Tansley review

Development and physiology of the brown alga *Ectocarpus siliculosus*: two centuries of research

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Summary

Brown algae share several important features with land plants, such as their photoautotrophic nature and their cellulose-containing wall, but the two groups are distantly related from an evolutionary point of view. The heterokont phylum, to which the brown algae belong, is a eukaryotic crown group that is phylogenetically distinct not only from the green lineage, but also from the red algae and the opisthokont phylum (fungi and animals). As a result of this independent evolutionary history, the brown algae exhibit many novel features and, moreover, have evolved complex multicellular development independently of the other major groups already mentioned. In 2004, a consortium of laboratories, including the Station Biologique in Roscoff and Genoscope, initiated a project to sequence the genome of *Ectocarpus siliculosus*, a small filamentous brown alga that is found in temperate, coastal environments throughout the globe. The *E. siliculosus* genome, which is currently being annotated, is expected to be the first completely characterized genome of a multicellular alga. In this review we look back over two centuries of work on this brown alga and highlight the advances that have led to the choice of *E. siliculosus* as a genomic and genetic model organism for the brown algae.

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I. Introduction

The brown algae belong to the division Heterokonta and are therefore only very distantly related to the three most intensely studied eukaryotic groups, the animals, fungi and green plants (Baldauf, 2003; Davis, 2004; Fig. 1a). This independent evolutionary history has furnished brown algae with many novel metabolic, physiological, cellular and ecological characteristics, including a complex halogen metabolism, cell walls containing many unusual polysaccharides and high resistance to osmotic stress. Developmental processes are particularly interesting in this group, which evolved complex multicellularity independently of the three other aforementioned major groups. From a more applied point of view, the evolutionary history of the brown algae also underlies the high commercial value of several members of the group in the sense that they have evolved novel biomolecules such as polysaccharides and defense elicitors that have a wide range of applications in industry (Klarzynski *et al.*, 2000; McHugh, 2003).

It is important to note, however, that whilst the independent evolutionary history of the brown algae is the source of much of the interest of this group, it can also be seen as a handicap because the well developed model organisms from the plant and animal lineages are of limited relevance to brown algal biology. Specialized brown algal models have been developed in specific domains, for example members of the fucoids for cell biology approaches (see references in Corellou *et al.*, 2005), but a polyvalent model organism that allows access to a wide range of questions at the molecular level has been lacking. This situation is changing with the development of genomic and genetic tools for the filamentous brown alga *Ectocarpus siliculosus*. The sequencing of the genome of this alga has recently been completed and the sequence is currently being annotated (http://www.cns.fr/externe/English/Projets/Projet_KY/organisme_KY.html). It is therefore an opportune moment to look back at the emergence of *Ectocarpus* as a model organism.

Research on *Ectocarpus* began in the 19th century with descriptions of species and taxonomy, followed by studies aimed at unravelling reproduction and life history. Other major aspects that have been studied include the sexual pheromones and infection of *Ectocarpus* by viruses. Research has also been carried out on ultrastructure, photosynthesis and carbon uptake, gamete recognition and resistance to anti-fouling agents. Several eukaryotic parasites of *Ectocarpus* have been described. A proposition to adopt *Ectocarpus* as a general model organism for the brown algae was made in 2004 (Peters *et al.*, 2004a). This proposition was based partly on this alga's long history as an experimental organism, but also took into account several features that make *Ectocarpus* an interesting model for genetic and genomic approaches. These features include its small size, the fact that the entire life cycle can be completed in Petri dishes in the laboratory (Müller *et al.*, 1998), its high fertility and rapid growth (the life cycle can be completed in 3 months), the ease with which genetic crosses can be carried out and the relatively small size of the genome (200 Mbp compared with 1095 and 640 Mbp for *Fucus serratus* and *Laminaria digitata*, respectively; Le Gall *et al.*, 1993; Peters *et al.*, 2004a).

Here we present an overview of the work that has been carried out on *Ectocarpus* over the last two centuries and discuss how the availability of a number of genomic tools, in particular the complete genome sequence, is expected to accelerate research in many domains of brown algal biology in the coming years.

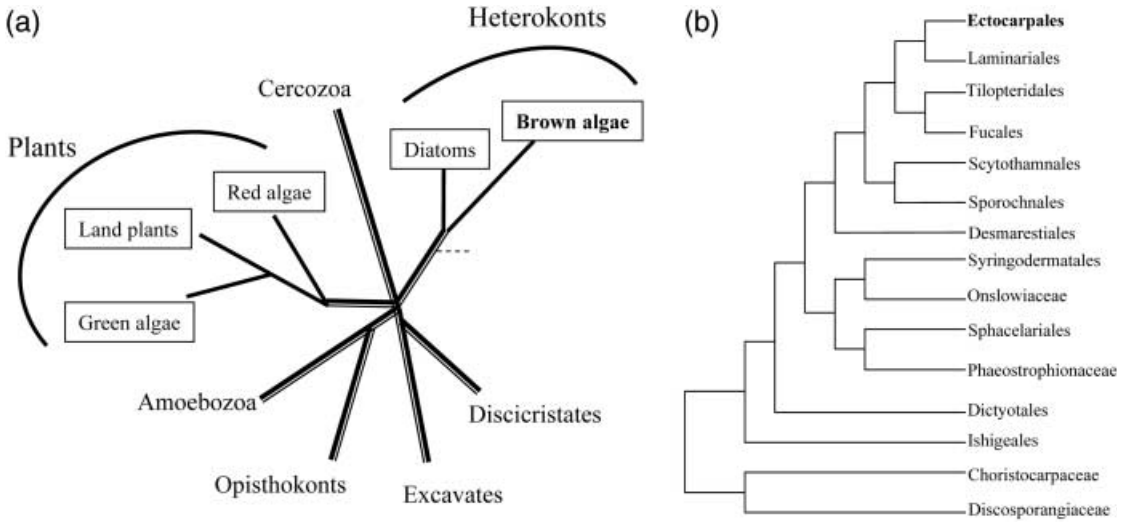

Fig. 1 Phylogeny of brown algae and Ectocarpales. (a) Position of brown algae within the eukaryotes (adapted from Baldauf, 2003). Brown algae belong to the heterokont phylum, which is phylogenetically distant from land plants and the green and red algae. Photosynthetic organisms are framed. (b) Position of the Ectocarpales (in bold) within the brown algae (adapted from Kawai *et al*., 2007).

