ULTRASTRUCTURAL AND EXPERIMENTAL STUDIES ON THE DEVELOPMENT OF *FELDMANNIA* SP. (ECTOCARPALES, PHAEOPHYCEAE).

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and finally to my parents, sister and Terry to whom this work is dedicated.

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DECLARATION

I declare that this dissertation is my own, unaided work. It is being submitted for the degree of Master of Science in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any University.

Sandra Duff

2nd day of August, 1989.

ABSTRACT

Feldmannia sp., a small filamentous member of the Ectocarpales, was isolated from Rocky Bay, Natal. The morphology and development of the thallus under controlled conditions has been described.

Use was made of both optical and electron microscopy, in an investigation of the development of plurilocular sporangia, the development of motile cells, their release and the subsequent development of the sporelings into mature reproductive plants.

Ultrastructural studies on plurilocular sporangia have revealed stages in development of the motile cells. Pronounced changes occurred just prior to motile release, where the cytoplasm pulls away from the cell wall and flagella and intraplastidial eyespots become apparent. Possible stages in locule cell wall formation were also observed. Localization of growth regions has been made by treating growing filaments with the optical brightener Calcofluor White M2R.

The influence of photoperiod, photon fluence, light quality and temperature on changes in mean cell numbers and the onset of a reproductive phase has also been examined. Extreme conditions inhibit growth. The following growth parameters have been found to promote growth and reproduction. Photoperiods of 12L:12D, 16L:8D, and 14L:10D, photon fluences of 85 to 114$\mu Em^{-2}sec^{-1}$, and temperatures ranging from 20-30°C proved to be conducive to good growth. Blue and green light was found to enhance growth.

At no stage were any unilocular sporangia, usually associated with the sporophyte generation, produced, which may indicate that the original stock may be a gametophyte.

I. INTRODUCTION

The Phaeophyta are a large group of algae, constituting a high percentage of the total marine flora, particularly in the northern hemisphere. They occupy a diversity of niches and associated with this, a wide range of thallus forms. The range of forms includes the large kelps, which may reach 70 metres in length (e.g. Laminaria sp. Lamouroux), to the smaller encrusting forms (e.g. Ralfsia sp. Berkeley), which may be as small as a millimetre. Other forms include the fine, filamentous forms (e.g. Ectocarpus sp. Lyngbye). Some members may be epiphytic (e.g. Streblonema sp. Derbes et Solier), while most are free living, often attached firmly to the substrate by elaborate holdfast systems. Common to all brown algae is the presence of the pigments xanthophyll, fucoxanthin and violaxanthin, which gives them their characteristic colouring.

There are a range of life histories in the brown algae. More is known about the life histories of the larger, more common members such as the Laminariales and Fucales, since more research has been conducted on these orders. Central to understanding the life history in the algae, is a knowledge of the stages of development throughout the life of the algae, under the full range of environmental conditions to which the algae are naturally exposed. Knowledge of the developmental stages of an alga involves observations on the establishment and germination of spores\gametes, mode of growth and branching of the vegetative thallus, stages in the development of reproductive structures and the mechanism of release of motile cells. Early studies on life histories (e.g. Ectocarpus siliculosus Dillw., Knight 1929) relied on observations of developmental stages at the light microscope level. These studies have now been supplemented with the advent of the electron microscope, resulting in a better understanding of our knowledge of the ultrastructure of reproductive structures. Ultimately, determination of the life history strategy often provides an indication of the evolutionary status of the alga within a genus.

In this investigation Feldmannia sp. a filamentous member of the

Ectocarpales, has been studied. Emphasis has been placed on the development and ultrastructure of reproductive structures, as well as the influence of different environmental factors on growth and reproduction of the species.

1. LITERATURE REVIEW

1.1. Division Phaeophyta

The approximately 1500 species belonging to 250 genera recognised in this division are largely marine, traditionally occupying a position along the shore somewhere between the Chlorophyta and Rhodophyta. Characteristically the Phaeophyta are brown, but this is not always a reliable taxonomic feature. More reliable taxonomic criteria require the use of the light microscope, chemical analysis using electrophoresis, and in some cases the transmission electron microscope. These include:

- the pigments chlorophyll a and c, ß carotene, violaxanthin and fucoxanthin (Bold & Wynne 1978),
- the storage product laminarin (Bold & Wynne 1978),
- cell walls comprising an inner cellulosic layer and an outer slimy layer, both walls containing alginic acid (Clayton & King 1981),
- pyrenoids which are often associated with a sheath of polysaccaride reserves and are often of the stalked variety (Bold & Wynne 1978),
- chloroplasts containing thylakoids in groups of threes, girdle lamellae and a genophore or DNA zone beneath the girdle lamellae are present (Clayton & King 1981),
- gametes or asexual zoospores are asymmetrical, often reniform in shape and possess dimorphic, laterally or sub-apically inserted flagella. The long propelling front flagellum bears mastigonemes whilst the posterior flagellum is smooth (Bold & Wynne 1978).

Life histories in the Phaeophyta range from the simple diploid

haplobiontic to the diploid diplobiontic type. In the more 'primitive' orders sexual reproduction involves isogamy or anisogamy. The more 'advanced' orders have oogamous reproduction and produce well defined antheridia and oogonia (Table 1.). Traditionally, the order Ectocarpales, having an isomorphic life history, was considered to have been the ancestor of other orders (Fritsh 1945, Papenfuss 1953 in Clayton 1988), More recently evidence has been put forward to suggest that marine, benthic Chrysophytes, probably parenchymatous in form, may have been the ancestors of the brown algae (O'Kelly & Floyd 1985). Clayton (1988) suggests that the possibility of an ancestor having a heteromorphic life history is not out of the question.

The Phaeophyta have many different modes of asexual reproduction. Gametophyte and sporophyte generations of many species reproduce by means of asexual zoospores and vegetative multiplication by fragmentation, special propagules and basal parts of thalli, growing out to produce new fronds.

Table 1.: Characteristics of the orders of the Phaeophyta
(Taken from Clayton & King 1981).

SEXUAL REPRODUCTION- ISOGAMOUS OR ANISOGAMOUS; GAMETAMGIA- PLURILOCULAR; GENERATIONS ISO- OR HETEROMORPHIC	
Primary sites of cell division leading to growth: Description	Order
- intercalary, or apical;	1.ECTOCARPALES
- localised at the base of apical hair-like filaments;	2.CUTLERIALES
- prominent apical cell.	3.SPHACELARIALES
SEXUAL REPRODUCTION- OOGAMOUS; GAMETANGIA- ANTHERIDIA AND OOGONIA	
Primary sites of cell division leading to growth:	
- localised at the base of apical hair-like filaments; generations heteromorphic;	4.DESMARESTIALES
- intercalary or apical meristematic regions, and meristoderm; generations heteromorphic;	5.LAMINARIALES
- apical cells and/or adjacent tissue (except Durvilleae). Antheridia and oogonia borne on the thallus surface, generations isomorphic;	6.DICTYOTALES
Antheridia and oogonia in conceptacles, gametophyte generation only.	7.FUCALES

1.2. Order Ectocarpales

This most primitive of the orders of the brown algae (Fritsch 1945) is commonly filamentous and is characterised by its simple vegetative form and reproductive structures. Classification of families, genera and species is based on morphological and anatomical features, e.g.

- relative dominance of the prostrate and erect filament systems,
- the presence of hairs and pseudohairs,
- the presence of ascocysts or paraphyses,
- the shape of plastids,
- the shape of plurilocular sporangia and size of the loculi,
- whether or not the plurispores are of one type,
- diffuse growth meristems, which may be apical or intercalary.

The life history in the Ectocarpales theoretically involves an alternation of a haploid gametophyte and diploid sporophyte (Figure 1.). Muller (1975) has shown that modifications to the typical life cycle (Figure 1.) are evident and that gametophytes could be haploid and sporophytes could be haploid, diploid or triploid. The gametophyte and sporophyte are to some degree heteromorphic, depending on the combination of unilocular and plurilocular reproductive structures between generations.

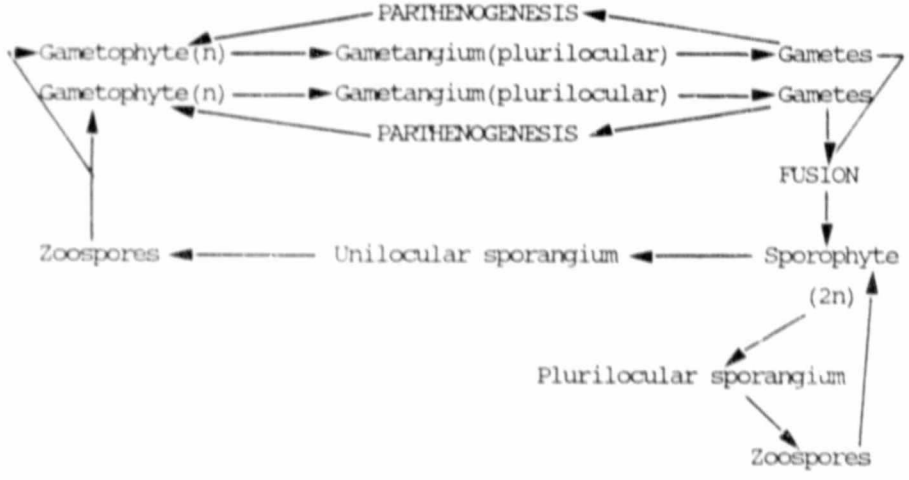

Figure 1.: The life cycle of Ectocarpus siliculosus (Dillw) Lyngbye (Bold & Wynne 1978)

In some members of the Ectocarpales only the sporophyte generation has been observed (Pedersen 1974, Clayton 1984). Müller (1962 & 1963) found that a plurilocular sporangia-bearing Ectocarpus Lyngbye placed in temperatures below 14°C, produced unilocular sporangia, while, at 16°C both unilocular and plurilocular sporangia were produced, and above 19°C, only plurilocular sporangia were produced. A number of studies however, have shown the inability of plurilocular sporangia-bearing plants to produce unilocular sporangia when subjected to a range of environmental conditions. For example, Sorocarpus micromorus (Bory) Silva (Pedersen 1974) was cultured under varying photon fluences, temperatures and photoperiods and none of the conditions produced plants with unilocular sporangia. Studies on two clones of Myriotrichia clavaeformis Harvey (Pedersen 1978) revealed that the clone with only plurilocular sporangia never formed unilocular sporangia, although cultivated under the same conditions which gave abundant unilocular sporangia in the other clone.

