simple or branched processes and a chloroplast of variable morphology, generally containing one or more pyrenoids; the Polyblepharidaceae includes naked forms capable of exhibiting changes in the shape of the protoplast and provided with two or more flagella; and the Phacotaceae characterized by cells that are usually enveloped in a calcified sheath or capsule separated from the cytoplasmic membrane by an intervening mucilaginous space.

Family: Chlamydomonadaceae
Genus: *Chlamydomonas*

Occurrence

Most species of this alga are freshwater forms, or grow in ditches, tanks, ponds and lakes. Some are marine whereas others occur on moist, terrestrial habitats. Quite a few grow in brackish water and in waters polluted with sewage or industrial wastes. *Chlamydomonas* also occurs in airborne dust. *C. nivalis* accumulates large quantities of haematochrome and is found on snow in alpine and arctic regions, causing 'red snow'.

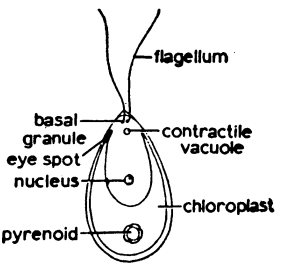

Fig. 6-1. *Chlamydomonas*, structure as seen under light microscope.

Nutrition

Most species are obligate phototrophs but *C. dysosmos* is a facultative heterotroph and can

grow in the dark in presence of acetate as a carbon source.

Morphology

Light Microscopy. *Chlamydomonas* is a motile unicellular alga. It is generally oval but variations in its shape are also noticed. The cell is provided with a cellulosic wall and gives rise to two anteriorly inserted whiplash flagella, each originating from a basal granule located in the anterior papillate or non-papillate region of the cytoplasm (Fig. 6-1). Two, rarely more, contractile vacuoles are found near the bases of the flagella and a prominent cup or bowl-shaped chloroplast is present in each cell. The chloroplast contains at its posterior end a pyrenoid with a starch sheath, and towards one side of its anterior end is found an eye spot. The single nucleus remains suspended in the colourless portion of the cytoplasm.

Variability. *Chlamydomonas* exhibits great variability in cell shape (Fig. 6-2), insertion of flagella, cell wall, chromatophore (Fig. 6-3 A–G), pyrenoid, and in various other structural and reproductive features. A

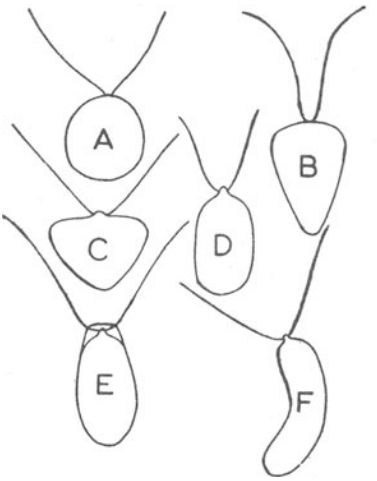

Fig. 6-2. *Chlamydomonas*, some cell shapes. A, *C. incerta*; B, *C. conica*; C, *C. gyroides*; D, *C. biconvexa*; E, *C. pseudocostata*; F, *C. lunata*. (After Ettl, 1965.)

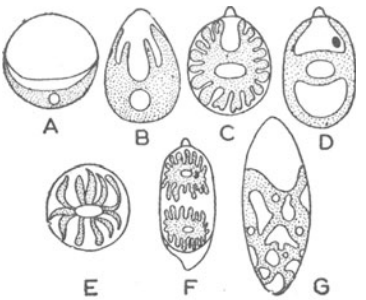

Fig. 6-3. *Chlamydomonas*, chloroplast morphology. A, *C. ametastatos*; B, *C. polydactyla*; C, *C. corrosa*; D, *C. regularis*; E, *C. arachne*; F, *C. zygnemoides*; G, *C. diffusa*. (After Ettl, 1965.)

detailed study of such variability and the importance of such features in the taxonomic delimitation of different species has been described by Ettl (1965).

Electron Microscopy. The flagellum shows the typical 9 + 2 arrangement of the component fibrils. The cytoplasmic, mitochondrial, nuclear and chloroplast membranes are all double unit structures. The chloroplast contains bands composed of variable number of photosynthetic thylakoids

which are not organized into grana-like structures (Fig. 6-4). A colourless, granular matrix separates the two consecutive bands of a thylakoid. The chloroplast thylakoids are neither derived from chloroplast envelope nor from pyrenoid lamellae but originate by incorporation of fresh material into pre-existing membranes. The eye spot consists of two or three, more or less parallel rows of linearly arranged fat droplets. Also present in the cytoplasm are endoplasmic reticulum and dictyosomes (Golgi bodies).

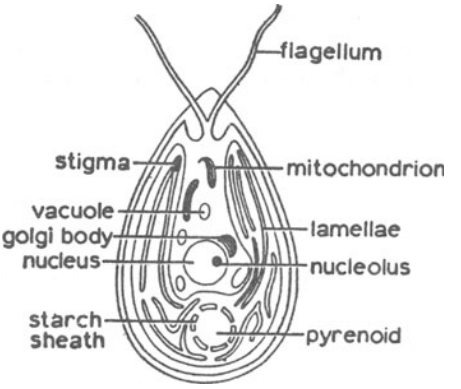

Fig. 6-4. *Chlamydomonas*, ultrastructure. (After Sager, 1965.)

Function

Motility is accomplished by the lashing action of flagella, the energy for which is derived from ATP (adenosine triphosphate). However, it has been reported that the flagella of a paralyzed mutant of *Chlamydomonas* fail to beat even if the alga is fed with ATP; this is thought to result from the absence of the enzyme ATPase in the paralyzed mutant.

In *C. reinhardii* the flagellar apparatus can produce two different kinds of motion, viz., a breast-stroke which propels the alga forward, and an undulating beat which produces trailing, backward motion.

The eye spot is apparently not an organ of **phototaxis** since mutant strains of the alga lacking an eye spot are still phototactic. The removal of water or other material from the cell is a function generally assigned to the contractile vacuoles. The inability of mutants of *Chlamydomonas*, lacking contractile vacuoles, to grow in solutions of high osmotic pressure suggests that contractile vacuoles are involved in the osmotic regulation of the cell.

Reproduction

Asexual. During favourable environmental conditions, the alga discards its flagella and enters into a non-motile phase, its protoplast undergoes two, three or four successive mitotic divisions (all longitudinal) resulting in the production of 4, 8, or 16 uninucleate daughter protoplasts (Fig. 6-5 A). Each of these acquires a pair of flagella and a wall before being released from the mother cell through gelatinization or rupture of the mother wall. The zoospores gradually develop into mature *Chlamydomonas* cells.

Under adverse conditions, the daughter protoplasts, instead of growing into zoospores, continue to divide, each producing a group of 2 to 4 aplanospores within its own newly formed gelatinous wall. Meanwhile, the original parent wall also gelatinizes and the entire groups of aplanospores remain embedded within a common gelatinous matrix. Such a phase, which may

be quite short-lived, is called a **Palmella stage** (Fig. 6-5 B). With the recurrence of a favourable growing period, each aplanospore changes into a zoospore which grows in size to become a *Chlamydomonas* plant. In *C. nivalis*, the aplanospores develop into hypnospores.

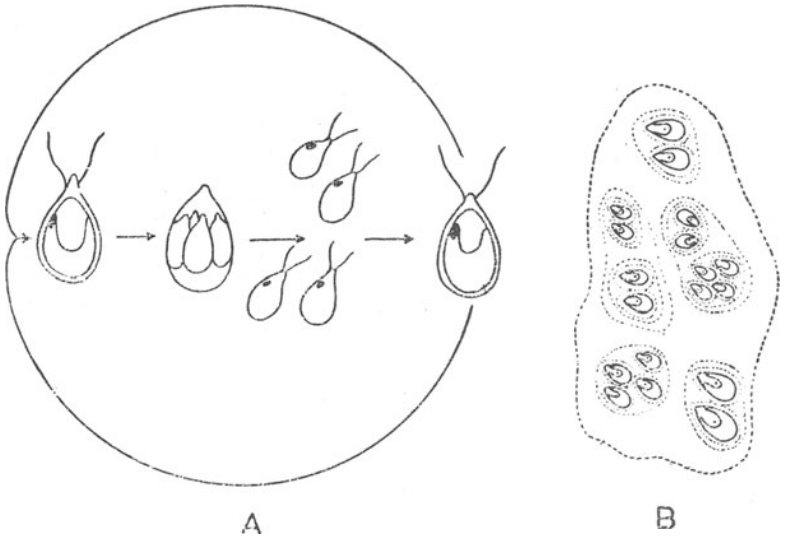

Fig. 6-5. A, diagram showing zoospore production; B, Palmella stage.

Sexual. It ranges from isogamy to oogamy (Figs. 6-6; 6-7 A, B) and the sexually reproducing alga may be homothallic (*C. gynogama*, *C. media*) or heterothallic (*C. reinhardii*). Anisogamy and oogamy have been described on the basis of observations with materials collected from natural habitats.

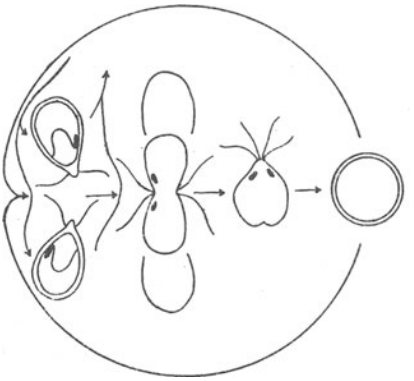

Fig. 6-6. Isogamy.

Therefore, virtually nothing is known about the nature of genetic or environmental factors controlling sexual reproduction in anisogamous or oogamous species of *Chlamydomonas*. Much experimental work, however, has been

carried out on the isogamously reproducing heterothallic species.

In heterothallic *C. moewusii* adult individuals themselves copulate. In other cases, gametes are generally formed by repeated divisions of the protoplast of a mother cell and are therefore smaller in size.

In *C. debaryanum*, the sexual reproduction is isogamous involving a union between biflagellate gametes. *C. braunii* shows anisogamy (Fig. 6-7 A). The male zoogametes (microgametes) are smaller in size and are produced in greater numbers per cell than the female zoogametes (macrogametes). As a rule, the male cell produces eight microgametes whereas the female produces only four macrogametes. This difference between two kinds of gametes is further advanced in the oogamous *C. coccifera* (Fig. 6-7 B) where the female cell, instead of dividing to produce gametes, discards its flagella and directly functions as an immobile female gamete, the egg. The male gametes, on the other hand, are produced by repeated divisions of the protoplast of the cell into 16 small, biflagellate microgametes.

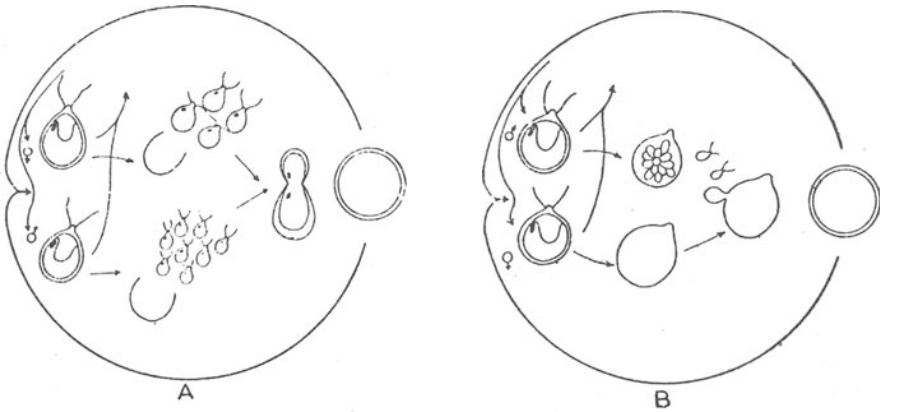

Fig. 6-7. *Chlamydomonas*. A, *C. braunii*, diagram showing anisogamy; B, *C. coccifera*, illustrating oogamy.

Control of Sexuality. In *C. reinhardii* and *C. moewusii* (=*C. eugametos*), two main kinds of factors—genetic and environmental—are known to affect the onset and course of sexual reproduction.

Genetic Factors. A pair of sex-determining **allelic genes** designated + and − exist in the diploid zygote. The complementary genes segregate in the ratio of 1 : 1 during meiosis in the zygote, thus giving rise to half + type and half − type individuals (Fig. 6-8). Mating can occur only between gametes of complementary sexes, i.e., + and −.

The radiation-induced non-flagellate mutants of *Chlamydomonas* are completely asexual suggesting that, for initiation of gametic union, the presence of flagella is indispensable.

Environmental Factors. In general, nitrogen deficiency induces gametogenesis. Ammonium nitrogen is very specific in the inhibition of sexuality (Fig. 6-8). Even the differentiated gametes lose their gametic nature in the presence of ammonium nitrogen and behave as vegetative cells. The male

and female cells differ in their response to light; male gametes (— mating type) are produced in the presence of light and female gametes (+ mating type) in its absence (Wiese, 1965). The presence of calcium is essential for

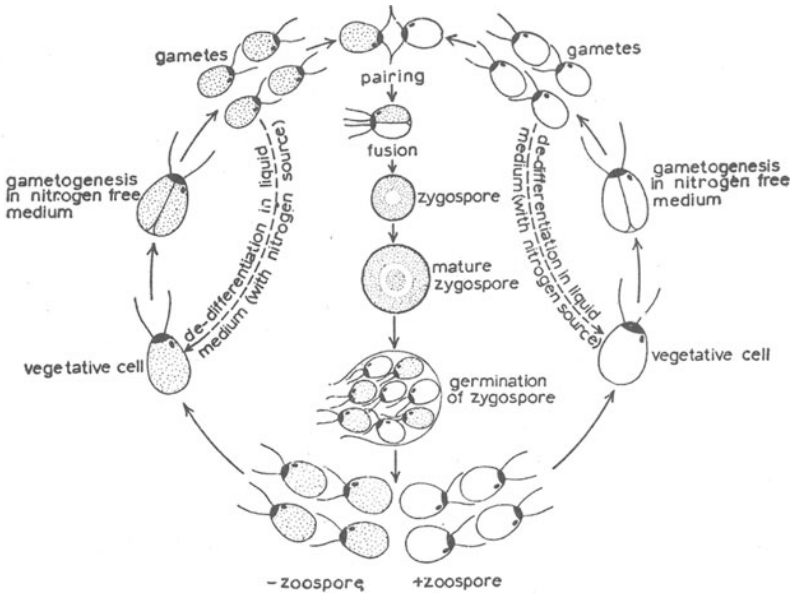

Fig. 6-8. *Chlamydomonas reinhardii*, diagram illustrating the experimental control of sexual cycle.

mating. The intensity of light and temperature also affect the percentage of mating gametes. With the increase in light intensity from 800 to 6000 lux, there is a corresponding increase in the number of zygotes (Trainor, 1958). Further, when grown under continuous illumination, the yield of zygotes in *C. chlamydogama* was less than one half of that grown under alternating light and dark periods of 12 hr each. The number of zygotes at 27°C was higher than at 22°C, even though the duration and the intensity of light were the same in both cases.

Zygote Formation. This includes five distinct phases: (1) gametic differentiation, (2) clumping, (3) pairing, (4) plasmogamy, and (5) karyogamy.

The differentiation of vegetative cells into gametes is conditioned by such factors as nitrogen deficiency, high light intensity and high CO_2 concentration. Clumping (Fig. 6-9 A) involves the clustering or aggregation of + and — gametes through agglutination and entanglement of their flagellar tips. The initial attraction for the clumping of complementary gametes is probably due to the extrusion from their flagellar tips of sexually compatible strands (Brown, Johnson and Bold, 1968). It is believed that this agglutination results from the presence of complementary mating type substances (known as isoagglutinins) of a glycoproteinaceous nature in the flagellar tips (Wiese,

1965). After clumping, the pairing phase starts in which the individual gametes within a clump pair off (Fig. 6-9 B) each pair consisting of a + and a — gamete still united at their flagellar tips. The factors leading to the formation of the gametic pairs are not known. Subsequent to the pairing phase comes the gametic fusion during which the flagellar union progresses downward towards the papillate surface of the two gametes. The gametic pair then establishes a connection or passage through the fusion of the two papillae, and this is followed by the release of flagellar union, thus making the gametic pair temporarily motile (Fig. 6-9 C). Immediately after this, the flagella are discarded and the cytoplasmic fusion is followed by the fusion of the gametic chloroplasts (Brown, Johnson and Bold, 1968). Karyogamy follows plasmogamy, the two nuclei coming to lie in the equatorial region where they fuse.

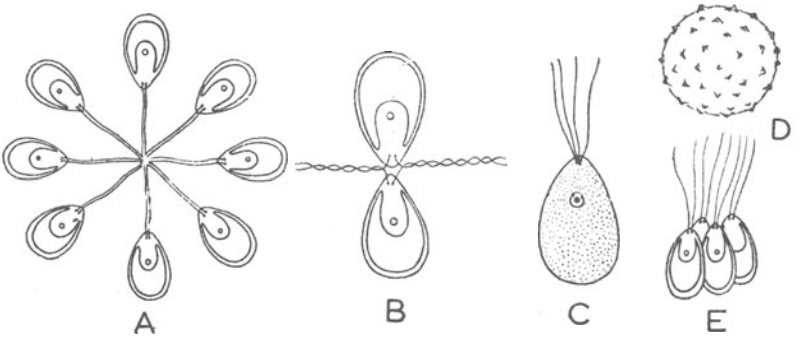

Fig. 6-9. *Chlamydomonas*, stages in sexual reproduction.

The zygote (Fig. 6-9 D) is normally a thick-walled structure which germinates after a certain resting period, the meiosis taking place during the first nuclear division. The haploid zoospores (Fig. 6-9 E) resulting from zygote germination give rise to *Chlamydomonas* plants.

Chromosomal and Non-chromosomal Genes. Cytological investigations have failed to reveal the morphology or number of chromosomes in *Chlamydomonas*. The genetical system of the alga is very interesting in view of its having both chromosomal (*c*) and non-chromosomal (*nc*) genes affecting the same phenotype (Sager, 1960). Thus, it has been shown that sensitivity, resistance, or dependence on streptomycin are governed by *c* as well as *nc* genes (Sager, 1962). The requirement for acetate in some strains is also known to be determined by *nc* genes. Recombination for both *c* and *nc* genes has been demonstrated by Sager (1965). The *nc* genes are thought to be present on the DNA of the plastids.

Life cycle of *Chlamydomonas reinhardii*. *C. reinhardii* is a convenient experimental subject and some workers have recently grown it in synchronous culture. Since most of the cells in such cultures are at the same stage of division or development at any given time, uniformly homogeneous material can be obtained for the study of sexual process at the molecular level.

There are two strains of *C. reinhardii* in respect of the number of zoospores produced per zygote. The strain which produces 8 zoospores is referred to as the octet strain and the one forming 4 zoospores as the quartet strain. The heterothallic octet strain has a simple life cycle with all those characteristic features of the sexuality typical of higher plants. The synchronization of mitotic cell division is achieved by simply growing the cells in alternating light-dark periods. The products of mitosing cells differentiate into zoospores in the presence of a nitrogen source but into gametes in the absence of nitrogen. When transferred to fresh medium containing combined nitrogen, the gametes can dedifferentiate into zoospores. Karyogamy occurs after 12 hr of gametic union and the zygotes undergo a maturation period of a few days. The mature zygote germinates and divides meiotically on transfer to a fresh culture medium.

Three different kinds of DNA have been detected in *C. reinhardii.* These are the α, β and γ which differ from each other in buoyant densities and guanine-cytosine content. The α-DNA represents the chromosomal DNA, the β occurs in chloroplasts, and the source of γ is still unknown. During maturation of zygote, the γ-DNA is replaced by a new type known as 'maturation DNA or M-DNA'. In addition to M-DNA, the zygote contains α- and β-DNA.

When the + and − gametes differing from each other in respect of two contrasting pairs of genetic markers are crossed, 4 pairs of zoospores, in which the two cells of each pair have identical genotype, emerge. This suggests that crossing over takes place at the 4-stranded stage. This fact together with the observation that only one round of DNA replication occurs between gametic union and emergence of 8 zoospores, suggests that gametes contain double the amount of DNA present in the zoospores.

Genus: *Volvox*

Occurrence

It is a freshwater planktonic form occurring as green balls of pin-head size in temporary and permanent pools and ponds. The spring and rainy seasons are the usual periods of their active vegetative growth.

Morphology

The colonies (coenobia) generally have a spherical or ellipsoidal contour, and are motile (Bonner, 1950); the movement is brought about by the joint action of the flagella of individual cells. The coenobium consists of a mucilaginous matrix within the periphery of which lie all the cells in a single layer (Fig. 6-10). The central portion of the colony is hollow and devoid of cells but is filled with mucilaginous substance. Each individual cell has its own mucilaginous sheath which may be confluent with the sheaths of adjoining cells. The coenobia of *V. aureus* are composed of 500–3000 cells whereas those of *V. globator* have up to about 20,000 cells. Other species may have 500–50,000 cells per coenobium. In their general morphology, the indivi-

dual cells of *Volvox* resemble *Chlamydomonas* and *Sphaerella* in that a cell has a pair of anteriorly inserted flagella and a cup-shaped or laminate chloroplast containing one or more pyrenoids. Two or more contractile vacuoles

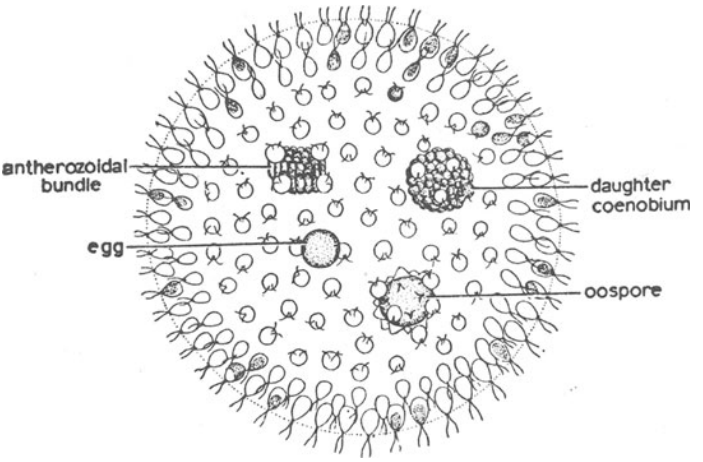

Fig. 6-10. *Volvox aureus*, coenobium.

and an eye spot are found in the anterior region. The cells in the posterior region of the coenobium are usually larger than those in the anterior region. Most species have prominent cytoplasmic strands connecting the cells of a colony but *V. mononae* and *V. tertius* lack such connecting strands. The nucleus possesses cytologically distinct chromosomes, the haploid number for *V. globator* and *V. aureus* being 5.

Reproduction

In contrast to *Chlamydomonas*, which discharges all its vital functions (including reproduction) and normally does not die, the cells of a *Volvox* colony show pronounced functional specialization.

Asexual. Most of the cells of a coenobium are vegetative but a few in the posterior region are reproductive (gonidial) which enlarge and lose their flagella.

Two to 50 asexual gonidia are produced in a coenobium. Before they divide, the gonidia are slightly pushed into the interior of the colony. The first two divisions are in planes perpendicular to each other (Fig. 6-11 A) but subsequent divisions are longitudinal. The daughter protoplasts at the 8-celled stage are in the form of a curved plate called the **Plakea stage** (Fig. 6-11 B). At the 16-celled stage, cells are arranged within the periphery of a hollow sphere with an opening toward the anterior end. The cell division phase in the developing coenobium continues until it attains a specific number of cells. At the end of this phase, the peripheral row of almost spherically arranged cells which are still naked, have their anterior ends pointing towards the centre of the developing coenobium. The

coenobium then undergoes a complete inversion, i.e., inside-out turning, through the opening, with the consequence that the anterior ends of cells, which were previously facing the centre of the sphere, now face outward. Each cell of the daughter coenobium finally acquires a cell wall and a pair of flagella at its anterior end. The daughter coenobia are ultimately released after the disintegration of the parent coenobium.

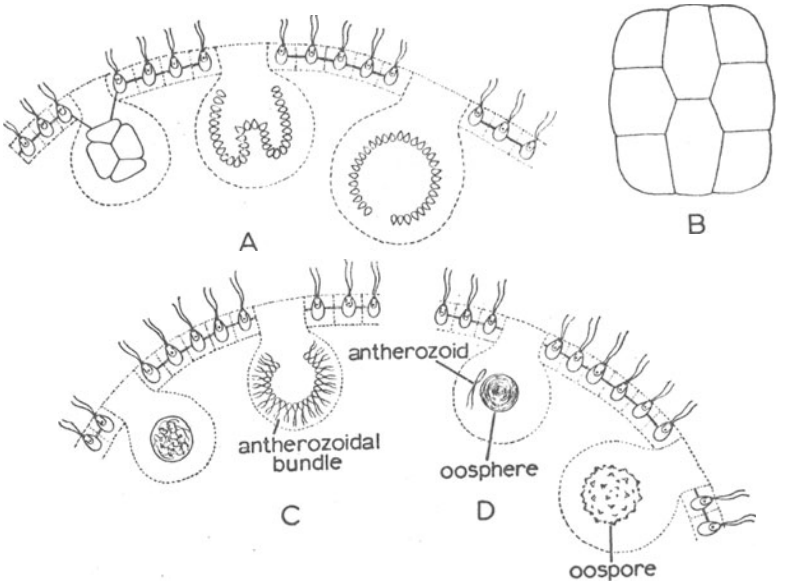

Fig. 6-11. *Volvox*. A, part of colony showing asexual reproduction; B, Plakea stage; C–D, stages in sexual reproduction. (After Smith, 1955.)

Sexual. This is oogamous. Species reproducing sexually may be monoecious (*V. globator*) or dioecious (*V. aureus*). Since the monoecious forms are commonly **protandrous**, fertilization between antherozoids and eggs from different individuals is effected. The gonidium-like male and female cells are respectively called antheridia and oogonia.

Each antheridium undergoes repeated cell divisions in a way similar to that in the development of an asexual gonidium into a daughter coenobium. A mass of naked, biflagellate fusiform antherozoids, ranging in number from 16 to 512 per antheridium, is ultimately produced (Fig. 6-11 C).

Vegetative cells destined to become oogonia enlarge, lose their flagella and become rounded or flask-shaped, with much of the oogonial portion projecting into the interior of the colony. The entire contents of the oogonium finally form a single non-flagellate egg (Fig. 6-11 D) with a beak-like protrusion toward one side, through which the antherozoid enters the oogonium.

During fertilization the entire mass of antherozoids is set free as a unit colony; this bundle remains intact until it approaches an egg. The individual antherozoids are then set free and one of them fuses with the egg,

leading to the production of the oospore (Fig. 6-11 D). The oospore subsequently secretes a smooth or spiny wall of three layers; it also accumulates much haematochrome that colours the zygote orange red. The oospore constitutes the perennating stage in the life history of *Volvox*.

During oospore germination, the two outer wall layers gelatinize and the inner layer forms a vesicle in which the zygote protoplast migrates. The zygote nucleus divides meiotically and of the resulting 4 nuclei, three degenerate but the fourth uninucleate protoplast develops into a coenobium through asexual reproduction.

The alga is haploid with its diploid phase restricted to the zygote.

A *Volvox* colony contains only two types of cells, the somatic and the reproductive. The culture filtrate from male *Volvox* has been found to induce the formation of antheridia in the male strain and of oogonia in the female strain. The nature of the filtrate component active in triggering the process of sexual reproduction seems proteinaceous (Starr, 1968).

Order: CHLOROCOCCALES

This order includes unicellular and colonial forms in which the vegetative thallus is non-motile and no vegetative cell divisions occur. Vegetative cells lack flagella, contractile vacuoles and eye spot. The colonial members arise as a consequence of the union of zoospores or autospores inside the parent cell wall or soon after their liberation from the parent cell. The thallus morphology, the features of asexual reproduction (zoosporic or non-zoosporic) and, to some extent, the tendency of the cells to become coenocytic have formed the basis of classification of the order into families; the two main series of forms in this order, namely, the azoosporic Chlorococcales and the zoosporic Chlorococcales are illustrated here with reference to the representative genera *Chlorella* and *Hydrodictyon*.

Family: Chlorellaceae
Genus: *Chlorella*

Occurrence

It is a cosmopolitan, ubiquitous alga occurring in freshwater, brackish water and terrestrial habitats. *C. lichina* is an algal symbiont of the lichen *Calicium chlorina*. Some chlorellas, e.g., *Zoochlorella*, grow as symbionts in *Hydra* or other aquatic animals.

Morphology

Chlorella is a unicellular non-motile alga. The spherical, subspherical or ellipsoidal cells are bound by a true cellulosic wall. Each cell has a bell-shaped or cup-shaped parietal chloroplast with or without a pyrenoid (Fig. 4-1 D). There may be a hyaline cavity toward one side of the chloroplast and in this cavity, or in the colourless central cytoplasm, are located the single nucleus, the mitochondria and the Golgi bodies. The photosynthetic

thylakoids lack grana-like organization. Toward the end of growth phase and the beginning of reproductive phase, the cells become multinucleate.

The fine structure of *Chlorella* cells has been found to be highly plastic and polymorphic. Changes in environmental conditions such as light quality, duration and intensity, temperature, and chemical composition of the growth medium, greatly influence the micromorphology of the chloroplast, pyrenoid and cell wall.

Reproduction and Life Cycle

It reproduces exclusively by the formation of (normally four) asexual autospores. Motile cells, zoospores or gametes are not produced.

Tamiya (see Morimura 1959) succeeded in synchronizing the population of *C. ellipsoidea* by subjecting the cells to alternating light-dark cycles. With the technique of synchronous culture, four phases (Fig. 6-12) in the life cycle of *Chlorella* were identified: (1) the growth phase during which the autospores grow in size at the expense of the photosynthetic products; (2) the early ripening phase in which the cells of the growth phase prepare themselves for cell division; (3) the post-ripening phase in which the cells divide twice either in the dark or light; and (4) the division phase during which the parent wall gelatinizes or ruptures liberating the autospores which in the very young stage are known as dark nascent cells (D_n).

The growth and early ripening phases are both light- and temperature-dependent, whereas the post-ripening and autospore liberation phases are only temperature-dependent. As shown in Fig. 6-12, the growth and early

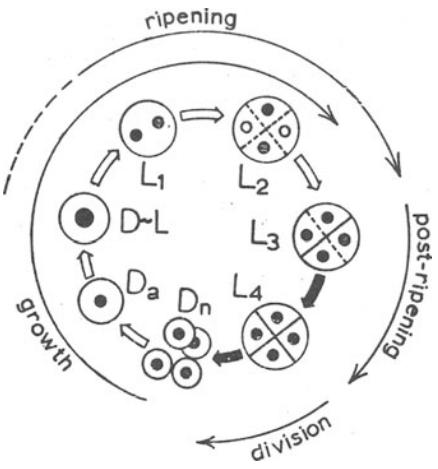

Fig. 6-12. *Chlorella ellipsoidea*, life cycle as studied by the technique of synchronous culture. (After Tamiya, 1963.)

ripening phases are further subdivided into five stages distinguished on the basis of photosynthetic activity, DNA contents and division performance in the dark. The D_a (dark active) cells are uninucleate, photosynthetically

very active and are derived from D_n cells. D∼L (dark to light transitional) cells are also uninucleate but are larger in size and photosynthetically less active with comparatively higher DNA content than the D_n cells. L_1 cells are larger with more DNA than D∼L cells. All these cells, i.e., D_n, D∼L, and L_1 fail to divide when they are incubated in the dark. The L_2 cells are binucleate and are capable of incipient division in the dark. L_3 cells always become tetranucleate when kept in the dark or light and produce four autospores. L_4 cells are tetranucleate autospore mother cells which liberate the autospores or D_n cells, independently of light. (L_1, L_2, L_3, L_4 are light cells.)

The cell division of *Chlorella* has a specific requirement for sulphur because it cannot occur in its absence. Under photosynthetic conditions it requires nitrogen in addition to a sulphur source, whereas in the dark the cell division can proceed normally with sulphur alone (Hase, 1962).

Importance

The alga grows very fast under a variety of conditions and its photosynthetic pigments and reserve products are similar to those of higher plants. These attributes have made it a favourite object of extensive study on the mechanism of photosynthesis. Biochemical analyses of its cells have revealed that they may be very rich in proteins (about 50%), fats (about 20%), carbohydrates (about 20%), amino acids, vitamins and minerals. In view of this, many laboratory and pilot plant studies on the feasibility of growing *Chlorella* in mass cultures for use as human food or animal feed, have been conducted in Japan, Germany, the United States and Israel. However, the prohibitive cost of growing, harvesting and processing has prevented its commercial exploitation.

Another possible use is in the regulation of oxygen and CO_2 supply in nuclear submarines and space vehicles.

Family: Hydrodictyaceae
Genus: *Hydrodictyon*

Occurrence

Popularly known as 'water net', it grows in the plankton and benthos of freshwater ponds and lakes. The common species, *H. reticulatum*, is found free-floating in certain ponds in spring and summer. It is sometimes so abundant that it almost covers the entire pond surface. Another species, *H. indicum* Iyengar, is common in India.

Morphology

The alga is a macroscopic non-motile coenobium consisting of a network of pentagons or hexagons with each corner of the polygon resulting from the union of three large cylindrical cells (Fig. 6-13 A). Each coenobium is typically cylindrical, closed at both ends, and may consist of a few hundred to several thousand cells in different species. The mature coenobium may

sometime be as long as 30 cm. The young cells are generally uninucleate with a parietal, band-shaped chloroplast having a single pyrenoid. As the cells grow and enlarge they become coenocytic and the band-shaped

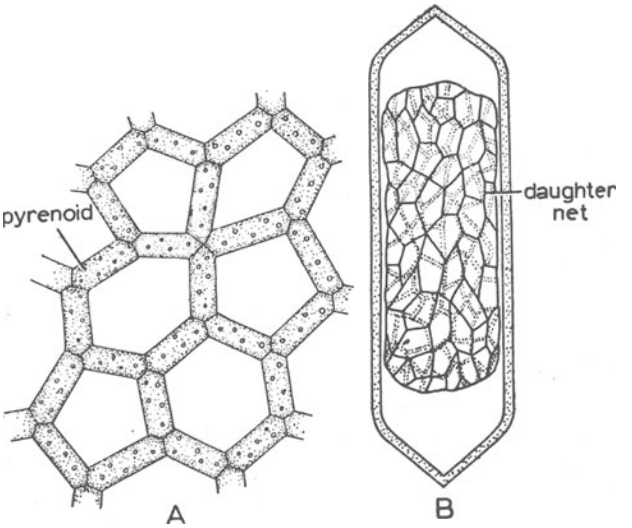

Fig. 6-13. *Hydrodictyon.* A, part of coenobium showing polygonal meshes; B, formation of new net inside a parent cell.

chloroplast becomes reticulate and forms many pyrenoids. Mature cells, which may sometime measure 2–3 mm in length, contain a large central vacuole which displaces the cytoplasm toward the periphery of the cell.

Reproduction

Vegetative. This is sometimes effected by fragmentation of a net into two or three nets.

Asexual. It is brought about by the production of a large number (up to 20,000) of tiny biflagellate zoospores within any vegetative cell of the thallus. These are not liberated but remain within the mother cell and for a short while may exhibit some movement. The flagella are then withdrawn and the zoospores elongate into tiny cylindrical cells which come together in groups of five or six and dispose themselves in such a manner as to form a characteristic miniature net of *Hydrodictyon* (Fig. 6-13 B). The parental cell wall ultimately softens, liberating the young *Hydrodictyon* coenobium which later grows to adult size without undergoing any cell division.

Sexual. It is isogamous and involves the fusion of biflagellate isogametes (Fig. 6-14 A, B) produced in large numbers from ordinary vegetative cells. Unlike the zoospores, the gametes are liberated from the parent cell through a hole in the wall and gametic fusion (Fig. 6-14 C) takes place in water. The zygote is thin-walled and green in colour. It undergoes almost immediate

germination by dividing meiotically and produces four haploid zoospores (Fig. 6-14 D). Sometimes, however, the zygote may perennate. The zoospores escape from the zygote wall and develop individually into non-motile polyhedral cells (Fig. 6-14 E). With the advent of a favourable growing season each zygote or polyhedron divides to produce numerous zoospores which arrange themselves appropriately so as to form a daughter *Hydrodictyon* net. Sometimes, a polyhedron produces a disk net which in turn produces zoospores. These then align to form a cylindrical net. The cells of the net are observed to grow in size only after they have established contacts with adjacent cells.

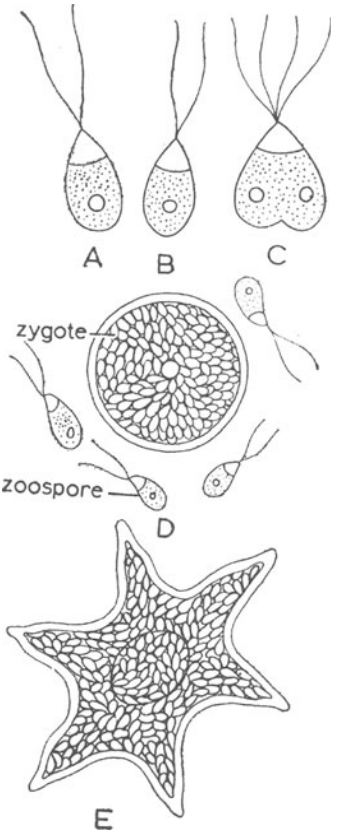

Fig. 6-14. *Hydrodictyon*, stages in sexual reproduction. A–B, gametes; C, zygospore; D, zygote with its germination product (zoospores); E, a polyhedron.

Order: Ulotrichales

This order includes unbranched filamentous and parenchymatous forms showing little structural or functional specialization among vegetative cells except that the lowest cell of the thallus may be modified to serve as an organ of anchorage. Depending on cell structure, the order has been classified into three suborders, the Ulotrichineae, the Prasiolineae and the Sphaeropleineae. Ulotrichineae have uninucleate cells with a single, parietal, band- or girdle-shaped chloroplast and are segregated into four families on the basis of wall structure and thallus organization; the Ulotrichaceae include simple filamentous forms with cells having a one-piece simple cell wall and a girdle-shaped chloroplast; the Microsporaceae have one filamentous genus in which the cell wall is composed of overlapping H-shaped pieces; in Cylindrocapsaceae the cells are elliptical with a thick, stratified wall and in old filaments some cells may divide obliquely so that some parts of the filament become multiseriate; the Ulvaceae contain forms with parenchymatous thalli and are retained in the Ulotrichineae because like *Ulothrix* their young stages are generally filamentous.

The Suborder Prasiolineae with a single Family Prasiolaceae includes only one membranous or thalloid genus *Prasiola* whose cells contain an axile stellate chloroplast.

The Sphaeropleineae is also a monogeneric Suborder represented by

Sphaeroplea, an unbranched filamentous alga made up of large coenocytic cells each provided with several band-shaped or discoid chloroplasts.

The prevailing modes of sexual reproduction and life cycle are: (1) isogamous and haploid (Ulotrichaceae and *Microspora*); (2) isogamous and isomorphic (Ulvaceae); and (3) oogamous and haploid (*Cylindrocapsa* and *Sphaeroplea*). *Prasiola* exhibits an interesting type of life cycle in that the thallus is either completely diploid, producing diploid zoospores; or is partly diploid and partly haploid, with the gametes being produced from the cells of haploid tissue (Cole and Akintobi, 1963). In certain species of *Prasiola* patches of gametophytic tissue develop upon the diploid sporophyte following meiosis.

Family: Ulotrichaceae
Genus: *Ulothrix*

Occurrence

The alga occurs in flowing waters, ponds, pools, lakes and in reservoirs where the water is continuously renewed. It also grows on stones and pebbles lying within the spray zone of waterfalls. When young, it generally grows attached to submerged objects. *U. zonata* is found in cold water streams, *U. flacca* is marine and *U. implexa* is a lithophyte in estuaries.

Morphology

The plant body is unbranched uniseriate filament composed of short, cylindrical, quadrate or squarish cells (Fig. 6-15 A) of which the basal cell is colourless and modified to function as a holdfast. The growth is diffuse, every cell (except the basal holdfast) being capable of division. The vegetative cells are uninucleate, possess a cell wall and a girdle-, collar- or ring-shaped chloroplast having one or more pyrenoids (Fig. 6-15 A, B). The ring-shaped chloroplast may be closed or open at one end (Fig. 6-15 C, D).

Reproduction

Vegetative. A filament fragments into two or more pieces, each of which then grows into a new plant.

Asexual. Each cell except the basal holdfast produces up to eight, rarely 16, zoospores and usually there exists an apical-basal gradient in their formation. The mother protoplast divides mitotically (Fig. 6-15 E), producing naked and stigmatic zoospores which resemble morphologically the unicellular Volvocales and which are liberated from the parent cell through a pore in the lateral wall (Fig. 6-15 F). Depending upon the number of divisions, the mother protoplast may produce quadriflagellate macrozoospores (Fig. 6-15 G), quadriflagellate microzoospores (Fig. 6-15 H) or biflagellate microzoospores (Fig. 6-15 I). The liberated zoospores swim about for some time, anchor themselves to some solid object by their anterior ends, withdraw flagella and secrete a cell wall. Each then divides into two cells, the lower forming the holdfast and the upper dividing and redividing to produce

vegetative cells of the filament (Fig. 6-15 J). The filaments formed from macrozoospores are much stronger and healthier than those derived from biflagellate microzoospores. Quadriflagellate microzoospores on germination produce intermediate filaments.