II. Diversity and taxonomy, distribution and ecology

1. Diversity and taxonomy

Dillwyn (1809) published the first valid description of *Ectocarpus* (using the name *Conferva siliculosa*) based on material collected by W. J. Hooker on 'rocks in the sea at Cromer and Hastings'. These English localities lie in Norfolk and East Sussex, respectively. Type material, collected by Hooker in 1807, is housed at BM (BM000685585 and BM000685588) under the name *C. confervoides*. Lyngbye (1819) described the genus *Ectocarpus* based on material from Denmark and cited *C. siliculosa* Dillwyn as basionym. The correct nomenclature, therefore, is *E. siliculosus* (Dillwyn) Lyngbye (see Silva *et al.*, 1996 for further details). *Ectocarpus siliculosus* is the type species of the order Ectocarpales, which includes most of the smaller brown algae. Originally regarded as phylogenetically primitive, molecular systematics has shown the Ectocarpales to belong to a group of brown algal orders that evolved quite recently. They are closely related to the large and highly differentiated Laminariales, which are major components of coastal marine floras (Rousseau & de Reviers, 1999; Draisma *et al.*, 2003; Cho *et al.*, 2004; Kawai *et al.*, 2007, Fig. 1b). Many species have been described in *Ectocarpus* (www.algaebase.org, April 2007, listed 392 taxa of *Ectocarpus*, of which 98 are flagged as 'current'; numerous strains are publicly available at the Culture Collection of Algae and Protozoa (CCAP) in Oban, UK; Gachon *et al.*, in press). However, only *E. fasciculatus* Harvey (1841) is currently recognized as a second, well defined species, based on morphology (Russell, 1966, 1967a), crossing studies and sequence analyses (Stache-Crain *et al.*, 1997). Crossing experiments have shown that the taxon *E. siliculosus* may represent a species complex (Stache-Crain *et al.*, 1997) and ongoing, refined analyses are expected to resolve this complex, increasing the number of recognized species. Identification of different species of *Ectocarpus* based on morphology is difficult because of the plasticity of the commonly examined features (habit, branching pattern, size of sporangia). In addition, the two generations of a species may differ considerably (Müller, 1972a; Kornmann & Sahling, 1977).

2. Distribution

Ectocarpus siliculosus is distributed worldwide in temperate regions, but does not occur in the tropics and south of the Antarctic convergence (Stache, 1990; Wiencke & Clayton, 2002). It occurs in fully marine and in low-salinity habitats (e.g. five practical salinity units (psu) in Finland) and has even been recorded at a freshwater site in Australia (West & Kraft, 1996) and in a salt-polluted river in Germany (Geissler, 1983). Records of *E. fasciculatus* are mainly from the North Atlantic but there are some from Korea, Chile, South Georgia and South Africa (www.algaebase.org, 2007). On the shore, *Ectocarpus* occurs from high intertidal pools to the sublittoral. It is found on abiotic substrata (rocks, wood, plastic, ship hulls) and epiphytic on macrophytes or free-floating (Russell, 1967a,b, 1983a,b). As a result of its ability to grow on a range of abiotic substrates, *Ectocarpus* is a common fouling alga (Morris & Russell, 1974).

3. Ecology

There have been a limited number of ecophysiological and ecological studies on *Ectocarpus*. Growth rate is dependent on temperature, and there is evidence that temperature also influences the life cycle, at least in some strains (Müller, 1963; Bolton, 1983; see Section III.2: Sporophyte and gametophyte architecture). The thermosensitivity of different strains suggests that there is genetic heterogeneity within the *Ectocarpus* genus (Bolton, 1983). A similar result was obtained for osmo-acclimation (Thomas & Kirst, 1991a,b; see Section V.2: Abiotic stresses). In the field, *Ectocarpus* is a short-lived annual which may dominate the ectocarpoid flora on kelps (Russell, 1983a,b). Despite the commonness of *Ectocarpus*, there are few data concerning phenology in the field (mostly to be extracted from floristic works) and nothing precise on seasonality or habitats of the two generations.

The availability of the *E. siliculosus* genome sequence is expected to facilitate the analysis of the ecology of this species by providing a basis for the development of molecular markers. Particularly important challenges in this respect will include the identification of the sex locus and of genes specifically expressed in the two generations, as these will provide molecular tools that can be used to investigate several aspects of the life cycle under field conditions. Molecular markers will also allow the exploration of genetic polymorphism among *Ectocarpus* species from multiple locations across the globe.

III. Development

1. Life cycle and reproduction

Male and female gametes are morphologically identical in *Ectocarpus* (isogamy) but differ with respect to their physiology and their behaviour: female gametes settle sooner and produce a pheromone whilst male gametes swim for longer and are attracted to the pheromone produced by the female. Studies on the reproduction of *Ectocarpus* began with the observation of sexual fusions involving the attraction of male gametes to settled female gametes from field thalli of *E. siliculosus* in Naples, Italy (Berthold, 1881). These findings were hotly debated until Sauvageau (1896, 1897) and Oltmanns (1899) succeeded in repeating the experiment. Gamete fusions in *Ectocarpus* were later used by Hartmann (1934) to support his erroneous theory of relative sexuality (see Müller, 1976a