1.3. Genus Feldmannia Hamel

Feldmannia was first described by Hamel (1939) and named after the French phycologist Jean Feldmann. Ravanko (1970) did not recognise this genus. She regarded it as a stage in the development of other genera within the Ectocarpales, which have diverged morphologically due to environmental conditions. This 'variant theory' was challenged by Clayton (1972), who argued that divergent morphologies observed in members of the Ectocarpales was the result of contaminants, which in nature grow in close association with species of the Ectocarpales.

Feldmannia is now accepted as a valid genus by Clayton (1974), Abbott and Hollenberg (1976) and others and there is general agreement on the following morphological characteristics used to identify the genus;

(i) large degree of branching near the base, producing a tufted appearance;

(ii) meristematic regions occurring near the base and occasionally secondary and tertiary zones at distal ends of filaments;

(iii) usually epiphytic but whose base may become endophytic as in the case of Feldmannia padinae (Buffh) Hamel;

(iv) plastids of lower cells are discoid to rod-shaped, but more elongated and rectangular in upper cells;

(v) plurilocular sporangia frequently stalked, occasionally sessile and mostly occurring below the meristem.

There is some disagreement in the thallus features put forward by Abbott and Hollenberg (1976) and Clayton & King (1981). Abbott and Hollenberg (1976) propose that sterile, hair-like, filaments are usually present in members of this genus, while Clayton & King (1981) attributes this feature to the genus Ectocarpus.

Vegetative reproduction by fragmentation has not been observed, however, occasionally stolon-like filaments are formed which may give rise to new plants.

1.4. The taxonomy of the Order Ectocarpales

Members of the filamentous, brown algae have very few distinctive taxonomic characters, making classification into orders, genera and species difficult. It is not surprising then that numerous alterations to the existing classificatory schemes have been made over the years. A review of the changes in the classification of the Ectocarpales from its inception to its present status was made by Clayton (1974).

The Ectocarpales were originally divided into a large number of families, first by Thuret (in Le Jolis 1864), then by Kjellman (1872 in Hamel 1939), however, the filamentous Phaeophyta did not as yet have individual taxonomic status. The family Ectocarpaceae was erected by Oltmanns (1904 in Clayton 1974) and comprised all brown algae producing unilocular and plurilocular sporangia. The order Ectocarpales was recognised independently by Oltmanns (1922 in Clayton 1974) and Setchell and Gardner (1922 in Clayton 1974). Kylin (1933 in Clayton 1974) proposed that the Ectocarpales contain only those species which were entirely filamentous and he removed all

the pseudoparenchymatous forms to the Dictyosiphonales. Since there are species morphologically intermediate in form in the Ectocarpales, Chordariales, Scytosiphonales, and Dictyosiphonales, a complete acceptance of the filamentous nature as characteristic of the Ectocarpales, has not been achieved.

Russell (1964) suggested that life histories could be used as a taxonomic characteristic to clarify the status of these forms which are intermediate between the Ectocarpales, Scytosiphonales, Chordariales and Dictyosiphonales. His need for review of the existing ordinal status was fuelled by discoveries that some species of Scytosiphonales are linked, by life history, to encrusting members of the Ectocarpales. Streblonema anomalum Setchell & Gardner was shown to have an alternative existence as a species of Scytosiphon C.Agardh. (Loiseaux 1973). However, due to the variability in the life histories of these algae, the use of life histories as a taxonomic criterion seems unreliable (Clayton, 1984).

Classification of the orders within the Phaeophyta, presently relies on anatomical features of the thallus. The uniformity of chloroplast ultrastructure, as found by Evans (1966, 1968 in Clayton 1987), Hori (1971, 1972 in Clayton 1987),and Asensi et al.(1977 in Clayton 1987), has been put forward as a potential taxonomic tool. Many species, however, have not yet been investigated and studies on Scytothamnus australis (J.Agardh) Hooker & Harvey and S.fasciculatus Hooker & Harvey (Clayton 1986) have revealed changes in chloroplast morphology during sporogenesis. This stresses the need for caution in using chloroplast ultrastructure in the taxonomy of brown algae.

Life histories in some orders e.g. Fucales, Sporochnales and Laminariales are so uniform that they have become a useful taxonomic character (Clayton 1980). Use of life histories in classification of other orders is less accurate, since often this is based on limited experimental studies and making assumptions e.g. that the possession of uni'ocular sporangia denoted a diploid sporophyte. Orders that have a large number of members with gaps in their life histories e.g. Ectocarpales, should obviously not be classified according to their life history (Clayton 1986). Studies of S.australis and

S.fasciculatus (Clayton 1986) revealed how easy it is to overlook sexual reproduction in the life history and assume that the life cycle is asexual. Sexual reproduction only occurred in highly specific environmental conditions and the process of isogamy was infrequent. The documentation of numerous asexual life histories in the Ectocarpales (Pedersen 1983, Wynne & Loiseaux 1976, both in Clayton 1986) need careful reassessment and experimentation before they can be accepted as the complete life history (Clayton 1986).

Filamentous, brown algae were originally all classified in the genus Ectocarpus. Many genera, however, have since been erected.

The taxonomy of the Ectocarpales has recently been revised by Clayton (1974). Her attempts at obtaining some form of taxonomic order has led to studies on thallus development, life histories and more recently ultrastructural studies on members of the Ectocarpales. Since as yet no better means of classifying genera in the order has been proposed, the morphological characteristics as defined by Clayton (1974) will be maintained. To quote:

" The filamentous, brown algae have few enough taxonomic characters and those which are valid should be retained (Clayton 1974)."

Studies on individual members of the genus have been conducted and the earliest comprehensive study on Ectocarpus was that of Knight (1929) on Ectocarpus siliculosus. Using light microscopy she presented detailed accounts of the general morphology of the species, the structure and functioning of sporangia, cytology, speculations on the life history, geographical distribution and sexuality. While subsequent studies have found minor errors in her interpretations of observations, such as her documentation of unilocular gametangia, which are now known to be unilocular sporangia, her accurate accounts have laid the ground work for more recent studies on this genus (Baker & Evans 1973, Markey & Wilce 1976a,1976b; Toth 1976a, 1976b).

1.5. Reproduction in the Ectocarpales

As mentioned earlier, the structure and development of reproductive structures in the Ectocarpales is considered primitive (Fritsh 1945). There are two types of reproductive organs:

i) The plurilocular gametangia or sporangia which are derived by mitosis, and associated with cross wall formation.

ii) Unilocular gametangia or sporangia, where during development, cross walls are not formed.

Nuclear divisions in the unilocular sporangia are generally meiotic, but in some instances zoospores formed retain the same level of ploidy as the parent algae (Müller 1975).

In the Ectocarpales, plurilocular structures occur in both the asexual and sexual phases and as such may be either sporangia giving rise to diploid zoospores or gametangia producing haploid gametes. These structures are mostly isomorphic between phases and a knowledge of the life history is necessary before distinguishing between the two. Generally, the plurilocular sporangium is more common and occurs more often in the life cycle than the plurilocular gametangium (Clayton 1974, Fletcher 1981). Both the sporangia and gametangia develop from lateral branches and may be stalked or sessile.

In the majority of members of the Ectocarpales, unilocular reproductive structures are described as sporangia. The plurilocular sporangia and gametangia contain many locules each possessing a single motile cell. In contrast, the motiles produced in unilocular organs develop in a single unpartitioned organ. These unilocular structures are not as common as the plurilocular structures and were thought not to be an obligate phase in the life history (Knight 1929).

Using the light microscope, Knight (1929) noted that the plurilocular sporangia of Ectocarpus siliculosus arose as papillae cut off by the formation of cross walls at right angles to the long axis of the plant. Each tier underwent longitudinal divisions until as many as 600 loculi were evident in the mature sporangium. Throughout their

development, the nucleus, chromatophores and partition walls were evident. A more detailed description of the stages in the development of the plurilocular sporangium or zoospore formation (zoosporogenesis) was the result of the studies on Ectocarpus parvus Saunders (Lofthouse & Capon 1975) using electron microscopy. This study has been summarised in Figure 2.

The early development of a unilocular gametangium commences as a spherical swelling in the cell wall of a vegetative cell (Knight 1929). The unilocular gametangium gradually increases in size with increased nuclear divisions until hexagonal masses of protoplasm separated by cleavage planes can be recognised in the mature organ.

Using the light microscope, the observed release of motile cells was preceded by a bulging of the tip of the sporangium (La Claire & West 1979). This eventually bursts to release the motiles. In plurilocular structures, the locule walls do not appear to be completely broken down. For example in Scytosiphon, the motiles appear to stream through a central column to the exterior of the reproductive structure (Clayton 1984). A central column was found in cross sections of E.parvus (Lofthouse & Capon 1975).

A number of ultrastructural changes take place in both the unilocular and plurilocular structures just prior to the release of motile cells. In the plurilocular structures, spaces develop separating the motile from the cell wall (Scytosiphon Clayton 1984; Cutleria hancockii Dawson. La Claire & West 1979; Pylaiella littoralis M(L)Kjellman. Markey & Wilce 1976a). These spaces are thought to be filled with mucilage. In unilocular structures the motiles round up before release and are surrounded and permeated by mucilage (Pylaiella littoralis Toth 1976a). Other ultrastructural changes occurring before the release of motile cells include the production of intraplastidial eyespots and a pair of flagella in each motile cell.