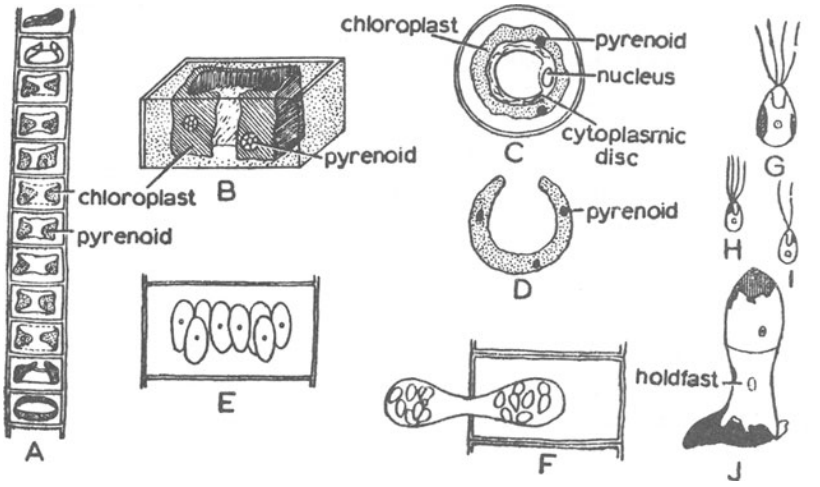

Fig. 6-15. *Ulothrix*. A, part of filament; B, three-dimensional view of a cell with ring-shaped chloroplast; C, cross section through a cell with ring-shaped chloroplast; D, a horse-shoe-shaped chloroplast; E, division of protoplast of a cell; F, liberation of swarmers in a mucilaginous vesicle through a lateral pore; G, quadriflagellate macrozoospore; H, quadriflagellate microzoospore; I, biflagellate swarmer; J, germling with holdfast.

Asexual reproduction also takes place by aplanospores. The daughter protoplasts instead of forming zoospores, differentiate into non-motile thin-walled aplanospores which may either be released from the parent cell or remain enclosed within it during the unfavourable growth period. With the onset of favourable periods, each aplanospore gives rise to a zoospore, or germinates directly into a filament.

Sexual. Almost all the experimentally investigated species are sexually heterothallic and gametic union (Fig. 6-16 A, B) always occurs between morphologically similar gametes of opposite, + and − mating types. Up to 32 or 64 gametes per cell are formed and in the mode of formation and morphology, they resemble the biflagellate microzoospores. The zygote (Fig. 6-16 C) is a thick-walled resting structure and on germination (Fig. 6-16 D) its nucleus divides by meiosis during which the + and − sex factors segregate giving rise to haploid zoospores or aplano-

Fig. 6-16. *Ulothrix*, sexual reproduction. A–B, fusing gametes; C, quadriflagellate zygote; D, germination of zygote and production of meiospores.

spores, half of which are of + type and the other half of — type. Up to 16 zoospores or aplanospores may be produced from a germinating zygospore, though four is the usual number. Reduction in chromosome number during germination of the zygote nucleus has been observed.

The rare parthenogenetic development of a gamete into a vegetative filament and also the occasional gamete-like behaviour of the biflagellate zoospores have also been recorded.

Order: CLADOPHORALES

This order includes freshwater and marine filamentous algae, mostly branched, having large multinucleate cells which are thick-walled and possess reticulate chloroplasts and many pyrenoids. Most members occur in relatively clean, flowing water of streams. These algae generally do not occur in absolutely quiet waters.

Reproduction takes place by fragmentation, by bi- or quadriflagellate zoospores and by biflagellate isogametes. The life cycle involves an alternation between isomorphic generations.

Family: Cladophoraceae
Genus: *Cladophora*

Occurrence

A large genus with about 150 species, *Cladophora* occurs in diverse freshwater, brackish water and marine habitats. Plants may be free floating or attached on rocks, aquatic angiosperms or shells of molluscs. Some species grow so prolifically during spring and summer as to form dense mats, blankets or ropy aggregates, blocking and clogging the streams. Other species produce sponge-like balls in deep water. *C. glomerata* is a typical freshwater species, often the most abundant filamentous alga found in streams throughout the world. *C. crispata* becomes attached to the walls of municipal water reservoirs. Massive growth of various species of *Cladophora* in canals impedes the flow of water through water distribution channels.

Most species prefer hard waters having relatively high pH (about 7.5 to 9.5).

Morphology

Cladophoras are profusely branched and, when young, are attached to the substratum by septate rhizoidal downgrowths from the basal region of the filaments or their lowermost cells (Fig. 6-17 A). The rhizoidal branches are often perennial and help in perennation of the alga through unfavourable conditions.

Cells are large, cylindrical, multinucleate, and have an elaborate chloroplast which may form a continuous reticulate network in younger cells but may become parietal in older stages (Fig. 6-17 B, C). In specimens collected

from shaded habitats the chloroplast occurs as a dense reticulum whereas in those from well exposed localities, the chloroplasts are clearly separated from

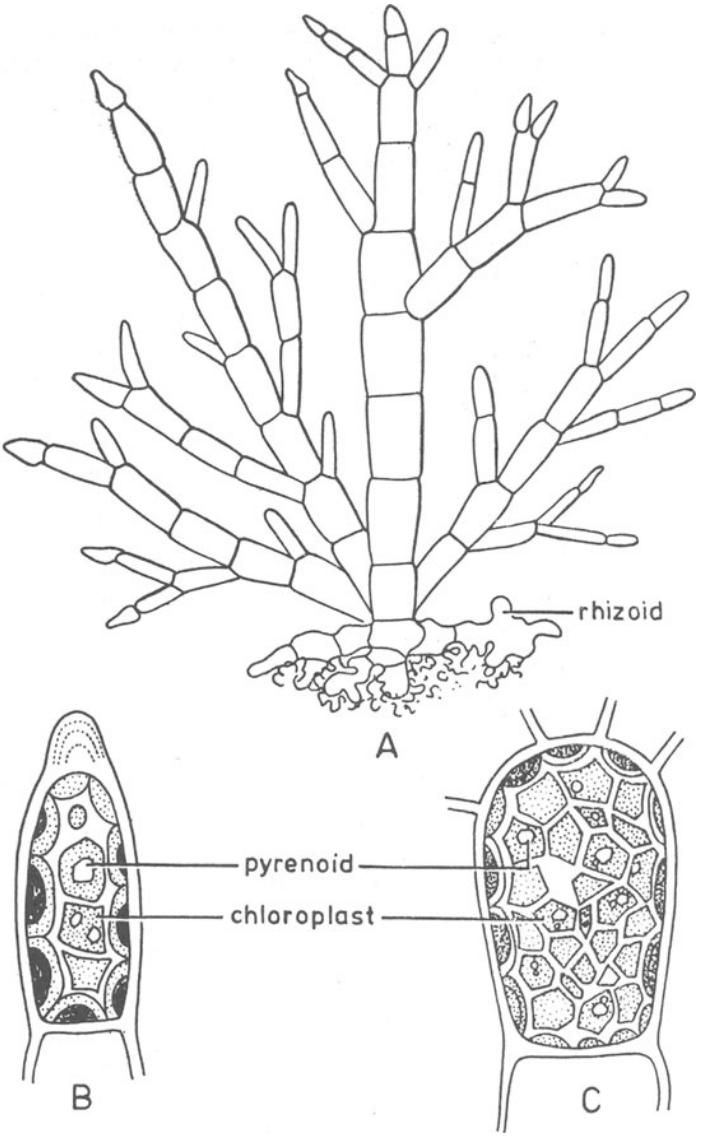

Fig. 6-17. *Cladophora jongionum* Van den Hoek. A, habit; B, C, apical and intercalary cells with parietal chloroplasts.

each other (Whitton, 1970). Many pyrenoids occur in the chloroplast. Cytoplasm is located centrally in the cell and contains many nuclei. Unlike most uninucleate plant and animal cells, in this alga mitosis and cytokinesis are quite independent of each other and this is why the cells are multinucleate.

The branching (see Fig. 6-18 A) of the thallus is usually dichotomous and rarely trichotomous. Alternate (lateral) branches are also known. The branches originate in a characteristic manner, just beneath the septum between two cells. A bulge appears near the upper end of a cell (Fig. 6-18 B). A portion of the cell contents of the parent cell passes into the bulge which is then cut off by a septum. This septum is parallel to the long axis of the

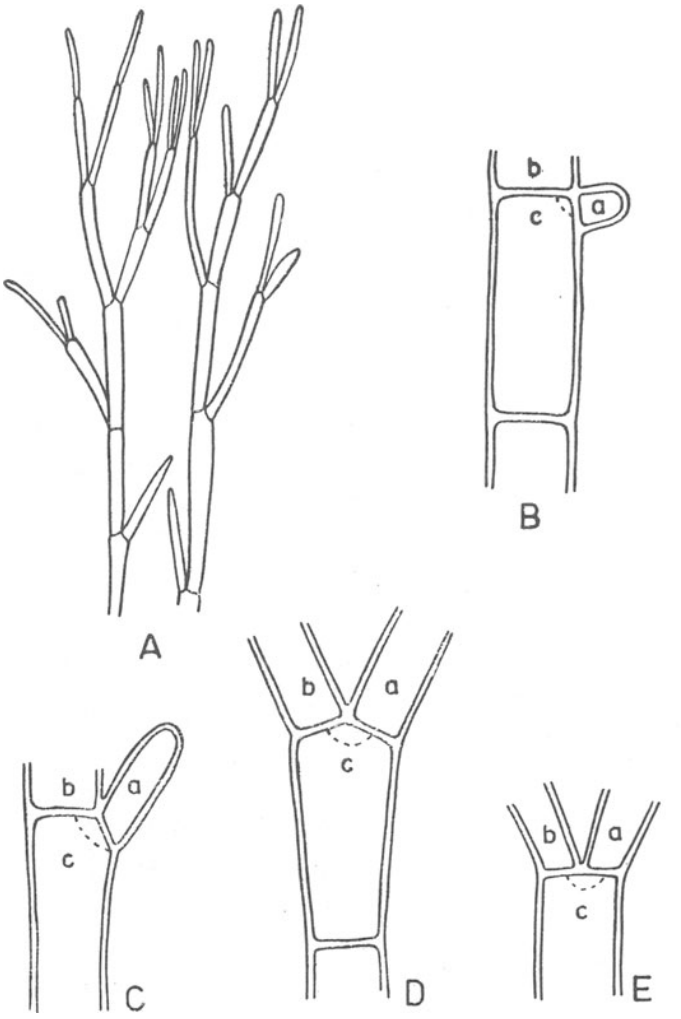

Fig. 6-18. A, *Cladophora* sp. showing branches; B–E, successive stages in evection.

filament and at right angles to the septum between the two parent cells. The cell bulge is then moved upward and outward as a result of localized active growth of the parent cell membrane just beneath the bulge (Fig. 6-18 C). Consequently, the 90° angle between the new and old septa increases progressively (Fig. 6-18 D) until, in some cases, the two septa align in a

straight line (Fig. 6-18 E). By this stage the new bulge will have grown into a new cell or even a small branch and the mode of branching will appear dichotomous (Fig. 6-18 E) instead of lateral. Such a process of branch formation has been styled *evection* and is characteristic of many species. Occasionally, two such buds may be formed instead of one and then the branches will appear trichotomous.

Unlike *Ulothrix*, the mode of growth in *Cladophora* is both apical and intercalary. The relative extent of apical and intercalary growth in different species, however, varies widely; in *C. rivularis* it is almost entirely intercalary.

The cell wall is thick, stratified and composed of layers of microfibrils (mostly cellulosic) arranged at right angles to each other. The microfibrils of the lateral walls are often continued into those of the cross septa. The microfibrillar layers frequently become so compact with the amorphous layers that the lamellar details of the outer wall become obscure.

The branches appear coarse and stringy to the touch and as there is little secretion of mucilage, they harbour many diatoms and other microorganisms as epiphytes.

Reproduction

Vegetative. A filament may fragment into two or more pieces, each of which may form a new plant. The smaller branches often become detached from the parent thallus and then grow into new plants.

Asexual. The protoplasts of apical or subapical cells break into uninucleate segments which are transformed into a bi- or quadriflagellate zoospore. Mature zoospores are released through pores formed in the lateral walls of the cells. Zoospore formation occurs in ordinary, unmodified cells.

Following a period of motility and dispersal, the zoospores settle down on some substratum and germinate to give rise to *Cladophora* plants which are morphologically identical with the zoospore-producing parent thalli. The reduction division in some species has been observed to occur at the stage of zoospore formation.

Sexual. The apical or subapical cells produce biflagellate isogametes. Fusion of gametes from + and − strains occurs outside the parent cells. The zygote germinates after a rather brief rest period to grow directly into a diploid plant. The alternation of generations is isomorphic.

Fortnightly peak periods of gametogenesis and zygote formation have been reported for this alga and it is thought that such periodicity may be connected in some way with lunar tide cycles.

Many species have been shown to be polyploids. The number of nucleoli and chromocentres has also been correlated with the degree of polyploidy.

Seasonal Growth Cycle. Whitton (1970) has reviewed the seasonal growth behaviour of *C. glomerata* in eutrophic waters of temperate rivers. The perennating filaments attached to rocks start producing new branches in spring. After a maximum of growth and branch formation in summer there is a

7. (54-22/1972)

slowing down of vegetative growth. A second period of rapid growth occurs in early autumn. It is thought that such growth occurs from zoospores produced in the preceding summer. During late autumn, the upright parts of thalli become detached, leaving behind the basal fragments still attached to rocks. Thick-walled akinetes are occasionally formed.

In standing water, on the other hand, thalli and akinetes survive the winter on the bottom of the ponds under a layer of silt. Zoospore release occurs during May-June.

Order: CHAETOPHORALES

The heterotrichous habit, i.e., the differentiation of branching filaments into erect and prostrate systems, is the most outstanding singular feature of this order. These plants may further be characterized by the frequent production of hairs (e.g., in Chaetophoraceae) or setae (e.g., Coleochaetaceae) or by the formation of differentiated reproductive organs like sporangia and gametangia (e.g., Trentepohliaceae). The hairs have a single or a group of narrow elongated cells with well-marked tapering apices whereas the setae or bristles are the outgrowths of the wall with pointed ends. Certain members, e.g., Trentepohliaceae, exhibit a marked terrestrial or subaerial tendency. *Fritschiella tuberosa* (Chaetophoraceae) is exclusively terrestrial, its prostrate system is buried in moist soil and serves as a means of perennation.

Except for *Aphanochaete*, *Chaetonema* and *Coleochaete*, other members of the Chaetophorales show isogamous sexual reproduction.

According to Fritsch (1935), the heterotrichous habit represents the highest type of vegetative organization in the green algae. He has postulated that the Chaetophorales represent the surviving descendants of ancestral forms from which higher land plants originated in the remote past. Of the Chaetophorales, *Fritschiella* seems to stand nearest to the probable ancestral forms because of its heterotrichous habit coupled with a terrestrial mode of life.

Family: Chaetophoraceae
Genus: *Fritschiella*

Occurrence

Fritschiella tuberosa Iyengar is an interesting monotypic tropical alga widespread in India, Nepal, South Africa and many other countries. It grows on moist soils of drying up rain water pools. It is either terrestrial or amphibious but generally does not grow in free water.

Morphology

First described by the late M. O. P. Iyengar from South India, this species was later studied in detail by R. N. Singh from Uttar Pradesh and other places.

This alga is probably the most highly differentiated species among the

green algae. It is heterotrichous and filamentous and is endowed with the potentiality of almost every conceivable kind of somatic advancement possible in a heterotrichous organism.

The thallus is differentiated into four distinct regions (Fig. 6-19 A): (1) the rhizoidal system, consisting of colourless, branched or unbranched,

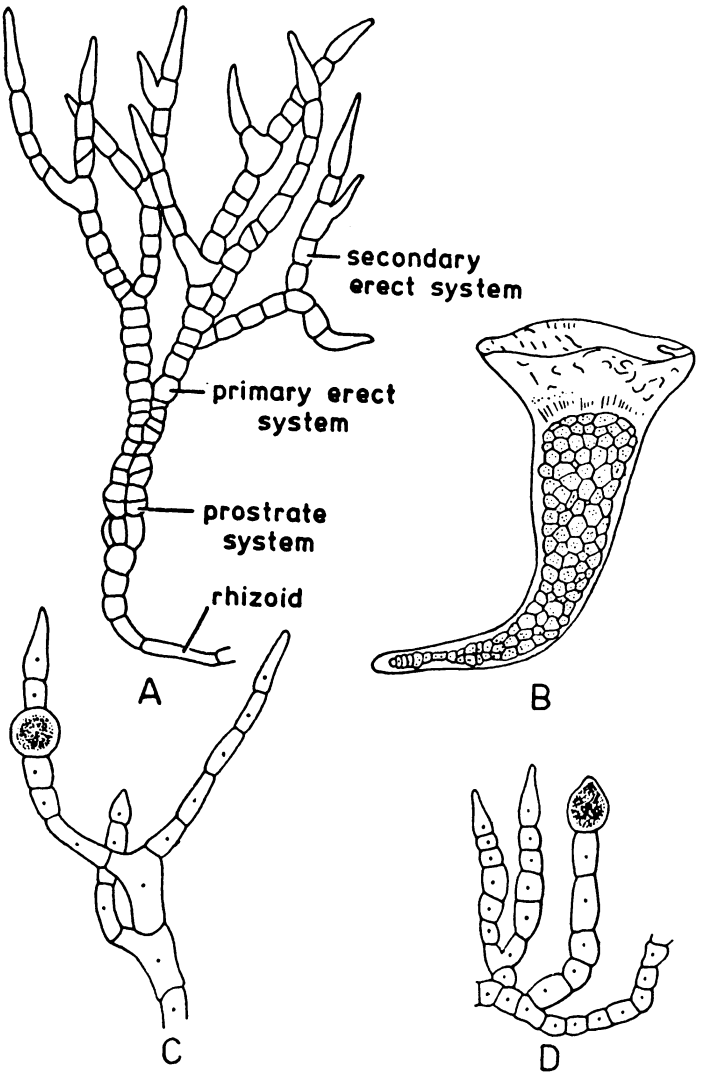

Fig. 6-19. *Fritschiella.* A, habit; B, funnel-shaped, perennating, tuberous body; C, intercalary sporangium; D, terminal sporangium (after Patel and Patel, 1969).

multicellular rhizoids comparable structurally and functionally with the rhizoids of bryophytes; the rhizoids not only anchor the plant to the substratum but also serve to absorb soil nutrients; (2) the prostrate system, composed of uni- or multiseriate (pseudoparenchymatous) clusters of rounded

or irregular cells with dense contents (proteins, starch, haematochrome, etc.); it is generally filamentous in the early stages of development in young plants; sometimes its filaments become further differentiated into nodal and internodal regions; cells or filaments of the prostrate system give rise on their lower side to rhizoids and on the upper to the primary erect system; they are meant for food storage, perennation, and reproduction; (3) the primary erect system, having uni- or biseriate cells very similar to the cells of the prostrate system; these are partly photosynthetic and partly conducting in function; and (4) the secondary erect (projecting) system, consisting of freely branched uniseriate filaments with green, elongate, uninucleate cells, each having a parietal chloroplast with a few pyrenoids; this system is probably meant exclusively for photosynthesis.

The rhizoidal and prostrate systems are subterranean, being completely embedded beneath the surface of soil. The primary erect system is generally partly embedded and partly projecting above ground level. The secondary erect or projecting system is fully exposed to light and air.

Perennation

During adverse periods the erect and rhizoidal systems commonly degenerate and disappear and the alga perennates by means of its prostrate system. Unmodified cells of prostrate system tide through adverse periods as such but the nodal regions of prostrate system, being capable of independent existence, may get detached from the parent thallus through death and decay of internodal regions, and thus not only serve the function of perennation but also vegetative propagation. Yet another mode of perennation is by the formation of specially modified tuber-like bodies (Fig. 6-19 B) enclosed by cell walls having a dark and thick cuticle. Formed in prostrate system, these tubers are commonly either funnel-shaped or club-shaped (Fig. 6-19 B). Each such tuber may be up to 1 mm in length.

Reproduction

Asexual. It takes place by formation of bi- or quadriflagellate zoospores, one or two of which are normally formed from a mother cell. Such zoospores are structurally similar to those of *Ulothrix*. Patel and Patel (1969) have recorded differentiated intercalary and terminal sporangia (Fig. 6-19 C, D) which are somewhat larger in size than the ordinary vegetative cells. However, the nature and fate of the products of such sporangia are not known.

Sexual. An interesting feature of *F. tuberosa* is the combination of a highly differentiated vegetative thallus with an equally simple and primitive mode of sexual reproduction, viz., isogamy.

Biflagellate isogametes are formed from cells of prostrate system (Singh, 1954). After fertilization the zygote germinates to produce a diploid plant ($2n = 8$) which is morphologically identical with the parent, gametophytic plants. Meiosis is believed to occur during formation of asexual spores by the sporophyte. The haploid zoospores give rise to gametophytic plants

($n = 4$). Thus, the alga exhibits an isomorphic alternation of generations.

Fritschiella has attracted considerable attention in connection with speculations on the algal ancestry of higher plants (see Singh 1954). It has been strongly advocated that the ancestors of the early land plants were green algae very similar to the present day *Fritschiella*. The reason why such ancestors are sought among the green algae is that the basic physiological, biochemical and metabolic characteristics of the higher plants (from bryophytes onwards) can only be compared with those of the Chlorophyceae. Within the Chlorophyceae, *Fritschiella* is considered as coming nearest to the hypothetical ancestors on the basis of its following attributes:

1. terrestrial or amphibious habitat;
2. rhizoidal system strongly resembling the rhizoids of bryophytes both structurally and functionally;
3. prostrate system being very much like the subterranean rhizomatous structures of certain pteridophytes and angiosperms;
4. reproduction being mostly confined to prostrate system as in Anthocerotales in which reproductive bodies are endogenous and embedded in thallus;
5. tropical distribution (it is widely believed that the first transmigrants were tropical forms); and
6. the high degree of thallus differentiation indicating the existence of a strong potentiality for further elaboration in a chaetophoraceous alga in the direction of evolution of an early algal transmigrant form.

Family: Coleochaetaceae
Genus: *Coleochaete*

Occurrence

Coleochaete, the sole genus belonging to the Family Coleochaetaceae, has some 10 species which grow epiphytically on the leaves and stems of freshwater higher plants such as *Hydrilla* and *Potamogeton*, or as endophytes in *Nitella*.

Morphology

C. pulvinata is heterotrichous with the branched filaments of the erect system organized into a more or less hemispherical cushion. In *C. scutata* (Fig. 6-20 A) the erect system is absent and the prostrate system is discoid showing radial arrangement of its congregated branches thus giving rise to a pseudoparenchymatous disc which grows by means of apical (marginal) meristems. The branching of thallus may result from lateral outgrowths or **dichotomy** of the apical meristems. The cells are uninucleate, and contain an irregular parietal chloroplast with one or two pyrenoids. *Coleochaete* differs from other members of the Chaetophorales in its chromosome number and morphology.

Some or most of the cells of the thallus produce setae, each of which is composed of a cytoplasmic thread completely or partially enclosed within a

basal sheath. The setae are generally regarded as outgrowths of the plasma membrane emerging through a pore in the cell wall. They readily break off after some time, leaving only the basal sheaths.

Reproduction

Asexual. Single, ovoid, biflagellate zoospores are produced in ordinary vegetative cells. They possess a lateral chloroplast but are devoid of an eye spot. After a brief movement, the zoospores withdraw their flagella, secrete a wall and divide in a horizontal plane; the upper cell forms the seta and the lower divides in one or two planes at right angles to each other to form the primary initials which by their activity produce a discoid thallus (*C. scutata*). In heterotrichous forms (*C. pulvinata*) the germinating zoospores at first produce the branched prostrate system from which the branches of the erect system arise later.

Sexual. C. pulvinata is monoecious whereas *C. scutata* is dioecious. The sexuality is of an advanced oogamous type and is accompanied by a kind of fruit formation not found in any other green algae. The heterotrichous species bear the sex organs on the branches of the erect system.

In *C. pulvinata* antheridia are produced terminally on branches, sometimes on the same branch that bears an oogonium. Antheridial initials arise as colourless outgrowths from the terminal cells of a branch (Fig. 6-20B) and one antheridium gives rise to a single biflagellate oval or spherical, naked gamete.

The antheridia of *C. scutata* are produced from some intercalary cells lying within the periphery of the thallus. At the time of antheridial formation, an intercalary cell divides into a number of daughter cells each of which finally differentiates into an antheridium. Frequently the antherozoids of *C. scutata* are green rather than colourless.

The oogonia of *C. pulvinata* are also produced terminally and singly but their seemingly lateral position is due to growth and production of branches from the cells beneath the oogonium (Fig. 6-20 B). Each oogonium is a flask-shaped structure with a swollen base containing an egg and a slender neck or trichogyne. This type of oogonium strongly resembles the carpogonium of the Rhodophyta. Discoid species have typically papillate oogonia lacking trichogynes.

During fertilization an antherozoid enters an egg through an apical opening formed in the trichogyne or in the papilla of the oogonium. The resulting oospore enlarges in size and certain branches from the neighbouring cells of a fertilized oogonium grow out forming a rather loose pseudoparenchymatous envelope or jacket that completely encircles the oospore. The oospore plus the envelope is called a **spermocarp** (Fig. 6-20 C) which is analogous to an angiospermic fruit.

After perennation, when the oospore germinates, its nucleus divides meiotically followed by a few mitotic divisions, producing a 16- to 32-celled structure (Fig. 6-20 D). Each of these cells forms a biflagellate zoospore

(Fig. 6-20 E) which grows into a new plant. *Coleochaete* is haploid, with oospore representing the only diploid stage in the life cycle.

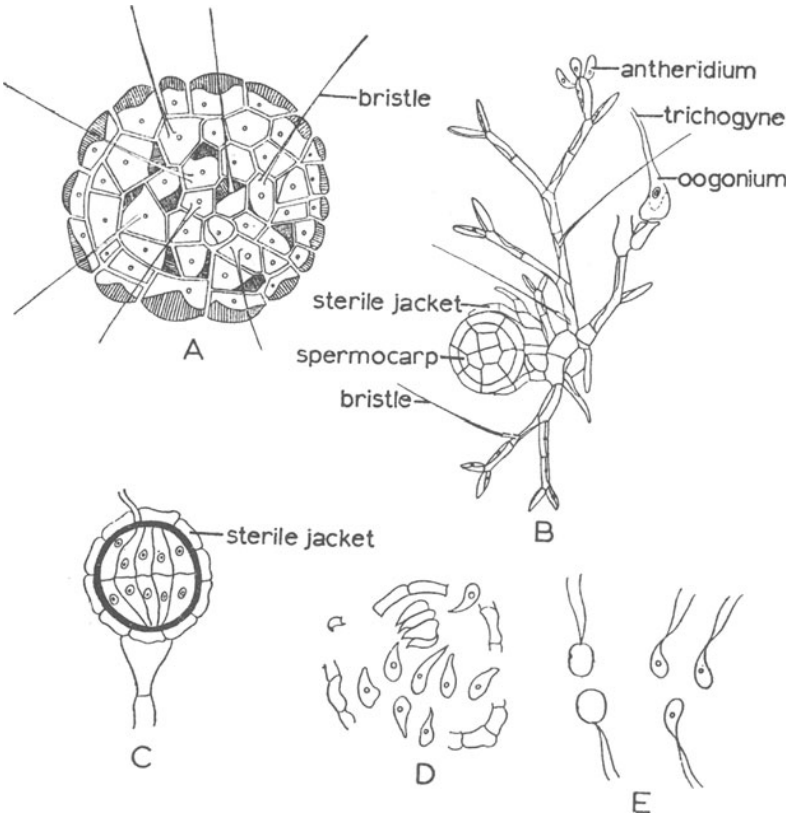

Fig. 6-20. *Coleochaete*. A, *C. scutata*; B, *C. pulvinata*; C, spermocarp; D, liberation of meiospores (zoospores) from germinating spermocarp; E, zoospores.

Order: Oedogoniales

The two main distinguishing features of this order are: (1) the mode of cell division leading to the formation of characteristic caps on certain cells, and (2) the production of multiflagellate zoospores or gametes.

The single Family Oedogoniaceae comprises the three genera *Oedogonium*, *Bulbochaete* and *Oedocladium* which differ from one another in thallus organization. *Oedogonium* is an unbranched, filamentous form growing attached to the substratum when young but often becomes free-floating at maturity. *Bulbochaete* is a profusely branched alga with unilateral branches lying on alternate sides and almost every cell bearing a characteristic attenuated hair with a bulbous base; the terminal cells are generally provided with a pair of such hairs. The *Oedocladium* is terrestrial, and heterotrichous with a prostrate system bearing numerous branched, septate and colourless rhizoids on its lower side and branches of the erect system on its upper side.

Family: Oedogoniaceae
Genus: *Oedogonium*

Occurrence

Oedogonium is a widespread freshwater alga that grows epiphytically in ponds, pools, and shallow tanks.

Morphology

The filaments are unbranched and consist of cylindrical cells except for the basal cell which is modified into a holdfast. Each cell is provided with a thick wall differentiated into an inner cellulosic, a middle pectic and an outer chitinous layer. The terminal cells of the filaments are generally rounded or acuminate and the intercalary cells often show an apical-basal polarity. Some or most of the cells at the distal end exhibit parallel striations or annular scars and are known as cap cells (Fig. 6-21).

Cells are uninucleate and when mature have a central vacuole containing cell sap; and an elaborate, reticulate chloroplast containing many pyrenoids (Fig. 6-21). A sheath of starch granules surrounds each pyrenoid.

The cells of *Oedogonium* are similar to those of other green algae except that their chloroplasts contain microtubules each made up of two helically wound subunits. Such elements have also been observed in the chloroplasts of zoospores, zoospore germlings and eggs (Hoffman, 1967). These microtubules are considered to provide structural support to the massive reticulate chloroplast and perhaps also to facilitate its growth and development.

Vegetative Cell Division. As the cell enters the division phase, the nucleus moves from the lateral position to the centre. Soon a transverse ring of wall material appears on the inner face of the lateral wall just below the apical end of the cell. The nuclear division, the growth of the ring in thickness and the formation of a groove enclosing the growing ring occur concomitantly. An unattached floating septum is formed between the two daughter nuclei. The middle and outer wall layers external to the groove then rupture, permitting free elongation of the ring which forms a new

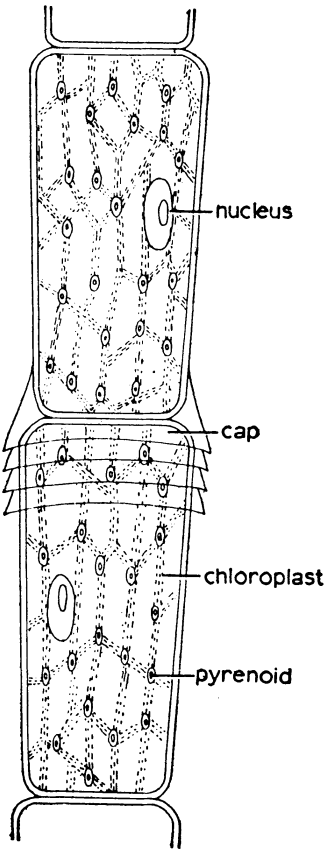

Fig. 6-21. *Oedogonium*, part of vegetative filament showing cell structure and a cap cell with four caps.

piece of cell wall lying between the cap and the sheath. Ultimately, the floating septum moves upward and becomes fixed near the terminus of the old cell wall. The new cell has the wall formed from the thickened ring and the newly synthesized piece. The membranous striation of the ruptured parental wall at the anterior region of the upper daughter cell is the cap and the cell bearing it is known as a cap cell. The number of caps on a cell indicates the number of cell divisions that have taken place (Fig. 6-21).

A recent ultrastructural study (Fig. 6-22) of the mode of cell division in *Oedogonium borisianum* (Hill and Michalis, 1968) has revealed that: (1) the ring (Fig. 6-22 A) below the cap originates as a three-layered structure by a method which excludes the possibility of its origin from an infolding of the innermost wall layer; (2) the ring grows in size with a gradual addition of vesicular material from the cytoplasm; (3) the mother wall adjacent to the fully-formed ring splits off; (4) the single, peripheral nucleus migrates to the centre and karyokinesis occurs; this is followed by the formation in the

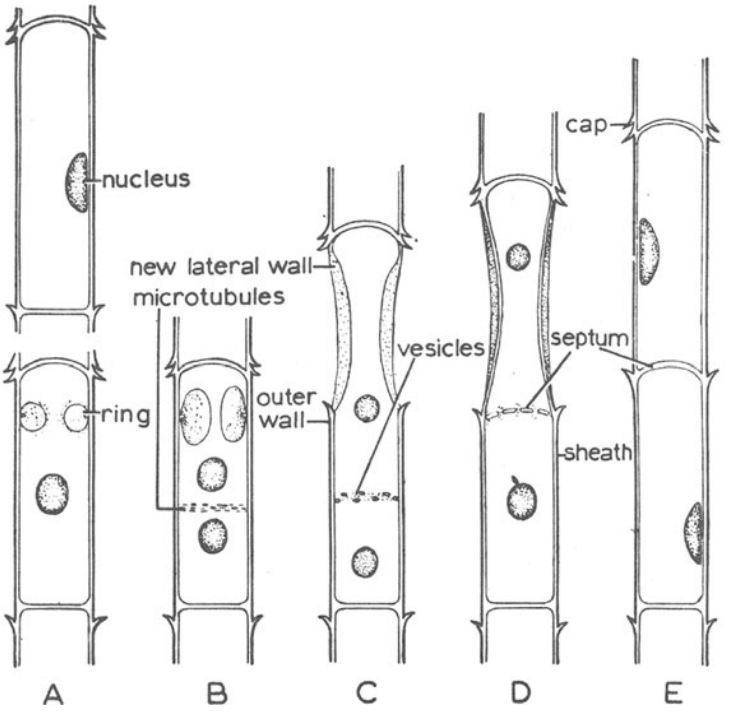

Fig. 6-22. *Oedogonium borisianum*, stages in cell division.

internuclear region of a row of microtubules (Fig. 6-22 B) in a plane parallel to that of the future cross wall; (5) concurrent with this development the ring expands into a cylinder (Fig. 6-22 C) which becomes the cuticle of the upper daughter cell. The septum, which for some time remains unattached to the lateral wall, later migrates upward to the base of the cylinder (Fig. 6-22 D); (6) soon a new lateral wall is formed between the cuticle and plas-

malemma of the upper daughter cell; (7) finally, the upward migrated transverse septum grows into a mature cross wall which unites with the newly formed wall on either side (Fig. 6-22 E). The mode of cross wall formation rules out the possibility of cell division either by cytokinesis or by annular furrowing of the cytoplasm.

Reproduction

Vegetative. Fragmentation is the usual method of propagation but under certain conditions the alga may also propagate by akinete formation.

Asexual. It occurs by the formation of multiflagellate zoospores produced singly within a cap cell. During their formation the cell contents contract slightly and develop a semicircular hyaline area on one side of the protoplast. A ring of basal granules appears at the base of the hyaline area and from each comes out a single flagellum, thus forming a ring of flagella around the base of the hyaline area. The basal granules remain connected together by fibrous strands. After the development of the zoospore, the cell wall near the cap region opens apart and the single zoospore moves out of the cell in a mucilaginous vesicle which soon gets dissolved liberating the zoospore (Fig. 6-23 A, B). The zoospore moves to and fro for some time,

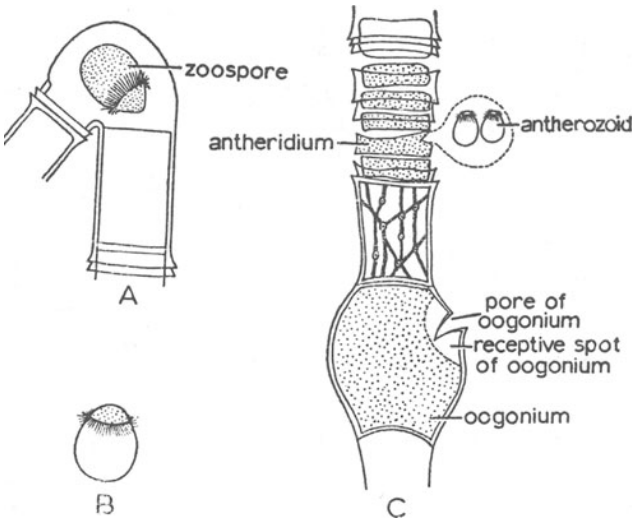

Fig. 6-23. *Oedogonium.* A, formation and liberation of zoospore; B, one zoospore with crown of flagella; C, oogamous reproduction in a homothallic macrandrous species.

settles down on a suitable substratum, and attaches itself to the substratum with its hyaline flagellar end. Thereafter the flagella are withdrawn and the quiescent zoospore elongates considerably. The attached end of the growing zoospore may differentiate into a smooth or lobed holdfast depending on the smoothness or roughness of the substratum respectively.

Sexual. Reproduction is oogamous and is of two kinds: (1) **macrandrous**, and (2) **nannandrous.**

Macrandrous forms may be monoecious producing antheridia and oogonia on the same plant (Fig. 6-23 C) or dioecious, producing antheridia and oogonia on different individuals. In nannandrous forms the sexual plants are dimorphic—the oogonia being formed in filaments of normal size whereas antheridia are produced in filaments, known as dwarf males (Fig. 6-24) or nannandria, which are extremely small and are always found attached to oogonia proper or to the underlying cell.

Macrandrous Forms. The antheridial mother cell may be terminal or intercalary and in either case it divides vegetatively into an upper antheridial cell and a lower sister cell. The latter undergoes a series of vegetative divisions to produce a row of two to many antheridia. An antheridium may give rise to a single multiflagellate antherozoid or it may undergo a single transverse or vertical division followed by the transformation of each of the two daughter cells into an antherozoid. Although quite similar to the zoospores, the antherozoids are smaller and may have fewer flagella.

The oogonia are produced from cells which act as oogonial mother cells. Each of these cells divides into an upper cap cell functioning as oogonium proper, and a lower supporting cell. The latter may again behave as an oogonial mother cell, thus forming a row of two or more consecutive oogonia. Or, it may remain vegetative, in which case the oogonia are solitary. The oogonium, which is filled with reserve food, enlarges to some extent and assumes a more or less spherical shape. A colourless region, called the receptive spot, appears at one point near the side wall and through it an antherozoid finds its way into the egg.

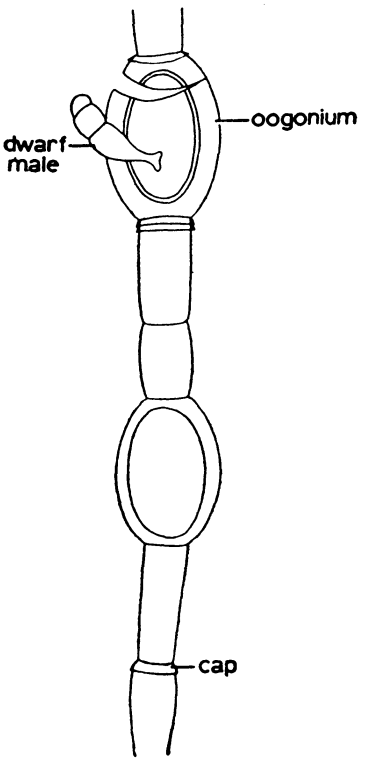

Fig. 6-24. *Oedogonium*, a nannandrous species.

Nannandrous Forms. Each dwarf male or nannandrium is a few-celled filament with a basal stalk cell and two to three antheridial cells. Dwarf males are derived from antherozoid-like zoospores, known as **androspores**, which are formed singly within antheridia-like cells called **androsporangia**. A species that bears both oogonia and androsporangia is designated **gynandrosporous**, whereas the one that bears them on different filaments is termed **idioandrosporous**. The changes that take place during the development of androsporangia and androspores are similar to those occur-

ring in the production of antheridia and antherozoids. After their liberation from androsporangia the androspores swim about for some time and then only one or a few of them may settle on an oogonium or a suffultory cell. The androspore then germinates into a dwarf male. Each antheridium of the dwarf male commonly produces two antherozoids lying one above the other.

Oogonial development in nannandrous species is similar to that in the macrandrous species. In certain heterothallic, nannandrous forms, some hormone is excreted by oogonial mother cells that attracts the androspores to the oogonia. A gelatinous envelope is formed around the developing oogonia and the attached dwarf male, and this serves to trap the spermatozoids so that the sperms remain in the immediate vicinity of the oogonium.

The oospore becomes thick-walled. After remaining dormant for some time it germinates and its nucleus divides by meiosis to produce four protoplasts each of which forms a multiflagellate zoospore which grows into a new haploid *Oedogonium* plant. In some heterothallic species two of the four zoospores develop into male filaments whereas the other two develop into female filaments.