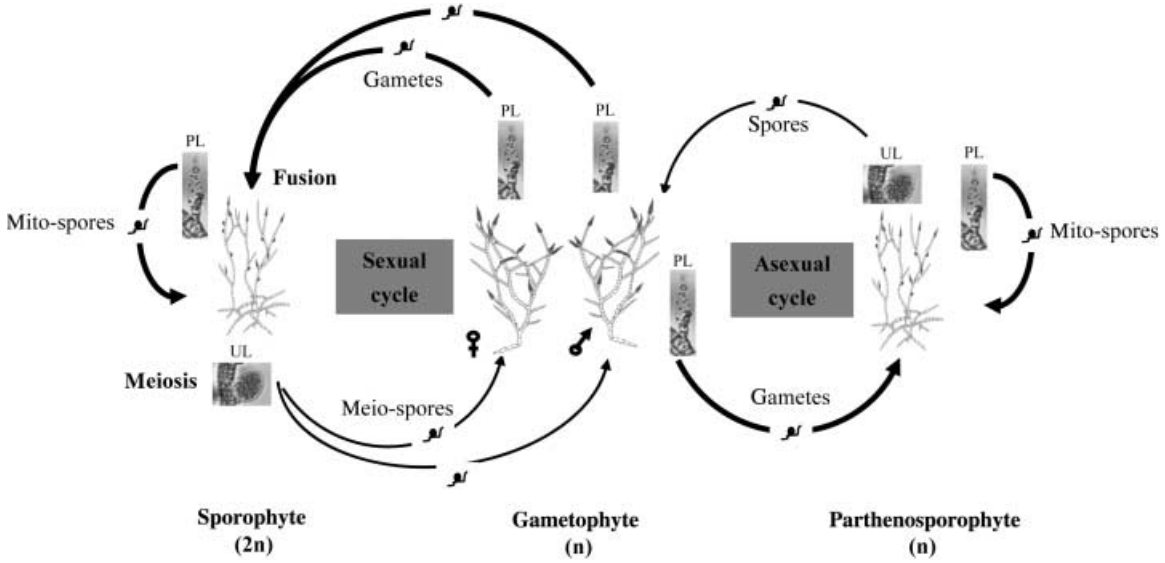

Fig. 2 Life cycle of *Ectocarpus siliculosus*. Diploid sporophytes produce meiospores (by meiosis) in unilocular sporangia (UL). Meiospores grow into male or female gametophytes (dioecism). Gametophytes produce gametes in plurilocular gametangia (PL). Fusion of gametes produces a zygote that grows into a diploid sporophyte, completing the sexual cycle. Unfused gametes may grow parthenogenetically and form a parthenosporophyte, which is indistinguishable from the diploid sporophyte. Both sporophytes and parthenosporophytes can reproduce themselves asexually by the production of mitospores in plurilocular sporangia.

for details). Knight (1929) identified the young, unilocular sporangium as the site of meiosis, Papenfuss (1935) and Kornmann (1956) published major contributions on the life history of *Ectocarpus*, and Boalch (1961) developed refined culture techniques. The entire life history of *E. siliculosus* from Naples was finally unravelled by Müller (1964, 1966, 1967, 1972b) using clonal cultures and chromosome counts. It is schematized in Fig. 2. The basic life history of *E. siliculosus* involves an alternation between the sporophyte and dioecious gametophytes, and sex determination is genotypic (Müller, 1967). Male and female gametophytes are morphologically indistinguishable. One of the problems with understanding the life history was that sporophytes and gametophytes are difficult to distinguish morphologically. Another problem was that zoids from plurilocular reproductive organs have different functions according to the generation forming them: on sporophytes they contain asexual zoospores that directly reproduce the sporophyte, while on gametophytes they contain gametes. Further complications include the parthenogenesis of unfused gametes, which develop into haploid parthenogenetic sporophytes morphologically indistinguishable from diploid sporophytes, and heteroblasty (different fates) of spores from unilocular sporangia, developing either into gametophytes or into sporophytes. Furthermore, life cycle generation is not determined rigidly by ploidy (Müller, 1967).

An important challenge for the future will be the characterization of the genetic mechanisms that control life cycle progression in *Ectocarpus*. This will require the development of methodologies for positional cloning of mutated loci and of genome-wide methods to analyse gene expression throughout the life cycle. Work is currently ongoing in several groups to develop these techniques.

2. Sporophyte and gametophyte architecture

Ectocarpus siliculosus is a small filamentous alga that grows to *c*. 30 cm in length in nature, but it may become fertile in the laboratory at 1–3 cm.

Sporophyte development is initiated with the germination of the zygote. The first division produces two cells of identical developmental fate (Peters *et al.*, 2004b). Subsequent mitoses lead to the formation of a basal (or prostrate) filamentous structure, defining the early sporophyte (Fig. 3a). Phaeophycean hairs, that is, hyaline filaments devoid of plastids developing from a basal meristem, are absent in *Ectocarpus* but present in the sister genus *Kuckuckia.* However, in *Ectocarpus* the distal end of filaments, or of plurilocular sporangia, may be less pigmented and resemble a hair; such structures may be referred to as pseudo-hairs (Cardinal, 1964; Pedersén, 1989). If the growth conditions are favourable (Ravanko, 1970), erect filaments (called 'upright' filaments) emerge after a few days, contributing to the establishment of an overall filamentous architecture (Fig. 3c).

The typical structure of a vegetative cell is illustrated in Fig. 4. Features common to all brown algal cells include a chloroplast surrounded by four membranes, arranged as two double-membraned envelopes (chloroplast envelope, CE). The second envelope is loosely associated with the chloroplast and forms part of the chloroplast endoplasmic reticulum (CER). The lamellae of the chloroplast are composed of three thylakoids,

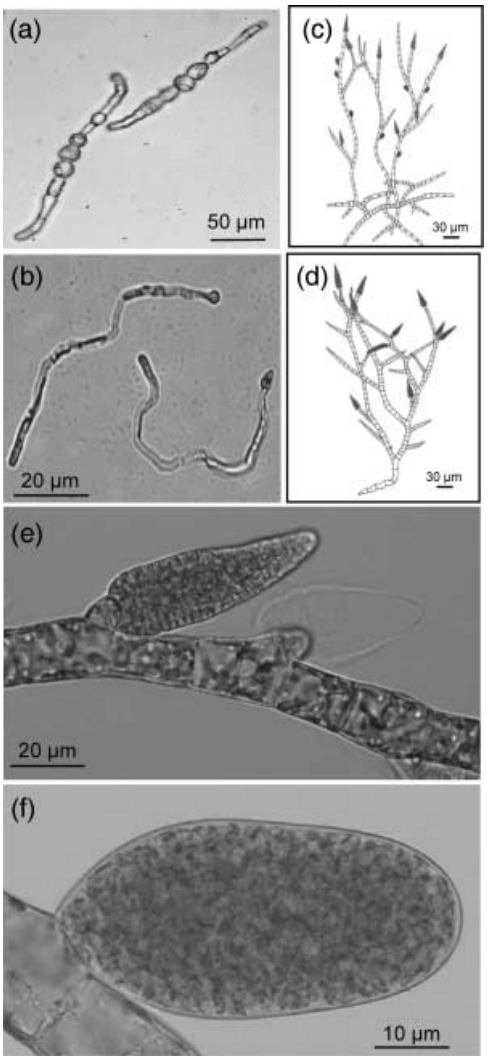

Fig. 3 Morphology of *Ectocarpus siliculosus*. Photographs of 1-wk-old vegetative sporophyte (a) and gametophyte (b) and schemes representing the whole body of the mature sporophyte (c) and gametophyte (d) after 6 wk of growth. (e) Plurilocular sporangium and gametangium (occurring on the sporophyte and the gametophyte, respectively) before (left) and after (right) release of zoids. (f) Unilocular sporangium from sporophyte. Sporangia and gametangia can be either sessile or pedicellate (Kim & Lee, 1992).