The ultrastructure of the apical cells within the plurilocular sporangia of E.parvus revealed poorly defined membrane systems and organelles (Lofthouse & Capon 1975). These differences, when

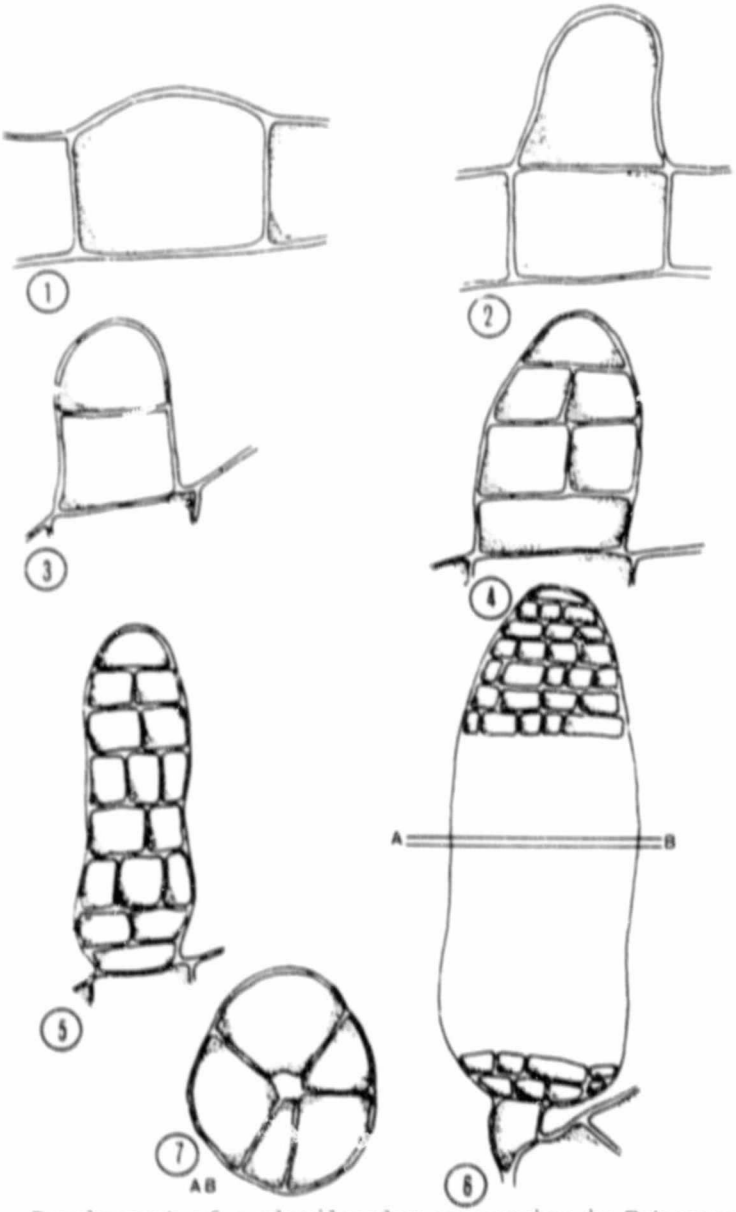

Figure 2: Development of a plurilocular sporangium in Ectocarpus parvus (modified from Lofthouse & Capon).

1. A vegetative cell showing convex bulging of the lateral wall.
2. Newly formed cell wall between sporangial initial and vegetative cell.
3. The two celled stage.
4. The six celled stage.
5. Nine rank stage.
6. Mature plurilocular sporangium.
7. Cross section revealing a central supporting column.

compared with other developing motiles elsewhere in the sporangium, led these authors to presume that the apical cells are non-viable.

The process of liberation of motile cells is thought to be the same in both unilocular and plurilocular structures. Knight (1929) observed that release of sporangia was caused by perforation of the cell wall at the terminal end and the creation of a 'suction pressure' enabling the zoospores to "ooze" out in a mucilaginous mass. More recent observations seem to indicate that internal pressure is created within the sporangium, resulting in the passive liberation of the motile cells. Furthermore, discharge of motile spores does not seem to be related to the turgor pressure of the spores themselves, but rather to hydration of the mucilage surrounding the motiles in both plurilocular and unilocular organs. Using histochemical staining techniques Toth (1976a) has shown that the mucilage in Pylaiella littoralis is carbohydrate in composition and carbohydrates are prone to hydration. Release of motiles of intertidal algal could then be synchronised with the incoming tide.

There are a number of theories as to the origin of the mucilage surrounding the motile cells in the Ectocarpales. In an few instances, vesicles in developing motiles were observed releasing their contents into the space between the motile and the cell wall (Baker & Evans 1973, Markey & Wilce 1976a) and it was suggested that the contents of these vesicles constitute the mucilaginous matrix (La Claire & West 1979). However, this does not explain the extensive thinning of locule walls in many species. It was suggested that the vesicles could also be a source of enzymes responsible for the break down of the cell wall and the resultant break-down products form the matrix (Clayton 1984). In Scytosiphon (Clayton 1984), there was unequal erosion of the outer sporangial walls. The inner layer composed of alginic acid being eroded far more than the outer, persistent, cellulosic layer. Furthermore, this may lead to the observation that the locule walls are composed largely of alginic acid and that the break down of this component produces the matrix. The remaining cellulosic component, would be sufficiently thin to easily break by mechanical stress, such as that caused by the swelling of the matrix.

After release, the motiles swim for up to 24 hours before settling on a substrate and responding to external stimuli such as light (Wynne 19 in Fletcher 1987, Baker and Evans 1973). Motiles of members of the Ectocarpales are typical of all phaeophytes, possessing two sub-apically inserted heterokont or unequal flagella. The posterior, shorter flagellum is smooth whilst the anterior flagellum bears mastigonemes.

The flagellum is divided into a number of regions; the basal body, transition zone and the flagellar shaft, which in some cases bears mastigonemes or hairs. All of these regions are traversed by microtubules, which are arranged in the axoneme, which in turn are arranged in different patterns depending on where they occur along the flagellum (Figure 3.).

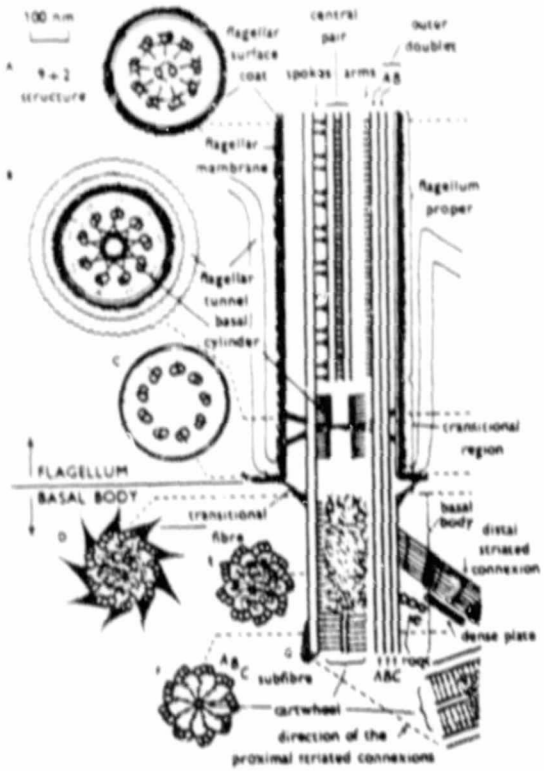

Figure 3.: Diagrammatic reconstruction of the flagellar shaft, transition region and basal body of Chlamydomonas reinhardtii Dangeard (from Cavalier-Smith 1974 in Moestrup 1982).

The axoneme arrangement of the basal bodies is characterised by a 'cartwheel' pattern and variation from this arrangement is reportedly rare (Moestrup 1982). The precursors of the basal bodies are the centrioles which are not thought to play an active role in mitosis (Pickett-Heaps 1971 in Moestrup 1982). Basal bodies are thought to be involved in flagellar movement, however examples of motiles without basal bo[illegible] have questioned this theory.

The brown algae, like other heterokont algae, lack a transitional helix. The absence of this structure indicates that it may be of little importance in flagellar functioning and its function may be taken over by other flagellar parts.

The flagellar shaft is characterised by nine outer doublets of mictotubules which surround a central pair of single microtubules. This arrangement is more commonly known as the '9+2' arrangement. The acronema or hairpoint and mastigonemes are features of the shaft; the former restricted to the tip and the mastigonemes may at maturity be distributed, helically, in one row, along the entire length of the flagellum.

Typically, brown algal mastigonemes possess an expanded base, with a hollow centre, which gives rise to a shaft region. The shaft comprises two zones, a proximal fibrillar part and a distal tubular region, commonly bearing two hairs (Russell 1973).

The acronema is in essence a continuation of the two central axonemes in the '9+2' arrangement. The delicate nature of the acronema and its sensitivity to mechanical stress, means that it has been lost in numerous specimen preparations. In Ectocarpus sp., only 1% of the cells had an acronema (Moestrup 1982). A number of functions has been ascribed to this region; initial contact between the mating gametes in Chlamydomonas reinhardtii and the attachment to the substrate e.g. in Chorda tomentosa (Toth 1976b). In the latter, Toth (1976b) suggests that the acronema is covered with a sticky protein which enhances adhesion to the substrate.

Muller (1988) and Kawai (1988) have observed zooid flagella with

epiflourescence microscopy under blue and blue-violet light excitation. The front flagellum was completely invisible, while the posterior flagellum shows a bright, blueish green fluorescence over its entire length, including the swelling near the base. Maximum fluorescence emission at wavelengths of 515-520nm, upon excitation of 440nm by the exciter filter, indicates that the autofluorescent substance is considered to be a flavin (Kawai 1988). It is present in brown algal flagellate cells which have eyespots and are phototactic. Because the flagellar swelling in the posterior flagellum is a presumptive photoreceptor for phototaxis in these groups, it is suggested that the flavin located in the posterior flagellum, acts as a photoreceptor pigment in phototaxis.