Order: CONJUGALES

This order includes both unicellular and simple, unbranched filamentous forms which differ from other green algae in: (1) elaboration or complexity of chloroplast, (2) absence of flagellated stages, and (3) occurrence of sexual reproduction through conjugation involving the fusion of amoeboid gametes.

The cells commonly have a few, large and elaborate chloroplasts which are in the form of: (1) a flat axile plate, as in *Mesotaenium* and *Mougeotia*; (2) a pair of axile stellate chloroplasts, as in *Cylindrocystis* and *Zygnema*; and (3) one or more spiral, ribbon-like chloroplasts, as in *Spirotaenia* and *Spirogyra*. The unicellular forms, popularly known as "desmids", contain an axile (rarely parietal) chloroplast.

With rare exceptions, the chloroplasts contain large and well-defined pyrenoids. The cells have a single, conspicuous, centrally located nucleus. Many members have unusually a large number of rather small-sized chromosomes.

The usual methods of reproduction are vegetative and sexual, though rarely asexual reproduction occurs by the formation of akinetes and aplanospores.

Vegetative multiplication in the unicellular desmids is brought about by cell division. In filamentous forms fragmentation is often attended by the formation of special types of septa which may be replicate or semireplicate.

Sexually, the unicellular Conjugales reproduce by the formation of a papillate bridge between two cells through which the contents of one, designated as male, pass into the other, termed as female. The contents of both the conjugating cells may sometimes pass into a conjugation tube and gametic fusion may take place within the tube rather than within one of the two cells,

Family: Zygnemaceae
Genus: *Spirogyra*

Occurrence

A freshwater submerged or free-floating alga, it occurs in various water bodies of both temporary and permanent nature. The filaments are slimy to touch because of an outer mucilaginous wall layer.

Morphology

The plant body is an unbranched filament consisting of a uniseriate row of cylindrical cells with the basal cell frequently developing into a branched, or highly-lobed, anchoring organ in those species which grow attached to the substratum. The cell wall consists of two layers, an inner cellulosic and an outer pectic layer. The septa between the cells may be plane, semireplicate or replicate, and as in higher plants, their formation is always followed by the secretion of a cellulosic layer along both of its sides. A large vacuole containing tanniferous vesicles lies in the centre of the cell. The cytoplasm forms a thin layer lining the cell wall, and the single nucleus situated in the central part of the cell is connected with the peripheral cytoplasm through a number of radiating cytoplasmic strands. One or more helical or spiral, ribbon-shaped chloroplasts, with smooth or serrated margins and containing several pyrenoids, each ensheathed with starch grains, are present in the cell.

According to Dawes (1965) *Spirogyra* shows that: (1) the inner layer of the cell wall as well as the wall of the conjugation tube consists of cellulose-I microfibrils; (2) the chloroplast contains photosynthetic bands each consisting of 4–12 thylakoids, numerous starch ensheathed pyrenoids and pyrenoid-like bodies lacking starch sheath (karyoids or protopyrenoids); and (3) there are other cellular organelles such as nucleus with a complex nucleolus, numerous Golgi bodies and mitochondria, and endoplasmic reticulum.

Some species found in freshwater streams grow attached by means of rhizoids occurring in the form of small or large protuberances, or rods or rosettes. In a physiological study of rhizoid formation in *Spirogyra fluviatilis*, Nagata (1973) has observed that in laboratory cultures, rhizoid formation was restricted to the terminal cells of a filament. Out of various stimuli inducing rhizoid formation, light proved to be the most important since no rhizoids could be initiated in the dark. Red light was much more effective in inducing rhizoid differentiation than green or blue light. The fact that the effect of red light was completely reversed by subsequent exposure to far-red light indicates the involvement of phytochrome in rhizoidal differentiation.

Nuclear Cytology

Among the polycentric (having several centromeres) chromosomes there are invariably two or more chromosomes termed nucleolar organizing chromosomes (Fig. 6-25 A–E) which contain a distinct nucleolus organizing region (Fig. 6-25 A). During nuclear division, the nucleoli disappear (Fig. 6-25 B–E) by metaphase and reappear by interphase. The interphase nucleolus is highly

differentiated containing a nucleolar organizing region in a highly coiled form. This region remains coated with a deeply stainable substance known as

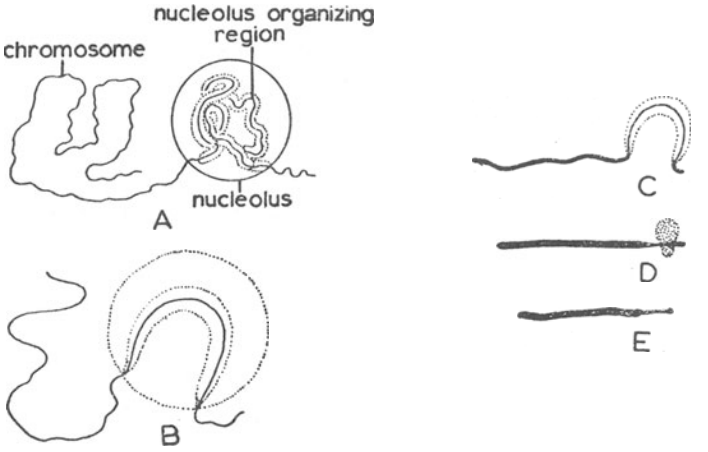

Fig. 6-25. *Spirogyra*, stages in nucleolar organization. A, coiled nucleolar organizing region of the chromosome inside the nucleolus at interphase; B, uncoiling and contraction of nucleolar organizing chromosome at early prophase; C–D, dispersal of nucleolus at mid and late prophase; E, disappearance of nucleolus. (After Godward, 1966.)

organizing track. The complex nucleolus results from the fusion of a number of nucleoli depending upon the number of nucleolus organizing chromosomes.

Reproduction

Vegetative. It is the most common method of reproduction and involves fragmentation of filaments.

Sexual. Species showing lateral conjugation are homothallic whereas those showing scalariform conjugation are generally heterothallic. In scalariform conjugation (Fig. 6-26 A–D) the filaments of + and − strains associate in pairs and in each pair the side walls of some or all cells facing each other form papillate outgrowths. The latter extend towards each other and finally fuse thus establishing a conjugation canal. The entire protoplasmic contents of the − type cell go into the + type through the canal by amoeboid movement (Fig. 6-26 D). When the conjugation begins, the two filaments are in contact with each other. As the papillae of the conjugation canal are formed and grow to form the canal, the two mating filaments are pushed away from each other. It has been observed that during the formation of the conjugation tube, one papillate outgrowth penetrates into the other. The dissolution of the cross wall at the junction of the two papillae is affected by the formation in the cytoplasm of a large number of vesicles or contractile vacuoles which migrate to the junction and release substances that dissolve the cross wall (Dawes, 1965).

After the completion of conjugation one of the two fusing filaments has

empty cells and the other contains dark, spherical or oval structures, the zygotes. This type of sexual reproduction involving migration of the — gamete into the + is physiologically anisogamous even though the two fusing gametes are morphologically isogamous. In some species, the contents of both — and + cells move into the conjugation tube and the fusion between gametes takes place within the conjugation canal. Such species are morphologically as well as physiologically isogamous.

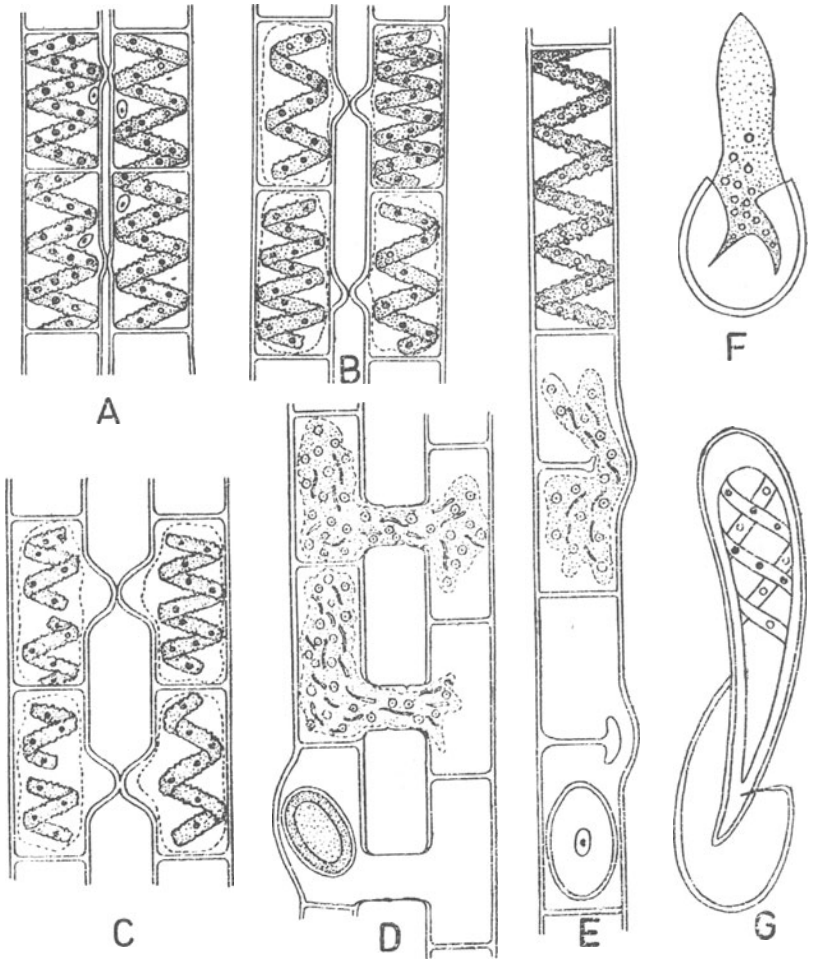

Fig. 6-26. *Spirogyra.* A–C, successive stages in scalariform conjugation; D, physiological anisogamy and formation of zygospore in one of the two conjugating cells; E, part of filament showing stages in lateral conjugation; F–G, early and late stages in germination of zygospore.

In lateral conjugation (Fig. 6-26 E), the septum at the point of its attachment with either side of the lateral wall dissolves, the lateral wall bulges out slightly thus creating a passage between the adjacent cells. Soon, the contents of one cell start exhibiting amoeboid movement and finally move into

the next cell through a narrow lateral passage. If the filaments are examined at this stage, empty cells are seen in the male and zygote-containing cells in the female.

Rarely, some cells of a filament may act as male and others as female with respect to another member of a filament pair. In this case, each of the conjugating filaments contains male as well as female cells. It has also been reported that three filaments A, B and C (instead of two) may conjugate, with the central filament B behaving as male toward A and as female toward C.

The zygospore is always a three-layered, thick-walled structure. It germinates after perennation and its nucleus divides by meiosis. Three of the resulting four nuclei degenerate and the surviving uninucleate protoplast undergoes repeated transverse divisions giving rise to a single *Spirogyra* filament (Fig. 6-26 F, G).

Genus: *Zygnema*

Occurrence

Commonly found free-floating in ponds and streams, *Zygnema* is a widely distributed freshwater alga. Sometimes it may grow submerged, living entirely under water, attached to stones, pebbles and other objects.

Morphology

Like *Spirogyra*, *Zygnema* is also filamentous. It differs from *Spirogyra* mainly in plastid morphology. Cells of *Zygnema* contain a pair of axile stellate chloroplasts (Fig. 6-27), each harbouring a single central pyrenoid with

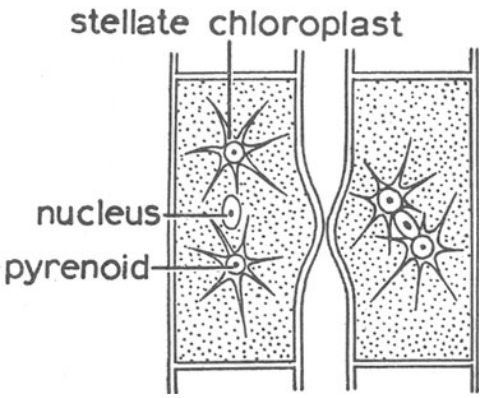

Fig. 6-27. *Zygnema*, cells showing pair of axile stellate chloroplasts.

radiating starch grains. The cells are uninucleate and the single nucleus lies embedded in the middle of the cytoplasm that separates the two chloroplasts. The nucleus contains one nucleolus with one nucleolar organizing chromosome.

Reproduction

Vegetative. It is brought about by the fragmentation of filament.

Asexual. In clonal cultures, three species of *Zygnema*, i.e., *Z. sterile*, *Z. spontaneum* and *Z. sp.* have recently been reported to lack sexual reproduction, and multiply solely by the formation of akinetes (*Z. sterile*) or aplanospores (*Z. spontaneum* and *Z. sp.*). Filaments resulting from aplanospore germination in fresh culture medium are normal and unbranched but become branched when grown in old culture medium.

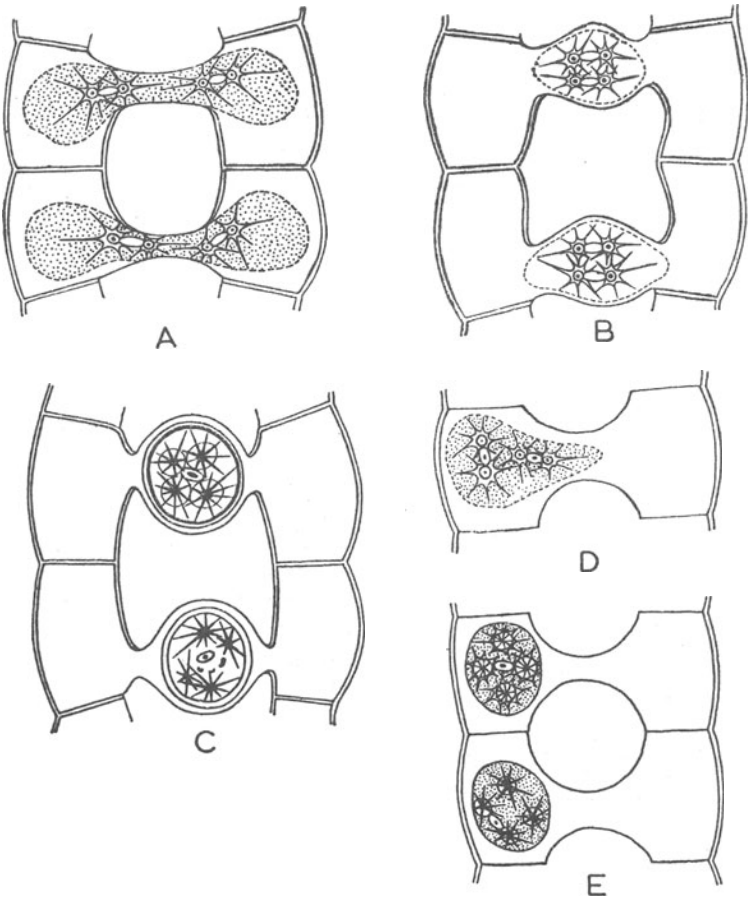

Fig. 6-28. *Zygnema.* A–E, stages in sexual reproduction.

Sexual. Reproduction is always morphologically isogamous. Most species are physiologically isogamous (Fig. 6-28 A–C) but some are anisogamous (Fig. 6-28 D, E). Both scalariform and lateral conjugation are known.

Class: CHAROPHYCEAE

The Class Charophyceae includes a single Order Charales having six living

8. (54-22/1972)

genera, all placed in the Family Characeae. Popularly known as stoneworts, the Charophyceae constitutes a distinctive and isolated group of highly differentiated and elaborately organized green thallophytes that—because of their uncertain affinities—have presented greater difficulties in classification than any other group of plants. The class is characterized by such distinguishing features as: (1) enclosure of antheridia and oogonia by jackets of sterile cells; (2) biflagellate and spiral-shaped nature of the antherozoids; (3) non-formation of zoospores; and (4) germination of zygote into a protonemal stage which gives rise to a mature plant.

The thallus of the Characeae is differentiated into nodes and internodes with whorls of branches of limited growth being produced from each node.

Despite these distinctive features, the photosynthetic pigments, the food reserves, the cell wall, the flagellar morphology and the life cycle of these algae are typically chlorophytean. Further, a thallus differentiated into nodes and internodes and producing branches only from the nodal cells is also found in *Draparnaldiopsis*. In view of these similarities, the Charophyceae are classified here along with the Chlorophyceae in the Phylum Chlorophyta.

Besides living genera, some fossil members of the Charales have been recorded from as far back as the Devonian. All the living members are submerged aquatics growing attached by means of multicellular rhizoids to the muddy or sandy bottom of shallow, brackish or freshwater lakes, ponds or streams. Some species of *Chara* become heavily encrusted with calcium carbonate.

Order: CHARALES
Family: Characeae
Genus: *Chara*

Morphology

Usually 6–10 inches tall, the main axis and branches of *Chara* are differentiated into nodes and internodes (Fig. 6-29 A). Each node bears a whorl of several branches and consists of a pair of central cells surrounded by a peripheral group of 6–20 cells. The internode is always composed of a single elongate cell which may be corticated. The corticating cells are also long and are derived from both the upper and lower nodes. Branched multicellular rhizoids are formed from the lowermost one or two nodes. The branches of the first order, produced from the nodes of the main axis, are known as primary laterals. These are also called laterals of limited growth because of cessation of growth in these branches after they have formed a few nodes and internodes. Single-celled branches called secondary laterals arise from the nodes of primary laterals. Sometimes, from near the axils of the oldest whorls of the primary laterals (pseudoaxillary position), branches of unlimited growth come out with the same degree of differentiation as found in the main axis.

Cell Structure

The nodal cells contain dense cytoplasm, a single nucleus and a few discoid or elliptical chloroplasts but in the internodal cells there is a large central vacuole, hundreds of nuclei and many discoid chloroplasts, the latter two occupying the peripheral cytoplasmic region. The chloroplast lacks pyrenoid.

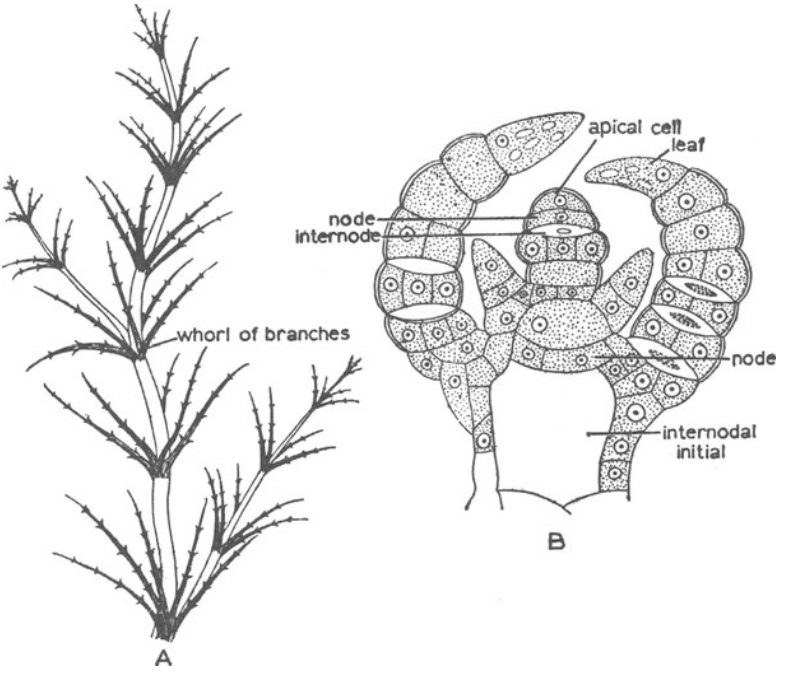

Fig. 6-29. *Chara*. A, part of vegetative thallus showing habit; B, vertical section through apex of thallus.

On the basis of its streaming nature, the cytoplasm is divided into a stationary exoplasm and a rotating endoplasm which continually streams up and down along the longitudinal plane. The streaming of the cytoplasm is thought to result from alternating contraction and expansion of the protein fibrils which remain fixed to the cell wall.

Growth

The main axis and primary laterals grow by means of a dome-shaped apical cell (Fig. 6-29 B) which cuts off a transverse segment at its posterior end; the resulting segment divides transversely into an upper nodal initial and a lower internodal initial. The latter does not divide further but elongates considerably. The first division of the nodal initial is in a vertical plane producing two semicircular cells, but all subsequent divisions occur in curved planes, each intersecting the preceding plane of division in such a way that a group of 6–20 peripheral cells enclosing a pair of central cells is produced.

Each peripheral cell divides into an apical initial of the primary lateral and a basal nodal cell and, therefore, the number of peripheral cells at the node determines the number of primary laterals produced at that node. Each basal nodal cell further divides into an upper and a lower cortical cell. Thus an internodal region becomes corticated with corticating cells, one half of which are derived from the upper node and the other half from the lower node.

The nodal initials of primary laterals also exhibit the same pattern but the apical initials of secondary laterals located at the nodes of primary laterals do not divide further and only elongate into single-celled, scale-like structures.

Reproduction

Vegetative. Reproduction in some cases takes place by means of amylum stars which are stellate aggregates of cells densely filled with starch grains and produced from the lower nodes. Other means of vegetative propagation are by the formation of bulbils upon rhizoids and on lower nodes of cells, or by the formation of protonemal outgrowths derived from the nodes.

Sexual. All species of *Chara* show a highly advanced type of oogamy. The antheridial filaments are borne within a structure called the **globule** whereas the oogonium is present within the **nucule.** Both are situated at the base of secondary laterals (Fig. 6-30 A).

A mature globule is a hollow spherical body and consists of an outer, a middle and an inner group of cells termed respectively as shield cells, manubrial cells and capitulum cells (Fig. 6-30 B). A capitulum generally divides and gives rise to a group of two to four secondary head cells or capitula from each of which is produced a pair of antheridial or spermatogenous filaments (Fig. 6-30 B). Each cell of the spermatogenous filament forms a spiral biflagellate sperm (Fig. 6-30 C, D). In an electron microscope study of *C. corallina*, Moestrup (1970) has reported the presence of scales on the flagella of its spermatozoids. Cells of spermatogenous filaments generally divide synchronously.

The nucule is an oblong structure consisting of five spirally coiled tube cells which form a sterile envelope around the oogonium and of five-lobed corona cells that project beyond the apex of the oogonium (Fig. 6-30 A).

Development of Sex Organs

Globule. It develops from an adaxial peripheral cell at the node of a primary lateral. This cell divides into two, the lower forms the pedicel and the upper acts as an antheridial initial (Fig. 6-31 A). The latter divides thrice to give rise to an octant stage. Each cell of the octant undergoes two periclinal divisions resulting in a radial row of three cells (Fig. 6-31 B, C) with the outer cell functioning as the shield cell, the middle as the manubrium, and the inner as the primary head cell. Subsequently, the shield cells expand laterally and the manubria elongate vertically. By this time, the primary head cell has already divided and the resulting secondary head cells bifurcate vertically into a pair of cells, each developing into a spermatogenous filament. In a mature globule, the large number of spermatogenous filaments radiate

in the hollow cavity.

Nucule. This is also produced from a peripheral cell close to the antheridial initial. It divides into a row of three cells, of which the lower

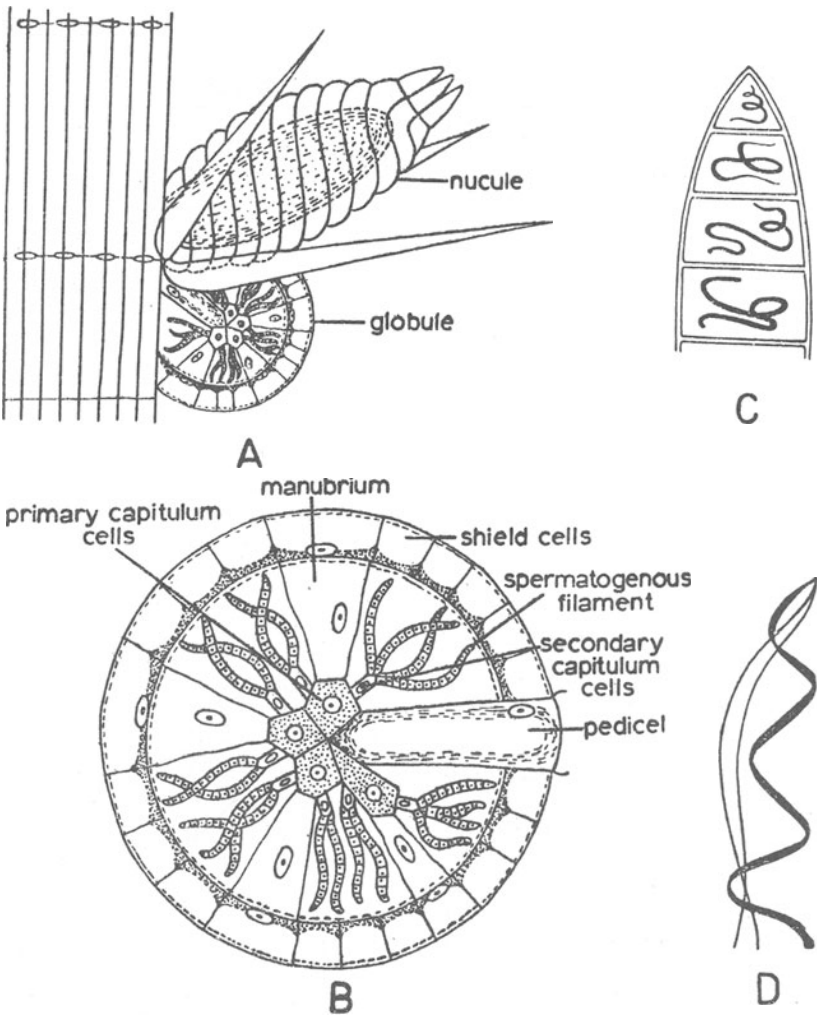

Fig. 6-30. *Chara.* A, portion of a plant bearing nucule and globule; B, structure of a mature globule; C, spermatogenous filament; D, biflagellate spiral sperm.

forms the pedicel, the middle the sterile jacket or sheath cells, and the upper the oogonial mother cell (Fig. 6-31 D). The middle cell divides repeatedly to form a group of five sheath cells enclosing a central cell. Meanwhile the oogonial mother cell forms a lower stalk cell and an upper oogonium. The five sheath cells undergo a single transverse division to form a lower tier of five tube cells and an upper tier of five coronal cells (Fig. 6-31 E). Finally the tube cells elongate and coil spirally around the oogonium thus pushing up the coronal cells at the top of the oogonium. A large quantity of food

reserves accumulates in the oogonium which differentiates into an egg.

At the time a nucule matures, the spiral tube cells separate from one another and the resulting space in between them provides an opening for the entrance of sperms, one of which finds its way inside the oogonium and fuses with the ovum.

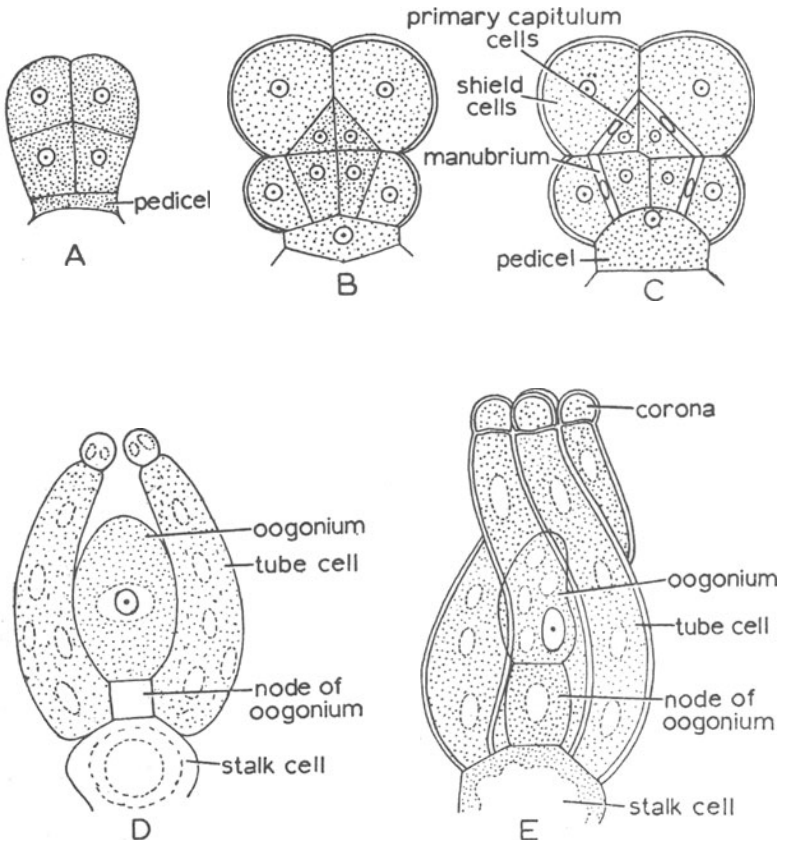

Fig. 6-31. ***Chara.*** **A–C, development of globule; D–E, development of nucule.**

The wall of the zygote thickens and after perennation the oospore germinates; the nucleus migrates to the anterior region where it probably divides meiotically. A septum is then laid down forming a uninucleate upper cell and a degenerative, trinucleate lower cell (Fig. 6-32 A). The upper cell undergoes one mitotic division in a vertical plane. Of the two daughter cells, one acts as the protonemal initial and the other as the rhizoidal initial. These initials form the filamentous protonema and the colourless rhizoids respectively (Fig. 6-32 B), both with nodes and internodes. The plant arises from one of the appendages borne on the second node of the protonema.

Because of the suspected occurrence of meiosis during germination of zygote (and not during gametogenesis), the life cycle of *Chara* is believed to be of the haploid type.

While studying the influence of chemical substances on the regulation of growth of protonema and adult thalli of *Chara*, Imahori and Iwasa (1965) found that thiamine, pyridoxal, gibberrellic acid and kinetin differentially

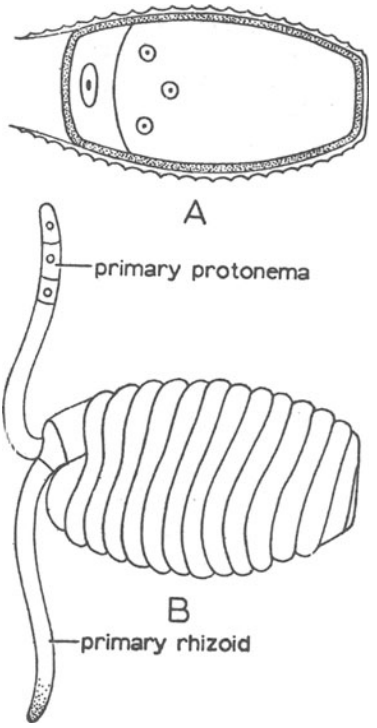

Fig. 6-32. *Chara*. A, longitudinal section of germinating oospore showing nuclear division; B, surface view of germinating oospore. (After Smith, 1955.)

promoted the growth of the protonema whereas vitamin-B_{12} and nicotinamide favoured the growth of the mature thalli. Various amino acids administered individually or in mixtures increased the growth of both protonema and of adult thalli.

Phylogeny

This has long been a hotly debated subject and the available fossil records are of little help in suggesting any clues to the origin of this Class. In respect of photosynthetic pigments, food reserves, cell wall composition, flagellar morphology of spermatozoids, and zygote germination, the Charophyceae resemble Chlorophyceae. The differentiation of the main filament into nodal and internodal cells, formation of corticating filaments, production of lateral branches of limited growth and participation of the laterals of limited growth in reproduction are features that are shared also by the chaetophoralean genus *Draparnaldiopsis*. These traits have been emphasized by many phycologists to suggest a possible derivation of the Charophyceae from the

Chaetophorales (Desikachary and Sundaralingam, 1962). But the apical growth and the complex nature of the male and female sex organs point against any possible origin of Charophyceae from the Chaetophorales. Papenfuss (1955) on the basis of parallelism in the development of sex organs has emphasized an affinity between bryophytes and Charophyceae. Bold (1967) considers that morphologically the sex organs of Charophyceae are in no way different from those of bryophytes.

TEST QUESTIONS

1. Give reasons for the inclusion of such morphologically diverse forms as *Chlamydomonas, Sphaeroplea, Ulva, Fritschiella* and *Chara* in the Chlorophyta.
2. Name the organisms responsible for 'red snow' and 'red rust of tea'. How does the mode of nutrition of an algal symbiont in a lichen differ from that of a parasitic alga such as *Cephaleuros*?
3. Compare the different types of chloroplasts met with in Chlorophyceae.
4. Why are *Chlorella* and *Hydrodictyon* included in the Order Chlorococcales and not in Volvocales?
5. In what ways does the nuclear cytology of Chlorophyta differ from that of Euglenophyta?
6. "Chlorophyta comprise a group of unicellular and colonial algae full of evolutionary potentialities." Discuss this statement.
7. Can you offer some evidences to show that in *Chlamydomonas*: (a) the presence of flagella is essential for gametic union; (b) the contractile vacuoles are the organs of osmoregulation; and (c) the eye spot is probably not the primary photoreceptor organ in phototactic responses?
8. Explain the terms: complementary sex factors; flagellar agglutination; genetic recombination; linkage groups and tetrad analysis.
9. What will happen if the + and − strains of *Chlamydomonas moewusii* are: (a) grown in a medium lacking ammonium nitrogen; (b) kept in the dark; or (c) cultured in a calcium deficient medium?
10. Mention the importance of resistance and biochemical markers in the genetical analysis of any organism.
11. Devise an experiment to demonstrate the recombination for non-nuclear genes in *Chlamydomonas.*
12. What is cell synchrony and what advantages does it offer in physiological and biochemical investigations?
13. How would you prove that *Volvox* is more than a mere aggregation of *Chlamydomonas*-like cells, and segregation of vegetative and reproductive functions in *Volvox* occurs during its embryonic stage?
14. What could be the probable functions of light, temperature, sulphur and nitrogen in the regulation of life cycle in *Chlorella*?
15. Why is *Chlorella* a favourite organism in researches on photosynthesis and as a potential food source?

16. Compare the asexual reproduction of *Volvox* with that of *Hydrodictyon.*

17. Discuss the affinities and systematic position of *Chara.*

18. What is a nannandrium ? In what way does the sexual reproduction of macrandrous *Oedogonium* differ from that of nannandrous species ?

19. Elucidate the genetic significance of the degeneration of three out of four nuclei produced during the germination of a zygote in *Spirogyra.*

20. Compare the different modes of perennation met with in green algae studied by you.

21. Do you think that the protonemal stage of *Chara* is homologous with the prostrate system of a heterotrichous alga ?

22. Distinguish between: (a) zygospore and oospore; and (b) oogonium and archegonium.

23. Describe the cell structure of *Spirogyra.* List five substances which *Spirogyra* must obtain from water if it is to grow vigorously.

24. Draw a labelled diagram of a mature vegetative cell of *Spirogyra.* Give at least four features in which this cell differs from: (1) a cell in the meristematic zone of a root apex; and (2) a typical parenchyma cell in the pith of an angiosperm.

25. *Pleurococcus* commonly occurs on tree barks and other terrestrial substrata whereas *Euglena* cannot grow in such habitats. What explanation can you offer to account for this variation ?

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7

Xanthophyta

GENERAL CHARACTERS

Commonly known as yellow-green algae, the Xanthophyta include one Class, Xanthophyceae, characterized by the following general features: (1) the photosynthetic pigments consist of chlorophyll-a and -e, β carotene and xanthophylls; of these, β carotene is usually present in fairly high concentrations; (2) the food reserves are oil, lipid, and a glucose polymer termed **leucosin** or **chrysolaminarin**; (3) the cell walls when present are generally composed of two equal or overlapping halves, e.g., *Tribonema* (Fig. 7-1) and are composed mainly of pectic substances with smaller amounts of cellulose; in some members the cell walls may be silicified; and (4) the flagellated cells possess two anteriorly inserted flagella of unequal length, the longer being pantonematic and the shorter acronematic.

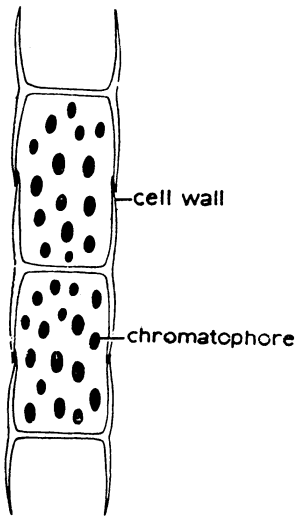

Fig. 7-1. *Tribonema*, portion of filament showing cell wall made of overlapping H-pieces and discoid chromatophores.

The Xanthophyceae are widely distributed in aquatic, terrestrial and subaerial habitats but the maximum representation is in freshwater habitats.

The Xanthophyceae exhibit well-marked parallelism with Chlorophyceae in thallus structure which include motile, palmelloid, dendroid, filamentous and siphoneous forms. However, the yellow-green algae in general do not reach the level of evolutionary development attained in the green algae. Thus, the advanced, elaborate pseudoparenchymatous and parenchymatous habits met with in the Chlorophyta are not seen in the Xanthophyta.

The usual methods of propagation and reproduction are vegetative, asexual and sexual. Sexuality is rather rare and has been established in only three genera. In *Tribonema* fusion takes place between two biflagellated zoogametes one of which discards its flagella and becomes non-motile during or shortly before fusion, thus indicating a sort of oogamy. In *Botrydium*, both isogamous and anisogamous forms of sexuality are known. *Vaucheria* is oogamous.

The formation of flagellated structures during the life cycle of most of the Xanthophyceae together with the occurrence of predominantly flagellated unicells in primitive members of the class, suggests a flagellated ancestry for the Xanthophyceae.

The Xanthophyceae resemble Chrysophyceae in their flagellation, amoeboid habit and food reserves; and Bacillariophyceae in food reserves and cell wall composition. The class is unique in having a preponderance of pectic substances in the cell wall and presence of chlorophyll-e in some members. Round (1965) and Bold (1967) consider Xanthophyceae, Chrysophyceae and Bacillariophyceae to be closely related and accordingly include them in a single Phylum, the Chrysophyta (or Chrysophycophyta).

The vegetative cells of certain members of Xanthophyta, now segregated into a distinct Class called Eustigmatophyceae, contain an exceptional kind of stalked pyrenoid which is polygonal, projects from the inner face of the chloroplast, is enveloped by flat plates of some unidentified food reserve, and is devoid of photosynthetic thylakoids (Hibberd and Leedale, 1970). Another distinguishing feature of these algae is that zoospores have only one, subterminally inserted, emergent flagellum having a flagellar swelling at its proximal end. This flagellum has stiff hairs.

Order: HETEROSIPHONALES
Family: Botrydiaceae
Genus: *Botrydium*

The most primitive genus of siphoneous Xanthophyceae, *Botrydium* strongly resembles the green alga *Protosiphon* in its habit and habitat.

Occurrence

It is a terrestrial alga found on muddy or damp soils near the banks of temporary or permanent pools, ponds and streams. It grows as pin-head vesicles which often form a thick, yellowish-green coating over the soil surface.

Morphology

The thallus is unicellular, coenocytic and consists of a lower profusely branched, colourless rhizoidal portion and an upper, globose or cylindrical vesicle which may measure a few mm in size. The rhizoids are subterranean and serve the function of thallus anchorage. The vesicle contains a central vacuole and a peripheral protoplasm with numerous minute nuclei

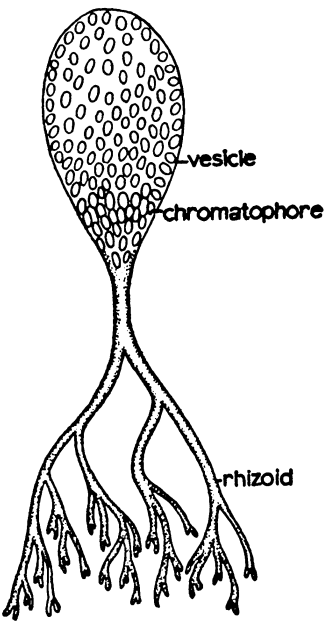

Fig. 7-2. *Botrydium*, thallus.

and discoid chromatophores (Fig. 7-2). The chromatophores possess naked pyrenoids and lie next to the cytoplasmic membrane whereas the nuclei occupy a position internal to the chromatophores. Rhizoids are also multinucleate with nuclei lying scattered in the vacuolated and non-vacuolated cytoplasm.

Occasionally the thalli become heavily encrusted with calcium carbonate. The cell wall is chiefly composed of cellulose. The photosynthetic food reserves are normally oil and leucosin.

Reproduction

Vegetative. It is rare and may be brought about by budding of mature vesicles.

Asexual. It takes place by means of biflagellate zoospores, or by the formation of aplanospores or hypnospores. High humidity and free water both stimulate zoospore formation (Erben, 1962). The vesicular coenocytic protoplast fragments into uninucleate parts, each developing into a pyriform biflagellate zoospore. The flagella are anteriorly inserted, the longer being pantonematic and the shorter acronematic.

Generally the zoospores differentiate directly into vegetative thalli. Sometimes their behaviour is facultative and they may also act as gametes. Under certain conditions, uninucleate or multinucleate aplanospores are formed instead of zoospores. During adverse environmental conditions, aplanospores transform into uninucleate or multinucleate hypnospores. Both uninucleate and multinucleate aplanospores germinate directly into new thalli. On the other hand, the uninucleate hypnospores behave like aplanospores in respect of germination but the multinucleate hypnospores produce uninucleate aplanospores or zoospores which then give rise to new thalli.