which are absent from the pyrenoid space (Bouck, 1965; Oliveira & Bisalputra, 1973). In *Ectocarpus* the chloroplast is ribbon-shaped. The size and number of chloroplasts may vary within the same organism (Ravanko, 1970). Other typical features of *Ectocarpus* include several prominent and pedunculated pyrenoids on the inner face of the chloroplast, which are used as a taxonomic marker for the Ectocarpales (Evans, 1966; Rousseau & de Reviers, 1999). Chloroplast endoplasmic reticulum and CE envelopes also surround pyrenoids, this time being tightly adjacent. A third external envelope, called the pyrenoid sac, surrounds the pyrenoid but has no connection with the reticulum system (Bouck, 1965). The nuclear envelope is continuous with the CER, which is itself in close vicinity to the Golgi apparatus (Bouck, 1965; Oliveira & Bisalputra, 1973). It has been hypothesized that these connections create a complex network of membranes allowing photosynthates to be efficiently transferred from the chloroplast to the Golgi apparatus, the latter being also in direct contact with the CER (Oliveira & Bisalputra, 1973). Cytoplasmic ER is dispersed throughout the cytoplasm and is mainly rough (Oliveira & Bisalputra, 1973). Osmiophilic bodies (OSB), which are thought to contain lipids, are dispersed throughout the cytoplasm and probably originate from the CER (Oliveira & Bisalputra, 1973). They have been observed within the cell wall and also external to it. Vacuoles can be either large structures occupying peripheral locations (Oliveira & Bisalputra, 1973) in fixed material, or most of the cellular space (as in land plant cells; Knight, 1929, confirmed by data from our laboratory, after staining with cresyl blue or neutral red, on sporophytic filaments). The nuclear region encompasses two centrioles, which are considered as a microtubule organizing centre (MTOC; Katsaros *et al.*, 1991). The chromosome number in the haploid nucleus is estimated to be *c.* 25 (Peters *et al.*, 2004a). Mitochondria are preferentially peripherally located. They are maternally inherited (Peters *et al.*, 2004b). The cell wall consists of a fibrillar matrix (see Section IV.1: Photosynthesis and carbohydrate metabolism) with several plasmodesmata distributed uniformly along the cross walls. Upon ageing, cell wall ingrowths occur, accompanied by a reduction in the size of the nucleus, mitochondria, ER and Golgi, followed by the disintegration of the chloroplasts and finally by autolysis of the cytoplasm (Oliveira & Bisalputra, 1977a,b).

Most of the reproductive organs are carried by upright filaments (Müller, 1964). Two types of reproductive organs are produced by the sporophyte: plurilocular and unilocular sporangia. Plurilocular sporangia are cone-shaped three-dimensional structures of variable size (Knight, 1929), composed of a large number of locules with different shapes (Baker & Evans, 1973b; Fig. 3e). These locules are generated by several successive mitoses and each gives rise to a single zoospore, which is released through the apex of the sporangium. The mitospores, which are biflagellate and competent for swimming shortly after their release (Müller, 1980), are a means of vegetative reproduction. Unilocular sporangia are born on the sides of branches (Fig. 3f, Baker & Evans, 1973a). A single meiosis occurs within the single thick-walled locule (Baker & Evans, 1973a) and this is followed by several mitoses, which generate about a hundred meiospores, half of which are female and half male (Müller, 1980). Müller (1963) and Ravanko (1970) reported that plurilocular sporangia were produced when the external temperature was relatively high (*c.* 20°C or summer), whereas unilocular sporangia were produced when the temperature was lowered to 13°C (mimicking winter conditions). However, this temperature dependence is not observed in many strains of *Ectocarpus* (A. F. Peters, unpublished data). Meiospores germinate to produce haploid, dioecious gametophytes

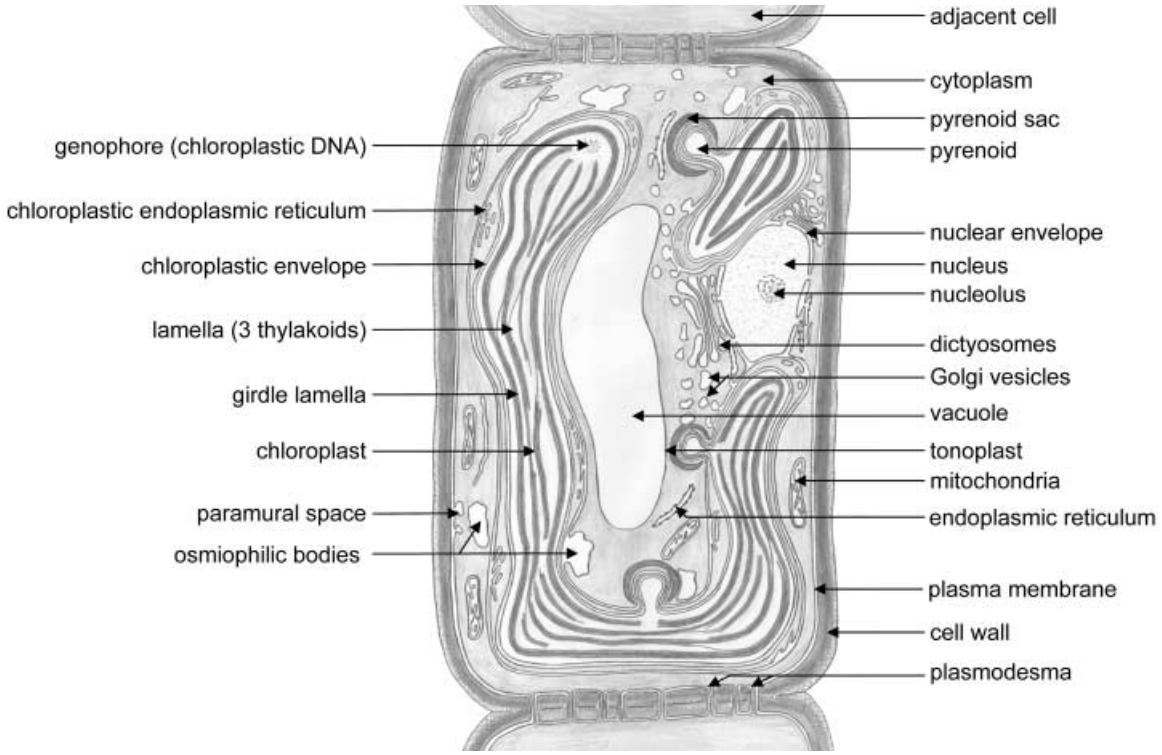

Fig. 4 General ultrastructure of a vegetative cell of *Ectocarpus siliculosus*. The general ultrastructure of a vegetative cell is similar in both prostrate and erect filaments (Oliveira & Bisalputra, 1973). The different compartments of the cell are illustrated (see text for details). Lines represent membranes and define subcellular compartments, except for thylakoids, drawn as a thick black line. Depending on their type and age, vegetative cell size varies from 10 to 35 μm in length, and 5 to 15 μm in width (under laboratory culture conditions).