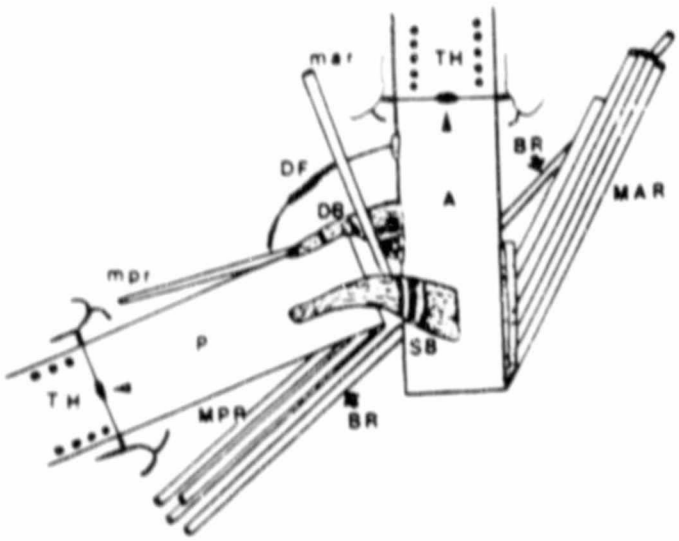

Figure 4: Diagrammatic reconstruction of flagellar apparatus in Giraudyopsis stellifer zoospores (O'Kelly & Floyd 1985).
Mar & Mpr = Major & minor bypassing rootlets
DF = Distal fibre TH = Transitional helices
DB & SB = Deltoid & strap-shaped striated bands
A & P = Anterior & posterior basal bodies

Studies on the flagellar apparatus of Giraudyopsis stellifer (O'Kelly and Floyd 1985) revealed the flagellar apparatus components in the vicinity of the basal bodies (Figure 4.). Essentially a number of

rootlets serve to anchor the flagella. Studies of motile cells of brown algae have revealed the presence of an anterior rootlet (Manton 1957, Baker & Evans 1973, La Claire & West 1979, Henry & Cole 1982a & 1982b in O'Kelly & Floyd 1985, Moestrup 1982, Clayton 1984), while the major posterior rootlet appears to be absent, with the exception of Phyllospora comosa (Moestrup 1982) and possibly Ectocarpus (Baker & Evans 1973). O'Kelly and Floyd (1985) suggested that it may be significant that species in which major post rootlets are present, belong to groups regarded as primitive in the Phaeophyceae, as recognised by Scagel (1967).

1.5.1. Organelle functioning at the ultrastructural level

While it is often possible to discern the functioning of larger structures associated with algae, such as flagellar action or the release of motile cells, it is far more difficult and challenging to determine organelle functioning at the electron microscope level. Since sequences cannot be observed simultaneously, but require numerous embedding runs followed by sectioning over time, speculation is often made about the missing steps in a process. A number of important processes required for 'normal' cell functioning will be discussed in turn.

Transport of food reserves and secretory precursors are thought to be intricately involved with the pyrenoid, nucleus, chloroplast envelope and the Golgi (Bouck 1965). More specifically, materials produced in the chloroplast are stored in the pyrenoid or passed through the surrounding endoplasmic reticulum to the Golgi and ultimately extruded from the cell. Evans and Holligan (1972) argue that the by-products of photosynthesis arise as osmiophilic bodies in the chloroplast. These bodies are then expelled in vesicles from the chloroplast, where they coalesce in the cytoplasm to form larger osmiophilic bodies. Oliviera and Bisulptra (1973) noted 'osmiophilic structured' bodies associated with the chloroplasts and traced the origins of these bodies to the chloroplast endoplasmic reticulum.

In the cytoplasm, the osmiophilic bodies accummulate at the cell

periphery and are excreted from the cytoplasm, to be incorporated into the cell wall (Baker & Evans 1973, Loiseaux 1973). The presence of osmiophilic material external to the cell wall (Baker & Evans 1973), suggests that it has been extruded from the cell. Armstrong and Boalch (1960 in Baker and Evans 1973) demonstrated that Ectocarpus plants release metabolites into the culture medium. Whether or not in the last example the metabolites were from the osmiophilic bodies is not known.

Golgi-derived vesicles are thought to play a role in the transport of the osmiophilic material within the cytoplasm (Loiseaux 1973). The origins of the Golgi were suggested to be formed by blebs, produced by the outer nuclear membrane (Loiseaux 1973). These blebs coalesced to give rise to the perinuclear Golgi.

Very little is documented in the literature on the formation of locule walls within a sporangium. Baker and Evans (1973) suggested that the abundance, at a stage when cell wall formation was taking place and location of plasmalemmasomes and lomasomes (also known as paramural bodies) in the region of the forming cell wall, suggests that they may be involved in cell wall formation. Plasmalemmasomes and lomasomes have been reported in other brown algae (Cole & Lin 1970 in Baker & Evans 1973) but their role is as yet not fully understood.

The process of flagellar formation has been studied in Pylaiella littoralis (Markey & Wilce 1976b). The first indication of flagellar formation is the occurrence of a pair of centrioles, close to the nucleus and perinuclear Golgi body. Basal bodies at the distal end of each centriole give rise to a basal plate. This basal body/basal plate complex constitutes the flagellar precursors. The next stage is the formation of the flagellar vesicle into which the part of the forming flagellum above the basal body extends. It is possible that vesicles bud off from the Golgi and coalesce to produce the flagellar vesicle. The smooth flagellum develops appressed to the edge of the flagellar vesicle for part of its length, while the tinsel flagellum develops away from the vesicle wall. The region of the smooth flagellum appressed to the vesicle periphery has a swollen flagellar

membrane and is thicker than the tinsel flagellum (Markey & Wilce 1976b).

Mastigoneme formation most likely occurs within the endoplasmic reticulum. Evidence of this is found in the structure and size of tubular elements in the endoplasmic reticulum (Moestrup 1982). At first the flagellar hairs appear to be irregularly arranged, but later on the hairs become aligned in one or more groups. The bases of the mastigonemes are apparently attached to the endoplasmic reticulum membrane and this led to suggestions that the hairs are added to the growing flagellum together with a piece of endoplasmic reticulum membrane, which is then incorporated into the flagellar membrane. In Pylaiella littoralis, mastigonemes reportedly move via the Golgi to the growing flagellum on their way from the endoplasmic reticulum. In Giffordia granulosa (Sm) Hamel swarmers, fine spokes between individual mastigonemes seemed to keep two mastigonemes separate from each other (Loiseaux 1973). It was also suggested that these spokes may play a role in directing orderly transfer of hairs from the cytoplasm to their final position on the flagellum. Similar spokes have not been detected in other brown algae (Manton 1957, Bouck 1965, Baker & Evans 1973, La Claire & West 1979, Clayton 1984).

Using fluorescently labelled hairs, Bouck (1965) was able to show that the hairs were added to the base of the flagellum. However, it is clear that several different systems are involved, but, details of the actual attachment process of hairs to the flagellum is not known.

The arrangement of mastigonemes on the flagellum seems to vary depending on the age of the flagellum (Moestrup 1982). As observed by Markey and Wilce (1976b) and Berkaloff and Rousseau (1979 in Moestrup 1982), the newly formed mastigonemes, after their release from the cytoplasm, are packed together in a proximal group on one side of the flagellum. Distal ends of the flagella are free of hairs. Eventually the hairs are distributed along the entire length of the flagellum. Negatively stained preparations of Giffordia granulosa (Loiseaux 1973) showed that the mastigonemes were arranged in two opposite rows, unlike that found in the developing zoid within the sporangium.

1.5.2. Organelle fine structure as a taxonomic tool

Similarities of motile cell organelles between algal groups has led to a number of attempts at using organelle fine structure as an aid to assessing systematic and phylogenetic relationships in algal groups. Traditionally, features associated with thallus morphology, vegetative growth, reproduction and life history in brown algae have formed the basis for their classification (Wynne 1981 in O'Kelly & Floyd 1985). More recently it has been suggested that the value of the latter attributes are limited with respect to the Chlorophyta (Stewart & Mattox 1975, Floyd & O'Kelly 1984, Mattox & Stewart 1984, O'Kelly & Floyd 1984c, all in O'Kelly & Floyd 1985). Furthermore O'Kelly and Floyd (1985) suggest that continued use of thallus morphology, growth regions and reproduction in classification may obscure details which support the parallel evolution of thallus form and construction in different brown algal lineages.

As mentioned earlier, chloroplast structure has been considered as being sufficiently uniform within groups to be used as a taxonomic feature (Evans 1966,1968, Hori 1971,1972 & Asensi et al 1977 all in Clayton & Shankly 1987). Other organelles suggested as being useful in taxonomy are eyespots (Dodge 1973), flagella (Moestrup 1982) and the pyrenoid (Dodge 1973).

Eyespots are categorised into types by Dodge (1973) according to their association with the chloroplast, for example, Type B eyespots form part of the chloroplast and are loosely associated with a flagellum. The latter eyespot type was identified in the Xanthophoeae, Chrysophyceae and Phaeophyceae and these classes also share similar pigment composition and flagellar structure. Difficulty in using eyespot type in classification arises where variation within a single class exists, such as in the Dinophyceae.

Pyrenoid variation was also noted within a single class (Dodge 1973) and so use of pyrenoids in taxonomy is also questionable. Uniformity of pyrenoid structure does exist in the Phaeophyceae, where most members possess a single-stalked pyrenoid.

Moestrup (1982) organised all heterokont algae into the Heterokontophyta, characterised not only by the structure and arrangement of the flagellar hairs, but also by the transition region and numerous cytoplasmic features. The Phaeophyceae are included within this phylum. Variation in flagellar structure was found to be minor, involving the loss of one row of flagellar hairs, or loss of the transitional helix.