Sexual. Biflagellate zoogametes, morphologically identical with zoospores, are formed in the same way as the zoospores. The fusing gametes may be similar or dissimilar and accordingly the sexuality is either isogamous or anisogamous. The zygote is believed to germinate meiotically. Normally four haploid biflagellate zoospores are produced from a germinating zygote and each of these gives rise to a new *Botrydium* plant. The life cycle involves a haploid multicellular vegetative phase and a diploid unicellular zygote.

Family: Vaucheriaceae
Genus: *Vaucheria*

Vaucheria represents the climax of siphoneous habit among the Xanthophyta. Asexual reproduction in this alga occurs through the formation of **synzoospores** (compound zoospores) whereas sexual reproduction is of a specialized oogamous type. These reproductive features together with the branched, filamentous, siphoneous thallus, form the basis of its placement as a separate monogeneric family, the Vaucheriaceae. Previously *Vaucheria* was included

in the Order Siphonales of Chlorophyta but the revelation of features such as the absence of chlorophyll-b, presence of chlorophyll-e, excess of carotenoids over chlorophylls, storage of food reserves in the form of oil, and the unequal length of the paired flagella of the compound zoospores, led to its removal from green algae and inclusion in Xanthophyta.

Occurrence

Species of *Vaucheria* occur widely in stagnant and flowing freshwaters, in shaded terrestrial habitats, and on damp soils and walls. The alga may occasionally exhibit a thick, deep green, felt-like growth on moist soils. Some species are marine.

Morphology

The thallus is generally a sparingly branched, cylindrical tube lacking cross walls or septa (Fig. 7-3 A) except during reproduction. In terrestrial species, anchorage to the substratum is brought about by rhizoid-like branches. The thallus contains an outer cellulosic cell wall, a central vacuole that runs continuously from one end of the thallus to the other, and a continuous layer of protoplast with the peripheral region containing many discoid chromatophores devoid of pyrenoids. Numerous minute nuclei lie internal to chromatophores (Fig. 7-3 B) which exhibit phototactic response, i.e., back and forth movement in response to weak, unilateral illumination. When the light intensity is strong, they show a lateral movement, away from

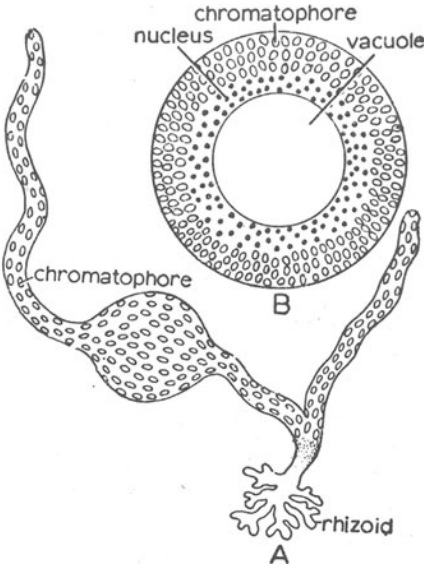

Fig. 7-3. *Vaucheria*. A, branched siphoneous filament with rhizoid; B, transverse section of the vegetative filament showing relative position of chromatophores and nuclei.

the light source (see Haupt 1962). In other words, the phototactic response is regulated in such a way as to permit maximum absorption of photosynthetic light by chromatophores, simultaneously protecting them against excessive photodamage.

The thallus grows in length by simple elongation of terminal portions of the branches.

Reproduction

Asexual. A variety of environmental factors affect zoospore formation (see Erben 1962). High humidity, transfer from running to still water, low light intensity or darkness, and dilution of the growth medium causing a reduction in the concentration of nutrients, hasten or induce sporulation. Light-controlled, diurnal periodicity in the induction of sporulation has also been reported in *V. sessilis.* It has further been observed that low light intensity enhances sporulation.

At the time of zoospore formation, apices of branches become delimited from the rest of the thallus by a septum and each of them develops into a club-shaped sporangium (Fig. 7-4 A). Subsequently, nuclei and chromatophores exchange their position so that the nuclei which were originally

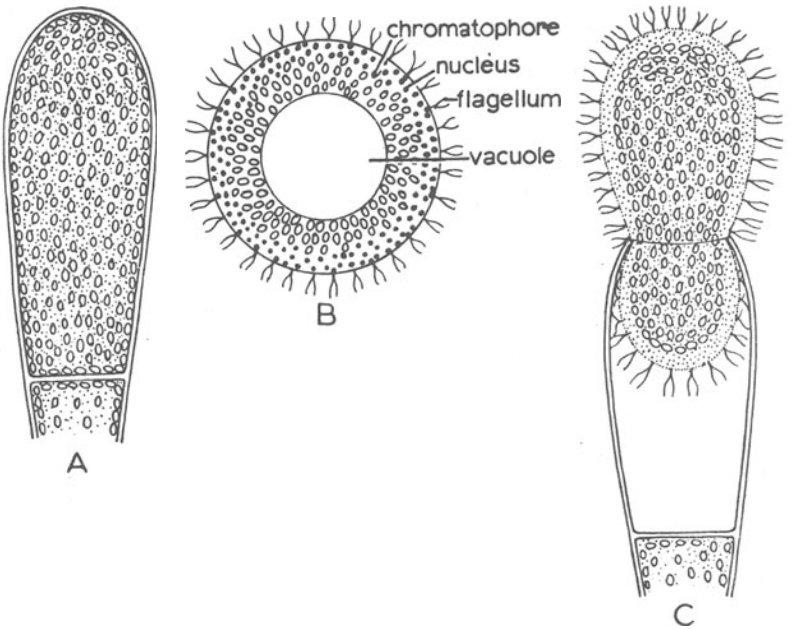

Fig. 7-4. *Vaucheria.* A, club-shaped zoosporangium; B, transection of a compound or synzoospore showing the relative position of chromatophores and nuclei; C, liberation of synzoospore. (Figs. A, C after Smith, 1955.)

placed internal to the chromatophores, now come to lie external to the chromatophores (Fig. 7-4 B). The sporangial protoplast then shrinks slightly and a pair of flagella of somewhat unequal length develops opposite each nucleus (Fig. 7-4 B). Both flagella seem to be acronematic. Zoospores are formed singly within the sporangia. At the time of liberation of zoospore an apical pore is formed by gelatinization of the sporangial wall and the zoospore gently glides out through this opening (Fig. 7-4 C). The naked

zoospores move freely for some time, settle down on a suitable substratum, discard their flagella and secrete a new wall. The germinating zoospore forms one to several germ tubes which develop into *Vaucheria* plant. Substances responsible for the differentiation of polarity which leads to the appearance of the germ tube, are believed to reside in the peripheral cytoplasm of the germinating zoospore.

The multiflagellate zoospore of *Vaucheria* is regarded as a compound zoospore or synzoospore (Fig. 7-4 B) resulting from failure of the sporangial protoplast to segment into biflagellate uninucleate zoospores as is the case in many zoosporic Xanthophyceae.

Terrestrial species commonly reproduce by the formation of non-motile aplanospores or hypnospores. Aplanospores are formed singly in club-shaped aplanosporangia (Fig. 7-5 A), and are liberated through apical pores (Fig. 7-5 B). Occasionally, aplanospore formation may even be induced in

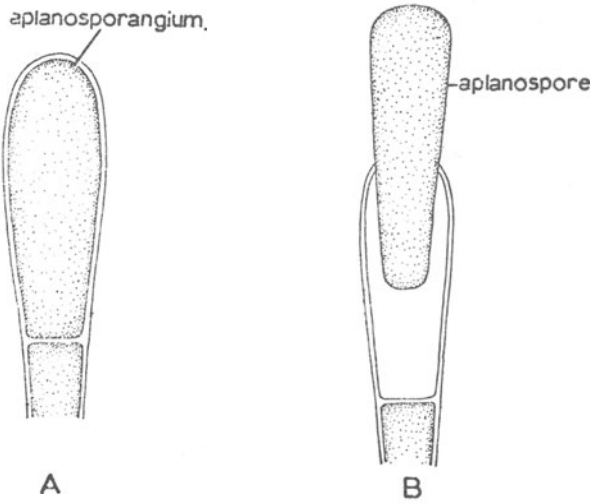

Fig. 7-5. *Vaucheria piloboloides.* A, club-shaped aplanosporangium; B, liberation of aplanospore.

aquatic species by their transference from running to still water or from light to darkness. During the formation of hypnospores the entire thallus segments transversely into a large number of coenocytic cells with each cell giving rise to a hypnospore which either germinates directly into a new thallus or divides into a number of small cysts. When the cyst germinates, the protoplast comes out through a pore in the cyst wall, exhibits amoeboid movement for a while and then grows into an adult thallus.

Sexual. Sexually reproducing species of *Vaucheria* are either homothallic or heterothallic bearing well-differentiated antheridia and oogonia. Sex organs may be sessile or stalked. In *V. geminata*, a homothallic species, sex organs are borne on special reproductive branches, producing a central curved antheridium surrounded by a peripheral group of 3–4 oogonia. Both antheridia and oogonia originate as protuberances (Fig. 7-6 A) which gradual-

ly grow in size and accumulate a large number of nuclei and chromatophores. The antheridia are generally tubular with their apices slightly curved or coiled (Fig. 7-6 B). The oogonia are commonly spherical or subspherical. At maturity the apical, curved portion of the antheridium becomes separated from its subtending branch by a transverse septum (Fig. 7-6 C). Its coenocytic protoplast divides into a large number of uninucleate, biflagellate (1 acronematic, 1 pantonematic) sperms (Fig. 7-6 C) which are usually liberated from the antheridium during early morning hours.

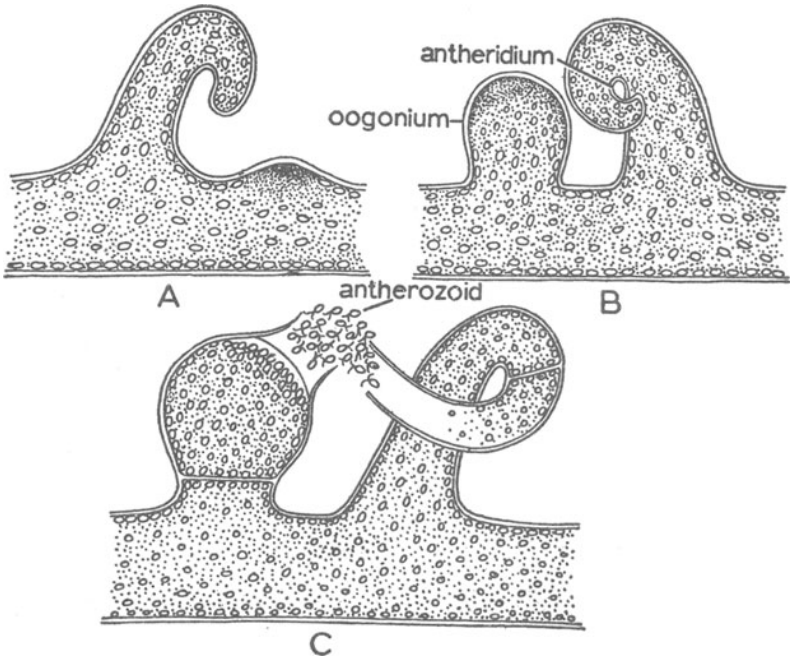

Fig. 7-6. *Vaucheria*. A, B, stages in the formation of sex organs (after Smith, 1955); C, release of antherozoids.

As an oogonium matures, all the nuclei except one migrate from the oogonium back into the main filament. Soon a transverse septum is laid down between the oogonium and the filament or the branch bearing it. Subsequently it develops a receptive region with an opening through which a sperm enters the oogonium.

Homothallic species produce both kinds of sex organs in close proximity, but generally they do not mature simultaneously thus preventing self-fertilization. The zygotes are thick-walled structures, rich in oil, and are commonly detached from the parent plant along with the oogonial walls. Occasionally the zygote is liberated from the oogonial wall while the latter is still attached to the parent plant. There is no cytological proof for the occurrence of meiosis during zygote germination, and no definite knowledge about the fate of the zygote. However it is believed that zygotic germination in *Vaucheria* is meiotic, and it directly gives rise to a new thallus. The

life cycle of *Vaucheria* seems to resemble that of *Botrydium* but the cytological proof is lacking.

TEST QUESTIONS

1. Which practical methods will you adopt to distinguish between: (1) *Protosiphon* and *Botrydium*; (2) *Tribonema* and *Microspora*; (3) *Vaucheria* and *Dichotomosiphon*; and (4) *Ophiocytium* and *Hydrodictyon* ?
2. Explain with reasons whether the thalli of *Botrydium* and *Vaucheria* are multicellular, acellular or unicellular.
3. Compare the zoospores of *Vaucheria* with those of *Oedogonium.*
4. What could be the reasons for the zoospores and sperms of *Vaucheria* to be respectively multiflagellate and biflagellate ?
5. Describe the characteristics of xanthophytes generally and of Heterosiphonales specifically. Name representative genera of the Heterosiphonales and discuss their salient features and interrelationships.
6. Do you think that the frequent occurrence of two-piece silicified cell walls in some members of the Xanthophyceae has any phylogenetic significance ?
7. Why is starch not found around the pyrenoids of Xanthophyceae ?
8. Enumerate the features that have been employed in the classification of Xanthophyceae.

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8

Bacillariophyta

The Bacillariophyta, popularly called diatoms, comprise a homogeneous assemblage of unicellular and colonial forms that differ generally from other algae in possessing highly sculptured and symmetrically ornamented cell walls. The main characters that collectively differentiate the Bacillariophyta from other phyla are: (1) the diploid nature of vegetative cells; (2) the presence of chlorophyll-c together with fucoxanthin, diatoxanthin and diadinoxanthin in chromatophores, the usual brown colouration being due to the preponderance of carotenoid pigments over chlorophylls; (3) the silicified nature of cell walls which consist of two highly perforated overlapping pieces sometimes bearing structures like spines and bristles; (4) the storage of oil and chrysolaminarin and not starch in food reserves; and (5) the reduction in size of cells occurring during vegetative multiplication compensated by the production of spores known as auxospores.

OCCURRENCE

Cosmopolitan and ubiquitous in distribution, the diatoms form a major component of the planktonic vegetation. They are very important as primary producers in the food web of aquatic ecosystems. The most common genera of freshwater habitats are *Synedra*, *Melosira*, *Navicula*, *Nitzschia* and *Asterionella*. Species of *Cocconeis*, *Gomphonema* and *Eunotia* grow epiphytically on certain freshwater algae such as *Cladophora* and *Oedogonium*. *Triceratium* and *Hyalodiscus* occur in the littoral and sublittoral zones as epiphytes on seaweeds.

MORPHOLOGY

The thalli are either unicellular or colonial. In respect of their shape and valve morphology the unicellular diatoms have been classified into two orders, the pennate diatoms (Pennales) with isobilateral symmetry, e.g., *Pinnularia* (Fig. 8-1 A) and the **centric** diatoms (Centrales) with radial symmetry, e.g., *Cyclotella* (Fig. 8-1 B). *Triceratium* (Fig. 8-1 C) has three planes of mirror symmetry. Further classification of the Centrales is based on the presence or absence of bristles or horns on the cell surface. The Pennales are classified according to the presence or absence and number and morphology of the **raphes** on the valves.

Colonial diatoms may be organized into uniseriate filaments, as in some species of *Melosira*. Or, there may be extensive branching of the mucilage

with the stalks bearing at their free ends groups of cells organized into fan-like structures as in *Licmophora flabellata*. Sometimes a colony results

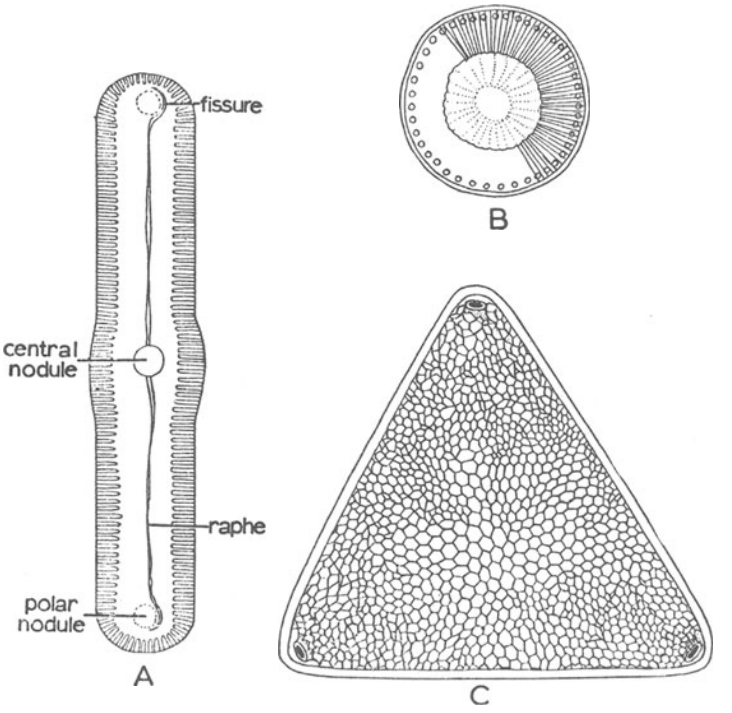


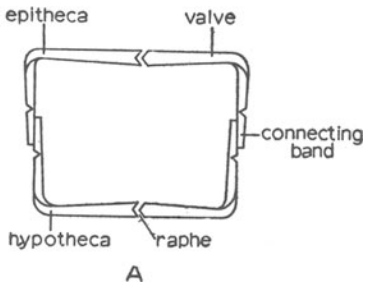
Fig. 8-1. A, *Pinnularia*, valve view; B, *Cyclotella*; C, *Triceratium*.

from the linkage of the cells through bristles or horns present on the valve surface as in *Chaetoceros*. Stellate colonies result from the union of cells at their basal ends through localized production of mucilage as in *Asterionella*.

CELL STRUCTURE

Cell wall (frustule) consists of two overlapping halves, the upper half is known as **epitheca** and the lower **hypotheca**. Each theca is further divided into two parts—the main surface and its incurved margins termed valve and connecting band, respectively (Fig. 8-2 A, B). The epitheca and hypotheca can be compared to a box, the lid corresponding to the former and the main body to the latter. The two connecting bands represent incurved sides of the lid and the main body whereas the valve relates to the top or bottom of the box. When fitted together, the connecting band of epitheca overlaps that of hypotheca and the two bands remain united in the overlapping region called girdle, by a cementing organic substance present between them. Accordingly, a cell can be seen from two different aspects, the **valve view** and the **girdle view**. Most diatoms appear rectangular in girdle

view but in valve view their shape is variable. The line connecting the middle of the two valves constitutes the pervalvar axis, and the plane along



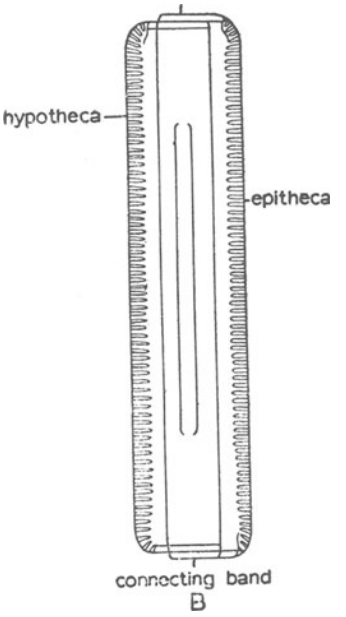


Fig. 8-2. *Pinnularia*. A, transverse section showing relative position of epitheca, hypotheca, connecting band, valve and raphe; B, girdle view showing relative position of epitheca and hypotheca.

which the cell divides (at right angles to the pervalvar axis) is called the valvar plane (Fig. 8-3). The ornamentations are generally confined to the valve portion of the silica wall.

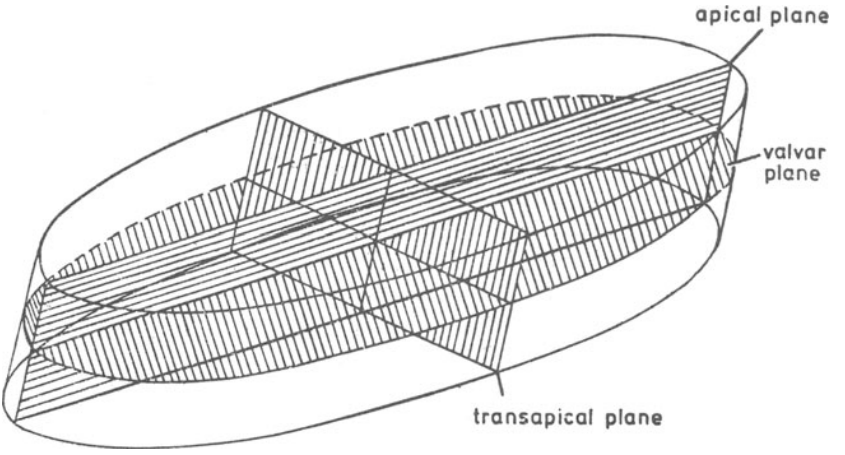


Fig. 8-3. Diagram illustrating the three planes of symmetry in a pennate diatom. (After Reimann *et al.*, 1966.)

The diatom cell walls are made up of crystalline silica (α-quartz) and an organic component of unknown nature. Silica is an absolute requirement for the multiplication of diatoms and the cytoplasmic membrane is consider-

ed instrumental in the formation of silicified wall (Lewin, 1962). In *Navicula pelliculosa* the wall consists of a silica shell or layer with an outer coating of organic material termed organic skin (Reimann *et al.*, 1966). The formation of silica shell is intracellular and occurs by deposition of silica inside the vesicles enclosed by a three-layered membrane, the **silicalemma** (Fig. 8-4). Apart from silicalemma, there is a more or less thick layer of acid polysaccharides lining the silica shell from inside. Silicic acid uptake has been studied in certain pigmented and colourless diatoms. It involves some cell surface factors, the absence of which impairs the process of silicic acid absorption. The composition and origin of organic skin is still unknown.

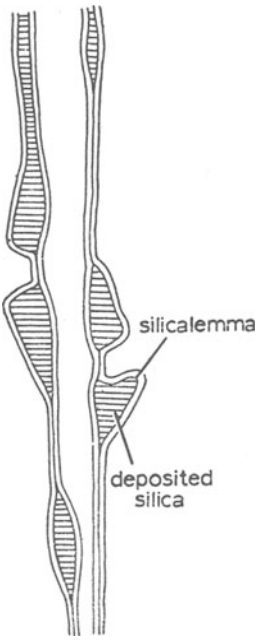


Fig. 8-4. *Navicula*, graphic representation of silicalemma formation. (After Reimann *et al.*, 1966.)

VALVE MORPHOLOGY

The fine lines or markings found on the surface of diatom valves vary enormously in their microscopic details, so much so that in the past they created much confusion in the formulation of terminology. However, according to Hendey (1959), four types of secondary structures are present on diatom valves: (1) the **punctae**, which are fine perforations arranged in regular rows corresponding to the markings or striae on the valve surface of silica walls; (2) the **areolae**, which are cavity-like depressions, coarser and larger than the punctae and are generally provided with sieve membranes; (3) the **canaliculi**, which are tubular canals running through the valve surface; and (4) the **costae**, which are specially thickened regions of the valve resulting from heavy accumulation of silica and represent the valvar ribs. The ribs constitute the backbones of the cell wall. Hendey has also classified the diatom walls into two types based on their nature: (1) the laminar wall, a single silicified layer; and (2) the locular wall, basically made up of two parallel wall layers with a number of loculi in between them.

In pennate diatoms the markings are arranged longitudinally, one on either side of the elliptical or oblong valves, e.g., *Pinnularia* (Fig. 8-1 A) whereas in centric diatoms they are distributed concentrically with reference to a central point on the more or less circular valves, e.g., in *Cyclotella* (Fig. 8-1 B).

In *Pinnularia* which is an oblong unicell, the valve view presents two planes of isobilateral symmetry—the apical and the transapical. The apical axis joins the two poles of the valve and the transapical axis passes along the transverse axis of the valve. In the axial area of some pennate diatoms, e.g., *Navicula*, there is a longitudinal slit termed raphe which is interrupted

in the middle by a **central nodule** formed by internal thickening of the valve (Fig. 8-5). Each pole of the valve also contains a nodule (polar nodule) which is formed in the same manner as the central nodule (Fig. 8-5). The centric diatoms lack a raphe.

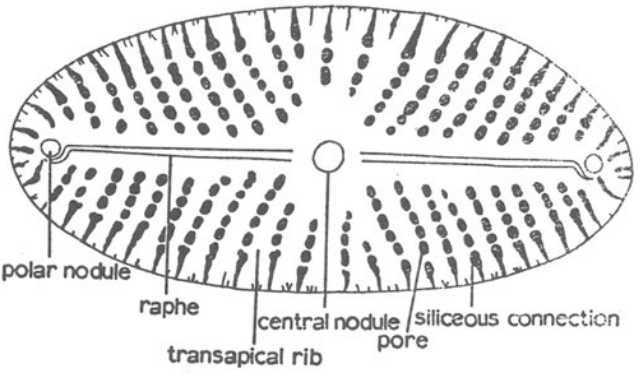


Fig. 8-5. *Navicula*, valve view illustrating details of cell wall structures. (After Reimann *et al.*, 1966.)

Navicula, like *Pinnularia*, is isobilaterally symmetrical with valve markings consisting of fine pores bridged from inside by sieve membranes containing numerous fine pores. The raphe situated in the central rib is also called apical rib. The rows of pores situated on the lateral sides of apical rib separated from one another by solid silica ribs are termed transapical ribs. The pores within a row are separated by siliceous connections which link the adjacent transapical ribs (Fig. 8-5). In valves which lack a raphe, the axial area may give the appearance of a false raphe or **pseudoraphe**. *Navicula* and *Pinnularia* possess two raphes each, one on each valve, whereas in *Achnanthes* only one of the two valves has a raphe. A pseudoraphe is characteristic of *Synedra* and *Fragilaria*.

PROTOPLAST

The cytoplasm of the diatoms is bounded by a membrane and surrounds a central vacuole. The cells are uninucleate. In the Pennales the nucleus in most species lies in a massive plasmatic bridge which separates the vacuoles; in the Centrales it normally occupies a position within the peripheral layer of the cytoplasm. Nuclear division shows two interesting features: (1) the spindle formation is intranuclear and the metaphase chromosomes arrange themselves in an equatorial ring rather than on an equatorial plate, and (2) the spindle is an elongated cylinder with flattened ends, a feature common to Chrysophyta and Pyrrophyta. In *Lithodesmium undulatum* certain plate-like structures constituting a spindle precursor are found along the nuclear membrane. During mitotic prophase in sperm-producing cells, a mitotic spindle consisting of long microtubules appears.

between precursor plates and nuclear wall. Gradually the nuclear wall degrades and a compact spindle is formed. Subsequent stages in nuclear division are similar to those in higher plants. The Centrales generally have numerous discoid chromatophores and the Pennales have one or two large plate-like frequently lobed chromatophores. Naked pyrenoids occur in some diatoms, e.g., *Nitzschia*. Electron microscopy of the diatom cell has demonstrated the presence of endoplasmic reticulum, Golgi bodies and mitochondria.

LOCOMOTION

Pennate diatoms possessing a raphe exhibit a gliding movement whose mechanism is not clearly understood. The protoplasm exhibits streaming and, believably, as a result the cytoplasm comes in contact with the external medium through the raphe. The friction caused by such contact brings about the movement of the cell. It has been suggested that the osmotic pressure of the cell, which is equivalent to about 4–5 atmospheres, is sufficient to force the entry of the cytoplasm into the complex canal systems of the raphe thus causing the streaming of the cytoplasm through the raphe. The movement is generally jerky but at times it may be creeping and steady. The gliding movement or cytoplasmic streaming has been explained by the hypothesis that protein fibrils of the protoplasm attached to its outer surface undergo alternate contraction and expansion. If the fibrils are on the inner surface, their beating will cause the peripheral cytoplasm to stream and if they are located in the outer surface, their beating will result in gliding movement.

Recent researches have established that cytoplasmic streaming, associated with the raphe system, plays an important role in the gliding motion of diatoms. The motile cells possess a fibrillar system under the raphes, and certain crystalline granules in the cytoplasm that produce some mucilaginous substance which is secreted through the raphes, sticks to the substratum and, during locomotion, is left behind as a trail. Some capillarity mechanism which causes a pressure gradient along the raphe, based on the secretion of the mucilaginous substance, possibly provides the motive force for locomotion in gliding movement.

The problem of floatation and sinking of planktonic diatoms has also aroused considerable interest in recent years. One theory proposes that accumulation of oil and lipids in old nitrogen-deficient diatoms gives the necessary buoyancy. This has been refuted on the basis that increase in thickness of the silica wall parallels the accumulation of fats and this offsets the reduction in specific gravity due to the fat. An alternative suggestion has been made by Gross and Zeuthen (1948) who consider that by excluding metal ions from their cell sap the diatoms decrease their specific gravity and hence keep floating. Lund (1959), however, contradicts the presence of such a mechanism in freshwater diatoms since their habitats are so low in ionic concentration that loss of ions from the cell sap may not be of much help in maintaining the buoyancy.

REPRODUCTION

There are well-documented evidences for the occurrence of vegetative and sexual reproduction in diatoms. Although the production of non-motile or biflagellate zoospores for some members of the Centrales, e.g., *Biddulphia*, has been claimed, there is still some doubt as to whether the spores are actually formed by the diatoms, or whether the supposed spores are in fact only chytrids that parasitize the diatoms. If indeed they are spores, their fate is unknown.

VEGETATIVE

The two thecae of the parent cell slightly gape apart before division. The nucleus divides mitotically and karyokinesis is followed by cytokinesis of the protoplast into two uninucleate parts along the valvar plane. Soon, new valves and later connecting bands are deposited along the freshly formed protoplasmic surfaces. The parental hypotheca serves as epitheca of one of the two daughter cells, whereas the parental epitheca remains as epitheca of the other daughter cell. Accordingly, the newly formed wall pieces always serve as hypotheca. Such a division leads to the production of two daughter cells of unequal size; the cell containing the parental hypotheca as epitheca is always smaller than its sister cell by about twice the thickness of the connecting band. Thus the progenies of a diatom become progressively smaller during successive cell divisions.

SEXUAL

In the Pennales an enlarged spore, termed auxospore, develops from the zygote resulting from the union of two amoeboid gametes. In the Centrales, however, fusion of a flagellated sperm occurs with a non-motile egg. Consequently, sexuality in the former is generally isogamous and that in the latter oogamous. Since the zygote enlarges to become an auxospore, the production of auxospores is a compensatory measure for the vegetatively reproducing diatoms to regain their normal size.

In the Pennales, two cells come to lie very close with their girdle view facing each other. A secretion of mucilage then envelops the conjugating pair. The diploid nucleus of the copulating cells undergoes meiosis and produces 3 or 4 haploid nuclei of which generally one or two form gametes and the others degenerate. The gametes move out of the parent cells and gametic union takes place in the mucilaginous envelope (Fig. 8-6 A–G). If each cell of the copulating pair gives rise to one gamete a single zygote results, when two gametes are produced from each partner then two zygotes are formed. Sometimes, physiological anisogamy occurs; in this case one gamete is mobile and the other stationary, one zygote being formed within each member of the copulating pair.

In Centrales, the cell functioning as female slightly extends with its

elongated nucleus to become an oogonium. Two meiotic divisions of the oogonial nucleus take place and at each division one of the daughter nuclei degenerates so that the protoplast contains a single haploid nucleus which

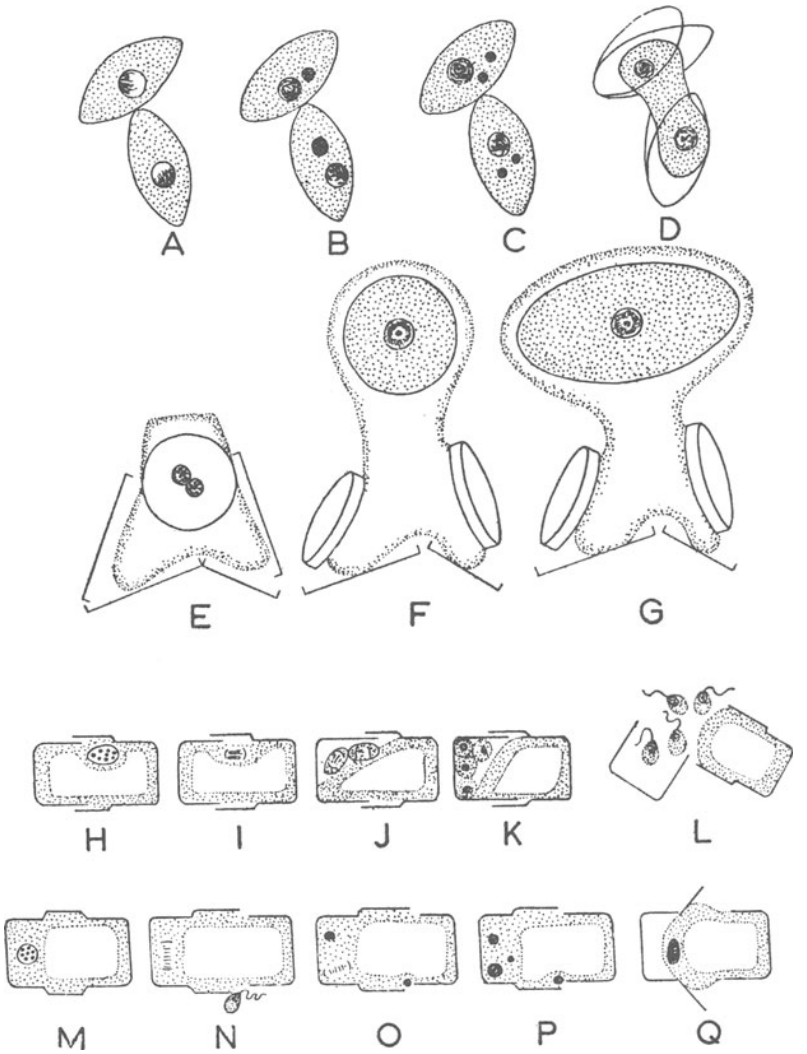


Fig. 8-6. A–G, *Cocconeis*, stages in auxospore formation. A–C, meiosis and degeneration of all nuclei except one in each frustule; D, fusion of gametes; E, zygote; F–G, development of auxospore from zygote. H–Q, *Melosira*, auxospore formation in a centric diatom, H–L, formation of antherozoids; M–Q, stages in fertilization and formation of auxospore. (After Smith, 1955.)

ultimately becomes the egg nucleus. The cell acting as spermatogonium divides meiotically to produce four uniflagellate spermatozoids. In *Cyclotella tenuistriata*, a vegetative cell functions directly as a spermatogonium

but in *Melosira varians* a vegetative cell divides mitotically to produce 4–8 spermatogonia (Fig. 8-6 H–L).

At the time of fertilization, spermatozoids gather around an oogonium and one of them fuses with the egg. The zygote escapes out of the female cell, enlarges to a characteristic size and becomes an auxospore which secretes a new wall and is now a diploid vegetative cell (Fig. 8-6 M–Q).

Schultz and Trainor (1968) have recorded uniflagellate and biflagellate spermatozoids, and auxospores in cultures of *Cyclotella meneghiniana* and *C. cryptica* and found that spermatogenesis and auxospore formation are stimulated with increasing concentrations of sodium. Both light and temperature have been reported to regulate sexuality in some diatoms.

PHYLOGENY

Because of the siliceous nature of the diatom cell walls, well preserved fossils of Bacillariophyta are available. The Centrales have been reported from the Jurassic and the Pennales from early Tertiary. The fossil evidence, therefore, suggests that the Centrales are more primitive from which the Pennales might have originated. The fact that most of the centric diatoms are marine planktonic forms in contrast to the Pennales which are predominantly freshwater, also indicates a centric ancestry for the Pennales.

The presence of fucoxanthin and chlorophyll-a and chlorophyll-c links the Bacillariophyta with Phaeophyta, and the characteristic food reserves relate the diatoms to Xanthophyta and Chrysophyta.

TEST QUESTIONS

1. What will happen when you attempt to grow diatoms in a nutrient medium with or without silica ?
2. How can you establish that silicon uptake in diatoms is an energy requiring process ?
3. What reasons can you offer to account for the progressive reduction in the size of vegetatively reproducing diatoms ?
4. Why do some algologists assign diatoms the rank of a Phylum whereas others group them as one Class in the Phylum Chrysophyta ?
5. Out of the Pennales and Centrales, which one do you consider more primitive and why ?

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9

Phaeophyta

The Phaeophyta (brown algae) are characterized by five chief distinguishing features: (1) the photosynthetic pigments include chlorophyll-a and chlorophyll-c, β carotene, fucoxanthin, violaxanthin, diatoxanthin and other xanthophylls, and in general there is an excess of carotenoid over chlorophyll pigments; fucoxanthin is present in sufficient quantity to mask the green colour of chlorophylls and to impart its own brown colour to these algae; (2) the excess photosynthate is commonly stored in the form of **laminarin** and mannitol, rarely as fat droplets; (3) certain whitish granules, called **fucosan vesicles,** are usually present in the cell; (4) the cell wall is composed of cellulose, fucinic acid and alginic acid; and (5) the flagellated structures have a pair of laterally inserted unequal flagella, of which the larger one is anterior and pantonematic whereas the smaller one is posterior and acronematic.

OCCURRENCE

With a few exceptions, e.g., *Pleurocladia, Heribaudiella, Bodanella* and a few species of *Ectocarpus* which are freshwater forms, all other brown algae are marine. They generally inhabit the colder waters but members of the Encoeliaceae (Ectocarpales), Dictyotales and *Sargassum* are markedly warm-water forms.

Depending on the topographical features of the land-sea boundary, the littoral region is divided into three major zones: sublittoral—always submerged with an approximate maximum depth of 100 metres; intertidal—submerged during high tide and exposed during low tide; and supralittoral —not very well-defined, and beyond which the highest tides or tide sprays rarely reach. According to the nature of the shore, tides and algal associations, the intertidal belt is further divided into three minor zones: the upper, middle and lower.

The sublittoral zone includes thick forests of such algae as *Laminaria,* with subflora of *Dictyota, Cutleria, Alaria, Himanthalia* and *Desmarestia.* In the intertidal belt the rockweeds (Fucaceae) grow in the upper zone, *Colpomenia, Iyengaria, Sphacelaria, Leathesia* and others in the middle zone and most of the Laminariales in the lower zone. Species of *Ectocarpus* form the subflora of the supralittoral zone.

RANGE OF THALLUS ORGANIZATION

The thalli range in structure from heterotrichous filamentous types through

pseudoparenchymatous uniaxial forms, to true parenchymatous forms. Neither unicellular nor simple filamentous brown algae are known and the simplest thallus organization in this Phylum is the branched, heterotrichous filament.

The vegetative body of the Laminariales and Fucales shows well-marked morphological and anatomical differences. In *Laminaria* the thallus is morphologically distinguishable into holdfast, stipe and blade. A transverse section of the stipe or blade shows the distinction of vegetative tissue into three regions: an outer epidermis, a middle zone of several-layered cortex, and multicellular central medulla. Some cells of the medulla produce vertically elongated 'hyphae', known as **trumpet hyphae,** that lack chloroplasts and discharge the function of water and nutrient conduction. *Nereocystis* and *Pelagophycus* (Laminariales) have certain cells that are similar to the sieve tubes of higher plants but are devoid of companion cells (Parker and Huber, 1965).

The vegetative thallus usually grows by intercalary or apical meristems. Trichothallic growth is characteristic of most Ectocarpales, Desmarestiales, Cutleriales and Sporochnales. Intercalary growth characterizes the Laminariales in which the meristem is located at the junction of the stipe and the blade. The Sphacelariales, Dictyotales and Fucales show apical growth.

CELL STRUCTURE

The cell wall is made up of an outer mucilaginous layer containing alginic and fucinic acids, and an inner cellulosic layer.

The cytoplasm generally contains many small vacuoles and a few refractile bodies of an unknown nature termed as the fucosan vesicles which are usually present at the site of high metabolic activity. The chromatophores are mostly parietal, frequently with more than one chromatophore per cell except in *Pylaiella fulvescens* which contains axile stellate chromatophores. The Ectocarpales show wide variation in the shape of their chromatophores which may be plate-like, ribbon-shaped or discoid. Projecting pyrenoids lacking photosynthetic thylakoids are present in some members of the Ectocarpales, Sphacelariales and Dictyosiphonales. The chromatophore lamellations in the Phaeophyta result from a rather close aggregation of generally three, rarely four, thylakoids that do not cohere into stacks or bands but run parallel to each other (Evans, 1966). In the biflagellate cells, the two basal granules, each with one flagellum, are laterally connected and in addition the anterior flagellum and the nucleus are also joined together through a fibrous structure comparable to a rhizoplast. *Dictyota* has monoflagellate sperms with two basal granules of which the posterior one does not give rise to a flagellum (Manton, 1965). The phaeophycean swarmers are commonly equipped with an eye spot and a chromatophore devoid of pyrenoids.