(Fig. 3b,d). These are filamentous organisms similar to the sporophyte, but with two important differences. Firstly, the meiospore germinates asymmetrically to produce a rhizoid and an upright filament, so no prostrate structure forms (A. F. Peters, unpublished data). Secondly, the thallus is more ramified than that of the sporophyte (Müller, 1980). Gametophytes produce only plurilocular gametangia and these are similar structurally to plurilocular sporangia on sporophytes. Gametes resemble mitospores in terms of their size and motility.

The developmental patterning varies greatly across the *Ectocarpus* species complex and is also dependent on the environment, growth conditions and even the age of the algae for some features (Ravanko, 1970). This plasticity is observed for the branching frequency and the number, shape, structure and positioning of the reproductive organs on filaments (Knight, 1929; Müller, 1980; Kim & Lee, 1992). Phytohormones, especially cytokinins, have also been reported to influence the development of *Ectocarpus* sporophytes (Pedersén, 1968, 1973).

As already mentioned, brown algae are interesting for developmental studies because they have independently evolved complex multicellularity. *Ectocarpus* is a relevant model to address this problem as it is closely related to complex algae such as the Laminariales and the Fucales. However, developmental processes in *Ectocarpus* are clearly simplified compared with its morphogenetically more complex sister families, which provides an advantage for the detailed dissection of these developmental processes. In particular, the simple growth pattern of the uniseriate, branched filaments represents an ideal system for the combined application of genetic and mathematical modelling approaches to understanding developmental patterning and then addressing the issue of the evolution of development and multicellularity.

3. Gametes and spores

Several electron microscopy studies of the motile cells (zoids) of *Ectocarpus* have been reported (Baker & Evans, 1973a,b; Lofthouse & Capon, 1975; Maier, 1997a,b). The typical structure of an *Ectocarpus* zoid is illustrated in Fig. 5.

Ectocarpus zoids correspond to the 'primitive' type of brown algal zoid according to Kawai (1992). Gametes and spores typically contain a single chloroplast with a pyrenoid (Baker & Evans, 1973a; Lofthouse & Capon, 1975; Maier, 1997a). As in the vegetative cells, lamella are composed of three thylakoids, and the nuclear envelope is in continuity with the chloroplast endoplasmic reticulum (Maier, 1997a). The nucleus of the male gamete is rich in heterochromatin. Several dictyosomes are present in gametes and spores (Baker & Evans, 1973b; Maier, 1997a). The Golgi apparatus of mitospores is very active both before and after release (Baker & Evans,

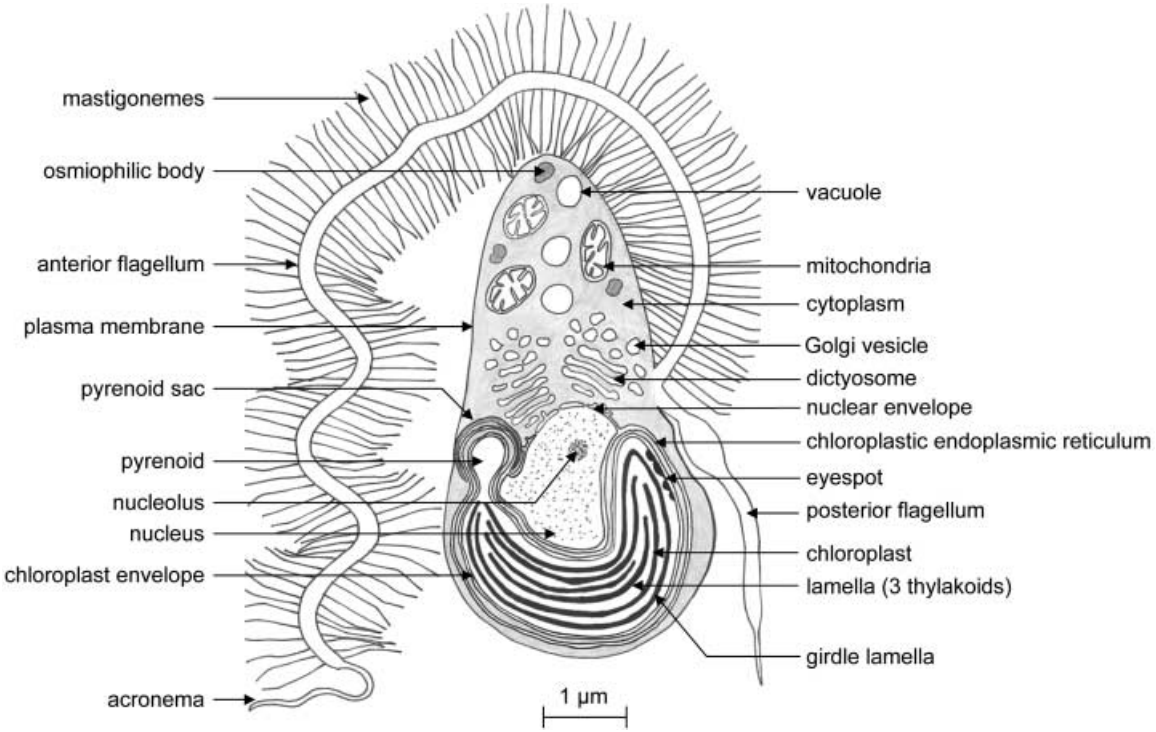

Fig. 5 General intracellular structure of *Ectocarpus siliculosus* zoids. A scheme representing the different compartments of an *Ectocarpus siliculosus* zoid cell from a plurilocular sporangium or gametangium is presented (see text for details). Lines represent membranes and define subcellular compartments, except for thylakoids, drawn as a thick black line.

1973b). This secretory activity may have important functions in the biosynthesis of the adhesive required for gamete adhesion and for the synthesis of new cell wall compounds during germination.

Ectocarpus gametes and spores are characterized by two flagella with lateral insertion. One is oriented forward and equipped with mastigonemes (hairs), and propels the cell with meandering beats. The second is oriented obliquely backwards and has no mastigonemes. Most of the time this posterior flagella is passively dragged, but occasional lateral beats induce abrupt changes in direction of up to 180° (Geller & Müller, 1981). Gametes and spores have a concave depression at the level of the eyespot into which the swelling of the proximal part of the posterior flagellum fits (Baker & Evans, 1973b; Kreimer *et al.*, 1991; Maier, 1997a). The possible function of the eyespot is to reflect and focus incident light on to the site of photoreception (Kawai *et al.*, 1990; Kreimer *et al.*, 1991). Zoids are capable of positive phototaxis and their posterior flagellum shows strong autofluorescence when irradiated by blue light (450 nm). The photoreceptor pigment is a flavoprotein, which is periodically shaded by a carotenoid stigma (Müller *et al.*, 1987; Kawai, 1988). The acronema (the whiplash tip) is extremely sensitive to mechanical stress and plays an important role in establishing the initial sexual contact between gametes. The details of the flagellar apparatus of both female and male gametes have been studied by electron microscopy (Müller & Falk, 1973; Maier, 1997b). Despite their different behaviours, no difference in their fine structure has been detected (Müller & Falk, 1973).