Hibberd (1979 in Moestrup 1982) uses flagellar structure in construction of the phylogenetic relations between algae (Figure 5.). Unlike Hibberd, Moestrup (1982) suggests that the brown algae may have been derived from the Chrysophytes or from heterokont fungi.

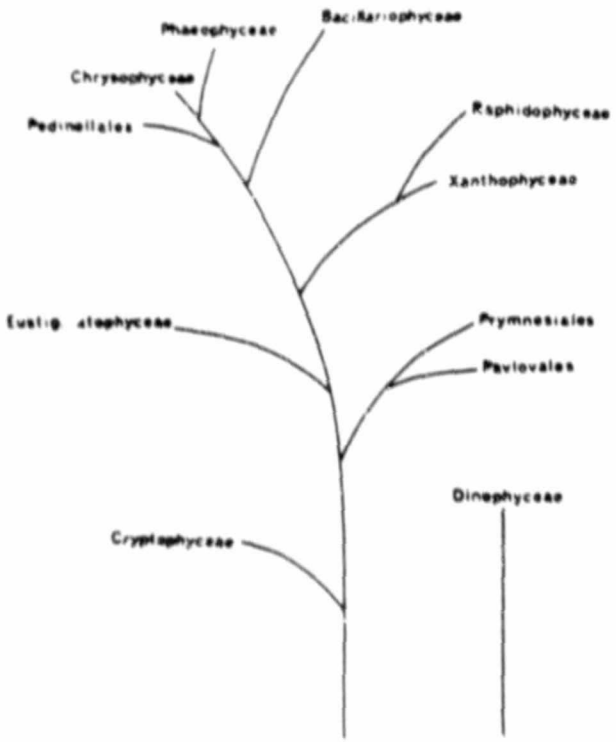

Figure 5.: Phylogenetic diagram based on flagellar structure (Hibberd 1979 in Moestrup 1982).

1.5.3. The concept of sexuality

Hartman (1925) introduced the concept of relative sexuality, which describes the ability of a gamete to act either as a male or female when mated with different partners. He inferred that the sex of gametophytes was determined early in its development by external factors.

While Müller (1975) argued that there was no data left to support the theory of sexuality in the plant kingdom, he noted that the apparently isomorphic gametes of Ectocarpus siliculosus differed behaviourally, acting either as 'male' or 'female' gametes. Female gametes released from gametangia tended to remain motile for up to twenty-four hours after release whereas male gametes settled soon after dehiscence. When the female gametes finally came to rest by fixing themselves to the substrate and withdrawing their flagella, they started secreting a sex hormone called 'ectocarpene'(Müller et al. 1971) which attracted the male gametes. For fusion to take place the male gametes retracted the front flagellum so that it could approach the female cell. After one male gamete had fused with the female, the zygote lost its attraction for the other male gametes. The chemical composition of the 'ectocarpene' has been ellucidated by gas chromatography.

Sexuality was also reported in an isolate of Giffordia mitchelliae (Harv) Hamel (Müller 1969). Here the female plurilocular gametangia had larger locules than locules of male plurilocular gametangia on the same plant. Large and small loculed gametangia and sexual activity have not been observed in subsequent studies on Giffordia (Amsler 1985).

1.6. Environmental control of reproduction

Algae, like higher plants, have developed elaborate reactions for seasonally synchronising the timing of spore or gamete production and release, and the phases of active growth and dormancy. This enables them to make use of seasonally varying ecological factors such as temperature, photon fluence, light quality, photoperiod and nutrient concentration. While temperature and day length and in some instances light quality act as triggers inducing reproduction or new growth, photon fluence and nutrients modify the amplitude of the reaction. Often there is an antagonism of reproductive and vegetative growth in a seasonal response sequence (Lüning 1981). For example, sorus development in a Laminaria species in short days involves a dramatic reduction of the growth rate (Lüning 1981).

Emphasis on the following discussion will focus on the major environmental parameters in turn and the influences these have had on controlling reproduction. The influence of nutrient concentration on growth and reproduction will not be reviewed. The discussion will not be limited to the Ectocarpales but will cite examples from all the algal groups.

1.6.1. Light climate

As we move from the shore margins into deeper waters, so the light quantity and quality undergo dramatic changes. An understanding of the way in which the life cycle and growth of seaweeds is related to light climate requires quantitative analysis of its responses to light. Most commonly, this goal has been approached by determination of the maximum/saturating and minimum light levels defining the limits within which major physiological processes can be operated.

In photosynthetic organisms, light provides not only energy for assimilation, but information which is used to adapt to the various light climates. Photoreceptors other than chlorophyll are responsible for the light absorption in these adaptive responses. In

algae a variety of photoreceptors are found, most abundantly phytochrome and/or cryptochrome (Dring 1988). As with higher plants, light may influence morphogenesis and metabolism (Dring 1988).

The light field, to which marine algae are subjected, is made up of the combined componen of light intensity, photoperiod and the quality or wavelength of light. While it is the combined action of these components that influence the growth and development of algae in nature, it is possible under culture conditions, to separate out these components and determine experimentally which is initiating a response.

1.6.2. Photon fluence

Photon fluence rate is the term used to describe the amount of light incident on a surface, as used by Ramus (1981) and $\mu molm^{-2}sec^{-1}$ is the Systeme Internationales (SI) unit of photon fluence rate, as described by Luning (1981). Throughout this thesis the term photon fluence rate will be used synonomously for the phrases light quantity and intensity.

There have recently been many studies on the rate of development of reproductive structures at different photon fluence rates. Most of these show that the rate or intensity of reproduction increases with increasing photon fluence providing other conditions are favourable eg. Hydrodictyon Roth (Neeb 1952 in Dring 1974), Porphyra C.Agardh (Kurogi et al.1962 in Dring 1974). The results have also often indicated that there is a threshold intensity below which reproduction does not occur e.g. Acetabularia crenulata (Terborgh & Thimann 1964), and Desmarestia Lamouroux (Chapman & Burrows 1970 in Dring 1974), and that reproduction is inhibited at high photon fluence rates eg. tetrasporophytes of Bonnemaisonia C.Agardh (Chen et al.1970a in Dring 1974) and Laminaria Lamouroux (Hsiao and Druehl 1971 in Dring 1974).

It has been argued that the effect of photon fluence on reproduction may be correlated with changes in photosynthetic activity and thus

when photosynthesis is inhibited at extreme low or high irradiances, reproduction is too. However, some results obtained indicate that photosynthesis was not saturated at energies which were saturating for reproduction (Kain 1966, Knaggs 1966).

The influence of photon fluence on the type of reproductive organ produced was illustrated by Müller (1962). He showed using a species of Ectocarpus that high light energies favour unilocular sporangium development, while low light energies favour plurilocular sporanguim formation. In Derbesia Solier, sporangia were produced in strong light, and vegetative propagules in weak light (Hustede 1964).

Light stimulates the liberation of motiles in some algae. Ectocarpus siliculosus gametes and zoospores are liberated from mature gametangia and sporangia within minutes of being removed from the dark and placed in the light (Clayton 1974). Egg liberation in Fucus requires at least eight hours of light; above this threshold the number of eggs released increases with the length of the irradiation period (Abe 1976).

Jones and Dent (1971) investigated the effects of photon fluence on the growth of algal spores and showed that an alga's pigment system may prove important in establishing photon fluence tolerances. Their results indicated that sublittoral species depend on a phycoerythrin -chlorophyll system for the utilization of light and can grow only at irradiances at which phycoerythrin is not destroyed. Intertidal species appear to have a second system which can function without phycoerythrin, at high photon fluence rates.

In some investigations, variation in photon fluence rates have been combined with variations in day length and it is evident that often the onset of a reproductive phase is related to the total incident energy or the radiation rather than to a specific irradiance or day length (Dring 1970). To measure the total quantity of light energy received per day, we measure the mean daily illuminance or MDI (Chapman and Burrows 1970 in Dring 1974). To calculate the MDI under laboratory conditions is easier than in the field, since a constant irradiance of light can be maintained. In the field the intensity

varies depending on the angle of the sun. MDI is calculated as follows:

$$MDI = \frac{I \times D}{24}$$

where I=illuminance in μ molm^{-2} sec^{-1}

D=day length in hours

24=no. of hours in a day.

1.6.3. Photoperiod

Photoperiodic responses are well known in higher plants and are associated with changes in day length and temperature, which often herald the onset of seasonal change. Initially it was thought that photoperiodic responses would not be evident in marine environments, as wide fluctuations in temperature and day length are not experienced, however, there is now adequate evidence that a photoperiodic response exists in seaweeds. Most of this evidence comes from studies on red algae (eg. Adey 1970, Lüning 1981, Guiry 1984, Guiry & Cunningham 1984), while comparitively fewer studies have concentrated on photoperiodic responses in brown algae (eg. Jaffe 1954, Byrne & Craigie 1971, Nakahara & Nakamura 1971, Dring & Lüning 1975, Terry & Moss 1980, Dieck 1987, Hoffmann 1985).

Photoperiodism can be defined as "the control of some aspect in the life cycle by the timing of light and darkness" (Hillman 1979 in Dring 1988). The criterion here is not the total amount of available light, but rather the effects of timing of the light in a twenty-four hour cycle. Lüning (1981) argues that an important criterion in pinpointing the presence of a photoperiodic response is the effect of a 'night-break'. A positive response to interrupted night regimes such as those found in Porphyra tenera Kjellm.(Dring 1967) and Bangia fuscopurpea (Roth)C.Ag.(Richardson 1970), suggests that a pigment similar to phytochrome of higher plants acts as a photoreceptor in

algae. Photoperiodic experiments on Ascophyllum nodosum (L) Le Jol (Terry and Moss 1980), revealed that a pigment with absorption in the blue region of the spectrum, was responsible for a photoperiodic response and not phytochrome.