The cells are uninucleate, with one or two nucleoli. Some members, e.g., *Fucus*, have in their resting nucleus many Feulgen-positive bodies called

chromocentres which arrange themselves linearly along the chromosome during late prophase but their identity is lost with the progressive contraction of chromosomes during metaphase; this state is maintained until the next interphase and prophase. The chromocentres are characteristic of only the Phaeophyta, but nothing is known of their function. Another feature is the presence of centrosomes at the poles of a dividing nucleus. The chromosome organization has advanced to such an extent that members of the Laminariales possess well differentiated *X* and *Y* sex chromosomes. Other subcellular organelles such as mitochondria, Golgi bodies, and endoplasmic reticulum are also present. Synaptonemal complexes have recently been reported in the meiotic nuclei of a few brown algae.

REPRODUCTION

VEGETATIVE

The commonest method is by fragmentation of a thallus into two or more parts, each of which regenerates into a mature new plant. In the Sphacelariales special reproductive branches known as propagules are formed which after detachment from the parent, give rise to new plants.

ASEXUAL

Except Tilopteridales, Dictyotales and Fucales, in all Phaeophyta zoospores are produced in well-defined sporangia borne on the sporophyte. The Ectocarpales and Sphacelariales produce unichambered or unilocular as well as multichambered or plurilocular sporangia. During the formation of zoospores, the diploid nucleus of the unilocular sporangium divides by meiosis, followed by a series of mitotic divisions of the resulting four haploid nuclei. Finally the cytoplasm becomes segmented into a number of uninucleate portions each of which subsequently acquires a pair of laterally inserted flagella. The zoospores produced from unilocular sporangia are haploid and on germination give rise to haploid, gametophytic plants.

In plurilocular sporangia, the nuclei never divide meiotically and therefore the zoospores are always diploid and serve as an accessory means of perpetuation of the sporophyte or the gametophyte. Other zoosporic Phaeophyta bear only unilocular sporangia. In Dictyotales the sporophyte forms tetrasporangia, and reduction division of the sporangial nucleus in these results in the formation of four haploid, uninucleate aplanospores or tetraspores. The Tilopteridales form single, quadrinucleate aplanospores, termed monospores, in each sporangium. Both tetraspores and monospores give rise to gametophytic plants. The Fucales lack asexual reproduction.

SEXUAL

Gametophytes bear only multichambered organs, the plurilocular game-

tangia, which form gametes that resemble zoospores in morphology. The Ectocarpales and Sphacelariales are mostly isogamous but the Punctariaceae, Cutleriales and possibly Tilopteridales are distinctly anisogamous. Several brown algae, e.g., the Fucales, Laminariales and Dictyotales, are oogamous. In Dictyotales, the antheridia and oogonia occur in sori but in Fucales they are produced in cavity-like depressions (**conceptacles**) on a fertile blade (**receptacle**).

A marked lunar periodicity in sexual reproduction has been observed in certain marine brown algae. The ova are released around the first quarter of the moon but there is no such rhythmicity for sporogenesis.

LIFE CYCLE AND ALTERNATION OF GENERATIONS

Three chief types of life cycle—isomorphic, heteromorphic and diplontic—are found in brown algae.

The Ectocarpales, Cutleriales, Tilopteridales, Sphacelariales and Dictyotales show an isomorphic life cycle in which both the sporophytic and gametophytic generations are morphologically similar. The Chordariales, Punctariales and Dictyosiphonales, included in the Order Ectocarpales by Fritsch (1945), as well as the Sporochnales, Desmarestiales and Laminariales, all exhibit heteromorphic alternation between a microscopic filamentous gametophyte and an elaborate, often macroscopic, sporophyte. In Chordariales the zygote germinates into a filamentous structure, designated as protonema if it generates the sporophyte as a lateral outgrowth, and as plethysmothallus if it forms zoospores in plurilocular sporangia for its own propagation. In the Fucales the plants are diploid and lack an alternation of generations. The only haploid stage in the life cycle is confined to the gametes. There is no independent, free living multicellular gametophyte in the Fucales.

CLASSIFICATION

Phaeophyta includes the single Class Phaeophyceae. On the basis of vegetative organization and sexual reproduction, Fritsch (1945) divided the Phaeophyceae into nine orders: Ectocarpales; Tilopteridales; Cutleriales; Sporochnales; Desmarestiales; Laminariales; Sphacelariales; Dictyotales; and Fucales.

Most other phycologists emphasize the three types of life cycle, and divide the Phaeophyta into three classes (or subclasses within the Class Phaeophyceae): Isogeneratae (including isomorphic forms); Heterogeneratae (comprising heteromorphic forms); and Cyclosporeae (constituting diploid forms). Within the Isogeneratae and Heterogeneratae, genera have been placed in different families, and families in different orders on the basis of differences in the nature of zoospores, sexuality, organization and growth of thallus.

PHYLOGENY

The Class Phaeophyceae comprises a group of multicellular algae some of which show a complex morphological and anatomical differentiation not seen in other algal phyla. The heterotrichous, uniaxial and multiaxial types of thalli met with in the Chlorophyta, Phaeophyta and Rhodophyta probably represent the parallel course of evolution that has taken place independently in the three phyla. The Phaeophyceae resemble Bacillariophyceae, Cryptophyceae and Pyrrophyta in having chlorophyll-c; and Chrysophyceae and Bacillariophyceae in having xanthophylls, fucoxanthin and neofucoxanthin. The three phyla Phaeophyta, Pyrrophyta and Chrysophyta (Class Bacillariophyceae in particular) probably belong to the same evolutionary line, showing predominance of brownish carotenoids over the yellow, and of carotenoid pigments over chlorophylls.

Oil and saturated fats are common food reserves in Xanthophyceae, Chrysophyceae, Bacillariophyceae and Phaeophyceae. In addition, laminarin is a distinctive reserve product of the Phaeophyta, and is chemically similar to leucosin. The Bacillariophyceae, however, differ from Phaeophyceae in having complex cellular morphology, with a two-piece silicified wall.

Both Xanthophyceae and Chrysophyceae resemble Phaeophyceae in their flagellar morphology but the presence of two-piece silicified wall and chlorophyll-e in certain members of the Xanthophyceae are two features unknown in Phaeophyceae.

Evolution is considered to have progressed along two different lines within the Phaeophyceae: one leads to isomorphic alternation of generations accompanied by advance of sexuality from isogamy to oogamy and, in vegetative organization, from simple to complex thalli; the other with heteromorphic alternation of generations shows a similar progression in reproduction and vegetative organization. The diploid Fucales are assumed to represent the evolutionary climax in the heteromorphic series, with the gametophyte reduced to the single-celled stage, i.e., the gamete.

Order: ECTOCARPALES

It has been differently delimited by various phycologists. Fritsch (1945) included in this order filamentous, pseudoparenchymatous and parenchymatous types derived from a heterotrichous filament. Emphasis on the heterotrichous character led to the inclusion of forms exhibiting isomorphic as well as heteromorphic alternation of generations in the order. Most other phycologists have, however, considered the life cycle of greater importance than the heterotrichous habit and have consequently raised those families in the Order Ectocarpales *sensu* Fritsch that exhibit heteromorphic alternation, to the rank of orders. This has led to the restriction of the Ectocarpales to the single Family Ectocarpaceae characterized by simple, heterotrichous members exhibiting isogamous sexuality and isomorphic alternation of generations.

In general, the prostrate system of Ectocarpaceae grows by an apical meristem but the development of the erect system is highly variable—*Ectocarpus siliculosus* shows diffuse growth, *E. lucifugus* apical growth, and *E. paradoxus* trichothallic growth. *Pylaiella*, an alga related to *Ectocarpus*, frequently shows longitudinal septation in its filaments as a result of which the thallus tends to become parenchymatous. *Streblonema* lacks an erect system.

Family: Ectocarpaceae
Genus: *Ectocarpus*

Occurrence

Worldwide in distribution, *Ectocarpus* grows in the littoral and supralittoral regions. In India it is mostly found in the supralittoral zone along the West and East Coasts.

Morphology

The thallus is made up of uniseriate filaments and is generally differentiated into a much branched erect system (Figs. 9-1, 9-2 A) and a prostrate system that is sparsely or profusely branched. Each branch generally arises from just below a septum. The main branches and their branchlets most often terminate in hairs consisting of numerous elongated, tapering, hyaline and much vacuolated cells. The plants are attached to the substratum by branched rhizoids produced from lower cells of lower branches and in some cases these descending rhizoids form a corticating layer around the lower cells of the main axis.

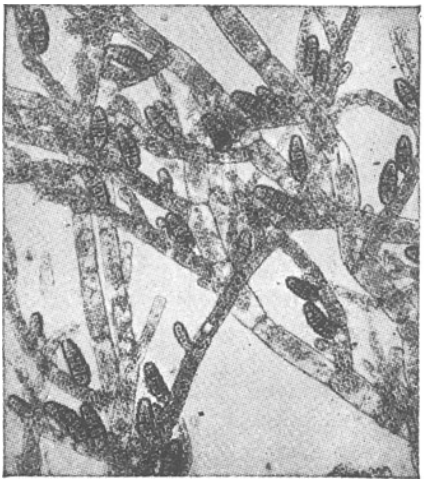

Fig. 9-1. *Ectocarpus*, photomicrograph showing habit and plurilocular sporangia.

The cells are uninucleate and have an irregular band-shaped, or many discoid chromatophores with smooth margin (Fig. 9-2 B). The chromatophore contains a projecting pyrenoid from which a new pyrenoid may develop by budding (Evans, 1966).

The growth and nutrition of *Ectocarpus confervoides* have been studied in unialgal, bacteria-free cultures (Boalch, 1961). The alga, which is obligately photoautotrophic, remains viable for over a year in light, but for only about three months in dark. Potassium nitrate, potassium phosphate, manganese chloride and a mixture of organic substances stimulate its growth in natural seawater medium in light.

Reproduction

Asexual. The sporophyte produces unilocular as well as plurilocular sporangia (Figs. 9-1, 9-3 A) terminally and singly on small branchlets. During the development of a unilocular sporangium, the terminal cell of a branchlet enlarges considerably, assumes a globose or ellipsoid shape (Fig. 9-3 B) with numerous chromatophores. Meanwhile the diploid nucleus of the unilocular sporangium undergoes a meiotic division and then many

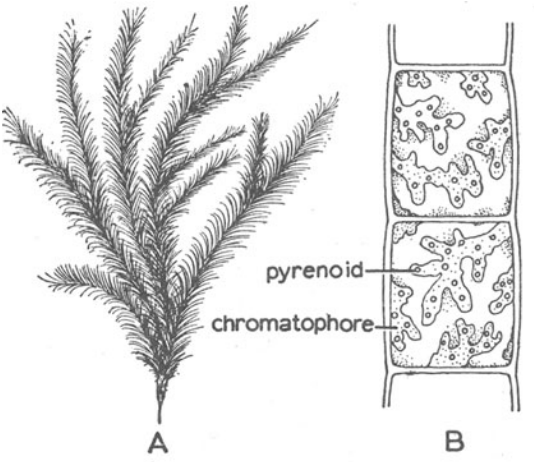

Fig. 9-2. A, *Ectocarpus* showing tufted habit; B, *Ectocarpus siliculosus*, cell structure.

mitotic divisions until 32–64 haploid nuclei are formed. After nuclear divisions, the sporangial protoplast divides into a number of uninucleate segments each with a single chromatophore corresponding to the number of haploid nuclei. Each segment is finally transformed into a haploid, biflagellate, pyriform zoospore in which the anterior longer flagellum is pantonematic and the posterior shorter flagellum is acronematic. After the protoplast of a sporangium has been used up in the formation of zoospores, a second sporangium may sometime regenerate from within the wall of the first sporangium.

The plurilocular sporangium (Fig. 9-3 C) also develops from a terminal cell which enlarges and accumulates numerous chromatophores. Subsequently it undergoes a series of transverse and vertical mitotic divisions producing several hundred small cubical cells arranged in definite tiers. Each cubical cell is diploid, uninucleate and forms a single biflagellate zoospore identical to the zoospores produced from a unilocular sporangium except that it is diploid. The haploid zoospores from unilocular sporangia are liberated *en masse* through a terminal pore but those from plurilocular sporangia generally come out one by one, rather in an orderly fashion,

through a terminal (Fig. 9-4) or lateral pore. After moving freely for some time the zoospores settle on some solid substratum and with their anterior end still attached to some object, grow into new plants. The haploid zoospores from unilocular sporangia form gametophytes or sexual plants whereas the diploid zoospores from a plurilocular sporangium produce sporophytes or asexual plants.

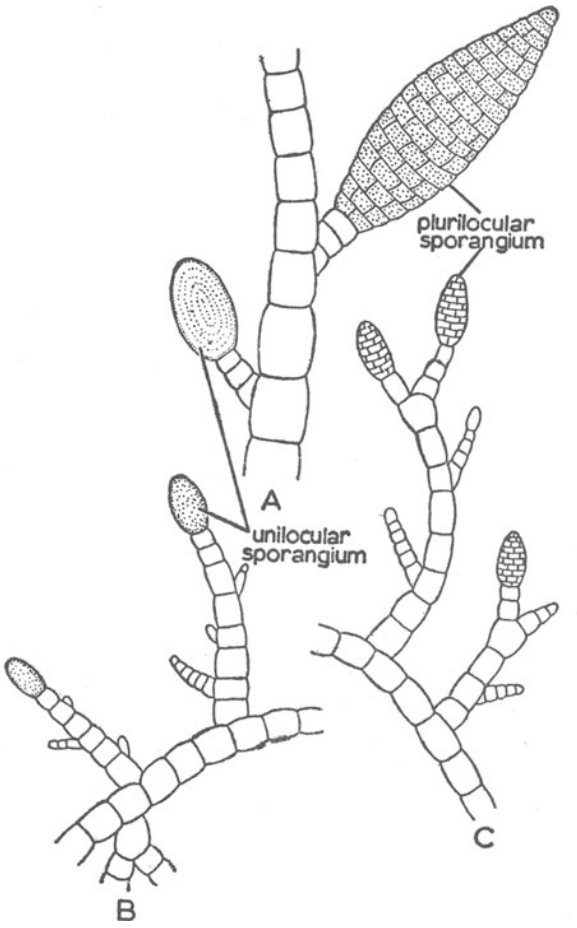

Fig. 9-3. *Ectocarpus.* A, *E. siliculosus* with unilocular and plurilocular sporangia; B, *E. landsburgii* with unilocular sporangia; C, *E. fasciculatus* with plurilocular sporangia.

Sexual. The plurilocular gametangia arise in a manner similar to plurilocular sporangia of the diploid sporophyte. In *E. siliculosus*, which is heterothallic, the loculi of all gametangia are of equal size. Consequently, the zoogametes produced singly from each locule are also of equal size and are morphologically identical with the zoospores. In such situations the sexuality is isogamous though sometimes it may be accompanied by physiological anisogamy since the male gamete may be more active and

motile than the passive and sluggishly motile female gamete. *E. secundus* forms two kinds of gametangia and gametes, the megagametangium with larger loculi and larger gametes, and the microgametangium with smaller

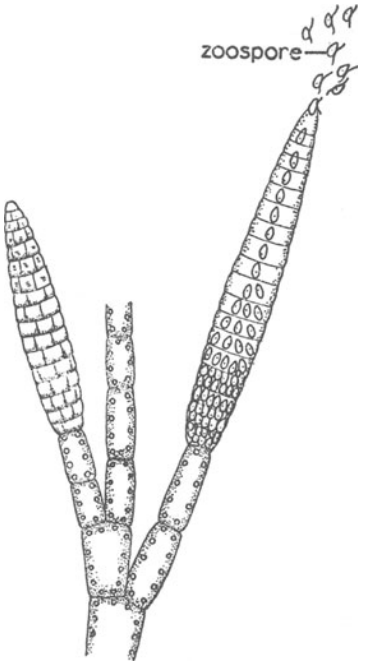

Fig. 9-4. *Ectocarpus* showing liberation of zoospores.

loculi and smaller gametes. Thus, *E. secundus* may be morphologically anisogamous (Fig. 9-5 A–C).

In *E. siliculosus*, several male gametes cluster around a single female gamete with their longer flagella attached to its surface (Fig. 9-5 D). The female gametes are known to produce a volatile sexual attractant (*cis*-1-[cycloheptadiene-2′, 5′-yl]-butene) which causes clumping of the spermatozoids (Muller *et al.*, 1971). This is believed to be the first authentic report of chemical characterization of a sex hormone in plants. Such a sexual union between zoogametes is unlike that observed in *Chlamydomonas* where several male and female gametes cluster together through agglutination of their flagellar ends. Ultimately one of the male gametes fuses with the female (Fig. 9-5 E–G). The zygote (Fig. 9-5 H) always germinates directly into a new plant and during its germination no reduction division occurs. The resulting plant is therefore diploid and represents the asexual sporophyte. If a gamete fails to unite with another gamete, it may germinate directly into a new plant.

Alternation of Generations

Sexual plants of *Ectocarpus* are dioecious and form only plurilocular gametangia. The zygotes upon germination do not divide meiotically. Hence the

plants produced from germinating zygotes are all diploid, and bear both unilocular and plurilocular sporangia which always form diploid zoospores. Since meiosis does not occur during their formation, they invariably produce

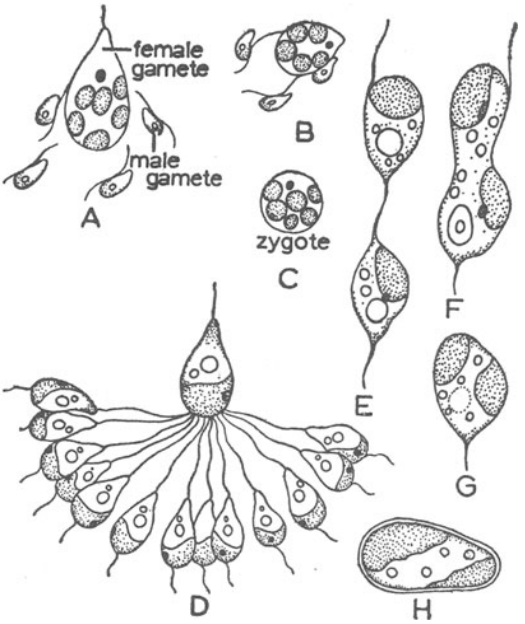

Fig. 9-5. *Ectocarpus*, sexual reproduction. A–C, *E. secundus*, stages in heterogamy; D, *E. siliculosus*, clump formation between one female gamete and many male gametes E–G, stages in fusion of male and female gametes; H, zygote. (After Fritsch, 1945.)

diploid sporophytic plants and serve as an accessory means of asexual reproduction. But during the formation of zoospores in unilocular sporangia, the first nuclear division is always meiotic and therefore such zoospores are haploid and germinate into haploid gametophytic plants.

The gametophytic and sporophytic generations of *Ectocarpus* are morphologically alike and indistinguishable and, therefore, its life cycle is isomorphic. However, recent researches have indicated that the life history may be much more flexible and variable than hitherto assumed. Muller's (1967) work on *E. siliculosus* has established that its life cycle is somewhat heteromorphic-diplohaplontic rather than strictly isomorphic. Germination of unfused gametes gives rise to haploid sporophytes which bear both unilocular and plurilocular sporangia. Similarly, diploid gametophytes and tetraploid sporophytes may also arise under certain conditions.

Order: FUCALES

The Fucales are characterized by the absence of alternation between multicellular sporophytic and free living multicellular gametophytic generations in the life cycle. The plants are all sporophytic or diploid. The gametophytic or haploid phase is restricted to the unicellular sperms and eggs produced within unicelled reproductive organs termed antheridia and oogonia.

The number of eggs produced inside an oogonium ranges from one, two, four to eight in various genera, but that of antherozoids within an antheridium is constant, being nearly 64 for all genera. The antherozoids of the Fucales have a shorter anterior and a longer posterior flagellum whereas in those of the other brown algae the arrangement is just the reverse. The sexual reproduction is oogamous and at the time of fertilization eggs and antherozoids are both extruded from the fertile cavities or the conceptacles.

Family: Fucaceae
Genus: *Fucus*

Occurrence

This alga grows attached to rocky substrata in the intertidal belt of the littoral zone. Species of *Fucus* are predominantly cold water forms and grow luxuriantly in the North Atlantic and Pacific shores.

Morphology

The plants, commonly less than 0.5 metre in length (the length depends on the degree of exposure), have complex vegetative organization. Externally

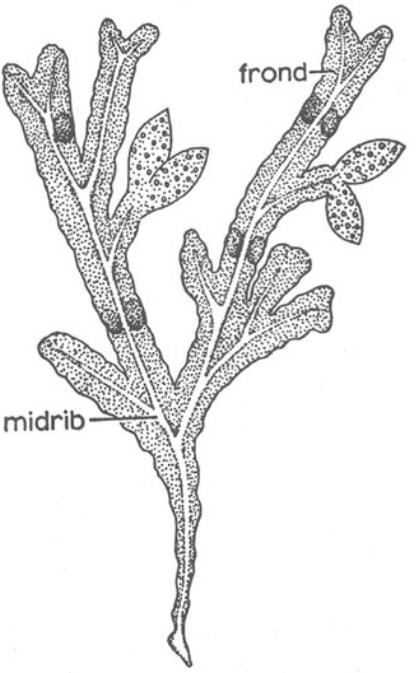

Fig. 9-6. *Fucus vesiculosus*, thallus morphology.

each plant is differentiated into a basal discoid holdfast that anchors it to the substratum, a short stipe and a flattened, dichotomously branched frond or blade (Fig. 9-6). The thallus is generally dark brown, and slimy

to the touch. The stipe is continued in the form of midrib in the frond of many species, though it never reaches the frond apex. *F. vesiculosus* contains a number of air bladders in pairs and these give buoyancy to the submerged plants. The mature thallus is in some respects similar to animal cartilage, the cells being embedded in a biphasic matrix composed of rigid fibres of alginic acid and cellulose.

The swollen tips of fertile branches harbour a large number of small, somewhat raised, flask-shaped cavities, the conceptacles (Fig. 9-7 A) which contain the antheridia and oogonia and open to the exterior through an **ostiole.** Lateral to the midrib are formed flat, wing-like expansions of the thallus with sterile conceptacles called **cryptoblasts** or **cryptostomata.**

Internally, the thallus is differentiated into three distinct regions—an outer single-layered epidermis called meristoderm, a central many-layered cortex, and an inner several-layered medulla. The cells of the medullary region are loosely arranged, some of them forming an anastomosing network of hyphae. These first-formed hyphae are smooth-walled and are called primary hyphae which may develop secondary thickening at maturity. The region of the cortex close to medulla has much elongated mucilaginous cells but the outer cortical cells and those of the epidermis are provided with chromatophores.

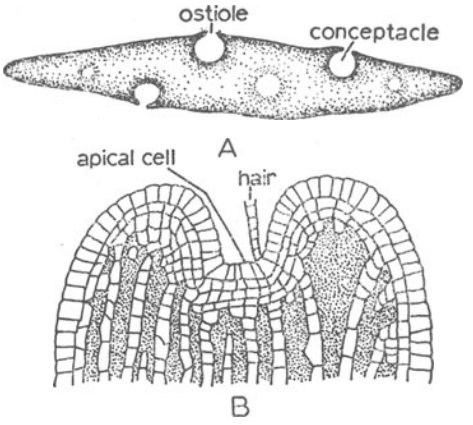

Fig. 9-7. A, *Fucus serratus*, transverse section through male receptacle; B, *F. vesiculosus*, vertical section through the apex.

The growth is initiated by an apical cell (Fig. 9-7 B) situated in the hollow depression at the branch apex. The apical meristem is like a truncated pyramid with three cutting faces—two lateral and one basal. The lateral segments derived from the meristem initially divide periclinally and of the resulting two cells, the outer one, by repeated divisions in various planes, organizes the epidermis and the cortex whereas the inner one, in association with the basal segment cut off by the apical meristem, forms the medulla. The epidermal layer as well as the inner cells of cortex retain meristematic potentiality, the former by cell divisions in vertical plane in-

creases the girth and the latter gives rise to secondary hyphae. Thus, in addition to primary growth, the alga also exhibits secondary growth.

In respect of functional differentiation, the epidermis and chromatophore containing cortical region are photosynthetic, the inner cortical region stores food reserves and the medullary cells are conductive in nature. The primary and secondary hyphae serve a mechanical function.

Reproduction

Vegetative. A thallus may fragment or dissociate into many parts, each of which grows into a new plant.

Sexual. Plants are monoecious (*F. spiralis*) or dioecious (*F. vesiculosus*). They may bear both antheridia and oogonia in the same or in different conceptacles on the same plant in monoecious species, and in separate conceptacles on different plants in the dioecious species.

A conceptacle develops from a superficial cell whose rate of growth and cell division slow down in comparison to those of neighbouring cells. Con-

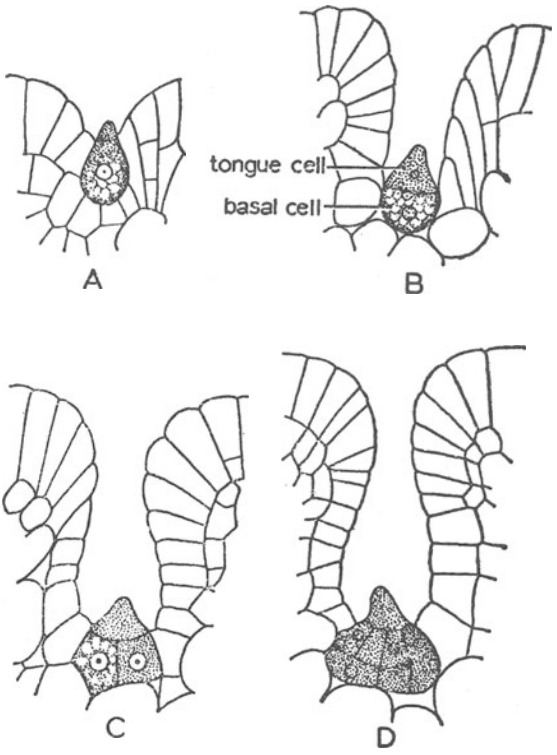

Fig. 9-8. A–D. *Fucus serratus*, early stages in the development of conceptacle.

sequently this apical cell comes to lie at the base of a flask-shaped cavity (Fig. 9-8 A). This development is followed by transverse division of the apical initial into two cells, a lower basal cell and an upper tongue cell

(Fig. 9-8 B). The latter may develop into a hair or degenerate. The basal cell, by a series of vertical divisions, gives rise to a layer of chromatophore-containing cells that line the floor of the cavity. These cells undergo one or two transverse divisions and form 2–3 layers (Fig. 9-8 C, D) of which the superficial layer is always fertile giving rise to antheridia or oogonia, or both.

Superficial cells which start functioning as oogonial mother cells divide into a lower stalk cell and an upper oogonial cell (Fig. 9-9 A). The first

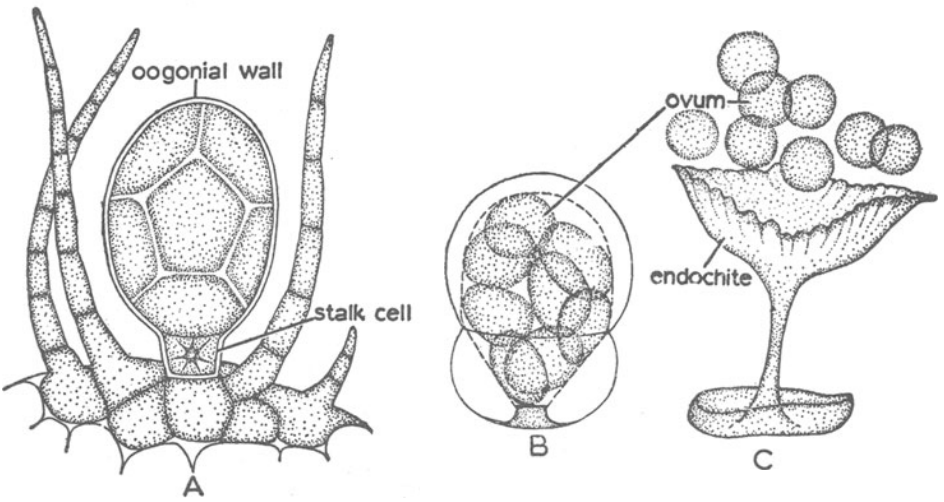

Fig. 9-9. *Fucus vesiculosus.* A, oogonium; B–C, formation and liberation of ova.

two divisions of the oogonial nucleus are always meiotic, invariably followed by a mitotic division of all the four haploid nuclei. The 8-nucleate oogonial protoplast cleaves into 8 uninucleate eggs (Fig. 9-9 B, C) enclosed inside a three-layered oogonial wall. The remaining cells of the superficial layer which fail to act as oogonial mother cells develop into multicellular hairs. Some of the peripheral cells near the ostiole also form unbranched hair-like appendages, which come out of the ostiole in the form of a tuft.

The antheridia are formed on lower branches (Figs. 9-10, 9-11 A) of the hairs produced from superficial cells lining the cavity. Like oogonial nucleus, the antheridial nucleus also divides meiotically, followed by four consecutive mitotic divisions of the four haploid nuclei. Then after the completion of nuclear division the antheridial protoplast fragments into 64 uninucleate, phototactic antherozoids bearing two lateral flagella of unequal length and containing a large nucleus that occupies most of the cell cytoplasm thus restricting the vestigial chromatophore, the well-developed eye spot and other cellular organelles to a small area within the sperm (Fig. 9-11 B). The antheridial wall is only two-layered.

During antherozoid development the eye spot originates *de novo*, as the vegetative cells do not have any eye spots. In a detailed study of the eye spot of *F. vesiculosus*, Bouck (1970) observed the following stages of develop-

ment: (1) appearance of electron-opaque granules along the chromatophore; (2) increase in size of the granules; and (3) shifting of chromatophore, along

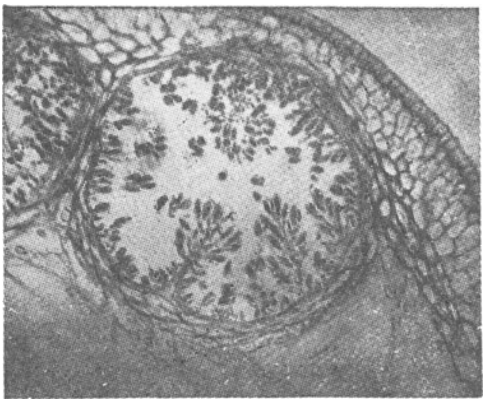

Fig. 9-10. *Fucus*, photomicrograph of transverse section through a male conceptacle showing antheridia.

with the eye spot, from centre of the cell towards its periphery, followed by fusion with the plasma membrane. Four distinct microtubules and a few fibrils arise near the base of the posterior flagellum between the chromatophore and the plasma membrane. It is believed that the granules of the eye spot arise from these microtubules and fibrils. Bouck (1970) considers that light is reduced in intensity by a shading device (the eye spot) and brought into focus by a focussing device (the flagellar base), and the photoreceptor is probably sandwiched between these two structures, and bound to the plasma membrane.

The internal structure of the sterile conceptacles (cryptoblasts) is similar to that of the fertile conceptacles except that they do not bear sex organs. The hairs show either basal or diffuse growth, and are unbranched. The cavity contains some mucilage.

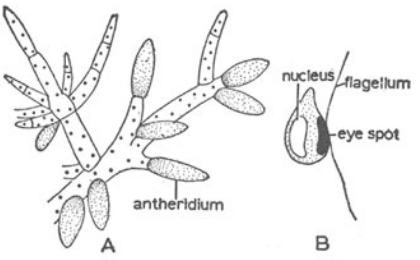

Fig. 9-11. *Fucus*. A, branched filament bearing antheridia; B, structure of antherozoid.

The wall layers of antheridia and oogonia are hydrophilic. They imbibe water at the time of liberation of antherozoids or eggs as a result of which

the outer wall layer swells up and bursts pushing out the antherozoids or the eggs through the hairs and ostiole to the exterior. The remaining wall layers also become dissolved in the surrounding water and liberate the antherozoids or eggs. In cultures, the clustering of antherozoids around a single egg has been seen (Fig. 9-12 A) and it results from a chemical stimulus provided by a hydrocarbon (probably n-hexane) present in the eggs of *Fucus*. Ultimately, one of the antherozoids finds its way into the egg, leading to the formation of the zygote.

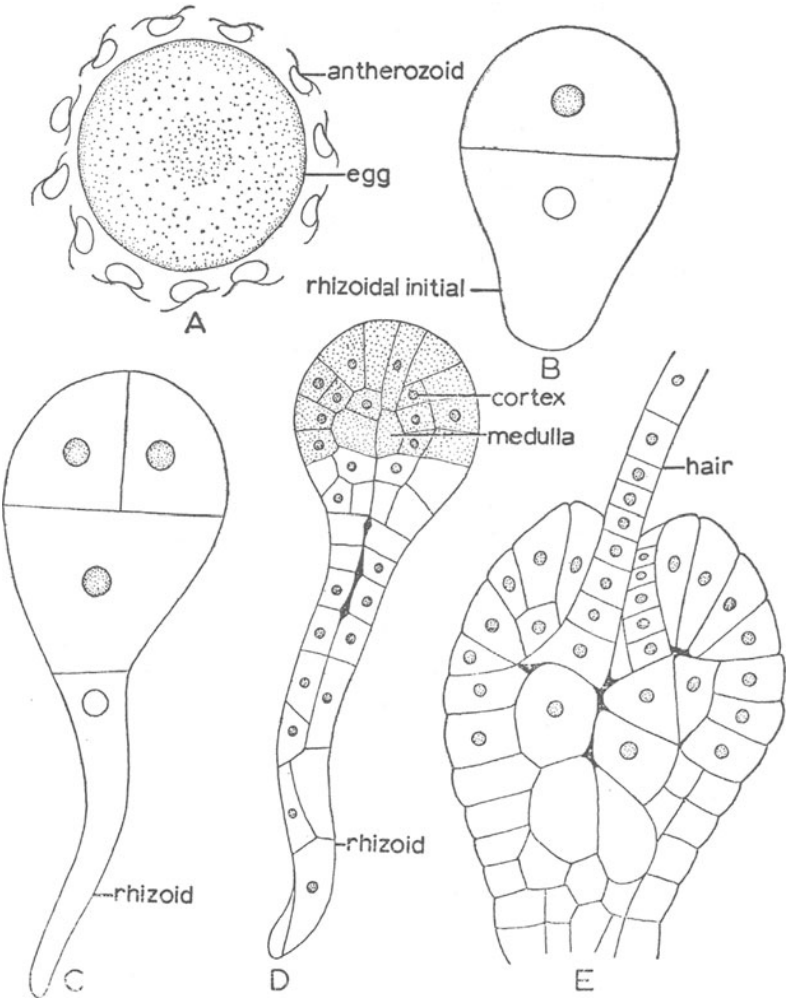

Fig. 9-12. *Fucus*, stages in thallus development. A–C, *F. furcatus* (after Smith, 1955); A, fertilization of egg; B, formation of rhizoidal initial; C, transverse section through two-day-old embryo; D–E, subsequent stages in embryo development.

The *Fucus* egg can show parthenogenetic development and in the laboratory parthenogenesis is induced by treating the eggs with dilute solu-

tions of acetic or butyric acid. During zygote germination (Fig. 9-12 B–E) or parthenogenetic development of the egg, the point at which the primary rhizoid develops is away from light indicating the existence of a light-controlled mechanism which regulates the development of a polarity gradient. Apart from visible light, ultraviolet light, hydrogen-ion concentration, temperature and auxin gradients, electrical field, mechanical deformation and the point of entry of the spermatozoid, all seem to control the development of polarity leading to the formation of rhizoids. A CO_2-pH gradient seems to control the auxin concentration and the region rich in auxin activity differentiates the rhizoid.

After the point of rhizoid formation has been determined, the zygote divides mitotically in a plane transverse to the axis defined by the rhizoid (Fig. 9-12 B). The upper cell of the two-celled embryo divides vertically whereas the lower divides transversely, thus forming a 4-celled embryo (Fig. 9-12 C). The upper two cells, together with the median cell, divide and redivide transversely and periclinally giving rise to a tissue differentiated into medulla and cortex (Fig. 9-12 D). The lowermost cell of the quadrant from which the primary rhizoid originated also forms secondary rhizoids. With the growth of the embryo, there differentiates an apical cell in a small depression which forms a filamentous hair (Fig. 9-12 E). Subsequently, the cells adjacent to the hair also grow into hairs and form a tuft on the upper surface. This development is followed by degeneration of all the cells of the hair except the basal cell of the first-formed hair. This basal cell finally functions as an apical initial of the *Fucus* thallus.

Family: Sargassaceae
Genus: *Sargassum*

Sargassum, like *Fucus*, is diploid and lacks an alternation of generations. The two genera mainly differ in their external morphology as well as in the number of eggs produced in an oogonium (eight in *Fucus* but one in *Sargassum*).

Occurrence

The alga, popularly called gulfweed, grows abundantly in tropical oceans in the Southern hemisphere. It may be free-floating or attached to rocks. The chief centres of its growth are the Australian and Caribbean coasts, Gulf of Mexico and the Sargasso Sea of south central Atlantic. It is also abundant on the West and East Coasts of India.

Morphology

Thalli of *Sargassum* are monopodially branched and the members of Sargassaceae are chiefly identified by the peculiar features of their lateral branch systems (Fig. 9-13 A, B). The plant body is usually differentiated into a holdfast, a short stipe and much branched long laterals which give rise at regular intervals to leafy laterals bearing cryptoblasts. From near the axil

of the leafy laterals, called primary branch, comes out a second order of branches, the first formed one or two branches of which transform into air bladders and the remaining branches serve as receptacles (Fig. 9-13 C, D) bearing both conceptacles and cryptoblasts. The 'leaves' have smooth or serrate margins and are often provided with a prominent midrib.

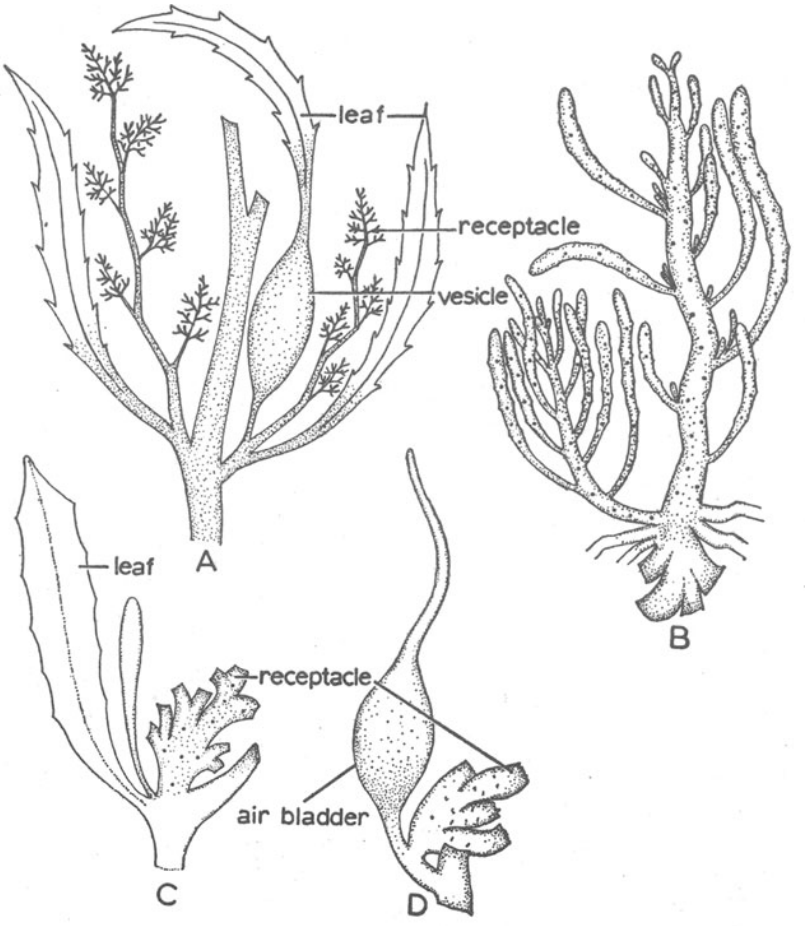

Fig. 9-13. A, *Sargassum longifolium*, part of fertile plant; B, *Sargassum swartzii*, part of plant; C–D, *S. swartzii*, portions with receptacle. (Figs. B–D after Chauhan and Thivy, 1964.)

A transverse section through a branch, leaf or stipe shows well-marked differentiation into three regions, the meristoderm, cortex and medulla. In some species, which are free-floating, the medulla may be almost absent. Unlike *Fucus*, the cortex of *Sargassum* neither contains mucilaginous cells nor hyphae. The air bladder consists of an epidermis, cortex and a central hollow cavity filled with air.

As in *Fucus*, growth and organization of the thallus is achieved through the activity of an apical cell with three cutting faces.

Reproduction

Vegetative. *S. natans* and *S. hystrix*, which grow free-floating in the Sargasso Sea, are known to multiply exclusively by vegetative means. It is interesting that free-floating algae of salt marshes, e.g., *Pelvetia* and *Bostrychia*, also multiply vegetatively.