The pheromone that attracts male gametes to female gametes is an unsaturated hydrocarbon. The substance initially identified was ectocarpene (all-cis-1-(cycloheptadiene-2′,5′-yl)-butene-1) (Müller *et al.*, 1971; Müller, 1976b, 1978; Müller & Schmid, 1988) but more recently Boland *et al.* (1995) have shown that a thermally labile cyclopropyl precursor, pre-ectocarpene, is more active by three orders of magnitude and is thus the actual pheromone.

There is evidence that cell-to-cell recognition between *Ectocarpus* gametes is mediated by N-acetyl glucosamine residues exposed on the plasma membrane of the female gametes and that these residues are specifically recognized by a receptor on the male gamete (Schmid, 1993; Schmid *et al.*, 1994a). In addition, the lectins concanavalin A (Con A) and *Aleuria aurantia* agglutinin (AAA) bind specifically to the anterior flagella of *Ectocarpus* gametes and the molecules with which they interact also may be involved in the gamete recognition process (Maier & Schmid, 1995).

Zoids from plurilocular and unilocular *Ectocarpus* sporangia share similar overall intracellular structures, but there are some important differences: plurilocular zoids are smaller and swim faster with more rapid changes of direction (Baker & Evans, 1973a). Zoids from unilocular sporangia are strikingly different

from the other *Ectocarpus* cell types (both plurilocular zoids and vegetative cells) in that the nucleus is physically separated from the chloroplast. Moreover, secretory activity is lower than in zoids from plurilocular sporangia (Baker & Evans, 1973b).

Gametes of *Ectocarpus* have also been used to study chemotaxis (Boland *et al.*, 1983), receptor modelling (Boland *et al.*, 1989) and phototaxis (Kawai *et al.*, 1990; Kreimer *et al.*, 1991). Protocols for isolation and biochemical characterization of plasma membrane and CER membrane have also been developed (Schmid *et al.*, 1992). Together with more recently developed molecular tools, these methods now offer access to a range of interesting biochemical events that take place during gamete interaction at fertilization, such as chemoreception, cell-cell recognition and fusion processes.

IV. Metabolism

1. Photosynthesis and carbohydrate metabolism

Marine environment constrains several aspects of photosynthesis in brown algae. First, carbon dioxide is not the main source of inorganic carbon (Ci). Indeed, seawater in equilibrium with air contains only 13 μM CO2, but ~ 2 mM anionic carbon, mainly in the form of HCO_3^- (Beer, 1994). Secondly, when seaweeds are immersed they do not receive the full light spectrum. Light absorption increases with water depth and varies according to the wavelength: red light (650 nm) is absorbed first, followed by purple (400 nm) and yellow (550 nm) light. By contrast, green (500 nm) and blue (450 nm) light display a strong penetration: at 10 m depth, red light is almost fully absorbed whereas the absorption of green and blue light is not significant.

Adapted to these conditions, brown algae differ from land plants by the pigment composition of their light-harvesting complexes (LHCs). Chlorophyll *c* (chl*c*) and the carotenoid fucoxanthin are indeed the main light-haversting pigments of brown algae. Their presence broadens the absorption spectrum toward the green light relative to the chlorophytes. Brown algae possess two different LHCs associated with photosystems I and II (PSI and PSII), but their pigment composition is controversial. Barrett & Anderson (1980) described a fucoxanthin chl*a*/*c* protein and a violaxanthin-enriched chl*a*/*c* protein. Conversely, Alberte *et al.* (1981) reported a chl*a*/*c* protein devoid of fucoxanthin and a second LHC containing chl*a* and fucoxanthin but not chl*c*. More recent analyses showed that the LHCs associated with PSI and PSII are in fact virtually identical with respect to their pigmentation and peptide composition. All complexes bound chl*a*, chl*c* and fucoxanthin in the proportion 6 : 2 : 7, but the LHC associated with PSI is significantly enriched in violaxanthin (De Martino *et al.*, 2000). As in plants, these accessory pigments transfer energy to chl*a* within the photosynthetic reaction centres (Grossman *et al.*, 1995). Violaxanthin does not participate in light-harvesting but is involved in photoprotection (Demmig-Adams & Adams, 1992; Lemoine *et al.*, 1995).

Under saturating red light, photosynthesis of *E. siliculosus* follows a circadian rhythm, with maxima at about noon, and can be stimulated by a pulse of blue light (Schmid & Dring, 1992). This stimulation is also observed in other Phaeophytes, but mostly absent in green or red algae (Schmid *et al.*, 1994b). Blue light induces several responses: an acidification at the surface of *E. siliculosus* (Schmid & Dring, 1993), accompanied by bicarbonate uptake (Schmid, 1998). A plasma membrane H^+-ATPase is thought to be activated, and the resulting acidification to increase the conversion of HCO_3^- to CO_2 in the extracellular space (Schmid & Dring, 1993). Recently, such blue light-induced H^+-ATPases have been identified in the brown alga *Laminaria digitata* (Klenell *et al.*, 2002). Blue light also triggers the mobilization of an internal carbon source, since photosynthesis is stimulated even in the absence of external Ci (Schmid & Dring, 1996). As a result, a C_4-like metabolism was initially proposed to exist in *E. siliculosus* (Schmid & Dring, 1996) but not all the enzymes necessary for a C_4 cycle were detected (Busch & Schmid, 2001) and the pool of intermediates seems to be too small to act as an organic carbon stock (Hillrichs & Schmid, 2001). The sequestration of a pool of Ci in the vacuole and its movement to the cytosol in response to blue light is now the favoured hypothesis (Schmid & Hillrichs, 2001). Analysis of the sequence of the *E. siliculosus* genome will help to confirm whether or not a C_4 pathway exists in the brown algae.

In contrast to the *Plantae* (Moreira *et al.*, 2000), the *Phaeophyceae* do not store the carbon assimilated by photosynthesis as insoluble starch granules, but instead as the soluble 1,3-β-glucan polymer (laminarin) localized in the cytosol (Craigie, 1974), and as mannitol, involved in osmo-acclimation (Davis *et al.*, 2003; see Section V.2: Abiotic stresses). Brown algae also produce complex polysaccharides which constitute their cell wall. They synthesize some neutral polysaccharides in common with land plants, such as cellulose (Carpita & McCann, 2000), but also unique anionic polysaccharides, such as alginates and sulphated fucans (Kloareg & Quatrano, 1988). *Ectocarpus* has not been well studied in this respect, but preliminary analyses in our laboratory confirm that all of the polysaccharides typical of brown algae are present in this genus (Estelle Deniaud, pers. comm.). The biosynthetic pathways of these brown algal polysaccharides are essentially unknown and the genome of *E. siliculosus* will be a much-anticipated asset to investigate these crucial metabolisms.