Dring (1984), however, regards that 'night-breaks' should not be regarded as an absolute diagnostic for photoperiodic responses. He bases his argument on the responses of flowering plants, where night breaks are not always effective in converting a long-day regime into a short-day regime (Vince-Prue 1983) and therefore there is no response to night breaks. He also argues that phytochrome does not seem to be the photoreceptor in algae and so we cannot use the definition of photoperiodism as used for higher plants. Rather there seems to be a range of different types of photoperiodic mechanisms evident in algae. The criteria then, used for establishing a photoperiodic response, should be the observation of a difference in development of an algal species in long and short days under identical temperature and nutrient conditions. This would usually involve reproduction of one or both phases in a life history, but vegetative development may also be controlled. This definition is favoured in this thesis.

It is often very difficult to distinguish between a response caused by light quantity and light period. An example of difficulty in determining which factor was illiciting the greater response is illustrated in Laminaria longicruris de la Pyl (Byrne & Craigie 1971). Sexual reproduction in this species was more prolific in a five hour day than in a twelve hour day, but brief interruptions of long dark periods with red light, reduced sexuality to the level recorded in long-day conditions. A positive photoperiodic response was detected in the gametophytes of Desmarestia (Nakahara & Nakamura 1971). Only vegetative growth was evident when grown in continuous light at 10°C, but one cycle of 10L:14D, followed by a return to continuous light, induced fifteen percent of the branches to become fertile and fertility was increased by eighty-five percent after four short day cycles (Nakahara & Nakamura 1971).

Lüning (1981) and Dring (1984) also found that a genuine photoperiodic response was characteristic of a heteromorphic life cycle. The most logical explanation of the coincidence of photoperiodism and heteromorphic life cycles may be that one of the two phases can only survive part of the year, so that the initiation of this phase has to be very accurately timed. This was shown to be the case in an asexual species of Monostroma arcticum shown to be non-photoperiodic in contrast to the hetermorphic species M.grevvillei (Thar.) Wittr. (Lüning 1980). While the ecological significance of photoperiodic responses in heteromorphic life histories is clear, more recently photoperiodic regulation of the isomorphic life history of a number of algae has been proven. These include Glossophora kunthii (C.Ag.)J.Ag.(Hoffman 1985); Dumontia contorta (Rietema & Breeman 1982); Gigartina acicularis (Wulf.)Lamour.(Guiry & Cunningham 1984). In most algae, photoperiodic responses are restricted to the sporophyte generation; up to now, the red algae Gigartina acicularis and Helminthora olivaricata are the only species where photoperiodic control has been reported for both generations (Guiry 1984, Guiry & Cunningham 1984).

1.6.4. Light quality

The light quality incident on algae changes with depth in the water column, causing the wavelength of light to be altered as it passes through the water. In general, green light travels the shortest distance, blue light travels to a greater depth, and red light travels the furthest. The wavelength of incident light is relatively constant, variation is introduced by tidal changes and seasonal fluctuations in water depth, although the latter is minimal.

Exposure to wavelengths other than those normally found in the field, may cause morphological and physiological changes, often associated with the reproductive phase.

Irradiation for one minute with a high intensity of red light in Trebouxia was sufficient to induce a four-fold increase in spore production and the effects of red light could be reversed by subsequent exposure to the same energy of far-red light (Giles 1970). There is at present no satisfactory alternative explanation for the antagonistic effect of red and far-red light in these algae, but the presence of phytochrome in these algae has yet to be confirmed.

Blue light has a specific effect on reproduction in a number of species. Male gametophytes of Laminaria saccharina remained vegetative indefinitely in red light, but twelve hours of irradiation with blue light was sufficient to induce egg production ten days later in 50% of the gametophytes (Lüning & Dring 1972). There are also reports that blue light stimulates spore liberation in Monostroma Thuret (Shihira 1958 in Dring 1988), Nitophyllum Greyville (Sagromsky 1961) and Desmotrichum undulatum (J.Ag.)Reinke.(Lockhart 1982 in Dring 1988), and inhibits zoospore formation in Protosiphon botryoides Klebs (Durrant et al.1968)

1.6.5. Temperature

Temperature has long been regarded as the major factor controlling the geographic distribution of marine algae. Temperature may control distribution of algae in two ways: extreme low or high temperatures may kill established plants or alternatively, prevent the reproduction of adult plants and establishment of juveniles (Dring 1984). The temperature limits for reproduction and for juvenile plants are usually narrower than those tolerated by adult plants, thus reproduction and establishment usually coincide with the most favourable conditions, while adult species must be able to withstand adverse conditions. This implies that if the distribution limits are determined by the temperature in the most adverse season, it is the tolerance of the mature plants which is critical, but if the limits appear to correlate with temperature in the most favourable season, the requirements for reproduction and establishment are important.

The physiological significance of the effects of temperature on reproduction are difficult to assess and often some other aspect of the alga's metabolism should be measured simultaneously to correlate with reproductive activity. Tseng and Chang (1956) demonstrated that spore production in Porphyra tenera can be divided into at least three phases: sporangium formation, spore formation and spore release, and that each phase has a different temperature optimum. Studies of spore production at different temperatures are therefore liable to reveal only a compromise between the optima for several processes.

Temperature may also have qualitative effects on reproduction. In species in which different spore types are produced by the same thallus, the temperature conditions may influence the type of spore which is formed. For example, Müller (1962,1963) has reported that Ectocarpus produced unilocular sporangia at low temperatures (below 10°C) and plurilocular sporangia above 13°C, whereas the situation appears to be reversed in Elachista Duby (Kornmann 1964).

Klebs (1896) showed that sudden temperature changes could induce spore formation and similar effects have recently been demonstrated in Derbesia (Sears & Wilce 1970), Fucus Linnaeus (Abe 1970) and Coscinodiscus (Werner 1971). In Porphyra tenera temperature increase resulted in decreased spore liberation, and vice versa, the intensity of effect being roughly proportional to the severity of the temperature change (Kurogi & Hirano 1956). Some of the other observed effects of sudden temperature changes may be attributable to their effects on an endogenous rhythm.

There are few detailed studies of the interaction of temperature with photon fluence. Ectocarpus (Müller 1962) sporangial development was not influenced by light at 13°C or 19°C, but at 16°C unilocular sporangia were formed preferentially at high photon fluence rates, while plurilocular sporangia were formed at low light energies.

The literature indicates that the timing mechanism for photoperiodic responses must be temperature compensated in some way, so that the critical day length is relatively constant at different temperatures

This is because the effect of temperature appears to be exerted on the process that is under photoperiodic control and not on the photoperiodic control mechanism itself. The sensitivity of critical day length to temperature has been examined in Gigartina acicularis. At 20°C, tetrasporogenesis appeared to be independent of day length, as the alga formed tetraspores in both 8 and 16 hour days. At 16°C however, there was a distinct short day response with a critical day length between 10 and 14 hours and the response to short days was inhibited by a night-break (Guiry 1984).

1.7. Germination and attachment of spores

The first and possibly, the most critical stage in the development of every independent phase in the life history of an attached alga, is the germination of the spore and the attachment of the young plant to a substrate. In spite of the extensive research devoted to seed and spore germination in most other plants, the germination of algal spores has received very little attention.

While no studies could be found on germination in the Ectocarpales in particular, the results available for other brown algal spores suggest that germination is rapid under a wide range of conditions. Neither extreme temperature nor light conditions appeared to lower the percentage germination. The apparent absence of the specific germination requirements, that are often associated with seeds, is perhaps to be expected when the smaller size and fewer food reserves, which could be used during a dormant period, are considered. Most spores produced by marine algae lack the resistant walls and low metabolic rates of spores and seeds of terrestrial plants and so are unable to delay germination until conditions are more favourable. It is anticipated, however, that environmental stresses will influence the further development of the alga after germination.

Although the environment has little influence on the rate of germination, both the pattern and direction of germination or polarity may be strongly modified by the environment. The polarity of developing embryos of Fucus and Pelvetia Decaisne & Thuret have been studied (Dring 1982) and while as many as fifteen physical,

chemical and biological factors may influence polarity, light is thought to be the most important stimulus for a zygote on the shore.

Rapid attachment of spores is particularly important for species in the intertidal or upper subtidal zones that are subject to wave action. Studies on the Fucales have shown that spores become attached even before the first visible signs of a rhizoid emerging. In Fucus (Dring 1982), unfertilized spores did not become attached to the substrate, while fertilized ones appeared to secrete a jelly identified as the polysaccharide fucoidan, at the point where the rhizoid was due to emerge.

An attachment material secreted after initial settling of zygotes in Ulva mutabilis Føyn has been studied at the electron microscope level (Bråten 1975). Associated with the production of the adhesive polysaccharide is the formation of the cell wall. Evidence based on the difference in stainability to ruthenium red (Luft 1971) (which stains acid mucopolysaccharides) indicated that the anchoring material secreted by young zygotes during the initial settling, is chemically different from the material secreted by the rhizoid cells, and the two substances seem to have different origins within the cell. A continuous secretion of cementing material takes place as the rhiziod grows.

III. AIMS OF THE PROJECT

Based on the literature survey, it appears that the stages in the development and the structure of thallus and reproductive organs, is relatively uniform within the order Ectocarpales. In this investigation, an attempt was made to determine whether Feldmannia sp. conforms to the already described developmental stages and the structure of reproductive organs, as described for closely related species. While there is an increasing amount of research on members of the Ectocarpales, aspects of the life cycle of Feldmannia sp. have been dealt with only briefly in descriptions of Hamel (1939), Knoeffler-Péguy (1970) and Clayton (1974). No research has been conducted on the South-African species of Feldmannia and so it was felt that a detailed investigation of this species was justified.