Sexual. Species are both dioecious and monoecious but, in contrast to *Fucus*, *Sargassum* forms special branches that bear conceptacles which may be fertile (Fig. 9-14 A) or sterile (Fig. 9-14 B). The conceptacles and antheridia develop in the same manner as in *Fucus*; oogonial development

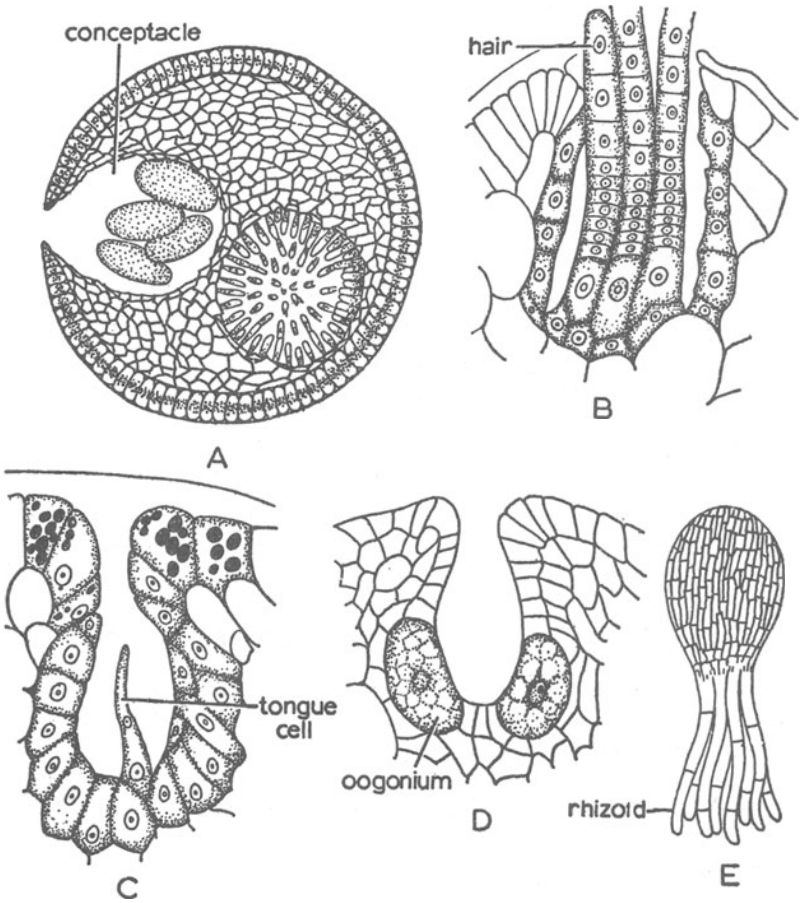

Fig. 9-14. A, *Sargassum swartzii*, transverse section through rachide showing compressed nature (after Chauhan and Thivy, 1964); B, *S. filipendula*, section of young cryptoblast; C, *S. filipendula*, section of young conceptacle; D, *S. linifolium*, section through a young female conceptacle showing embedded oogonia; E, *S. filipendula*, germling at rhizoidal stage.

(Fig. 9-14 C, D) also follows the same course up to the stage of formation of 8 nuclei, but 7 out of the 8 nuclei degenerate and the remaining nucleus along with the oogonial cytoplasm forms a single egg.

After maturation of the oogonium, the outer wall layer ruptures and the middle develops into a mucilaginous stalk which pushes the entire oogonium through the ostiole to the exterior while still attached to the mother plant. Fertilization occurs inside the oogonium and even the resulting zygote starts germinating within the oogonial wall. The remaining features of sexual reproduction and development of embryo into a germling (Fig. 9-14 E) are similar to those of *Fucus*.

TEST QUESTIONS

1. What explanations can you offer for the occurrence of brown algae in distinct zones along the littoral and sublittoral regions?
2. Both Chrysophyta and Phaeophyta are similar in respect of their pigmentation, food reserves and flagellar morphology, yet they are treated as separate phyla. Why?
3. In the absence of any unicellular algae in the Phaeophyta, how would you account for the ancestry of this Phylum? Explain, giving reasons.
4. It is generally considered that an alga with heteromorphic life cycle and heterotrichous habit would be nearest to the hypothetical progenitor of land plants. How about certain brown algae?
5. Give two most important and least variable features that have formed the basis of classification of Phaeophyceae into various orders.
6. Compare and contrast the anatomical features of *Fucus* or *Sargassum* with those of an angiospermic plant.
7. In what ways does the life cycle of Fucales differ from that of any angiospermic plant?
8. Support with arguments the hypothesis that the evolution in the life cycle of algae ended with a *Fucus*-like form.

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10

Rhodophyta

The Rhodophyta (red algae) owe their colour to the presence of excess of r-phycoerythrin in their chromatophores and this red pigment masks the colour of other photosynthetic pigments. The Rhodophyta are characterized by six main features: (1) the flagellated motile stages are totally absent; (2) the sexuality is highly specialized; the male gamete, called spermatium, is motionless and at the time of fertilization is passively transported to and lodged on the trichogyne of the female carpogonium; also, there are distinct post-fertilization developments not found in any other algal phyla; (3) chlorophyll-d, biliproteins (r-phycoerythrin and r-phycocyanin) and the xanthophyll taraxanthin are the characteristic pigments; besides, the chromatophores generally contain chlorophyll-a, α and β carotenes, lutein, zeaxanthin, neoxanthin and rarely other xanthophylls; (4) the reserve foods are floridean starch and galactoside floridosides and these do not accumulate within the chromatophore but outside it, in the cytoplasm; (5) the cell wall contains polysulphate esters of carbohydrates in addition to cellulose and pectin; and (6) the transverse walls in multicellular forms are generally provided with pits which permit cytoplasmic connection between adjacent cells.

OCCURRENCE

The unicellular red alga *Porphyridium* grows on damp soil. Except for a dozen genera, e.g., *Batrachospermum* and *Lemanea*, which are freshwater forms, others are exclusively marine and grow mostly in the intertidal and sublittoral regions. Members of Rhodophyta grow in almost all marine habitats, but their greater concentration occurs in the warmer seas.

Some of the calcareous algae, e.g., *Corallina* and *Lithothamnion*, possess stony thalli and are largely responsible for the formation of coral reefs. *Ceratocolax*, *Choreonema* and some others are parasites on macrophytic marine algae.

Visible light, particularly its blue and red regions, which are mainly effective in photosynthesis, seems to play a somewhat important role in the ecological distribution of the sublittoral marine algae since with increase in depth of water there is a corresponding decrease in the quantity and quality of incident light. At a depth of about 10 metres only 10 per cent of the blue-green and 1 per cent of the red light are received. At still deeper levels, further reduction in the quantity of these spectral regions takes place. Under such light conditions, mainly Rhodophycean members can photo-

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synthesize because r-phycoerythrin present in them can efficiently absorb the available blue-green light. They also exhibit complementary chromatic adaptation so that the colour of the incident light induces the development of a particular photosynthetic pigment which has maximum absorption of the incident light. Possibly for these reasons the red algae can grow at depths where few others can subsist.

RANGE OF THALLUS ORGANIZATION

Perhaps the most beautiful and most showy of the marine algae, forms such as *Plocamium* and *Delesseria* are a real delight to behold and collect. When mounted on a herbarium sheet and dried, a specimen of *Plocamium* looks like the creation of a painter. In general, not as large as the brown algae, the red algae are more delicate in texture and more slimy and soft to touch.

The Subclass Bangioideae, with the single Order Bangiales, comprises unicellular (*Porphyridium*), filamentous (*Goniotrichum*) and parenchymatous (*Porphyra*) forms. The thalli of Florideae are more elaborate, with two main types of organization—uniaxial and multiaxial—and in both a pseudoparenchymatous thallus results from the coalescence of filament branches. Uniaxial thalli have a single central or axial filament, which may be corticated, with a number of richly branched laterals organized to form a pseudoparenchymatous structure, e.g., *Dumontia* and *Batrachospermum*. In the multiaxial types, there are several central or axial filaments which, together with their branches, form the pseudoparenchymatous thallus, e.g., *Helminthocladia*.

The growth of the thallus in Bangioideae is diffuse whereas, with a few exceptions, in Florideae apical growth is the rule. An apical cell, by consecutive divisions, forms a transverse series of cells each of which further divides tangentially to produce a group of pericentral cells that surround a central cell. The pericentral cells may not divide further (as in Rhodomelaceae) or may divide vertically to form a cortex or the initials of the lateral branches which coalesce to form a compact structure with internal differentiation into an outer photosynthetic zone, an inner food storing cortex, and a central medulla containing vertically elongated hyphae, e.g., *Gelidium* and *Gloiosiphonia*.

CELL STRUCTURE

The cell wall is made up of an inner microfibrillar cellulosic layer and an outer pectic layer. In most cases it contains an outer coating of mucilage. Cells may either harbour a single, stellate, axile chromatophore with a central, naked pyrenoid (Bangioideae), or many discoid and parietal chromatophores devoid of pyrenoids (Florideae). The entire class seems uniform in the ultrastructure of the chromatophore which is a double membrane organelle with widely separated single thylakoids. A recent

electron microscopic study of *Porphyridium* sp. has revealed the presence of particles called phycobilisomes, containing phycoerythrin and phycocyanin. Phycobilisomes are arranged linearly on the surface of the photosynthetic lamellae, those containing phycoerythrin are spherical whereas those having phycocyanin are discoid. In addition to phycobilisomes, many scattered grains of floridean starch occur in the cell cytoplasm.

The Rhodophyta show considerable variation in the shape, size and number of nuclei. Cells of Bangioideae are always uninucleate with a single nucleolus, those of Florideae are more complex. In general, if the apical cells are uninucleate, the segments derived from them will also be uninucleate. In such forms as *Griffithsia* there may be as many as 4000 nuclei per cell. The shape of nucleus varies depending on its position in the cell. Thus, it is spherical when centrally situated, and flat when parietally located. Nuclei may migrate from one cell to another through a pit connection. Some red algae, e.g., *Porphyra*, exhibit a diurnal periodicity in their nuclear division. A few red algae have revealed the presence of dictyosomes, endoplasmic reticulum and tonoplast-bounded vacuoles (Brody and Vatter, 1959). Mitochondria have recently been reported in *Batrachospermum moniliforme*.

REPRODUCTION

Unicellular forms propagate vegetatively. In multicellular forms, asexual or sexual reproduction is more common.

ASEXUAL

This is brought about by non-motile spores which are given different names depending on the nature of the cells from which they are produced and their number within each sporangium. Accordingly, there are four chief types of asexual spores: (1) monospores, formed singly in monosporangia; (2) neutral spores, formed by direct transformation of vegetative cells into spores; these two types of spores are characteristic of the Bangioideae; (3) carpospores, formed either directly from the division products of the zygote, as in Bangioideae, or indirectly from the cells of certain filaments termed gonimoblasts that arise from the fertilized carpogonium or from an auxiliary cell containing the zygote nucleus; and (4) bispores, tetraspores, and polyspores produced respectively in twos, fours and multiples of fours within a sporangium of the diploid tetrasporophyte. Except for some red algae that form haploid carpospores growing into sexual plants, most red algae form diploid carpospores which on germination give rise to sporophytes or diploid plants, bearing diploid sporangia from which bispores, tetraspores or polyspores are produced after meiosis. These spores are therefore haploid and germinate to form gametophytes or haploid plants.

A tetrasporangium may be cruciate if the two divisions of the cell are at right angles to each other, tetrahedral if the two divisions are oriented in

such a manner as to form a group of four tetrahedrally disposed spores or zonate if there are three parallel transverse divisions.

SEXUAL

The motionless male cells or spermatia are formed singly within spermatangia produced in clusters either on special branches, as in *Polysiphonia*, or in definite sori, as in *Apoglossum*. The female cell, designated carpogonium, is a flask-shaped cell with a neck-like protuberance, the trichogyne. The egg nucleus is restricted to the basal portion. In Bangioideae, carpogonia are sessile but in Florideae they are stalked and produced terminally on a special branch, the carpogonial filament or procarp. During fertilization a spermatial nucleus passes down through the trichogyne and fuses with the nucleus of the egg. The sexual reproduction, from sex organ formation to fertilization, is remarkably uniform throughout the Rhodophyta, particularly the Subclass Florideae, which shows elaborate post-fertilization changes resulting in the production of a new generation, the carposporophyte, which is parasitic on the gametophyte. Such distinct stages in post-fertilization activities as the place of formation of gonimoblast filaments (either from fertilized carpogonium or from generative auxiliary cell, and in the latter case, the position and time of formation, if before or after fertilization of auxiliary cell) are so important and significant that they have provided the main basis for the classification of Florideae into various orders.

In Bangioideae, no new generation, like carposporophyte, is produced but the zygote divides directly into carpospores which germinate to form a *Conchocelis*-like vegetative phase. The general opinion among phycologists is that the mature plants of Bangioideae, e.g., *Porphyra*, *Bangia* and others are diploid and it is during the formation of carpospores that meiosis occurs. These carpospores produce haploid *Conchocelis*-like plants. Since there is no unequivocal cytological evidence for the occurrence of meiosis, whether during germination of zygote or formation of monospores or neutral spores, the controversy about the diploidy or haploidy of *Porphyra* or *Bangia* thalli is yet to be resolved. Some recent evidence indicates that *Conchocelis* may be diploid (Von Stotsch, personal communication). Morphologically, *Conchocelis* is regarded as the prostrate system and *Porphyra* thallus as the erect system of an originally heterotrichous alga.

In some Florideae, the gametophytes are haploid and the zygote nucleus may divide meiotically or more often mitotically in the carpogonium itself. In the majority, however, the zygote nucleus migrates into a well-differentiated cell, known as auxiliary cell, either through pit connections or through a tubular connection, the ooblast, specially established for this purpose between the carpogonium and the auxiliary cell. The division of the zygote nucleus in the auxiliary cell is always mitotic, hence the gonimoblast filaments produced are all diploid and so also are the resulting carposporophytes. Gonimoblast filaments produced from the carpogonia after meiosis are hap-

loid but in the others they are diploid. Accordingly the carposporophytic generation of the former is haploid whereas that of the others is diploid. Either terminal cells or all the cells of a gonimoblast filament may function as carposporangia. Each carposporangium forms a single carpospore which may be haploid or diploid depending upon the nature of the carposporophyte. Both carposporophyte and tetrasporophyte formed by the germination of the carpospores constitute the asexual generations.

Recent researches indicate that meiosis occurs in the developing apex of young plants of Nemalionales and not in the fertilized carpogonium.

LIFE CYCLE

Some red algae (e.g., certain Nemalionales) have two alternating haploid, gametophytic and carposporophytic generations. Remaining orders of the Florideae have three generations—a haploid gametophyte, a diploid carposporophyte and a diploid tetrasporophyte, one following the other in that sequence. The gametophytes and tetrasporophytes are two morphologically identical, free living generations, with an intercalation of a parasitic, diploid, carposporophytic generation. The products of the zygote nucleus form the carposporophyte whose carpospores give rise to tetrasporophytes. The gametophytic generation results from tetraspore germination.

CLASSIFICATION AND GENERAL CHARACTERS

Most phycologists divide the Class Rhodophyceae into two subclasses: (1) the Bangioideae (or Bangiophycidae) comprising the only Order Bangiales, and (2) the Florideae (or Florideophycidae) with 6 orders—Nemalionales, Gelidiales, Cryptonemiales, Gigartinales, Rhodymeniales and Ceramiales.

The Bangioideae include those forms which have simple thalli with cells containing single, axile chromatophores and are normally devoid of pit connections. The zygote divides directly into haploid carpospores. The thallus of the Bangioideae shows diffuse growth in contrast to the apical growth of the Florideae.

The division of the Florideae into 6 orders is based on features of post-fertilization development. Except for some Nemalionales, which do not have a free living diploid generation, all the other orders have two diploid generations, the carposporophytic and the tetrasporophytic. The latter is free living. The Nemalionales and Gelidiales have been segregated due to the lack of auxiliary cells in them. The Cryptonemiales and Gigartinales form auxiliary cells before fertilization whereas Ceramiales develop them after fertilization. In Rhodymeniales the auxiliary cells differentiate before fertilization but develop well and become easily recognizable only after fertilization. The Cryptonemiales differ from Gigartinales because their carpogonial branches always arise from special accessory branches, whereas in Gigartinales they are merely unmodified ordinary branches. In Rhodymeniales the auxiliary cell develops from the outer cell of a two-

celled branch but in Ceramiales it is formed from the supporting cell.

PHYLOGENY

A fairly homogeneous, well-circumscribed group, the Rhodophyta apparently show much closer relationship with the Cyanophyta than with any other algal phyla. The features shared by the two phyla are: (1) the absence of flagellated structures; (2) the formation of similar biliproteins (phycocyanin and phycoerythrin) as the accessory photosynthetic pigments; (3) the presence of pit connections; (4) the accumulation of fairly similar food reserves, e.g., floridean starch in Rhodophyta and cyanophycean starch in Cyanophyta; (5) the production of chemically similar mucilages in blue-green algae and in some Bangioideae and Nemalionales; and (6) similar patterns of fatty acid synthesis which differ from those of other algae in that the fat content does not increase with increase in the age of the thallus, and that the nitrogen starvation is not a factor in fatty acid accumulation (Fogg and Collyer, 1954). See also Table IV.

Table IV. Resemblances between Rhodophyta and Cyanophyta

1. Both have water soluble phycobilins, located on phycobilisomes, as accessory pigments
2. Free trehalose and galactose occur only in them
3. The food reserves, cyanophycean and rhodophycean starch, are elaborated by similar types of linkages and chemical routes
4. Linoleic acid and α-linoleic acid are present
5. Neither nitrogen starvation nor aging of thallus has any effect on the pattern of fatty acid accumulation
6. Xylans are the major components in the cellulose microfibrillar walls
7. Sulphated galactoses, uronic acids, glucose and xylose are the principal constituents of the mucilage
8. The organization of the photosynthetic thylakoids is similar, i.e., they occur singly and are widely separated
9. The patterns of isoenzymes involved in the elaboration of polyglucosides are similar
10. Both lack flagellated stages.

However, the biliproteins of Rhodophyta differ from those of Cyanophyta in their spectral properties. Cryptophyceae too are now known to possess biliproteins. The biliprotein allophycocyanin is probably identical with the phytochrome of higher plants, and future research may indicate the presence of this or other biliproteins in algal phyla other than Rhodophyta and Cyanophyta. In view of these, too much phylogenetic importance should not be attached to the biliproteins. See also Table V.

Table V. Differences between Rhodophyta and Cyanophyta

	Red algae	*Blue-green algae*
Cell structure	Eucaryotic	Procaryotic
Pigments	Chlorophyll-a and -d, zeaxanthin and neoxanthin	Only chlorophyll-a, myxoxanthin, myxoxanthophyll, oscillaxanthin
Food reserves	Rhodophycean starch, no proteins	Cyanophycean starch, and the proteinaceous cyanophycin
Cell walls	Cellulose and other carbohydrates present	Mucopolymers constitute the main component in certain forms
Motile stages	None	Non-flagellate multicellular motile stages, i.e., hormogonia formed in many forms, mature individuals of some species are also motile though non-flagellate
True sexuality	Present in most members	Absent, a primitive type of parasexuality, i.e., genetic recombination, occurs in some members
Chromosomes	Typically eucaryotic	True chromosomes not known, DNA fibrils not associated with histones but present in the nucleoplasm
Golgi bodies	Present	Absent
Endoplasmic reticulum	Present	Absent
Heterocysts and Nitrogen fixation	None	Many species have heterocysts and can fix atmospheric nitrogen
Susceptibility to virus infection	Not known	Cyanophages LPP-1 and SM-1 can infect a few species

The stacking together of photosynthetic thylakoids into distinct bands is regarded as an advanced feature and in this respect the Rhodophyta is the most primitive Phylum among eucaryotic algae since in the red algae the photosynthetic lamellae are single, unorganized, widely separated and not stacked into bands or multiple lamellae. Nonetheless, the Phylum Rhodophyta is morphologically as complex as Chlorophyta. Regarding the ancestry of the class, two alternatives have been suggested (see Klein and Cronquist 1967): (1) that they originated from some simple procaryotic blue-green algae, and (2) that they were derived from some archaic eucaryotic algae which themselves originated from blue-green algae. The fundamental disparity between the eucaryotic and procaryotic cellular organizations seems to rule out the existence of any direct link between the red and the blue-green algae. It would seem more plausible to speculate that the former probably originated from some primitive non-flagellated eucaryotic ancestor possessing biliproteins.

Order: NEMALIONALES
Family: Batrachospermaceae
Genus: *Batrachospermum*

Occurrence

It is an inland freshwater form widely distributed in tropical, subtropical and temperate regions. Slow-flowing streams, waterfalls and **oligotrophic** lakes are some of the habitats where it grows attached to stones. Most species prefer relatively well aerated 'clean' water streams. Some taxa are marine.

Morphology

Adult plants, which may be up to 15 cm long, are generally bluish-green, greyish-green, violet or olive-green, and soft and mucilaginous. To the naked eye, each plant appears as a branching chain of beads (Fig. 10-1 A). The alga is differentiated into a prostrate system that serves to anchor it to the substratum and an erect branched system, made up of whorls (Fig. 10-1 B), which floats freely in water. Many species are attached by rhizoids.

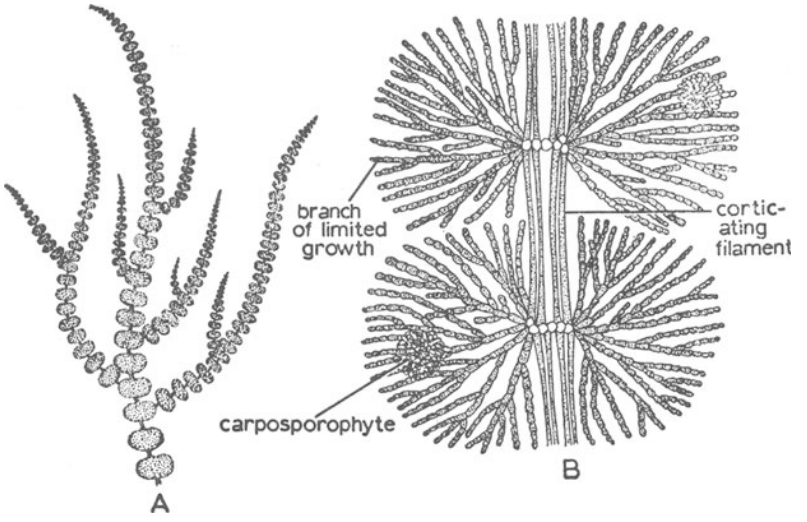

Fig. 10-1. *Batrachospermum moniliforme*. A, part of thallus; B, two whorls of branches (magnified).

The thallus is uniaxial. Its primary axis consists of a uniseriate row of large cylindrical cells, frequently terminating in a hemispherical apical cell. The axial filament is differentiated into nodal and internodal regions and is corticated. From the nodes arise two kinds of branches—the branches of limited growth which are produced in whorls, some branches often terminate in hairs; and those of unlimited growth which are generally formed singly and monopodially and like the primary axis, are further differentiated into nodes and internodes. The bead-like appearance of the alga is due to

the whorls of nodal branches. The cluster of branches at a node is called a **glomerule**. The nodal branches of limited growth are composed of moniliform cells and arise from just below septa of the axial filament, or from branches of unlimited growth whose cells are much longer than those of branch whorls. The cells are uninucleate and contain several parietal chromatophores, each with a single pyrenoid.

The growth of the axial filament and of the branches of unlimited growth occurs by means of an apical cell which cuts off segments transversely and produces a uniseriate row of cells. From each cell, near the septum, 4–6 lateral projections arise which soon become separated from the parent cell by a wall and function as initials of branches of limited growth. After the formation of the lateral branches, their basal cells give rise to corticating filaments. A branch of unlimited growth may also develop from one of these basal cells.

Reproduction

Batrachospermum reproduces sexually by the formation of spermatia and eggs, and asexually by carpospores.

The plants are monoecious or dioecious. The terminal or subterminal cells of branches of limited growth constitute the spermatangial initials. Each initial produces one or two spermatangia which are generally colourless and can therefore be easily distinguished from vegetative cells or branches.

The basal cell of a branch of limited growth cuts off a segment which is called the carpogonial initial. This initial undergoes 3 or 4 transverse divisions and forms a four to five-celled carpogonial branch whose terminal cell develops into a carpogonium. Each carpogonium is differentiated into a nucleate basal part and an enucleate neck or trichogyne, the latter being sometimes demarcated from the former by a median constriction.

The spermatia are carried passively along water currents to the carpogonia. During fertilization, the spermatium nucleus after migrating through the trichogyne, fuses with the egg nucleus (Fig. 10-2 A, B) of the carpogonium. After fertilization, the trichogyne shrivels and the zygote nucleus divides meiotically to form four haploid nuclei. At this time the fertilized carpogonium develops many small protuberances. Each of the four nuclei divides mitotically and one of the two resulting nuclei migrates into a protuberance which then becomes separated from the carpogonium and starts functioning as an initial of a gonimoblast filament. Thus a number of branched gonimoblast filaments (Fig. 10-2 C) arise from the base of the carpogonium. The terminal cells of the gonimoblasts later differentiate into carposporangia within which the carpospores are formed singly. The cluster of gonimoblast filaments along with associated carposporangia constitute the carposporophyte which grows as a parasite on the female gametophyte. Since meiosis occurs during division of the zygote nucleus, the carposporophyte and the carpospores are haploid. Some of the vegetative cells surrounding the carpogonium grow in the meanwhile and form a loose sheath

of sterile branches that encloses the carposporophyte which along with the sheath forms a characteristic fruit body known as **cystocarp.**

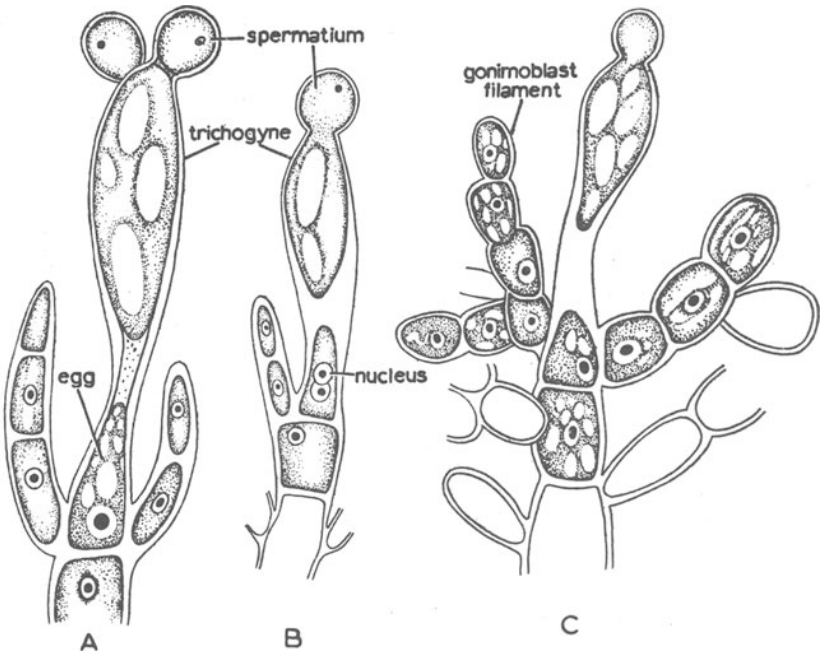

Fig. 10-2. *Batrachospermum moniliforme.* A–C, fertilization and post-fertilization stages.

Each carpospore on germination forms a branched, heterotrichous protonema-like filament. This constitutes a juvenile stage in the life history of *Batrachospermum* and was previously believed to be an independent algal genus *Chantransia.* Almost all freshwater species of *Chantransia* have been found to be the juvenile stage (Fig. 10-3 A) of *Batrachospermum.* The *Chantransia* thalli reproduce asexually by means of monospores formed singly in monosporangia (Fig. 10-3 B). Thus the monospores merely serve as an accessory means of multiplication of the *Chantransia* stage. The *Batrachospermum* plant arises from the *Chantransia* stage as a lateral outgrowth. Interpreted in terms of the heterotrichous habit, both the *Batrachospermum* phase and the *Chantransia* phase constitute the two systems of a heterotrichous plant, the former representing the erect system and the latter the prostrate system. A parallel situation is found in another red alga *Porphyra* which itself represents the erect system whereas the prostrate system is constituted by its *Conchocelis* phase.

Life Cycle

Batrachospermum includes three kinds of haploid somatic phases in its life cycle: (1) the free living gametophytic phase, (2) the parasitic carposporophytic phase, and (3) the free living *Chantransia* phase. The gametophytes

reproduce sexually through the formation of spermatia and eggs which unite to form zygotes. The zygote germinates meiotically forming a large num-

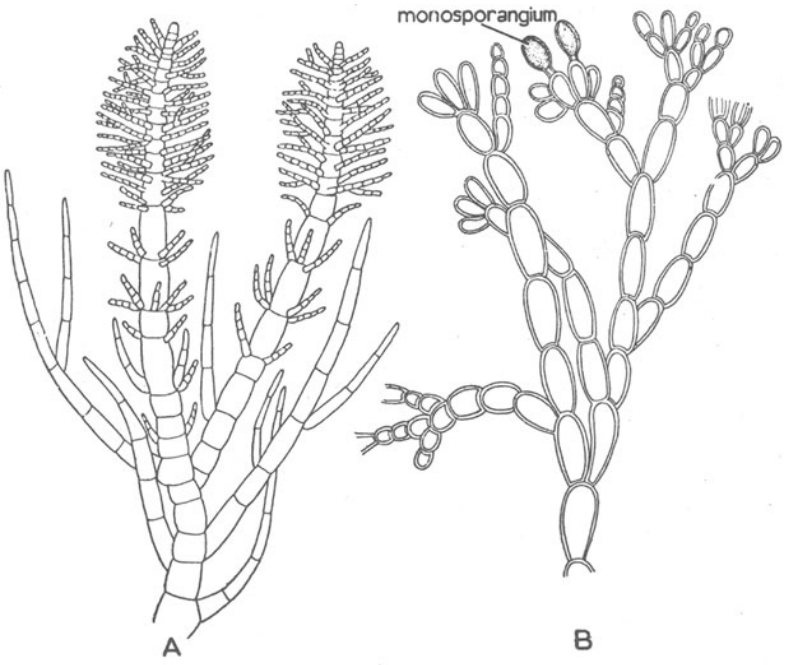

Fig. 10-3. A, *Batrachospermum ectocarpum*, *Chantransia* stage; B, *B. lochmodes*, monosporangia.

ber of branched gonimoblast filaments with their terminal cells functioning as carposporangia. Thus a haploid carposporophyte parasitic on the gametophyte results from germination of zygote. The carposporophyte in turn gives rise to *Chantransia* phase and *Chantransia* gives rise to *Batrachospermum* as a special branch. Both the carposporophytic and *Chantransia* phases are asexual, and the latter perpetuates asexually by monospores.

Cytologically the life cycle of the alga is haplontic in view of the zygote being the only diploid phase, and morphologically trimorphic because of the succession of three distinct morphological phases.

Order: CERAMIALES
Family: Rhodomelaceae
Genus: *Polysiphonia*

Occurrence

Polysiphonia grows extensively in the intertidal belt and sublittoral region. Most species are epiphytic on Fucaceae and other larger marine algae. *P. fastigiata* grows attached to the fronds of *Ascophyllum nodosum*; this species may be a semiparasite in view of the destruction of some of the host cells

near the point of its attachment. Some common species on the West Coast of India are *P. variegata*, *P. urceolata* and *P. platycarpa*. The plants grow in dense tufts (Fig. 10-4 A).

Morphology

The genus derives its name from the polysiphonous nature of the thallus which consists of an axial row of central siphons (Fig. 10-4 B) surrounded by a layer of 4–24 pericentral siphons. The plant body is heterotrichous with an erect system of branches and a filamentous prostrate system anchoring the plant to the substratum with the help of unicellular elongated rhizoids whose tips are flattened into lobed discs or haptera. The rhizoids arise from the pericentral cells facing the substratum. The thallus is dichotomously or laterally branched with two kinds of branches, the branches of unlimited growth made up of central and pericentral siphons and those of limited growth, known as trichoblasts, which appear like hairs, are dichotomously branched, tapering, uniseriate filaments made up of uninucleate colourless cells. Pit connections occur in between and among the central and the pericentral siphons (Fig. 10-4 C). The older parts of the thallus are generally corticated with cells cut off periclinally by pericentral cells of those regions. In surface view, under low magnification, the thallus of *Polysiphonia* seems to consist of a number of polysiphonous tiers. The orderly manner in which the tiers are arranged suggests a differentiation of filaments into nodes and internodes.

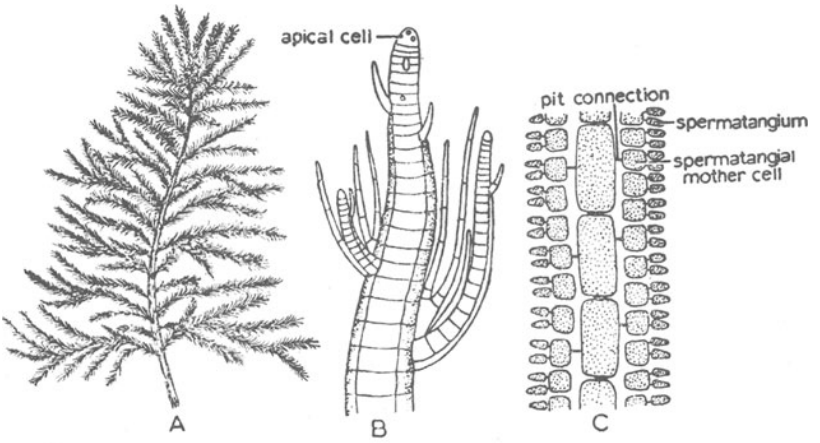

Fig. 10-4. A, *Polysiphonia nigrescens*, tufted habit; B, *P. sertularioides*, apex of thallus; C, spermatangia as seen in a section through fertile trichoblast (after Smith, 1955).

The thallus grows by means of an apical cell which by repeated divisions forms a row of axial cells. All axial cells excluding the first few cells undergo tangential vertical divisions and form a peripheral layer of a specific number of pericentral cells around the axial row of central cells. Both central and pericentral cells elongate into their respective siphons having

the usual pit connections. The trichoblast initial arises from an axial cell, 4 or 5 cells away from the apical meristem even before the pericentral cells are formed. Branches of unlimited growth also arise similarly but unlike the trichoblast initial, the initials of these branches behave exactly like the apical cell of the main axis and produce at first a transverse file of axial cells from which later the peripheral layer of pericentral cells arises. A branch of unlimited growth may sometimes arise in the axil of a trichoblast in which case its basal cell serves as the branch initial.

Reproduction

Polysiphonia is generally heterothallic or dioecious and includes three kinds of morphologically similar plants, the male gametophyte, the female gametophyte and the tetrasporophyte. The female plant also bears the diploid parasitic carposporophyte. Both carposporophyte and tetrasporophyte reproduce asexually by the formation of carpospores and tetraspores respectively.

Sexual. The male and female organs are borne on different plants. Spermatangia and carpogonia occur on small fertile trichoblasts. The male trichoblast, after becoming 2–3 cells long, forks; generally one, rarely both, of the branches so produced participates in the formation of spermatia. If only one branch forms spermatia, the remaining sterile branch repeatedly divides dichotomously. All cells except the two basal cells of the fertile branch divide and form central and pericentral cells. The latter function as spermatangial mother cells and produce one or a few spermatangia each along their free surfaces.

The two or three basal cells of the female trichoblast give rise to central and pericentral cells, and generally the middle cell of the three adaxial pericentrals produces on its free side a supporting cell (Fig. 10-5 A) which by successive transverse divisions organizes a 4- or 5-celled carpogonial branch (Fig. 10-5 B), the procarp. The terminal cell of the procarp transforms into a carpogonium with a swollen base containing a uninucleate egg and a long drawn-out enucleate trichogyne (Fig. 10-5 C–D). Meanwhile, the supporting cell also cuts off two sterile cells, one towards its base and the other towards its side. Both these cells form a few-celled sterile filaments (Fig. 10-5 E). Following these developments, fertilization occurs and a spermatium nucleus passes down the trichogyne and fuses with the egg nucleus in the swollen bases of the carpogonium (Fig. 10-5 F). This is accompanied by the cutting off of an auxiliary cell by the supporting cell of the carpogonium towards its upper side (Fig. 10-5 F). Soon a tubular connection is established between the auxiliary cell and the carpogonium base. The zygote nucleus then migrates to the auxiliary cell through this tube. By this time the trichogyne degenerates and the two pericentral cells of the trichoblast adjacent to the supporting cell start producing a sterile sheath, known as pericarp, around the developing carposporophyte; the pericarp has a terminal opening, the ostiole.

The divisions of the zygote nucleus in the auxiliary cell are always mitotic

and a mass of uninucleate gonimoblasts grows out from the upper side of the auxiliary cell. Terminal cells of gonimoblasts then form carposporangia. Meanwhile, the auxiliary cell, the supporting cell and the cells of the sterile

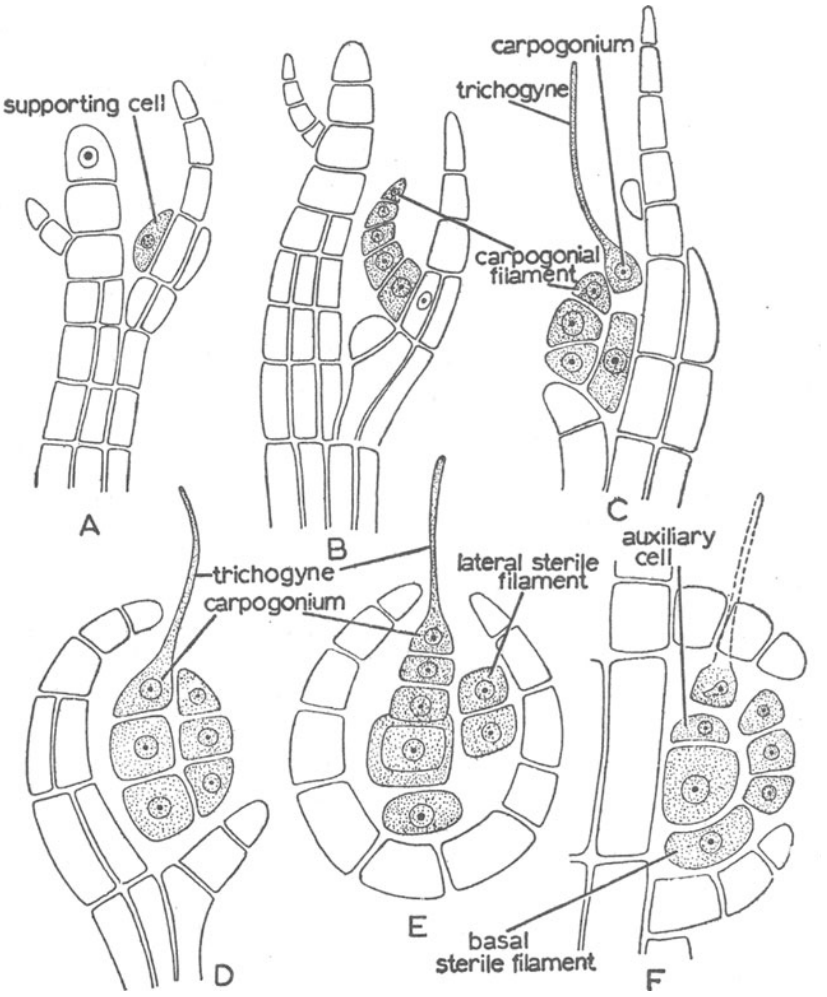

Fig. 10-5. *Polysiphonia flexicaulis.* A–F, longitudinal sections through successive stages of developing procarp (protoplasmic connections not drawn). (After Smith, 1955.)

filaments fuse to form a large placental cell which nourishes the developing carposporophyte. The carposporophyte with the pericarp and placental cell, is known as the cystocarp (Fig. 10-6 A).

Carpospores are diploid and are produced singly within carposporangia. The carpospore germinates into a new diploid free living plant, the tetrasporophyte. During germination, it divides transversely to form a four-celled uniseriate filament whose lowermost cell develops into a rhizoid and the uppermost as an apical initial of the tetrasporophyte. The tetrasporophyte initial functions exactly like the apical cell of the sexual plants, giving rise

to a branched thallus composed of central and pericentral cells (Fig. 10-6 B). The tetrasporophyte forms stalked tetrasporangia from certain pericentral cells (Fig. 10-6 C); the pericentral cell divides vertically into an outer cover

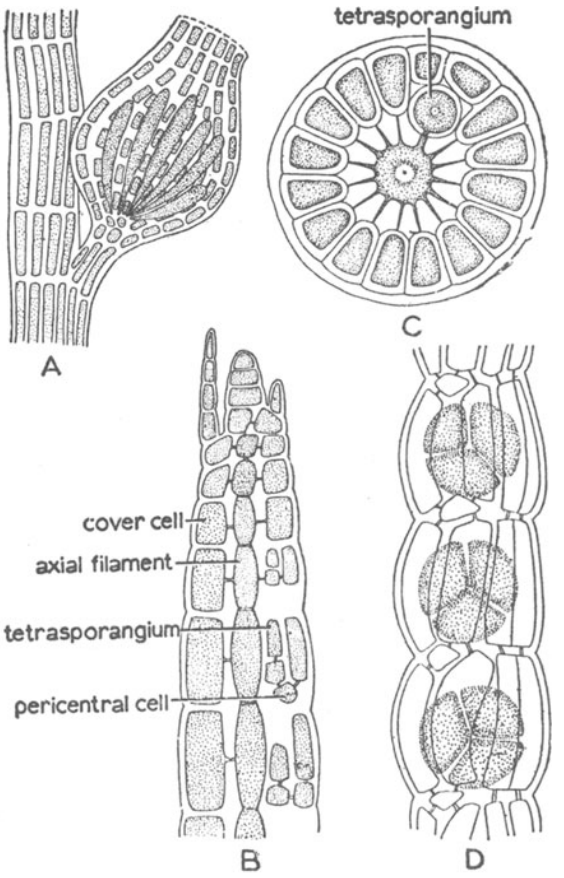

Fig. 10-6. A, *Polysiphonia nigrescens*, cystocarp; B, section through apex of tetrasporophyte; C, *P. fastigiata*, transverse section of developing tetrasporangium; D, old tetrasporophyte in surface view showing tetrasporangia. (Figs. B, D after Smith, 1955.)

cell and an inner sporangial mother cell. The former may not divide further, but the latter segments transversely into a lower stalk cell and an upper sporangial cell. The sporangial nucleus undergoes reduction division followed by cleavage of the sporangial protoplast into four tetrahedral uninucleate spores called tetraspores (Fig. 10-6 D).