2. Lipid metabolism

Worldwide, at least 50 species of brown algae are used as human food. Their lipid content has therefore attracted considerable attention from the viewpoint of both nutrition and pharmacology. A number of studies have been conducted to profile and quantify the fatty acids and the different classes

of lipids in these organisms, and to investigate whether the various lipid patterns correlate with the taxonomic position or any other characteristic of the brown algae. Brown algal polar lipids include several common glycolipids (monogalactosyldiacylglycerol, digalastosyldiacylglycerol, sulfoquinovosyldiacylglycerol) and phospholipids (phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phospahtidylglycerol and diphosphatidylglycerol). Interestingly, several reports have highlighted the high proportion of long-chain polyunsaturated fatty acids (LC-PUFAs) (Eichenberger *et al.*, 1993). Moreover, most of the Phaeophyceae contain the betaine lipids diacylglycerylhydroxymethyl-*N*,*N*,*N*-trimethyl-β-alanine (DGTA) and diacylglyceryltrimethylhomoserine, and either contain the common phosphatidylcholine phospholipids in surprisingly low amounts or do not produce them at all (Eichenberger *et al.*, 1993). All Ectocarpales contain phosphatidylcholine, but exhibit interspecific variation in their DGTA content: *E. fasciculatus* strains contain this lipid while *E. siliculosus* strains do not (Müller, 1995; Müller & Eichenberger, 1995). Diacylglycerylhydroxymethyl-*N*,*N*,*N*-trimethyl-β-alanine may therefore be used as a taxonomic marker (Müller, 1995), although an *E. fasciculatus* strain deficient in DGTA biosynthesis has been described. Genetic analysis of such a strain identified an autosomal locus necessary for the biosynthesis of this lipid (Müller & Eichenberger, 1997).

A novel phosphoglyceride, designated PX, was first isolated from *E. siliculosus*. It was shown to account for 2–4% of total lipids (Schmid *et al.*, 1994c), and to accumulate mostly in the plasma membrane of gametes. Since PX is rich in 20:4n-6 (arachidonic acid) and 20:5n-3 (eicosapentaenoic acid), it has been suggested that it could represent a potential reservoir for pheromone precursors. PX was subsequently detected in other brown algae (Ectocarpales, Fucales and Sphacelariales; Schmid *et al.*, 1994c).

Lipid metabolism plays also major roles in the control of defense mechanisms. Therefore, together with the genome sequence, it is likely that the efforts to screen mutants with altered resistance to pathogen attacks will lead to the phenotyping of plants impaired in lipid or fatty acid metabolic pathways, thereby potentially revealing novel specific traits of this metabolism in brown algae.

V. Interactions with the environment

1. *Ectocarpus* pathogens

Despite their small size and ephemeral life stages, filamentous brown algae have been frequently reported to be plagued by various pathogens, including viruses (Müller *et al.*, 1998) and eukaryotic parasites of different phylogenetic lineages: oomycetes, chytrids and hyphochytrids (Andrews, 1976; Küpper & Müller, 1999; Müller *et al.*, 1999) and by parasites related to the Plasmodiophorea (Karling, 1944; Maier *et al.*, 2000). In addition, numerous historical records described ectocarpoids with abnormal sporangia or vegetative cells suspected to contain unknown parasites (Rattray, 1885; Müller *et al.*, 1998).

The oomycete *Eurychasma dicksonii* has been described mainly in wild populations of *Pylaiella littoralis* (Küpper & Müller, 1999), but it displays a broad host range and infects various brown algae, including *Ectocarpus* (Müller *et al.*, 1999), in which it was initially described by Wright (1879). There is a current effort to set up a defined pathosystem using *E. siliculosus* and *E. dicksonii*, and *Ectocarpus* strains have been shown to exhibit differential susceptibility to a defined *Eurychasma* strain. Conversely, several *Eurychasma* strains exhibit different host specificities, suggesting coevolution of the two species (Gachon *et al.*, 2007). The molecular bases of resistance and virulence are under investigation.

Chytrids were described earlier by Petersen (1905), and the hyphochytrid *Anisolpidium ectocarpii* was described by Karling (1943) and Johnson (1957). Like *E. dicksonii*, *Chytridium polysiphoniae* (Chytridiomycota) is ubiquitous and can infect many hosts, including *E. siliculosus* and *E. fasciculatus* (Müller *et al.*, 1999). Interestingly, its negative effects on photosynthesis of its host was described at the cellular level in the related ectocarpoid *P. littoralis* using fluorescence kinetic microscopy (Gachon *et al.*, 2006). Recently, the 18S rRNA genes of *Chytridium polysiphoniae* and *Eurychasma dicksonii* were sequenced and used to clarify their phylogenetic affiliations (Küpper *et al.*, 2006). The plasmodiophorean *Maullinia ectocarpii* is an obligate intracellular parasite of *Ectocarpus* spp. (Maier *et al.*, 2000). However, the extent to which this infection occurs in nature and its effect on algal fitness are presently unknown.

Viral infections represent by far the most studied phenomenon in *E. siliculosus* (Müller, 1996; Müller & Knippers, 2001). Until the late 1980s, most reports of virus infections in brown algal tissues were based on electron microscopy studies, which sporadically described 'virus-like particules' (VLPs). Viruses were obtained in culture for the first time from a New Zealand strain of *E. siliculosus* after lysis of host cells, allowing evaluation of their infection potential (Müller, 1991; Müller *et al.*, 1990). Virus infections were found in approx. 50% of the individuals of a given natural population (Dixon *et al.*, 2000; Müller *et al.*, 2000) and were shown to occur worldwide in correlation with the cosmopolitan distribution of *E. siliculosus* (Müller, 1991; Sengco *et al.*, 1996).

The viruses that infect different ectocarpoid algae exhibit considerable variability in size and diameter and, in general, display a high degree of host specificity (Müller *et al.*, 1998). However, several instances of trans-specific infection have been described, for example between EsV-1 (*Ectocarpus siliculosus* virus-1) and *Kuckuckia kylinii* (Müller, 1992; Müller & Schmid, 1996) and also between EfasV-1 (*Ectocarpus fasciculatus* virus-1) and *E. siliculosus* (Müller *et al.*, 1996; Sengco *et al.*, 1996). Interestingly, EsV-1 and EfasV-1 are the most similar of the brown algal viruses in terms of their genome size (Müller *et al.*, 1996).