It was hoped that the species of the Feldmannia investigated would be determined. Since very little ground has been covered in the field of taxonomy in the South-African filamentous brown species, the expertise of Clayton (pers comm.) and keys from Australia (Clayton 1974), North America (Abbott & Hollenberg 1976) and Europe (Hamel 1939) have been relied upon. The taxonomic aspect, however, was intended to form a very small part of the investigation.

It was intended that the investigation would concentrate on thallus development and aspects of reproduction at the light and electron microscope level. This would include studies on stages from germination of zoospores to the initiation of sporangia on adult filaments. In addition, the development, maturation and release of zoospores was also studied. Particular emphasis was placed on the latter at the electron microscope level. Most of the detailed studies within the Ectocarpales have focussed on the development of motile cells within the sporangia (Knight 1929, Baker & Evans 1973, Loiseaux 1973, Lofthouse & Capon 1975, Markey & Wilce 1976, Toth 1976b, La Claire & West 1978, Clayton 1984 & 1986). These studies proved valuable in comparison with similar stages in this species of Feldmannia.

Since Feldmannia sp. was not relocated in the field, it was important that the environmental conditions most favourable for growth and the environmental extremes that Feldmannia sp. could endure, be established. This would provide further indication of its natural niche and geographical distribution. Since only plurilocular reproductive organs were evident on stock cultures, it was hoped that variations in environmental conditions would induce unilocular reproductive organ formation. In this way more information about the life cycle and ploidy of the species would be revealed. A change in phase under a photoperiod treatment, would indicate a positive photoperiodic response.

An attempt was made at determining the influence of different environmental parameters on thallus growth of Feldmannia sp. and on the production of reproductive structures. The value of experiments where one environmental parameter is varied while all others remain constant is great, as with reproduction, a single stimulus may trigger a response. This response may take the form of inhibited or enhanced production of reproductive structures, a change in the reproductive phase or the triggering of motile cell release. Experimental design in this investigation enabled the role of an individual stimulus eg. temperature, photon fluence, light quality and photoperiod to be pinpointed.

In essence then the following aspects were investigated:

(i) the taxonomic status of Feldmannia sp.,

(ii) the growth and development of the thallus and associated reproductive organs,

(iii) the development of motile cells within sporangia, at the transmission electron microscope level,

(iv) the environmental conditions that favour and inhibit growth and reproduction.

III. MATERIALS AND METHODS

3.1. The experimental organism

The alga used in this investigation was isolated by Professor R.E. Norris from algal material collected from Rocky Bay, Natal (Grid reference 30 30 CB, Figure 6.). The alga was isolated during part of a larger project on the Benthic Marine Algae of Natal and the catalogue number NAT 3686 was assigned to it. Feldmannia sp. was found growing epiphytically on Codiophyllum natalense Gray (Cryptonemiaceae, Rhodophyta). Confirmation of the genus name Feldmannia was made using descriptions by Hamel (1939), Knoepffler-Péguy (1970), Clayton (1974) and Abbott & Hollenberg (1976).

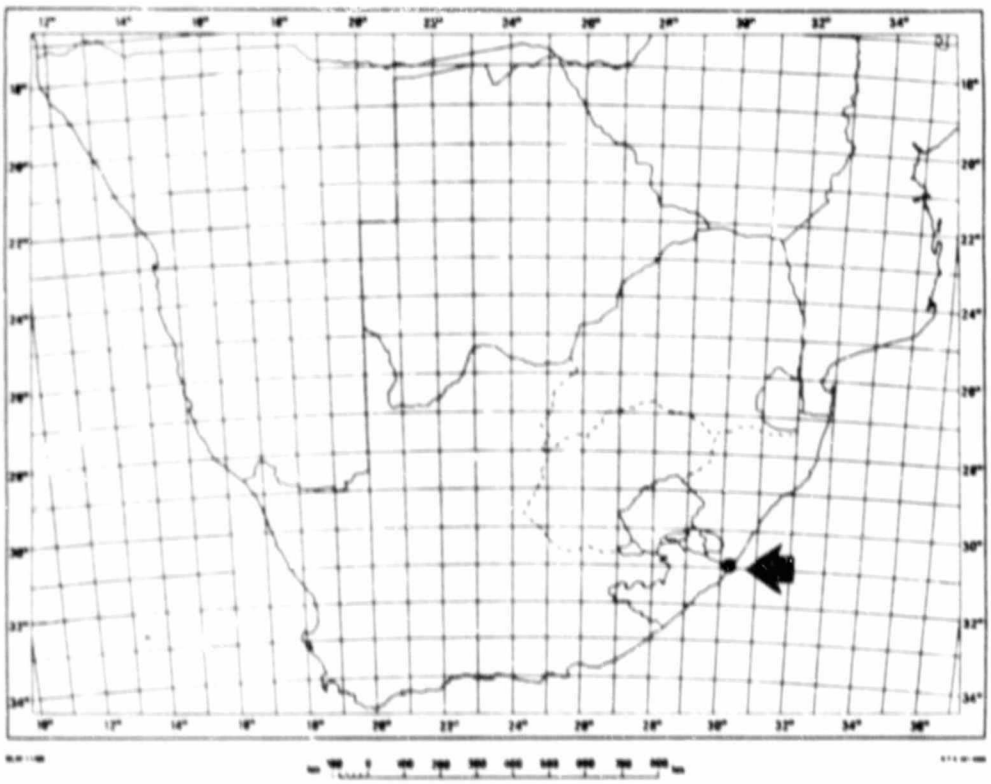

Figure 6.: Grid map of South Africa showing sample site.

3.2. Culture studies

3.2.1. Stock cultures

Stock cultures of Feldmannia sp. were placed in Repli-dishes containing enriched sea water medium and subjected to the following environmental conditions: temperature 20°C, photon fluence rate 90$\mu Em^{-2}sec^{-1}$, photoperiod of 16 hours light alternating with 8 hours of darkness. Stocks were maintained by removing newly released germlings and placing them in fresh medium. This subculturing was conducted fortnightly. Stock algae were used for experimental and microscopic studies.

3.2.2. Culture media

Feldmannia sp. was cultured in ½ strength Provosoli enrichment medium (E.S.medium) (section 8.1. in appendix) and ½ strength soil extract (section 8.1. in appendix) in sterilized sea water (section 8.1. in appendix), more specifically 10ml of P.E.S. and 5ml soil extract per litre of sterilized sea water. Two millilitres of Germanium dioxide was added to every litre of medium when algae were initially cultured to eliminate the growth of diatoms. Germanium dioxide interferes with silicon metabolism thereby inhibiting silicon frustule formation. The salinity of the medium using an A/C TO refractometer was found to be between 30-35 0/00 and the pH in the range of 8,39.

3.3. Light microscopy

A Zeiss compound photomicroscope, with bright field, Nomarski and phase-contrast optics and a Zeiss and Olympus inverted photomicroscope, were used during the light microscope studies. These included observations on stages in the development of the life cycle, measurement of filament sizes and to check on algal contaminants if present.

Specimens were photographed with the Zeiss compound and inverted microscopes using either black and white Agfapan Professional film (25 ASA) or Kodachrome colour film (100 ASA). Specimens were videod on the Zeiss compound microscope with an Hitachi video attachment.

A combination of exciter filter, barrier filter and dichroic mirror were attached to the Zeiss photomicroscope to observe fluorescence in algal filaments treated with the optical brightner Calcofluor White M2R, viz. exciter filter II (which transmits in the 390 - 420nm violet range of the spectrum), barrier filter LP450 (which transmits wavelengths of light above 500nm and protects the eyes) and dichroic mirror FT425. The arrangement of these three filters is illustrated in Figure 7. The dichrioc filter or chromatic beam splitter is able to reflect certain spectral ranges, while others can be completely transmitted. The barrier filter always had the highest wavelength to block out harmful light. Here 100 ASA colour film or 400 ASA black and white film were used to take photographs, since a slow film was necessary to increase the exposure time due to the small amounts of light emitted from the stained algae.

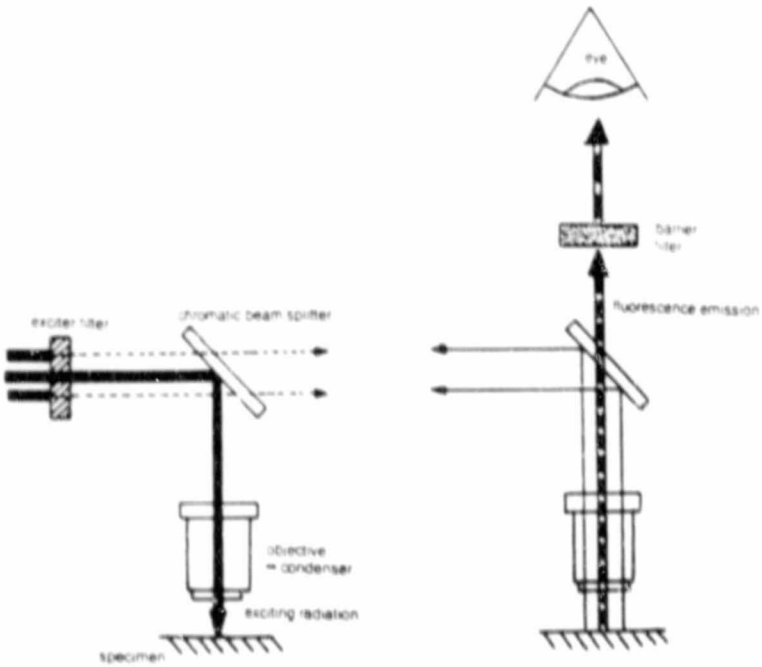

Figure 7.: The arrangement of filters attached to a photomicroscope used in fluorescence microscopy.

3.3.1. Staining techniques

Use of Periodic acid Schiff's stain (O'Brien & McCully 1981) procedure shows the presence of starch and some polysaccharides of cell walls by staining tissue red or magenta. Callose and cellulose are not stained, while, phenolics will stain red.

Sections, approximately 1 to 2um thick, of Spurrs embedded material of Feldmannia sp. were cut, using glass and diamond knives. The sections were placed in a drop of water on a glass slide, stretched and dried at low heat.