In *Polysiphonia* there is a pair of sex-determining allelic genes. The alleles segregate at the first meiotic division and give rise to spores, one half of which form male plants and the other half female plants.

Life Cycle

The sequence of somatic phases in the life cycle of *P. denudata* has been studied in cultures (Edwards, 1968). The plants are dioecious, and the diploid parasitic carposporophyte results from mitotic divisions of the zygote nucleus in the auxiliary cell. Carpospores germinate into diploid tetrasporophytes which are free living and are morphologically identical with sexual plants. Each tetrasporangium of the tetrasporophyte forms four haploid spores by reduction division, two of these spores produce male plants and the other two give rise to female plants. On the basis of alternation between morphologically identical free living generations, the life cycle of *Polysiphonia* is isomorphic. Cytologically, it is diplobiontic, and according to the number of generations, including the parasitic carposporophyte, it is isomorphic.

TEST QUESTIONS

1. Can you grow *Polysiphonia* in freshwater and *Batrachospermum* in marine habitats ? Give reasons for your answer.
2. What advantage does a red alga derive from the production of a carposporophyte ?
3. Why is the carposporophyte regarded as a parasitic generation ?
4. How do Bangioideae and Florideae differ in their post-fertilization changes ?
5. When the photosynthetic pigments (phycocyanin and phycoerythrin) of red algae are water soluble, why do they not leach out of the cells when the algae are growing in aquatic habitats ?
6. What probable function do the pit connections of red algae serve ?
7. While most of the marine Rhodophyta are red, those growing in freshwater are usually bluish-green, violet or purple. What explanation can you offer to account for this disparity ?
8. *Griffithsia* and *Vaucheria* both possess coenocytic cells, yet they are included in different phyla. Why ?
9. Compare the post-fertilization changes in Rhodophyceae and Ascomycetes.
10. What arguments can you advance to show that *Conchocelis* and *Chantransia* are the prostrate systems and *Porphyra* and *Batrachospermum* the erect systems of two heterotrichous organisms ?
11. Which features of post-fertilization changes have been employed in the classification of Florideae and why ?
12. Algae growing in exposed and sunny habitats are generally greenish or bluish-green whereas those found submerged in deep waters are usually red. How can you account for this difference ?
13. Compare the life cycles of *Batrachospermum* and *Polysiphonia*.
14. In what respects do red algae resemble, and differ from, blue-green algae ?

15. Differentiate between: (a) oogonium and carpogonium, and (b) spermocarp and cystocarp.

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12. (54-22/1972)

11

Field and Laboratory Techniques

FIELD STUDIES

SOIL ALGAE

For field studies of distribution and abundance of soil algae, the general ecological methods as used for higher vegetation are applied with appropriate modifications.

In tropical countries many blue-green algae, e.g., *Cylindrospermum*, *Nostoc*, *Tolypothrix*, *Scytonema*, *Camptylonema* and *Aphanothece* form characteristic patches or strata on the surface of soils of grass lawns, fallow agricultural lands, footpaths and crop fields. Their relative growth or abundance is estimated by means of microquadrats or microtransects.

Microquadrats are laid down by placing a 20-cm square frame in the field and drawing a corresponding outline on a graph paper. The contours and shapes of algal strata caused by different species are then drawn to scale on the graph paper.

If the topography of the habitat is uneven, a microtransect may yield more reliable information than a microquadrat. A transect is usually chosen to record some transition in vegetation. It is plotted by laying a string and then locating the algal patches in quadrats laid at fixed points along the transect. The actual contour of the habitat along the string and the location of different patches are then plotted to an appropriate scale.

The point frame centre method for the study of higher vegetation may likewise be applied to the ecological study of algae.

More advanced students might like to study the autecology of some selected perennial species. For this purpose, two or three suitable areas exhibiting the growth of the alga should be selected. Collections of alga as well as the soil should be made at regular, weekly or fortnightly, intervals.

Depending on the size of the stratum formed, blocks of soil of a certain fixed size (2, 5 or 10 mm^3) can be cut by means of a sharp blade and the algal growth estimated by extracting the pigments from the block in a fixed volume of 80% acetone. Pigments should be drawn out in at least three changes of acetone to ensure complete extraction. Measurement of optical density of the pigment extract in a cuvette of fixed path length in a spectrophotometer set at a wavelength of 490 nm (corresponding to myxoxanthin), against 80% acetone as the reference solvent, will give a rough estimate of blue-green algae present in the original block. For other algae, wavelengths

giving maximum absorption of some characteristic pigment, or of chlorophyll-a, may be used.

The algal growth should also be correlated with variations in soil characteristics by regular soil analysis. Simple and elementary soil tests are described here.

Moisture and Organic Matter Content

In a clean crucible weigh accurately about 5 gm of the soil. Place it (lid removed) in an oven maintained at 105°C for 24 hr. Replace the lid and transfer the crucible to a desiccator until cooled to room temperature. Reweigh. Express loss in weight on a dry weight percentage basis. This represents the moisture content.

Expose the crucible containing dry soil at first to gentle and then to strong heat until it glows red. Cool to room temperature in a desiccator and reweigh. The loss in weight this time represents the organic matter content of the soil. It is expressed as a percentage of the air dry soil.

pH

Prepare a 1 : 3 or 1 : 5 suspension of soil in glass-distilled water and shake vigorously for 10 min after adding 2–3 gm of barium carbonate ($BaCO_3$). Allow to settle for 5 min or filter, then pipette 10 ml of the clear supernatant and transfer it into a clean test tube. Add 0.2 ml of B.D.H. Universal indicator, shake, and read pH by matching the colour developed with the colour chart on the indicator bottle.

Nitrate Content

Shake vigorously for 15 min a 1 : 3 soil water suspension, allow to settle for 5 min, withdraw one drop of clear supernatant and to it add 7 drops of a 0.2% solution of diphenylamine in concentrated nitrogen-free sulphuric acid (H_2SO_4). Development of a blue colour indicates the presence of nitrates. Express the intensity of colour, corresponding to nitrate content, on an arbitrary scale of 1–4.

Carbonate Content

In a test tube containing 1 gm of soil, pour 5 ml of dilute hydrochloric acid (HCl). An effervescence indicates the presence of carbonates in soil. The degree of effervescence, correspoding to carbonate content, may be expressed arbitrarily as low, medium or high.

Base-Deficiency

Add 5 ml of a freshly prepared Comber's reagent (saturated alcoholic solution of ammonium thiocyanate) to a test tube containing 5 gm of soil. If a red colour develops, it indicates that the soil is base-deficient. The intensity of colour, proportional to base-deficiency, may be recorded on an arbitrary scale of + to + + + +.

Certain anaerobic reducing soils, however, do not give any reaction with

the Comber's reagent because the iron is present in the ferrous state. Hence, if no red colour develops following addition of reagent to soil, then a drop of hydrogen peroxide (H_2O_2) should be added to the mixture so as to oxidize ferrous to ferric iron. This oxidation results in the development of a colour. The change in colour intensity is primarily a function of the concentration of ferric iron present in the sample. Thus this test provides a simple method of finding out whether the iron present in the soil sample is in the reduced or oxidized state.

PHYTOPLANKTON

Samples of free-floating aquatic algae (the phytoplankton) may be collected from lakes, ponds, rivers and tanks by means of a plankton net. The relative proportions of different species represented in a plankton sample may be estimated by counting them in a haemacytometer. Freshwater phytoplankton may contain unicellular, colonial and simple filamentous algae, mostly belonging to the Chlorophyceae, Cyanophyceae and Bacillariophyceae. Marine phytoplankton is generally rich in diatoms and members of the Dinophyceae, though sometimes blue-green algae are also found.

In addition to phytoplankton, most samples will contain zooplankton. Two general characteristics of planktonic organisms should be noted: (1) they are small and free-swimming by means of flagella or free-floating; and (2) they have a large surface-volume ratio.

Freshwater phytoplankton, collected from relatively 'clean', nutrient-deficient or oligotrophic waters, exhibit a great diversity of algal species, though the concentration of each species or of the algae as a whole is very low. In contrast, polluted ponds and tanks, lakes or rivers that are rich in dissolved nutrients or are otherwise eutrophic, may at certain times, especially during summer, have luxuriant growth of one, or rarely a few species of algae, constituting what are known as "water blooms". Such blooms impart their characteristic colour to the water which appears like "pea-soup". Indeed the appearance of a bloom of the blue-green alga *Microcystis aeruginosa* in a lake or pond is regarded as indicative of pollution. One does not need a plankton net to collect such bloom algae; merely examining a drop of the bloom water under microscope will reveal thousands of cells or colonies of the alga.

The appearance of particular blooms, and the specific composition and proportion of different algae in a plankton sample, often show a marked correlation with physical and chemical factors of the environment such as pH of water, light intensity and availability (visibility), temperature, and nitrate, phosphate, oxygen or silica content. Methods of water analysis are beyond the scope of this book but the interested student may consult the works of Mackereth (1963), American Public Health Association (1965) and Strickland and Parsons (1965).

COLLECTION

As far as possible, algae should be collected fresh from nature and examined in the living state.

SOIL ALGAE

Commonly confined to the surface layers, soil algae should be collected by means of a diminutive rectangular shovel having a sharp blade. If the soil is damp, a small block can be removed directly by cutting with a scalpel. Algae growing attached to tree barks, damp walls, or other such substrata may likewise be collected by scraping with a scalpel.

It is often difficult to separate algae from soil particles. To obtain relatively clean samples, keep the soil block in a petri dish and add enough water to saturate it. Place a few coverglasses on the soil block and leave the dish open until the excess water has evaporated. Cover the dish with its lid and keep it in a north window. Within one or two days, the motile algae in the soil will creep up on the undersides of the coverglasses and begin to multiply. During the next few days, some other algae will also start growing on the coverglasses which can be removed periodically, placed on a drop of water on a slide, and examined under a microscope.

PHYTOPLANKTON

These can be collected by towing a plankton net of fine bolting silk. Certain smaller forms (nannoplankton or microalgae), however, are not retained in the net. These are collected by subjecting a sample of lake water to gentle filtration through a Millipore membrane filter of 0.45 or 0.80 μ pore size.

Reed stems, twigs of submerged weeds, soil samples and bark or stones may be collected and placed in polythene bags, tins or cartons. Add just enough water in the bottle to ensure a saturated atmosphere when it is closed. On return to the laboratory, the bottles should be opened and samples examined as soon as possible. Water samples should preferably be analyzed first and soil or bark algae observed last.

If it is intended to keep algae alive for any length of time, there should be no overcrowding. The larger the mass of algae gathered, the sooner they would die. When a concentrated sample of algae is obtained by means of a plankton net, some water should also be collected simultaneously. The plankton concentrate should be appropriately diluted with this water in the laboratory, otherwise the algae will tend to die quickly. Another precaution is to refrain from placing them indiscriminately in any kind of water, especially tap water, which is usually highly chlorinated or sometimes contains toxic amounts of zinc or copper.

Concentration and Enumeration

1. Allow a 250-ml sample of formalin-preserved phytoplankton to stand

undisturbed in a measuring cylinder for a day. Then carefully siphon off most of the supernatant. Swirl the sediment and the remaining fluid to make a homogeneous suspension.

2. Pour the suspension into a 25-ml volumetric flask. Rinse the cylinder with distilled water twice and add the rinses into the flask. Make up to indicated mark by addition of distilled water. Mix thoroughly.
3. Insert a Whipple eyepiece micrometer into the ocular lens of a microscope. This micrometer has its field of view divided into 100 squares, with each square further subdivided into 25 smaller squares. The area of each square can be determined under the low power of a microscope by calibrating against a stage micrometer.
4. Take a Sedgwick-Rafter counting chamber and carefully add 1 ml of plankton concentrate from the volumetric flask in such a way that the chamber is just completely filled without any air bubbles. Apply the coverslip supplied with the chamber. Gently place the chamber on the microscope stage and let it rest for 10 min so as to allow the algae to settle at the bottom of the chamber.
5. Now count the various algae individually in a Whipple field (square), repeating the counts at least 15 times and each time making the counts in a different, randomly chosen, square. Determine the number of individuals of each species observed in 1 ml volume. The field count volume can be calculated by multiplying the field area into 1 mm (depth of chamber) into the number of squares counted. To this the necessary correction regarding concentration factor ($\times 10$ in this case) should be applied to determine phytoplankton concentration in the original sample.

The foregoing method has proved useful for the larger algae found in phytoplankton. For smaller forms (nannoplankton), a Palmer Nannoplankton counting chamber can be used in place of the Sedgwick-Rafter chamber. The Palmer chamber is 0.4 mm deep and is designed for counting 0.1 ml volumes. It is calibrated against a stage micrometer under the high power of a microscope ($\times 40$ or 45) instead of at $\times 10$ as in the Sedgwick-Rafter chamber.

Estimation of Primary Productivity

Phytoplankton converts sunlight energy into chemical energy which can be utilized by other members of an aquatic ecosystem. Depending primarily on the kind of algal producers present and the nutrient concentrations, various ecosystems differ in respect of their primary productivity (defined as the rate at which the algal producers convert solar energy into chemical form).

A simple method of measuring primary productivity involves the establishment of small aquaria containing some algal culture medium (*vide infra*) and inoculated with a few species, preferably unicellular, of freshwater algae. These are then grown for 2–3 weeks in light of known intensity. If any (filamentous) algae grow attached to the bottom or side walls of the aqua-

rium, they can be gently scraped by means of a rubber-tipped glass rod. Water lost through evaporation should be replaced with distilled water daily. The algal suspension should be aerated 2 or 3 times every day (or continuously) by bubbling air into it. The following schedule is recommended:

1. Withdraw 20-ml aliquots of algal suspension from aquarium and centrifuge in clean tubes at about 3000g for 15 min. Discard supernatants.
2. Suspend pellets in 2–3 ml of distilled water and transfer quantitatively into accurately weighed clean and dry crucibles.
3. Dry crucibles in an oven at 105°C for 6 hr, withdraw, cool to room temperature in a desiccator and weigh. Record dry weights of algae by subtracting crucible weights.
4. Place the same crucibles in a muffle furnace at 600°C for 4 hr. Remove, cool in desiccator and reweigh. The difference between dry weight and ash weight in each case is an estimate of the biomass of the organisms present.
5. Repeat the above procedure at 24-hr intervals in order to estimate changes in biomass, representing primary production.
6. Calculate as follows:

$$\text{Daily production in aquarium (gm)} = \text{Aquarium volume (m}^3\text{)} \times \frac{\text{Daily production in sample (gm)}}{\text{Sample volume (m}^3\text{)}}.$$

Suitably modified, this method can also be adapted for estimating daily fluctuation in primary production in natural habitats such as ponds and lakes. The observed changes in biomass should be carefully correlated with changes in algal populations.

MARINE ALGAE

Seaweeds and other marine algae are best collected during a low tide. A rocky shore is most suitable for such collections since it supports a wide variety of different species occurring in rock-pools or as lithophytes.

PRESERVATION

When it is intended to store algae in the laboratory for subsequent morphological studies, they may be killed and preserved in a 4% solution of formalin (prepared by adding 4 ml of 40% formalin to 96 ml of distilled water; 40% commercial formalin is regarded as 100% for purposes of calculation). For preserving aquatic algae, appropriate quantity of 40% formalin may be added directly to the sample so as to obtain a final concentration of 4%.

Many terrestrial or subaerial algae can be preserved dry and stored in paper envelopes for long periods without any apparent damage or loss of viability.

For maintaining the algae in their natural (green) colour, any one of the following solutions may be employed. The algae are immersed in the preservative for a few days and then transferred to formalin acetic alcohol (FAA) solution.

1.	50% ethyl alcohol	90 ml
	40% formalin	4 ml
	Glycerol	3 ml
	Glacial acetic acid	3 ml
	Cupric chloride ($CuCl_2$)	9.5 gm
	Uranium nitrate (UNO_3)	1.5 gm

Although suitable for most green algae, this preservative can also be used for blue-green algae if 10 gm of copper acetate is substituted for the cupric chloride and uranium nitrate.

2.	Cupric sulphate ($CuSO_4 \cdot 5H_2O$)	0.25 gm
	Water	38 ml
	When completely dissolved, add	
	Glacial acetic acid	4 ml
	40% formalin	8 ml
	95% ethyl alcohol	50 ml
3.	Potassium chrome alum	10 gm
	40% formalin	6 ml
	Distilled water	500 ml

These solutions can also be used when certain rare algae have to be exhibited in their natural colours in a museum.

It is sometimes possible to preserve living algae on a slide for a few days. This is achieved by cutting a lens paper somewhat smaller than a square coverglass, and making a square hole in the centre and placing it on a slide having a drop of water. The living alga is then placed in the water drop in the centre of the square and covered with a coverglass. Since the lens paper is very absorbent the whole preparation can be easily kept moist. Alternatively, the alga can be placed in a few drops of water in a hollow cavity slide. A coverglass is then applied and its edges sealed with paraffin oil or Gold Size.

PREPARATION OF HERBARIUM SHEETS

It is fascinating to prepare herbarium sheets of seaweeds and the larger freshwater algae. The advantage of this method is that the specimen is preserved in its natural habit and colour.

Herbarium sheets of most marine algae, especially those having mucilaginous or gelatinous thalli (e.g., *Delesseria*, *Plocamium*, *Gracilaria*, *Porphyra* and *Ulva*) can be prepared with great ease. The specimen is floated in an enamel tray containing freshwater or seawater, as the case may be, and a piece of moderately thick herbarium sheet inserted in the water below the specimen. The alga is then spread and the tray tilted gently while the specimen is held on the sheet (Fig. 11-1). If the specimen is mucilaginous, it will stick to the sheet and any excess water on the latter may be drained off by keeping the sheet on an inclined plane for some time. The sheet is then placed between two dry newspapers or blotting papers and after a number of herbarium sheets of different algae have been prepared in this

way, they are all pressed in a herbarium press. After 24 hours the wet newspapers are replaced by fresh, dry sheets and pressed again. Five or 6 such changes are usually sufficient to absorb all traces of water from the sheets and specimens may then be mounted and labelled.

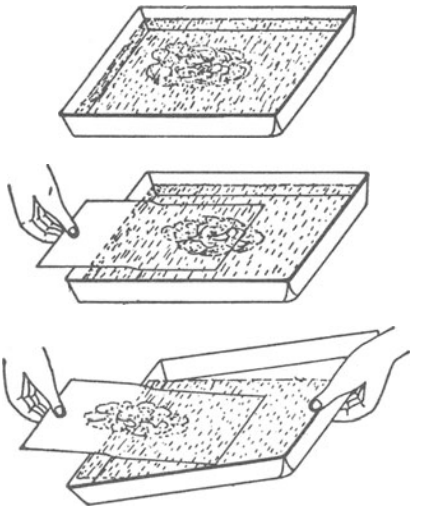

Fig. 11-1. Diagram illustrating the preparation of a herbarium sheet of an alga.

This method may be modified for non-sticky algae by affixing the specimen on to herbarium sheet by means of a few small pieces of cellotape.

PERMANENT PREPARATIONS

Permanent slides of algae have a limited use and as pointed out earlier, observations on living specimens should be preferred to making permanent preparations. Some of the more common methods are described here.

Schedule I

This method may be used for both freshwater and marine algae. It is suitable for morphological study and identification, but not for cytological study.

1. Place alga on a slide in a drop of freshwater or seawater.
2. Add a small drop of 40% formalin to fix. Drain out excess water.
3. Place a small lump of glycerine jelly on the alga. (Glycerine jelly is prepared by dissolving 5 gm of gelatine in 30 ml of water by gentle heat and then adding 0.125 gm phenol and 35 ml of glycerol.)
4. Transfer the slide to an incubator or oven (at 60°C) for a few minutes for the jelly to melt. Spread the algal material appropriately.
5. Apply circular coverglass. Keep the slide again in the oven for a short while. Wipe off excess jelly from around the coverglass and seal it with Gold Size. Store flat.

Schedule II

1. Place alga on a slide in a tiny drop of water, fix by adding a little of 40% formalin and then add a small drop of 10% glycerol.
2. Leave the slide in a warm, dust-free atmosphere for a few days so that the glycerine may concentrate.
3. Add a drop of glycerine jelly and apply a warm coverglass.

Schedule III

1. The fresh material may be fixed in any one of the following solutions:

(i) *Chrome-acetic Fixative*

10% aqueous chromic acid	2.5 ml
10% aqueous acetic acid	5.0 ml
Distilled water	92.5 ml

(ii) *Dioxan Fixative*

Dioxan	50 ml
40% formalin	5 ml
Glacial acetic acid	5 ml
Distilled water	50 ml

(iii) *Chrome-osmo-acetic Fixative*

Chromic anhydride	1 gm
Glacial acetic acid	3 ml
1% aqueous osmic acid	1 ml
Distilled water	100 ml

2. Wash the fixed material several times in tap water. Dehydrate gradually by passing the material through a large number of grades of ethyl alcohol (3%, 5%, 8%, 12% ... absolute alcohol, 2 changes in each grade). In the lower grades the material may be kept for 3 min; in the higher grades for about 5 min. After absolute alcohol the alga is mounted directly in a drop of Euparal on the slide and a coverglass applied.
3. If stained specimens are required, any one of the following stains may be used:
   (i) Aniline Blue (1% solution in 90% ethyl alcohol) used for filamentous green algae; stain for 5 min after 85% ethyl alcohol stage.
   (ii) Erythrosine Bluish (1% solution in absolute ethyl alcohol) used for staining gelatinous envelopes or sheaths; stain for 30 sec after 95% ethyl alcohol.
   (iii) Light Green (0.2% solution in 90% ethyl alcohol) used for cellulose cell walls; stain for 30–60 sec after 85% ethyl alcohol.
   (iv) Congo Red (0.2% solution in absolute ethyl alcohol) used for staining mucilage sheaths of Cyanophyta; stain for 60 sec after 95% ethyl alcohol.

Schedule IV

1. Smear the slide with a thin film of Mayer's albumin and allow it to dry for a few minutes. Place material on this in a drop of water and tilt the

slide to drain out water. Pass slide momentarily over a spirit flame.

2. Holding the slide in the left hand, pour 30% ethyl alcohol over it from a dropping bottle and immediately drain it out. Rinse the material similarly with 50%, 70%, 90% ethyl alcohol and give 2 changes of absolute ethyl alcohol. After the last change, immediately place a drop of Euparal on the material and apply a coverglass.

If necessary, the material may be stained at the appropriate stage.

Schedule V

This method is employed for making permanent slides suitable for cytological studies.

1. Fix the material in any of the fixatives described in Schedule III, or in a mixture containing equal volumes of glacial acetic acid and absolute ethyl alcohol. Material should be immersed in the fixative for 10–15 min.
2. Wash in 3 changes of water.
3. Mordant in 1.5% aqueous iron alum solution for 10–60 sec.
4. Wash thoroughly in running tap water for 2–3 min.
5. Stain the material in a drop or two of acetocarmine on a slide, or, in the case of unicellular forms, in a centrifuge tube; warm the slide gently over a spirit flame and apply a coverglass.
6. Dip the slide and coverglass horizontally in a petri dish containing 95% ethyl alcohol and allow them to remain there till the coverglass separates from the slide. Carry the coverglass and slide through two other petri dishes containing absolute ethyl alcohol, mount in Euparal and apply the coverglass again.

For further details of this method, and its modifications for application to different kinds of algae, consult Godward (1966).

Schedule VI

This method has been found useful for the Fucaceae (see Evans 1962; Godward 1966).

1. Fix material in 1 : 3 glacial acetic acid, absolute ethyl alcohol mixture for 10–18 hr; wash thoroughly in water.
2. Treat small fragments of thallus with a 1 M solution of lithium chloride for 10–15 min; wash in running water for 15 min.
3. Squash in water under a coverglass and irrigate the material with acetocarmine containing 3 drops of ferric acetate per 25 ml.
4. Warm the slide gently over a spirit flame and squash again; absorb away the excess stain by means of a dry blotting paper and ring the preparation with glycerine jelly.

Methods for the preparation of permanent slides of red algae have been described in detail by Dixon (quoted in Godward, 1966).

Schedule VII

This method is suitable for many filamentous algae.

1. Fix in any of the fixatives described in Schedule III, wash thoroughly in water.
2. Stain in Harris' haematoxylin which is prepared by dissolving 5 gm of haematoxylin and 3 gm of ammonium alum (aluminium ammonium sulphate) in 1000 ml of 50% ethyl alcohol by heating. To this, 6 gm of mercuric oxide is then added and the solution boiled for half an hour, filtered, cooled and then made up to 1000 ml in a volumetric flask by adding 50% ethyl alcohol. A few (8–10) drops of HCl are then added per 1000 ml of the solution.
3. Wash in water.
4. Examine under a microscope and if overstained, destain in acid water and wash thoroughly in tap water.
5. Dehydrate through a graded series of 50%, 70%, 90%, and 95% dioxan and water mixtures, allowing 30 min in each grade. Give two changes in absolute dioxan, allowing 10–12 hr in each.
6. Mount a few filaments in a thin drop of Canada Balsam dissolved in dioxan. Leave the slide aside for dioxan to evaporate, and apply a coverglass.

Schedule VIII (Personal communication from G. Russell)

This method is suitable for filamentous marine algae.

1. Fix the material in the following fixative for 12–24 hr:

Absolute ethyl alcohol	75 ml
Glacial acetic acid	25 ml
Concentrated solution of ferric chloride	3–4 drops

2. Wash in three changes of water and store in 70% ethyl alcohol.
3. Wash in distilled water and arrange on a slide in a few drops of 5% aqueous solution of sodium carbonate.
4. Warm the slide gently and then cover the material with an albumenized coverglass.
5. Squash between two thicknesses of filter paper.
6. Invert the slide in a ridged dish containing 20% acetic acid until the coverglass (along with the material) floats off.
7. Transfer the coverglass to a watchglass containing acetocarmine and heat to steaming for 7–10 min.
8. Transfer coverglass to 45% acetic acid and keep in this for 1–2 min until material becomes pale yellow.
9. Place in 95% ethyl alcohol and then give two changes of absolute ethyl alcohol.
10. Clear in Euparal essence and mount in Euparal.

HISTOCHEMICAL AND GENERAL METHODS

Temporary Fixation

Add a drop of dilute Iodine Potassium Iodide (IKI) solution to a 1–2 ml sample of algae; this also acts as a temporary preservative. (The IKI solution

is prepared by dissolving 2 gm of KI in 20–30 ml of water and then dissolving 1 gm of iodine. The solution is then made up to 100 ml with distilled water.)

Algae can also be fixed and temporarily preserved by exposure to osmic acid vapour.

General Staining

Different algae vary in their affinity for stains. More frequently employed stains are Methylene Blue, Gentian Violet or Acid Fuchsin (up to 1% aqueous solutions). The following simple procedure is recommended. Mount algae in a drop of water on a slide and apply a coverglass. Add a drop of the stain to one edge of the coverglass and let it diffuse to the opposite edge by removing water from the other side with a piece of dry blotting paper. In this way a spectrum of staining is obtained, the algae near one side of the coverglass are intensely stained and on the opposite side weakly stained.

Arresting the Movements

In the living state certain algae are actively motile or exhibit gliding movements. Others may exhibit a passive Brownian movement. To slow down such movements:

(i) add a tiny drop of IKI solution to a drop of algal suspension,
(ii) add a pinch of powdered gum arabic or dextran to a drop of algal suspension and apply a coverglass,
(iii) mix a drop of chloroform water (i.e., a drop of chloroform in 5 ml of distilled water) with a drop of algal suspension and apply coverglass, and
(iv) place a drop of algal suspension directly in the centre of a slide or petri dish containing a thin sheet of 2.5% aqueous agar, apply coverglass and observe under a microscope. This method will also stop Brownian movement.

Flagella

Most algal flagella cannot be seen under an ordinary light microscope without special staining. Following are the two commonly used methods to render flagella visible:

(i) add a few particles of lead of copying ink pencil to a drop of algal suspension, apply a coverglass and examine, and
(ii) fix algae in dilute IKI solution or by exposure to osmic acid vapour, apply coverglass and examine. If overstained, decolourize to desired degree by adding dilute sodium thiosulphate.

Cell Walls

The presence or absence of a true cell wall may be ascertained by microscopic observation of algal cells that had been placed in a plasmolysing solution, e.g., 10% sucrose, for a few minutes.

The presence of cellulose or related compounds in a cell wall may be

determined by treating the algae with Schultz's reagent (chlor-zinc-iodide) for 15–20 min, which gives a violet or blue colour with such compounds. Schultz's reagent used to be regarded as a specific stain for cellulose but it gives a positive colour response with other related compounds as well.

Alternatively, mix a drop of dilute IKI solution with a drop of algal suspension on a slide, followed either by treatment of the mixture with a drop of concentrated sulphuric acid, or by irrigation with 70% sulphuric acid. Cellulose turns blue.

Cell walls composed largely of pectic substances acquire a red colour when stained with a fresh, dilute aqueous solution of Ruthenium Red (one or two crystals in a small watchglass containing 2–3 ml water).

Gelatinous Envelopes and Sheaths

These are rendered conspicuous by staining with a very dilute aqueous solution of either Ruthenium Red or Methylene Blue. Alternatively, a trace of India ink or of Gurr's Negative Stain may be employed for the same purpose.

Chromatophores

Algal chromatophores are rendered conspicuous by treatment of the cells with boiling 8% aqueous silver nitrate solution for 4–5 min; this treatment turns the chromatophores brownish-black.

According to Friedmann (1966), the chromatophores assume a conspicuous and prominent appearance when viewed under a microscope which has a blue filter (e.g., the Kodak Wratten Filter No. 48) inserted between its light path. In visual work this filter has been reported to produce a surprisingly clear image and in photomicrography its use yields sharp and brilliant negatives. A further advantage of the blue filter is that it can be used equally well with different kinds of algae since most of them have a strong yellow component (i.e., the complementary colour to blue) in their chromatophore pigments.

Nuclei and Chromosomes

These can be demonstrated by the application of the iron alum-acetocarmine technique, as already described (Schedule V).

In temporary preparations, nuclei may sometime acquire a bluish colour when the cells are immersed in a very dilute aqueous solution of Methylene Blue, and then washed in water to remove the stain from the cytoplasm.

Starch and Other Reserve Materials

If a blue or violet colour develops after treatment of the material with IKI solution, starch is indicated. Iodine in chloral hydrate gives better results in this test.

Glycogen or cyanophycean starch generally gives a brownish colour on treatment with iodine, whereas floridean starch gives a reddish colour with this reagent.

Sulphated and non-sulphated polysaccharides in algal tissues may be detected by treatment with alcian dyes. Parker and Diboll (1966) have recommended the following schedule for localization of acid and sulphated polysaccharides in various marine and freshwater algae:

(i) fix algae in FAA or in 10% formalin in seawater or freshwater, dehydrate in tertiary butyl alcohol series, embed in paraffin under vacuum, cut sections (15 μ thick), and mount on slides,
(ii) stain 30–60 min in 0.5% aqueous Alcian Blue adjusted to pH 0.5 with NHCl,
(iii) wash in water, and
(iv) dehydrate, clear and mount.

The following procedure for histochemical localization of polysaccharides in marine algae has been suggested by McCully (1970):

(i) fix 2 mm^3 pieces of seaweed (e.g., *Fucus*) in 10% aqueous acrolein for 24 hr,
(ii) wash in water and dehydrate by passing through alcohol grades (10%, 20%, 30%, ..., 100%) at 0–4°C in a refrigerator,
(iii) infiltrate and embed in the resin glycol methacrylate and cut thin sections with a good microtome,
(iv) stain sections with a 0.05% solution of toluidine blue in benzoate buffer at pH 4.5 (alternatively, in dilute acid). Wash in tap water. Development of red colour indicates polysaccharides.

The red colour may be preserved by air drying the sections after staining and washing.

PREPARATION AND CLEANING OF DIATOM FRUSTULES

To study the frustules, make a diatom suspension and wash it by centrifugation; resuspend material in 10–20 ml of distilled water in a 50-ml quartz tube. Add 5–10 drops of hydrogen peroxide and expose the tube to ultraviolet radiation (short wavelength, germicidal) for up to 2 hours. Examine the irradiated suspension under a microscope.

If a quartz tube is not available, the peroxide containing sample may be exposed to ultraviolet light in an ordinary glass petri dish, with the lid removed during irradiation. This simple method for preparing clean frustules has proved convenient for taxonomical studies (Swift, 1967).

CHROMATOGRAPHY OF PIGMENTS

Chromatography is a convenient technique for separating individual components from a mixture of similar substances, e.g., amino acids, sugars and photosynthetic pigments. The mixture is extracted in a suitable solvent and the extract allowed to flow over the surface of a finely divided or coarse solid material. The different components of the mixture move in the solvent at different speeds thus becoming separated from each other in due course.

Simple chromatographic examination of algal pigments can prove helpful

in distinguishing various algal groups. The fat-soluble pigments, i.e., chlorophyll-a, chlorophyll-b, carotenes and xanthophylls can be easily extracted from any alga though *Chlorella* is particularly suitable for this purpose.

Concentrate a large population of *Chlorella* by centrifugation and suspend it in 10 or 20 ml of 80% aqueous acetone. Keep in refrigerator for 4–6 hours, filter or centrifuge to remove cell debris. The dark-green filtrate thus obtained is the solution of the photosynthetic pigments.

Cut a filter paper into small strips (2–3 cm wide and 10–12 cm long). Draw a pencil line across the paper strip nearly 2 cm above the bottom end. Develop a concentrated spot of the pigment extract in the centre of the pencil line (Fig. 11-2) and then dry the spot with a hot air blower. Place a few more drops of pigment extract along the pencil line and dry them in hot air. Repeat this step till the spot is concentrated.

Take a corked test tube having a hook underneath the cork (see Fig. 11-2) and place in it a few ml of a mixture of 90 parts of methanol + 10 parts of petroleum ether, or 5 parts of acetone + 95 parts of petroleum ether. Hook the paper strip to the cork in such a manner that its bottom end just dips into the solvent mixture (Fig. 11-2). Run the experiment for about an hour until the solvent front just reaches the top of the strip. Meanwhile watch the separation of individual pigments in the form of differently coloured bands along the paper strip.

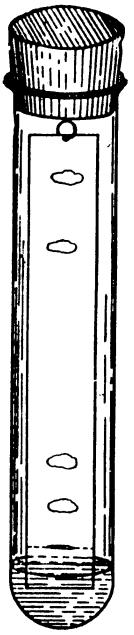

Fig. 11-2. Diagrammatic demonstration of ascending paper chromatography for separation of algal pigments.

CULTURE

Certain morphological and reproductive stages can best be demonstrated by suitable manipulation of live cultures of algae. Algal cultures may either be obtained from a Culture Collection, or raised in the laboratory. The three major Culture Collection Centres are:

(1) The Culture Collection of Algae, Department of Botany, Indiana University, Bloomington, Indiana, U.S.A.

(2) The Culture Centre of Algae and Protozoa, Storey's Way, Cambridge, England.

(3) The Culture Collection of Algae and Microorganisms, Institute of Applied Microbiology, University of Tokyo, Bunkyo-ku, Tokyo, Japan.

Unialgal cultures may, however, be isolated without much difficulty from

fresh material collected from nature. A number of culture media have been found suitable. The following three are likely to prove useful for the isolation and multiplication of most freshwater and soil algae:

1. Chu No. 10 (modified), for common freshwater and soil algae:

Calcium nitrate [$Ca(NO_3)_2$]	0.04	gm
Dipotassium hydrogen phosphate (K_2HPO_4)	0.01	gm
Magnesium sulphate ($MgSO_4 \cdot 7H_2O$)	0.025	gm
Sodium carbonate (Na_2CO_3)	0.020	gm
Sodium silicate (Na_2SiO_3)	0.025	gm
Ferric citrate	0.003	gm
Citric acid	0.003	gm
*A_5 Trace elements stock solution (optional)	1.0	ml
Glass-distilled water	to 1000	ml

*The A_5 Trace elements stock solution has the following composition in grams per litre of glass-distilled water:

Boric acid (H_3BO_3)	2.86
Manganese chloride ($MnCl_2 \cdot 4H_2O$)	1.81
Zinc sulphate ($ZnSO_4 \cdot 7H_2O$)	0.222
Molybdenum trioxide [MoO_3(85%)]	0.0177
Cupric sulphate ($CuSO_4 \cdot 5H_2O$)	0.079

2. Allen and Arnon's Medium (modified) for nitrogen-fixing blue-green algae:

Magnesium sulphate ($MgSO_4 \cdot 7H_2O$)	0.025	gm
Calcium chloride	0.05	gm
Sodium chloride	0.20	gm
Dipotassium hydrogen phosphate	0.35	gm
A_5 Trace elements stock solution	1.0	ml
Glass-distilled water	to 1000	ml

If 0.20 gm of potassium nitrate is added, this medium will also support the growth of many non-nitrogen-fixing blue-green algae.

3. ASM-1 Medium for freshwater planktonic algae:

Sodium nitrate	170	mg
Dipotassium hydrogen phosphate	17.4	mg
Disodium hydrogen phosphate	14.2	mg
Magnesium chloride ($MgCl_2 \cdot 6H_2O$)	40.7	mg
Magnesium sulphate	49.3	mg
Calcium chloride	22.2	mg
Ferric chloride ($FeCl_3 \cdot 6H_2O$)	1.1	mg
Sodium ethylene diamine tetra acetate (Na_2EDTA)	6.7	mg
Boric acid	2.5	mg
Manganese chloride ($MnCl_2 \cdot 4H_2O$)	1.4	mg
Zinc chloride ($ZnCl_2$)	0.4	mg
Cobalt chloride ($CoCl_2 \cdot 6H_2O$)	0.02	mg
Cupric chloride ($CuCl_2 \cdot 2H_2O$)	0.00014	mg
Glass-distilled water	to 1000	ml

13. (54-22/1972)

4. Marine algae Medium:

Smaller marine algae may be grown in either synthetic or natural seawater culture media. The latter have, however, been found to be more suitable than the former. In inland places far away from sea, the synthetic media may be used if seawater cannot be procured. The composition of a suitable culture medium, both natural and synthetic, for growing marine algae (e.g., *Ectocarpus*) is given here (see also Boalch 1961):

	Natural Seawater Medium	*Synthetic Medium*
Natural seawater	1 litre	
Glass-distilled water	—	1 litre
Potassium nitrate (KNO_3)	200 mg	200 mg
Potassium monohydrogen phosphate (K_2HPO_4)	35 mg	35 mg
Ferric chloride ($FeCl_3 \cdot 6H_2O$)	3 mg	—
Manganese chloride ($MnCl_2 \cdot 4H_2O$)	0.2 mg	—
Sodium chloride (NaCl)	—	30,000 mg
Magnesium chloride ($MgCl_2 \cdot 6H_2O$)	—	5000 mg
Calcium sulphate ($CaSO_4 \cdot 2H_2O$)	—	1000 mg
Sodium ethylene diamine tetra acetate (Na_2EDTA)	—	20 mg
Potassium chloride (KCl)	—	750 mg
Potassium bromide (KBr)	—	15 mg
Ferrous sulphate ($FeSO_4 \cdot 7H_2O$)	—	0.7 mg
Aluminium sulphate [$Al_2(SO_4)_3$]	—	0.25 mg
Cobalt sulphate ($CoSO_4 \cdot 9H_2O$)		0.03 mg
Cupric sulphate ($CuSO_4 \cdot 5H_2O$)		0.005 mg
Lithium chloride ($LiCl \cdot H_2O$)		0.05 mg
Sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$)		2.0 mg
Rubidium chloride (RbCl)		0.5 mg
Strontium chloride ($SrCl_2 \cdot 6H_2O$)		5.0 mg
Zinc sulphate ($ZnSO_4 \cdot 7H_2O$)		10.0 mg

(The pH of all the above media should be adjusted to 7.0–7.5 for green algae and 8.5–9.0 for blue-green algae.)

In all these media, phosphate should be autoclaved separately from the other components and mixed aseptically upon cooling.

The media may be solidified with 1 or 1.5% agar. It is advisable to sterilize agar in a small volume of water separately from the mineral salts, to mix the two components after autoclaving, and then to pour in sterile petri dishes.