The EsV-1 virus specifically infects the single-celled gametes or spores, that is, the only cells in the life history that lack a cell wall (Maier & Müller, 1998). Following infection, a single copy of the viral DNA appears to integrate into the host genome (Delaroque *et al.*, 1999). The viral DNA is then transmitted, via mitotic divisions, to all the cells of the developing alga. This has been confirmed by regenerating algae from protoplasts derived from virus-infected gametophytes (Kuhlenkamp & Müller, 1994). Despite the fact that they carry the integrated virus, vegetative cells do not produce viral particles (Müller *et al.*, 1998). Viral particles are only produced in reproductive organs (sporangia and gametangia) of mature algae from where they are released to infect a new generation of zoids. In addition to these cycles of re-infection, the viral genome can also be transmitted to progeny through meiosis, in which case it segregates as a Mendelian factor and is inherited by half of the progeny (Müller, 1991; Bräutigam *et al.*, 1995). The pathogenic character of viral infections has been unambiguously confirmed, but this association's main impact is on reproductive success. Plant sterility varies from partial (Müller *et al.*, 1990) to total (Müller & Frenzer, 1993), but no significant difference in photosynthesis, respiration and growth rate were observed in infected gametophytes or sporophytes (Del Campo *et al.*, 1997). This contrasts with the reduced photosynthetic performance of *Feldmannia* species infected with FsV (Robledo *et al.*, 1994).

The EsV-1 genome is a circular DNA molecule of a relatively large size (335 kbp) for a phycodnavirus (Van Etten & Meints, 1999; Van Etten *et al.*, 2002) with double-stranded regions interrupted by single-stranded regions (Lanka *et al.*, 1993; Klein *et al.*, 1994). Both EsV-1 and the related *Feldmania irregularis* virus (FirrV-1) have been sequenced (Delaroque *et al.*, 2001, 2003). EsV-1 contains approx. 231 genes with a wide range of predicted functions, including DNA metabolism, signalling, transposition, DNA integration and polysaccharide metabolism (Delaroque *et al.*, 2000a,b, 2003). It has also been proposed that the ability of the virus to integrate into its host's genome could be exploited to develop a transformation vector for a wide range of brown algae, including *E. siliculosus* (Henry & Meints, 1994, Delaroque *et al.*, 1999). However, the complex integration pattern of the virus into the algal genome will considerably complicate this task (N. Delaroque, pers. comm.). A microarray has been constructed to analyse EsV-1 gene expression (Declan Schroeder, pers. comm.) and it will be particularly interesting in the future to couple the analysis of viral and genome-wide host gene expression during viral infection.

The development of genomic tools provides a new context to investigate the possible genetic basis of the coevolution between some pathogens and brown algae. The search for inducers of defense responses and resistance against parasites is also still ongoing as, in contrast to kelps, *Ectocarpus* does not react with an oxidative burst upon recognition of alginate fragments (Küpper *et al.*, 2002a).

2. Abiotic stresses

Ectocarpus siliculosus is able to exploit a wide range of habitats and environmental conditions (see Section II.2: Distribution). This feature seems likely to be based at least as much as on a high intrinsic genetic variability as on a general physiological toughness, as illustrated by work carried out on copper and saline stress responses.

Interspecific variations in copper tolerance have been observed between different strains of *E. fasciculatus* and *E. siliculosus*, with the latter being the most tolerant (Morris, 1974). Differences have also been observed among *E. siliculosus* strains that are differently exposed to copper in their natural habitat (Russell & Morris, 1970; Hall, 1981). Cu^{2+} interferes with the general process of photosynthesis in brown algae, and particularly in *E. siliculosus*, by competing with magnesium for metal-binding sites in the chlorophyll molecules (Küpper *et al.*, 2002b). A study of the mechanism of tolerance to copper and other heavy metals suggested a co-tolerance to copper, cobalt and zinc, and provided evidence for an exclusion mechanism to explain the particularly low sensitivity of *E. siliculosus* copper-tolerant strains (Hall *et al.*, 1979; Hall, 1980, 1981). However, as yet there is no clear explanation for the intraspecific variation with respect to this trait within this species.

It has been suggested that the ability of some *E. siliculosus* strains to tolerate copper may be useful for the development of bioassays in which this alga is used for monitoring marine antifouling characteristics of copper-based materials (Hall & Baker, 1985, 1986). Copper chloride has been used to inhibit *E. siliculosus* infestations in tank cultures of *Gracilaria gracilis* (Van Heerden *et al.*, 1997).

Russell & Bolton (1975) reported the occurrence of salinity ecotypes within *E. siliculosus*. This study was extended by Thomas & Kirst (1991a,b), who showed that large differences in photosynthesis, accumulation of osmotically active compounds (mannitol; Davis *et al.*, 2003) and vitality occur between *E. siliculosus* isolates from different geographic locations following changes in salinity. They also observed that sporophytes were more salt-tolerant than gametophytes, irrespective of their level of ploidy.

Detailed investigations are necessary to decipher the physiological and cellular bases of salt and heavy metal tolerance in *E. siliculosus*. Mutagenesis and transcriptomic approaches will thus help to better understand the mechanisms involved in osmotic and oxidative adaptation, and to explain how these algae can cope with such a wide range of environmental conditions.

VI. Conclusion

Taken together, the above sections illustrate the broad range of phenomena that have been studied in *Ectocarpus* and provide an indication of the domains that could be further explored in the future. Notably, a large proportion of this past work, covering many diverse aspects of *Ectocarpus* biology, was carried out in

Dieter Müller's laboratory in Konstanz, and the efforts of this group have therefore laid the foundations for the development of *Ectocarpus* as a model organism.

The *Ectocarpus* genome project has federated a consortium of laboratories with an interest in this organism and these laboratories are currently developing several molecular tools. These include mutant screens, genetic transformation and genome-scale analysis of gene expression. Several developmental mutants have been isolated and positional cloning of some of the affected genes should be feasible in the near future. The availability of the genome sequence together with the ability to analyse gene function by forward and reverse genetic approaches will make it possible to address additional questions, many of which have been evoked in this review. Examples include the biosynthesis of diverse, brown algal-specific metabolites, such as lipids and complex cell wall components, and the genetic basis of resistance to biotic and abiotic aggression. The genome will also be an invaluable aide for the study of the ecology of *Ectocarpus*, by serving as the base for the development of neutral and selected molecular markers for the analysis of field isolates. In conclusion, the *Ectocarpus* genome sequence and the tool development associated with this project are providing access to a relatively unexplored branch of the eukaryotic tree and some exciting discoveries can be expected in this domain in the coming years.

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