Procedure:
OsO_4 was taken out of Spurr's sections by soaking sections for 30 minutes in a 1% sodium metaperiodate solution. Since the tissue was fixed in an aldehyde, there was a chance that the tissue elements would display a general background stain, in addition to the desired specific staining (O'Brien & McCully, 1981), so a blocking agent was used to avoid this. Slides were left in a saturated solution of DNPH in 15% acetic acid, in water for 30 minutes. Completeness of the blockade was established if a treated, rinsed slide was transferred to a Schiff's reagent bath (Section 8.3. in appendix) and no red or purple staining occurred. The blockade was followed by a rinse in running water for 10 minutes. The slides were placed in a 1% periodic acid solution for 20 minutes, washed for 5 minutes, and then placed in Schiff's reagent for 1 hour.
A transfer through 3 successive baths for 2 minutes each of 0,5% sodium metabisulfite in a 1% dilution of concentrated HCL followed. Slides were then washed in running water for 10 minutes, dried, and mounted in DPX and covered with a coverslip.

3.3.2 . Determining the movement of dye through the sporangial wall

Mature, sporangia-bearing filaments of Feldmannia sp. were placed in a depression slide in medium. A few drops of 1% Toluidine blue in pH buffer 4.4 were added to the medium and the filaments were placed into fresh medium after 10 minutes. The filaments were then mounted

on slides and covered with a coverslip and viewed under the Zeiss compound microscope.

3.3.3. Calcofluor White M2R

This stain binds to cellulose in the cell wall, but is excluded from the cytoplasm. The stain fluoresces light blue when observed under violet light and unstained areas, for example areas of new growth, do not fluoresce.

Filaments were submerged in a 0,01% aqueous solution of Calcofluor White M2R (Section 8.4 in appendix), made up in a ½ PES growth medium for 20 minutes. Following staining, filaments were washed in filtered sterilized sea water for 1 minute. The filaments were then returned to their culture dishes containing normal growth medium and left to continue growing.

Motiles released from sporangia, were left unstained, placed on a slide and covered with a cover slip. The slide was then observed under the Zeiss photomicroscope with fluorescence attachments emitting violet light, in an attempt to determine whether epi-fluorescence or autofluoresence occurred.

3.3.4. Attachment preferences of motiles

Various surface types and shapes of algae, shell and driftwood were used to establish whether or not Feldmannia sp. motiles had any substrate preference for attachment. The algae used included: Schottera micariensis (Phyllophoraceae, Rhodophyta)- a highly branched, filamentous form; Pterocladia capeloglossoides (Gelidiales, Rhodophyta)- a fine filamentous form; Chaetomorpha crassa (C.Agardh)Kuetzing (Cladophorales, Chlorophyta)- a tufted form; Cryptonemia luxurians (Cryptonemiales, Rhodophyta)-a dimorphic blade and fine filamentous form; Botryocladia madagascarensis Feldmann (Rhodymeniaceae, Rhodophyta)-a swollen blade like form; and Feldmannia sp.. These together with a shell and a piece of driftwood

were placed in an evaporating dish with ½ PES and ½ soil extract in sea water. Each evaporating dish was inoculated with a large number of motiles. The experiment was done in duplicate.

3.4. Transmission electron microscopy

Filaments of Feldmannia sp. possessing various stages of development of reproductive bodies were processed for electron microscopy. In an attempt to obtain a range of the stages involved, filaments from the same age stocks, were in some instances, embedded daily for 3 days.

There are essentially six steps involved before an adequate electron micrograph is obtained:

1) Primary fixation.
2) Post fixation.
3) Dehydration.
4) Embedding and polymerization.
5) Trimming and sectioning.
6) Staining.
7) Viewing.

The chemicals involved in steps 1-4 are highly toxic and must be used in a fume hood.

1) Primary fixation

This step is involved in fixing and stabilizing the proteins. Three fixation procedures, represented as a) Pienaar (1980), b) Clayton (1974) and c) Oliveira and Bisalputra (1973), were initially followed to obtain the best possible fixation procedure. While the procedure for all 3 methods will be given in detail, the method of Pienaar (1980) was eventually used for all electron microscopy. Clusters of filaments of Feldmannia sp. and other Ectocarpalean species were placed into different concentrations of gluteraldehyde (Section 8.2. in appendix) made up in sea water or cacodylate buffer (Section 8.2. in appendix) with caffeine or sucrose.

The procedure for making up the fixatives and the recipes of the constituents is outlined in the appendix (Section 8.2.).

2) Post fixation:

After fixation in glutaraldehyde the alga was washed for 10 minutes either in sea water, sodium cacodylate buffer, or sodium cacodylate buffer in a graded series of sucrose (Section 8.2. in appendix). The washed material was then post-fixed in 2% Osmium tetroxide (OsO_4) (Section 8.2. in appendix) for one to two hours at room temperature.

The washed material was post-fixed in 2% Osmium tetroxide (OsO_4) for:
a)& c) 1 hour
b) 2 hours

3) [illegible]

Dehydration of the material was by way of a graded ethanol series:

i. 10% aqueous ethanol for 10 minutes
ii. 20% "
iii. 50% "
iv. 70% "
v. 80% "
vi 90% "
vii. 100% " for 20 minutes (x2).

4) Embedding and polymerization

The material was then gradually impregnated with low viscosity Spurrs resin (Spurr 1969) (Section 8.2. in appendix) by increasing the concentration of the resin (Section 8.2. in appendix).

The algal filaments were arranged in the Spurrs resin at the bottom of aluminium embedding dishes. Filaments were also placed on a slide in Spurrs and covered with a cover slip to make permanent slides of

each embedding procedure. These dishes and slides were placed in an oven and polymerised at 70°C for 15 hours. Silica gel was always present in the oven to absorb any moisture that might slow down and affect polymerisation.

5) Trimming and sectioning

Filaments bearing sporangia were first observed under a compound microscope, cut from the polymerised resin blocks and glued onto resin stubs with araldite adhesive. Glass knives used for both trimming and sectioning were made on an LKB knifemaker. Sectioning was conducted on a Reichert-Jung Ultracut E microtome using both glass and diamond knives. Monitor sections were made to determine whether sectioning of the Spurr's block was through the sporangium and not just through the resin. After trimming, the stubs were returned to the oven overnight. Sections obtained were floated on water, expanded with chloroform and only silver and gold sections were picked up on 300 mesh copper support grids.

6) Staining

To improve the contrast of the ultrathin sections, positive staining with heavy metals was carried out. Drops of stain were pipetted onto parafilm on dental wax in a Petri dish, on which the grids were placed section side down. Two stains were used:
2% aqueous uranyl acetate (Reynolds 1963)(Section 8.2. in appendix) for 12 minutes and lead citrate (Reynolds 1963)(Section 8.2. in appendix) for 12 minutes. Sections were washed with distilled water between stains and after staining. The Petri-dish was covered to eliminate light, when uranyl acetate was used, and sodium hydroxide pellets were added when lead citrate was being used to remove carbon dioxide.

7) Viewing

Sections were viewed on either the 100S or 100C Jeol transmission electron microscope, operating at 80 KV. The spot size was occasionally adjusted to prevent break-up of very thin sections.

Embedding procedure for motiles

The procedure for embedding motiles released from plurilocular sporangia is different to that for the intact filaments, due to the motiles small size. Only those procedures different to those listed for the filaments, will be described.

1) Fixation

Zoospores of Feldmannia were micropipetted from the medium just after they were released from the sporangia. 9,5ml of culture medium containing zoospores was added to 0,5ml of 25% gluteraldehyde in a tapered centrifuge tube and thoroughly mixed. The fixation time ranged from 1 to 8 hours at room temperature (20°C).

After fixation the gluteraldehyde solution containing fixed motiles was centrifuged for 5 minutes at a low speed and 10 minutes at a high speed to concentrate the cells into a pellet. The material was given 3 washes in sterile sea water for 10 minutes each taking care not to resuspend the pellet.

2) Post fixation

The pellet was post-fixed in a 2% OsO_4 solution in sea water for 1 hour at room temperature. The pellet was resuspended in the OsO_4 solution to ensure a uniform post fixation.
After 1 hour a few ml of sea water was added and the solution plus cells was again centrifuged. The material was washed three times in sterile sea water.

3) Dehydration

The same procedure was used as outlined for intact algal filaments.

4) Embedding and Polymerisation
The material was gradually impregnated with Spurrs resin (Section 8.3.1. in appendix).

The pellet in the 100% Spurrs was placed in aluminium embedding dishes and polymerised at 70°C for 8 to 10 hours.
Trimming and sectioning of polymerised blocks and staining and viewing of sections followed the same procedure as that of the filaments.

3.4.1. Negative staining

Filaments bearing mature plurilocular sporangia were placed in hanging drop slides and flooded with fresh medium. Once motiles had been released, the filaments were removed from the slide leaving a small amount of medium containing motiles behind. Using a syringe some of this liquid was sucked up and drops placed on formvar coated grids. These grids, plus the cell suspension, were then osmicated for 30 seconds. The grids were then allowed to dry on the laminar flow bench for approximately 20 minutes. Distilled water was then placed on the grids to dissolve the salt crystals. The water was drawn off with dental absorbent fine points. Grids were washed 3 times and allowed to dry.

Preparations were stained for 20 minutes using pre-filtered, 1% aqueous uranyl acetate. Once again the grids were washed 3 times with distilled water and the excess water drawn off using a dental absorbent fine point. Grids were viewed on the 100S Jeol transmission electron microscope at 80 KV.

3.5. Scanning electron microscopy.

1) Fixation:

Zoospores were allowed to settle on glass cover slips in media and placed in 1 part 6% gluteraldehyde to 9 parts sterile sea water solution in a Petri-dish to maintain the correct orientation. All the material was fixed for 24 hours at room temperature.