For liquid culture, a small volume of the mineral salts medium is taken in an Erlenmeyer flask or a test tube which is plugged with non-absorbent cotton wool and then sterilized at 15 lbs/in^2 for 15 min. Corning test tubes (150 × 25 mm) with screw caps may be used if cotton plugging is desired to be avoided.

Fresh material collected from nature is observed microscopically to see if it is fairly unialgal or not. Some of it may be taken in a watchglass containing sterile water and teased and separated by means of sterile needles under a binocular dissecting microscope. The teased mass is transferred to a rubber-stoppered test tube containing a small volume of sterile water and shaken vigorously for 10–15 min so as to disperse the cells or filaments. A drop or two of the dispersed suspension is then added to a fresh watchglass containing a few ml of sterile water. From this, single cells or filaments are picked up by means of a fine capillary pipette under the binocular microscope and transferred to a second watchglass containing water. After three such washings, single cells or filaments are inoculated into culture tubes. At least a dozen tubes, flasks or petri dishes should be inoculated in this way since some of the cultures may fail to grow. Out of those that would grow, one or two may prove unialgal from the beginning; the others will have to be discarded.

Another method is to spread a drop of algal suspension on the surface of an agar plate and to incubate the latter for a few days until growth occurs. The plate is then examined under a microscope and areas showing the desired alga free (or remote) from unwanted organisms are selected. Small loops of agar from these selected areas are then picked up aseptically by means of a platinum or nichrome wire-loop, or a pipette and grown in liquid medium for subsequent examination.

Certain algae may best be isolated in water in which they grow in nature. This water may be sterilized by passing it through a sterile Millipore membrane filter (pore size 0.45 μ) and inoculated with single or a few cells or filaments of the alga collected from the same pond. Incubation of such crude cultures may be carried out by placing them in a north window, or by illuminating them with a 60-watt tungsten lamp. After the culture has established itself, it may be subcultured in a defined culture medium.

Many soil algae will grow in a biphasic soil water medium prepared by adding 6–10 ml of tap or pond water to 2–3 gm of soil in a cotton-plugged test tube and sterilizing in a steam sterilizer (without pressure) for a few hours. Sometimes addition of a few ml of Chu No. 10 medium to the soil water tubes may give better results, or the water may be entirely replaced by culture medium.

Although vigorous aseptic precautions are not needed for the crude and elementary culture work for demonstration purposes in a class room, yet it is advisable that the cultures are maintained in a unialgal condition and contamination prevented by adequate sterilization of glassware, media, inoculating needles, and pipettes. For work involving unialgal, bacteria-contaminated cultures, use of sterile rooms or inoculating hoods is unnecessary, and the inoculations or subculturing may be performed on a working bench in the laboratory.

Formation of zoospores, gametes, or other reproductive or vegetative stages in certain algae may be induced by modification of environmental and physiological conditions such as changes in intensity, quality or dura-

tion of light, pH, mineral balance (e.g., addition or depletion of a nitrogen source) and exposure to dry or wet conditions. Species of *Chlamydomonas* which form Palmella stages when grown on agar plates, can be brought into an active, motile phase by adding water. If this is done an hour before the commencement of a practical class, drops of liquid suspension from the agar plate can be taken for examination of living *Chlamydomonas* cells or zoospores. Likewise, if suspensions of cells from + and — strains of *Chlamydomonas* are obtained in this way and mixed, stages in conjugation and sexual fusion may readily be observed.

DEMONSTRATION OF SEXUAL CYCLE

Chlamydomonas is a good material for the study and demonstration of sexual stages in green algae (Hoshaw, 1961). The heterothallic species *C. reinhardii* and *C. moewusii* are especially suitable for demonstration of the sexual cycle. Cultures of their + and — mating types may be obtained from the Cambridge Collection or the Indiana University Culture Collection.

About a week before demonstrating sexuality, young subcultures of both mating types should be grown separately on agar medium (Chu No. 10 or soil water medium) in petri dishes (Fig. 11-3 A). When good growth has occurred, add 15–20 ml of sterile water on the surface of the agar culture and after a few hours, transfer 5–10 ml of the suspension into a sterile culture flask (Fig. 11-3 B) or tube and illuminate for 3–6 hr at 300–500 ft.c. The cultures are then covered with a black cloth or left in the dark overnight.

An hour before the practical class is scheduled to meet, the suspensions are transferred to light.

One drop each of the plus and minus mating type suspensions is then placed half an inch apart on a clean slide and the two drops are mixed by means of a needle. As soon as the drops are mixed, clumping of gametes starts and may be readily observed under low power of a microscope (Fig. 11-3 C). Numerous copulating pairs of gametes can be observed within a few minutes of mixing the two sexes.

After observing the clumping process, add a drop of IKI solution to the mixed suspension so as to kill the cells. Apply a coverglass and examine under high power of a microscope to observe quadriflagellate zygotes.

Mature zygospores can be successfully obtained when inocula from the complementary mating types are mixed and grown on the same place for 7–10 days. The plates are then stored in the dark for almost a month. Scrape off the mature zygospores from such plates by means of a sterile wire-loop and spread the scraping on the surface of a fresh agar plate in a few drops of sterile water.

Invert the inoculated petri dish over a petri dish bottom containing a few ml of chloroform to kill vegetative cells, if any, in the scrapings. Half a minute later, replace lid and incubate the culture plate in light for two days. Then apply a coverglass directly on the surface of the agar and ob-

serve the different stages of germinating zygospores under high power of a microscope (Fig. 11-3 D). Motile zoospores released from zygotes may be observed after the zygospores are covered with a drop of water.

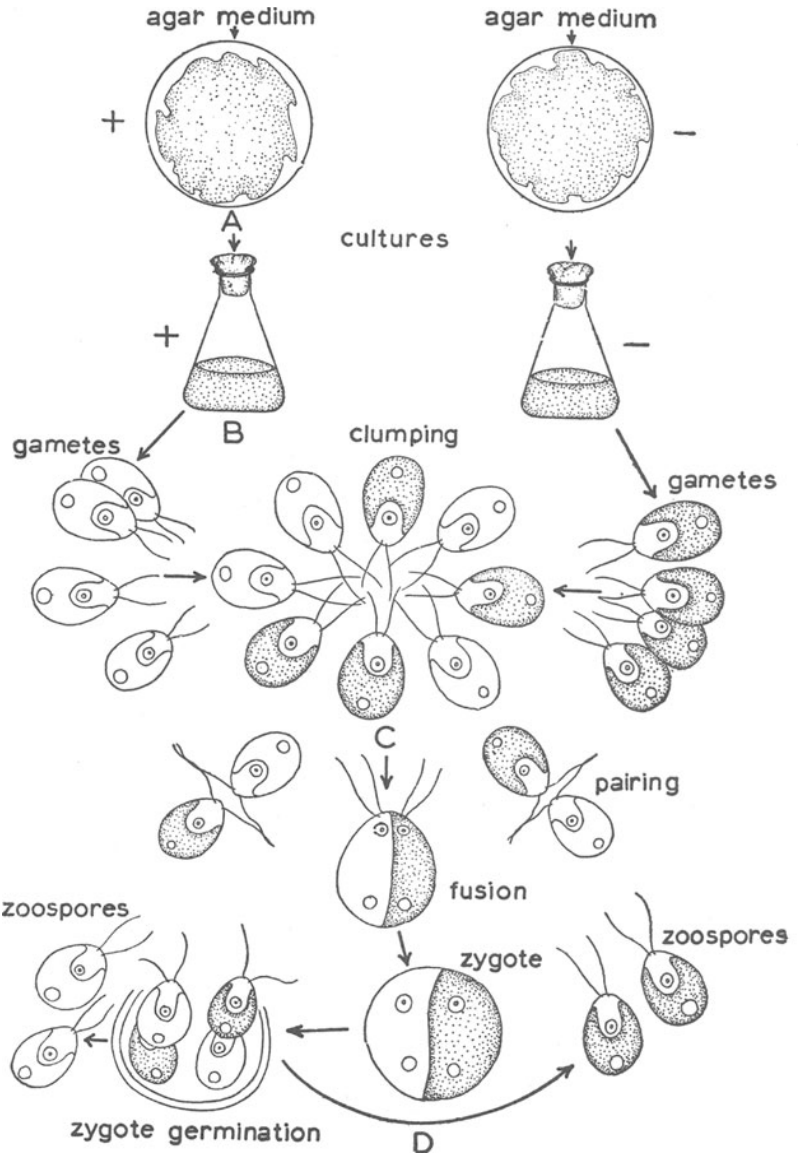

Fig. 11-3. *Chlamydomonas*, sexual cycle of heterothallic species. (After Hoshaw, 1961.)

Cultures of any suitable heterothallic macrandrous species of *Oedogonium* producing antheridia and oogonia on separate filaments can be used to demonstrate stages in the sexual cycle (Hoshaw, 1961). The formation of reproductive organs can be easily induced if freshly inoculated subcultures

are illuminated at 20–25°C, 300–500 ft.c. for a month or two, followed by incubation in very dim light. The following steps are suggested to demonstrate sexual phases: (1) add a few ml of soil water medium in a watchglass and place it in a petri dish; inoculate the medium with a small loopful each of male and female filaments and mix them gently; (2) add 10–15 ml of a 5% aqueous $NaHCO_3$ solution to the petri dish bottom, replace lid and keep the whole set-up at 25°C, 300–500 ft.c. (Fig. 11-4); the bicarbonate solution will enrich the CO_2 content of the atmosphere within the dish; (3) observe

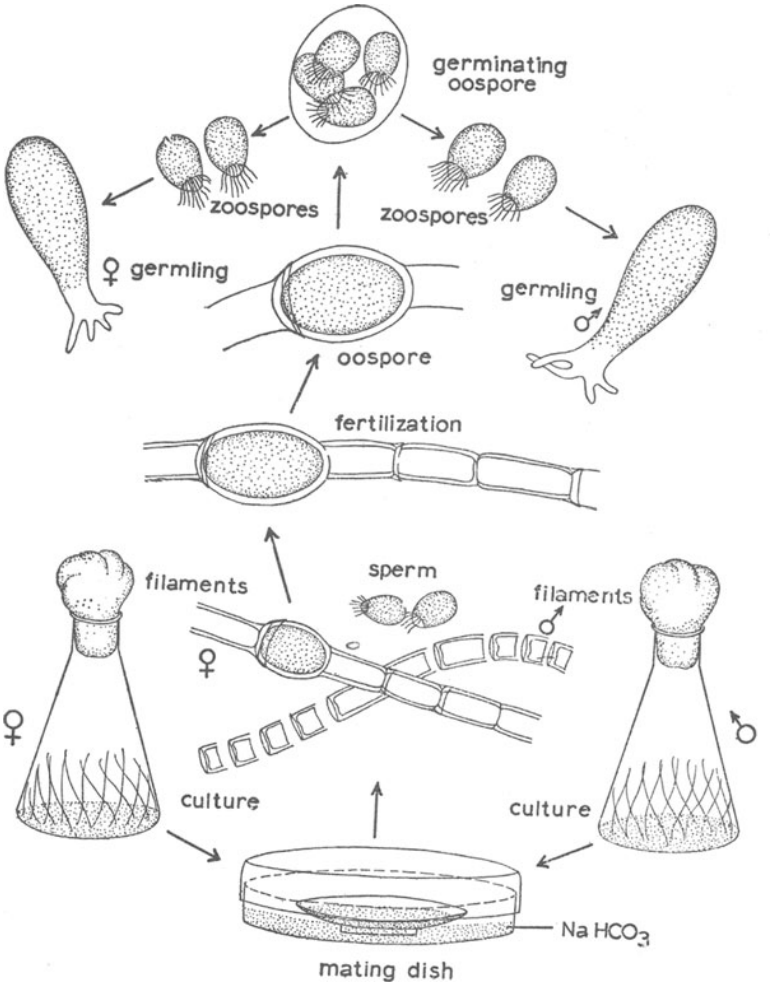

Fig. 11-4. *Oedogonium*, sexual cycle. (After Hoshaw, 1961.)

the filaments microscopically once every day and note the multiflagellate spermatozoids and the process of fertilization which may take place within three to five days after placing the mating dish in light (Fig. 11-4).

To demonstrate oospore germination, store the oospores dry for about a year. About a week before it is intended to demonstrate germination, the old and ripe oospores should be covered with soil water medium and incubated in light. They should be checked daily for the liberation of zoospores.

Sexual stages can also be readily demonstrated in the yellow-green alga, *Vaucheria sessilis*, grown in soil water medium containing a pinch of calcium carbonate. Freshly inoculated alga (in petri dishes containing liquid medium) should be incubated at low light intensity of about 300 ft.c. at room temperature. In such cultures, formation of antheridia and oogonia may be induced by giving light-dark periods of 16 hr–8 hr, respectively. When cultures become 12–15 days old, they may be continuously illuminated without any loss of sex organs.

GROWTH ESTIMATION

The growth of algae in relation to time can be estimated by recording the increase in cell numbers, optical density or dry weight at appropriate (fixed) intervals. In unicellular algae such as *Chlorella* or *Anacystis*, which grow in a well-suspended form, the cell count method is employed.

Cell Counting

This is done by means of a haemacytometer, originally devised for counting blood cells. Instructions for the use of different kinds of haemacytometers are supplied by their manufacturers and these should be consulted before using a particular make. A commonly used haemacytometer is a special type of glass slide which has a centrally located H-shaped groove which gives rise to two prominent ridges. Each ridge contains a big cubical chamber (1.0 mm long, 1.0 mm wide and 0.1 mm deep) which is partitioned equally into 25 smaller cubes by distinct narrow ridges, each consisting of 3 parallel lines. Some of the 25 chambers are again subdivided into 16 smaller cubes.

The length, breadth and depth of the area divided into 25 chambers are 1.0, 1.0 and 0.1 mm, or 0.1, 0.1 and 0.01 cm respectively. Hence its volume $= 0.1 \times 0.1 \times 0.01$ cm, i.e., 10^{-4} cc.

The volume of any one of the 25 chambers may also be calculated in the same way; it comes to 4×10^{-6} cc.

Similarly, the volume of one of the 16 smallest chambers will be 25×10^{-8} cc.

Having ascertained the volumes of the chambers, the cell numbers in a given suspension can be estimated by placing a drop on the H-shaped groove, applying the special kind of coverglass supplied with the haemacytometer, and counting the number of cells in the different chambers under a microscope. The cell numbers per cc of the suspension can then be calculated by multiplying by an appropriate factor.

Cell counting by means of a haemacytometer can be used to determine the growth curve of a unicellular alga such as *Anacystis nidulans*. Inoculate

culture flasks with a known number of exponentially-growing cells of *A. nidulans* and incubate the cultures at a constant temperature and light intensity (35°C, 1000–1200 lux). Withdraw small aliquots regularly at 24-hr interval and estimate cell numbers/ml. Continue the experiment till the cell numbers become constant or ultimately decline. Plot the $\log_{10}$ of cell numbers thus obtained against days after inoculation. A typical growth curve thus obtained may consist of: (1) a lag phase, (2) an exponential phase, and (3) a death phase (Fig. 11-5).

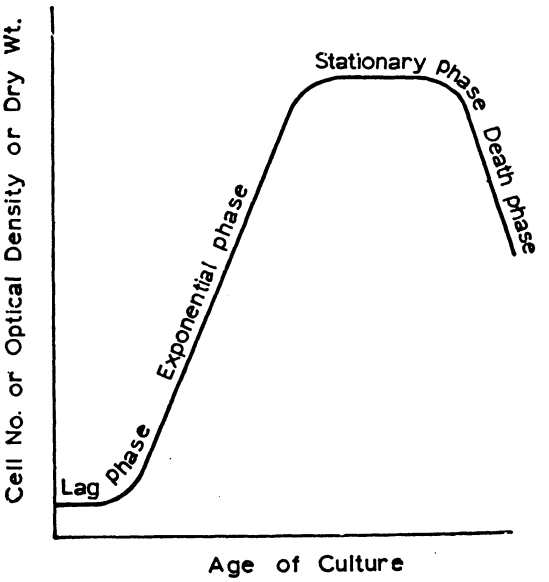

Fig. 11-5. Typical growth curve of an alga.

Optical Density Measurement

Growth estimation by optical density measurement is somewhat less reliable than cell counting. This is because the contents or proportions of different pigments are likely to vary with small changes in environmental factors and these changes are reflected in corresponding fluctuations in the optical density.

The optical density is generally determined in a photoelectric colorimeter (e.g., Bausch and Lomb Spectronic 20, Klett and Summerson colorimeter, and Zeiss Spekol spectrocolorimeter) set at an appropriate wavelength. Unicellular algae may be grown in optically matched test tubes of a size suitable for use in a given colorimeter. The optical density of the culture tubes can then be measured directly, using sterile medium (uninoculated) as the reference solvent. Since the increase in growth of the alga, at least up to the end of the exponential phase, is reflected in a corresponding increase in the optical density of its pigments, this method can be employed to estimate growth of the alga.

If the optical density of the culture tubes cannot be measured directly,

then 2–3 ml aliquots may be withdrawn aseptically from the culture tube or flask, and their optical density measured in cuvettes of 1 cm path length.

An appropriate cell number versus optical density calibration curve, at the particular wavelength used, must be plotted in order to correlate optical density with cell number.

Though somewhat unreliable, this method has the great advantage that growth in a large number of cultures can be estimated in a short time.

This method can also be applied to estimate the optical density of pigment extracts of algae instead of that of whole cells.

Dry Weight Determination

The growth of such algae as do not grow in homogeneous suspensions (e.g., filamentous forms) may be determined by harvesting the entire contents of a culture tube or flask by centrifugation. The algal pellet is suspended in distilled water, shaken for a few minutes and recentrifuged. The washed material is then quantitatively transferred to a weighed crucible. The crucible is later placed in an oven at 100°C for 10–12 hours and dried to constant weight. After removal from the oven, it is allowed to cool to room temperature in a desiccator and reweighed. The dry weight of the alga can thus be calculated.

TEST QUESTIONS

1. When algae can be cultured and studied in the laboratory, what is the need to study them in the field ?
2. Why is it not possible to make accurate counts of the filaments of *Ulothrix* or *Spirogyra* in a haemacytometer ? Explain.
3. What are oligotrophic, mesotrophic and eutrophic lakes ? Name some important factors that govern the distribution of aquatic algae.
4. What is the practical use of algal herbarium sheets and of formalin-preserved algae ?
5. In what ways do formalin-preserved *Ulothrix*, *Spirogyra* and *Oedogonium* differ from the respective fresh (living) forms ?
6. How does a blue filter help in the visual observation or microscopic study of the algal chromatophores ?
7. Compare the advantages and disadvantages of sterilization of algal culture media as carried out in an autoclave, a hot air oven, and a Millipore membrane filter.
8. Differentiate between unialgal and pure cultures. What is the importance of axenic and clonal cultures in algal research and for purposes of classroom demonstrations ?

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Glossary

acronematic (Gk. *akros*, tip; *nema*, thread). Flagella with slender and smooth surface and ending in a thin hair.

acyl lipid. Lipids joined to acids through anhydride bonds.

akinete (Gk. *a*, not; *kinein*, to move). A vegetative cell that becomes converted into a thick-walled non-motile resting spore; the wall of the cell becomes the wall of the spore.

algicide (L. *alga*, seaweed; *caedere*, to kill). A substance highly toxic to algae.

allelic genes (Gk. *allelon*, one another; *genos*, descent). A number of genes occupying the same locus on a linkage group or chromosome.

α-granules (Gk. *alpha*, first letter in alphabet; L. *granulum*, small grain). Submicroscopic granules rich in glycogen and found in the cells of blue-green algae.

androsporangium (Gk. *aner*, male; *sporos*, seed; *anggeon*, vessel). A sporangium producing androspore.

androspore (Gk. *aner*, male; *sporos*, seed). Antherozoid-like zoospore formed singly in an androsporangium; produces a dwarf male filament, as in Oedogoniaceae.

aneuploidy (Gk. *a*, without; *eu*, well; *aploos*, one fold). Chromosome number less or more than the exact multiple of haploid number.

anisogamy (Gk. *anisos*, unequal; *gametes*, spouse). Union between two morphologically dissimilar gametes.

antheridium (Gk. *anthos*, flower; *idion*, dim). Uni- or multicellular male gametangium.

antherozoids (Gk. *anthos*, flower; *zoon*, animal; *udos*, form). Sperms; male gametes.

aplanogametes (Gk. *a*, not; *planos*, wandering; *gametes*, spouse). Non-flagellate gametes showing amoeboid movement.

aplanospore (Gk. *a*, not; *planos*, wandering; *sporos*, seed). Non-motile spore in which the spore wall is not derived from the wall of its parent cell.

archegonium (Gk. *arche*, beginning; *gonos*, offspring). Multicellular female gametangium producing egg and consisting of neck and venter; includes sterile cells in addition to fertile egg.

areolae. Cavity-like depressions each covered with a perforated membrane, the sieve membrane.

asexual (Gk. *a*, without; *sexus*, sex). Lack of apparent sexual organs.

autospores (Gk. *autos*, self; *sporos*, seed). Non-motile spores resembling the parent cell in shape and structure.

autotrophic (Gk. *autos*, self; *trephein*, to nourish). Capable of producing the required food substances from inorganic raw materials.

auxospore (Gk. *auxein*, to increase; *sporos*, seed). Spores of diatoms.

benthos (Gk. *benthos*, depth of sea). Algae growing at the bottom of sea or lakes.

β-granules (Gk. *beta*, second letter in alphabet; L. *granulum*, small grain). Granules of proteinaceous nature confined to Cyanophyceae.

blepharoplast (Gk. *blepharis*, eyelash; *plastos*, formed). Granule lying at the base of a flagellum; gives rise to one flagellum.

canaliculi (L. *canaliculus*, small channel). Tubular canals present on the valve surface of diatoms.

carpogonium (Gk. *karpos*, fruit; *genos*, birth). Female gametangium in red algae; consists of a swollen base and an elongated neck or trichogyne.

carpospore (Gk. *karpos*, fruit; *sporos*, seed). Spore produced within a carposporangium of red algae.

carposporophyte (Gk. *karpos*, fruit; *sporos*, seed; *phyton*, plant). Carpospore-producing second generation plant of red algae; parasite on female gametophyte, arising directly or indirectly from zygote.

cellular (L. *cellula*, small room). Organisms made of conventional units of structure and function known as cells, each containing several proteins and both DNA and RNA; differ from **acellular**

entities which have very few proteins and either DNA or RNA but not both.

central nodule (L. *centrum*, centre; *nodus*, knob). A wall thickening in the centre of a valve in diatoms.

centric (L. *centrum*, centre). Refers to diatoms which are circular in valve view and have radial symmetry.

centrosome (Gk. *kentron*, centre; *soma*, body). A polar body at each pole of a dividing cell; the spindle is seen to diverge from these bodies.

chromatic adaptation (Gk. *chroma*, colour; L. *ad*, to; *aptare*, to fit). Capacity of some algae to synthesize pigments which are complementary to the quality of available light. Also known as **Gaidukov phenomenon.**

chromatophore (Gk. *chroma*, colour; *pherein*, to bear). Plastid containing chlorophyll-a and other pigments but not chlorophyll-b.

chromocentres (Gk. *chroma*, colour; *kentron*, centre). Feulgen positive bodies of unknown function found on the chromosomes of Phaeophyta.

chrysolaminarin. Leucosin; the food reserve of Chrysophyta.

cistron. Functional unit of gene which codes for a single polypeptide.

coccoid (Gk. *kokkos*, berry; *eidos*, form). Pertaining to habit; non-motile unicells.

coenobial. See **coenobium.**

coenobium (Gk. *koinos*, common; *bios*, life). Colony consisting of a definite number of cells arranged in a specific manner.

coenocytic (Gk. *koinos*, common; *kytos*, hollow). Multinucleate cell.

colligate (L. *colligare*, to bind together). Septum showing H-shaped structure.

colonial (L. *colonia*, form). Habit showing number of cells held together within an envelope.

conceptacles (L. *concipere*, to conceive). Cavity-like depressions on a receptacle of the Fucales that contain gametangia.

conjugation (L. *cum*, together; *jungare*, to yoke). Sexual union in Conjugales involving fusion between amoeboid gametes.

contractile vacuoles. Organelles of osmoregulation; also thought to play a role in the excretion of waste material.

costae (L. *costa*, rib). Thickened regions of the valve which separate the rows of areolae from each other.

cryptoblasts, cryptostomata (Gk. *kryptos*, hidden; *stoma*, mouth). Sterile conceptacles found in Fucales.

cyanophage (Gk. *kyanos*, blue; *phagein*, to eat). Virus that infects certain blue-green algae.

cyanophycin granules (Gk. *kyanos*, blue; *phykos*, seaweed; L. *granulum*, small grain). Proteinaceous food reserve occurring in granular form in cells of blue-green algae.

cyst (Gk. *kystis*, bladder). Resting cells with a thick envelope.

cystocarp (Gk. *kystis*, bladder; *karpos*, fruit). Fruiting body of red algae; aggregate structure consisting of carposporangia and sterile covering cells.

cytopharyngeal (Gk. *kytos*, hollow; *pharynx*, gullet). Pertaining to cytopharynx, i.e., gullet, reservoir and vacuoles in *Euglena.*

dendroid (Gk. *dendron*, tree; *eidos*, form). Tree-like habit with much branching. In certain colonial algae, tree-like habit is achieved by repeated branching of mucilaginous stalks.

derepression. Release of the inhibition of enzyme synthesis.

developmental (F. *developper*, to unfold). Changes that organism undergoes during its vegetative or body organization.

diaminopimelic acid. Amino acid with two amino groups; has a definite chemical structure.

dichotomy (Gk. *dicha*, two; *temnein*, to cut). Repeatedly bifurcating pattern of branching.

dictyosomes (Gk. *dictyon*, net; *soma*, body). Vesicular structures of unknown functions found in the cytoplasm of eucaryotic cells. Also known as **Golgi bodies.**

diffused centromeres. Chromosome containing many scattered centromeres along its length.

diffuse (L. *diffundere*, to pour). Pertaining to growth in which every vegetative cell is capable of growth and division.

dimorphic (Gk. *dis*, twice; *morphe*, shape). Where gametophytic and sporophytic generations are morphologically distinct.

dioecious (Gk. *dis*, twice; *oikos*, house). Location of male and female sex organs on separate plants.

diplohaplont (Gk. *diploos*, double; *haploos*,

simple; *on*, being). Where diploid and haploid generations alternate successively.

diplont (Gk. *diploos*, double; *on*, being). Diploid plant in which the only haploid stage consists of gametes.

encapsulated (Gk. *en*, in; L. *capsula*, little box). Pertaining to habit; a sac-like covering enclosing an organism.

endophyte (Gk. *endon*, within; *phyton*, plant). A plant living within another plant.

endoplasmic reticulum. Fine tubular or vesicular structures traversing the cytoplasm of eucaryotic cells.

endospore (Gk. *endon*, within; *sporos*, seed). Internally formed thin-walled spores of Cyanophyta, analogous to aplanospores.

epitheca (Gk. *epi*, upon; *theke*, box). The larger half of the diatom frustule which covers the smaller half (**hypotheca**).

eucaryota (Gk. *eu*, well; *karyon*, nucleus). Cellular organisms, in which genetic, respiratory and photosynthetic apparatuses are organized into nucleus, mitochondrion and chromatophore, respectively.

eutrophic (Gk. *eu*, well; *trophe*, nourishment). Pertaining to habitats rich in nutrients and organic matter for the growth of algae and other plants and microorganisms.

exospores (Gk. *exos*, outward; *sporos*, seed). Spores produced externally or outwardly as in Cyanophyta; analogous to aplanospores.

eye spot. Red-coloured spot (**stigma**) believed generally to have a visual function.

false branching. Branching resulting from the degeneration of a cell in a loop or from growth of free ends of trichome through filament sheath, as in some blue-green algae.

filamentous (L. *filum*, thread). Thread-like photosynthetic plants.

flagella (L. *flagellum*, whip). Fine, thread-like structures by the activity of which the cells move.

fucosan vesicles (L. *fucus*, seaweed; *vesicula*, small bladder). Sac-like structures in the cells of brown algae; contain phenolics and tannins.

gamete (Gk. *gametes*, spouse). A sex cell; two gametes of opposite sex unite to form a zygote.

gas vacuoles. Gas-filled cavities in cells of certain planktonic blue-green algae which disappear when subjected to pressure. Also known as **pseudovacuoles**.

girdle view. Side view of a diatom that reveals the junction of epitheca and hypotheca.

globule (L. *globulus*, small globe). Male reproductive organs of Charales having a jacket of sterile cells around the fertile cells; analogous to antheridium.

glomerule (L. *glomus*, ball). Cluster of branches at the node, as in *Batrachospermum*.

glucosamine. A glucose derivative containing amino group of the second carbon of the 6-carbon molecule.

glycolipids (Gk. *glykys*, sweet; *lipos*, fat). Compounds made of lipids and galactose.

gonimoblasts (Gk. *gonimos*, productive; *blastos*, bud). Filaments formed from the zygote in red algae; bear carposporangia.

gonospores (Gk. *gonos*, offspring; *sporos*, seed). Spores produced following meiosis; also called meiospores.

gynandrosporous (Gk. *gyne*, woman; *aner*, man; *sporos*, seed). Species bearing both oogonia and androsporangia on the same filament.

haematochrome (Gk. *haima*, blood; *chroma*, colour). Orange or red oily pigment related to carotene and present in resting cells and eye spots of certain algae.

haplont (Gk. *haploos*, simple; *on*, being). Haploid plant in which the only diploid stage is confined to the zygote.

heterocyst (Gk. *heteros*, other; *kystis*, bladder). Specialized enigmatic cell found in certain blue-green algae.

heterokaryosis (Gk. *heteros*, other; *karyon*, nucleus). Association of nuclei of different genetical constitutions in a vegetative cell.

heteromorphic (Gk. *heteros*, other; *morphe*, shape). Life cycle involving alternation between morphologically dissimilar generations.

heterothallic (Gk. *heteros*, other; *thallos*, young). Self-incompatibility; sexual fusion occurs only between gametes of different parentage or plants.

heterotrichous (Gk. *heteros*, other; *thrix*, hair). Thallus differentiated into a prostrate and an erect system of branch-

ing filaments.

heterotrophic (Gk. *heteros*, other; *trophe*, nourishment). Organisms dependent on exogenous organic sources for their metabolism and growth.

hologamy (Gk. *holos*, whole; *gametes*, spouse). Fusion of mature individuals, i.e., mature individuals directly act as gametes.

holophytic (Gk. *holos*, whole; *phyton*, plant). Plant-like mode of nutrition involving photosynthesis.

holozoic (Gk. *holos*, whole; *zoon*, animal). Feeding like animals by ingesting solid food.

homothallic (Gk. *homos*, same; *thallos*, young shoot). Self-compatible; fusion can occur between gametes derived from the same plant.

hormocyst (Gk. *hormos*, chain; *kystis*, bladder). Thick-walled hormogonium or multicellular akinete found in a few blue-green algae.

hormogone (Gk. *hormos*, chain; *gone*, generation). Short piece of trichome consisting of undifferentiated vegetative cells which are moniliform; hormogonium is generally motile and is meant for propagation.

hypnospore (Gk. *hypnos*, sleep; *sporos*, seed). Thick-walled spore; meant for perennation.

hypotheca (Gk. *hypo*, under; *theke*, box). Inner and smaller valve of a diatom frustule.

idioandrosporous (Gk. *idios*, distinct; *aner*, male; *sporos*, seed). Species bearing androsporangia and oogonia on separate filaments.

intercalary (L. *intercalaris*, inserted). Growth pattern in which newly formed cells are produced between two existing cells, e.g., of a filament.

isogamy (Gk. *isos*, equal; *gamos*, marriage). Fusion between morphologically and physiologically similar gametes.

isomorphic (Gk. *isos*, equal; *morphe*, form). Life cycle involving alternation between two morphologically similar generations.

laminarin. Polysaccharide food reserve in brown algae.

leucosin (Gk. *leukos*, white). Highly refractile polysaccharide with β-1,3 linkages which forms the food reserve in Chrysophyta and Xanthophyta; chemically similar to laminarin; sometimes also called chrysolaminarin.

loop-formation. Arch or dome formation; achieved by rejuvenation of growth and cell division in certain cells of the trichome of Scytonemataceae.

macrandrous (Gk. *makros*, large; *aner*, male). Filaments producing antheridia similar in size and morphology to those producing oogonia; sexually monomorphic plants, as in *Oedogonium*.

meiospores (Gk. *meion*; less; *sporos*, seed). Spores formed after meiosis in the zygote.

mitochondria (Gk. *mitos*, thread; *chondros*, grain). Cytoplasmic double-membraned organelles concerned with energy release in respiration.

mitospores (Gk. *mitos*, thread; *sporos*, seed). Spores formed after mitosis; may be haploid or diploid.

monoecious (Gk. *monos*, single; *oikos*, house). Plant bearing both male and female sex organs on the same individual.

monomorphic (Gk. *monos*, single; *morphe*, form). Formation of only one kind of plant in the life cycle.

mucopeptides. Compounds made of carbohydrates and amino acids, carbohydrates are N-acetylglucosamine and N-acetyl muramic acid; amino acids are glutamic acid, alanine, glycine, aspartic acid, lysine, or diaminopimelic acid.

multiaxial (L. *multi*, many; *axis*, axis). Formation of main axis of the thallus by a group of branched filaments.

multiplicative (L. *multiplicare*, to make manifold). Process leading to increase in number of an organism or its cells.

muramic acid. It is a glucosamine derivative containing carboxyethyl group at 3-0 position, e.g., 3-0-carboxyethyl-D-glucosamine.

nannandrous (Gk. *nanos*, dwarf; *aner*, male). Sexually dimorphic plants as in Oedogoniaceae with a dwarf male.

nucleolar organizing chromosome. Chromosome concerned with formation of nucleolus.

nitrogenase. Enzyme concerned with conversion of molecular nitrogen to ammonia.

nucule (L. *nucula*, small nut). Female reproductive organ of Charales.

oligotrophic (Gk. *oligos*, few; *trophe*. nourishment). Habitats relatively poor in nutrients.

oogamy (Gk. *oon*, egg; *gamos*, marriage). Fusion of a motile sperm with a large passive non-motile egg.

ostiole (L. *ostiolum*, little door). Opening or pore of a conceptacle of the Fucales.

ovum (L. *ovum*, egg). Female, non-motile gamete or egg cell.

oxidation pond. An enclosure for sewage designed to promote digestion of sewage with the help of oxygen released by algae during photosynthesis.

palmella stage (Gk. *palmos*, quivering). Temporarily non-motile sedentary stage in the life history of certain motile algae; cells remain passive and embedded in gelatinous matrix.

palmelloid (Gk. *palmos*, quivering; *eidos*, form). Palmella-like habit.

pantonematic (Gk. *pantos*, all; *nema*, thread). Flagellum in which the surface is covered with hair-like appendages.

parasexual (Gk. *para*, compared with; *sexus*, sex). Organisms showing genetic recombination not involving regular alternation between karyogamy and meiosis.

parenchymatous (Gk. *para*, beside; *engchyma*, infusion). Pertaining to a tissue composed of thin-walled and living cells.

periplast (Gk. *peri*, around; *plastos*, moulded). Delicate protective covering of flagellates that lack cell wall.

phototaxis (Gk. *photos*, light; *taxis*, arrangement). Movement oriented in response to light.

phycobilisomes (Gk. *phykos*, seaweed; L. *bilis*, bile; Gk. *soma*, body). Particles containing phycobilin, phycocyanin and phycoerythrin pigments.

plakea stage (Gk. *plakoeis*, flat cake). The curved plate-like, 8-celled stage in the development of a coenobium.

plane (L. *planus*, plain). Pertaining to septa with smooth flat surface.

plasmodesmata (Gk. *plasmor*, form; *desma*, bond). Cytoplasmic strands connecting adjoining cells and linking cytoplasm.

polar nodules (Gk. *polos*, pole; L. *nodulus*, small knob). Wall thickenings at the two poles in a diatom frustule.

polyhedral bodies. Polygonal particles found in cells of blue-green algae; their function and chemistry are not known.

polyphosphate granules. Polymers of inorganic phosphate stored in algal cells; occur in granular form.

polyploidy (Gk. *polys*, many; *aploos*, one fold; *eidos*, form). Cells containing three, four or more times the haploid number of chromosomes.

procaryota (Gk. *pro*, before; *karyon*, nucleus). Cellular organisms lacking membrane-bound genetic, photosynthetic and respiratory organelles.

propagule (L. *propagare*, to propagate). A few-celled branch serving as a means of vegetative propagation, as in Sphacelariales.

protandrous (Gk. *protos*, first; *aner*, male). Hermaphrodite organisms in which the male reproductive structure matures earlier than female.

pseudoparenchymatous (Gk. *pseudo*, false; *para*, beside; *engchyma*, infusion). Collection of cells, filaments or hyphae forming a tissue which resembles parenchyma.

pseudoraphe (Gk. *pseudo*, false; *raphe*, seam). False raphe; longitudinal space on the valve of a diatom bounded on both sides by striae.

punctae (L. *punctum*, point). Perforations in the wall of diatoms.

raphe (Gk. *raphe*, seam). Longitudinal cleft seen on the valve surface of a diatom.

receptacle (Gk. *recipere*, to receive). Tip of branch of thallus bearing conceptacles as in the Fucales.

recombinants. Offspring with new combination of genes different from either parent.

replicate (L. *replicare*, to fold back). A septum showing two circular infoldings, one on either side.

repression. Inhibition of enzyme synthesis.

reproductive (L. *re*, again; *producere*, to lead forth). Processes leading to the continuation of species or races.

rhizopodia (Gk. *rhiza*, root; *podos*, foot). Unicellular organisms capable of forming pseudopodia or false feet for locomotion or anchorage.

rhizopodial (Gk. *rhiza*, root; *podos*, foot). Type of habit in which unicellular organisms form pseudopodia as locomotory organs.

satellite chromosomes (L. *satelles*, attendant; Gk. *chroma*, colour; *soma*, body).

Short segments of chromosomes constricted from the rest of the chromosomes.

semireplicate (L. *semi*, half; *replicare*, to fold back). Only one side of septum wall showing circular infolding.

silicalemma (L. *silix*, flint; Gk. *lemma*, husk). Triple membrane system concerned with deposition of silica walls in diatoms.

simultaneous division. Nuclear and cytoplasmic divisions occurring concurrently.

siphoneous (Gk. *siphon*, tube). Tubular thallus in algae lacking septa or cross walls during vegetative phase of growth.

spermatium (Gk. *sperma*, seed). Non-flagellated naked male gamete of red algae.

spermocarp (Gk. *sperma*, seed; *karpos*, fruit). Fruiting body consisting of sterile jacket of cells around a fertilized oogonium, as in *Coleochaete*.

stigma (Gk. *stigma*, mark). Eye spot or a red spot of the flagellates found within the cell near or inside the chromatophore. It is believed to have a regulatory function in cell motility.

stipe (L. *stipes*, stalk). Stalk; portion of kelp between holdfast and blade.

subaerial (L. *sub*, under; *aer*, air). Referring to habitats that are well raised above soil surface, e.g., tree barks, rocks, and stones.

successive division. Nuclear and cytoplasmic divisions occurring one after the other.

synaptonemal complex. Characteristic structures corresponding to bivalents in meiotic nuclei and seen under electron microscope.

synzoospore (Gk. *syn*, with; *zoon*, animal; *sporos*, seed). Compound, multiflagellate and multinucleate asexual spore found in *Vaucheria*.

tetraspore (Gk. *tetras*, four; *sporos*, seed). One of the four non-motile haploid spores formed in tetrasporangium in red algae.

thylakoid. Structural unit of lamellar system forming a double membrane disc; a photosynthetic lamella.

transcription. Process in which DNA is copied into messenger RNA.

transduction (L. *transducere*, to transfer). Process of genetic recombination in which DNA of a donor bacterium is carried to the recipient by a virus.

transformation (L. *transformare*, to change in shape). Process of genetic recombination in bacteria affected by the incorporation of naked DNA from donor bacterium.

trichothallic (Gk. *thrix*, hair; *thallos*, young shoot). Intercalary growth in which meristem is located at the base of a hair.

trumpet hyphae. Long tubular achlorophyllous cells of medulla found in brown algae; conduct water and nutrients.

unduliseptate (L. *undulatus*, risen like waves; *septum*, partition). Septum with wavy margins.

uniaxial (L. *unus*, one; *axis*, axis). Plant axis made of a single filament or its branches.

vacuolar apparatus (L. *vacuus*, empty; *ad*, to; *paratus*, prepared). Includes two kinds of organelles, the contractile vacuoles and the cytopharyngeal apparatus consisting of gullet, reservoir, and a number of small contractile vacuoles surrounding the reservoir.

vacuole (L. *vacuus*, empty). Small, usually spherical space within a cell, bounded by a membrane and containing some fluid sap.

valve (L. *valva*, fold). One of the two parts of a diatom theca; the other is the connecting band.

valve view. Top surface of epitheca or hypotheca of a diatom.

vegetative (L. *vegetare*, to enliven). Formation of plant body lacking reproductive structures or organs.

water bloom. Luxuriant growth of one or a few species of algae in water, often imparting colour to water.

zoogamete (Gk. *zoon*, animal; *gametes*, spouse). Motile flagellate gamete.

zoospore (Gk. *zoon*, animal; *sporos*, seed). Motile flagellated asexual cell.

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