

## PROPOSAL OF *ECTOCARPUS SILICULOSUS* (ECTOCARPALES, PHAEOPHYCEAE) AS A MODEL ORGANISM FOR BROWN ALGAL GENETICS AND GENOMICS<sup>1,2</sup>

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**The emergence of model organisms that permit the application of a powerful combination of genomic and genetic approaches has been a major factor underlying the advances that have been made in the past decade in dissecting the molecular basis of a wide range of biological processes. However, the phylogenetic distance separating marine macroalgae from these model organisms, which are mostly from the animal, fungi, and higher plant lineages, limits the latter's applicability to problems specific to macroalgal biology. There is therefore a pressing need to develop similar models for the macroalgae. Here we describe a survey of potential model brown algae in which particular attention was paid to characteristics associated with a strong potential for genomic and genetic analysis, such as a small nuclear genome size, sexuality, and a short life cycle. Flow cytometry of nuclei isolated from zooids showed that species from the Ectocarpales possess smaller haploid genomes (127–290 Mbp) than current models among the Laminariales (580–720 Mbp) and Fucales (1095–1271 Mbp). Species of the Ectocarpales may complete their life histories in as little as 6 weeks in laboratory culture and are amenable to genetic analyses. Based on this study, we propose *Ectocarpus siliculosus* (Dillwyn) Lyngbye as an optimal choice for a general model organism for the molecular genetics of the brown algae.**

**Key index words:** algae; genetics; genomics; marine macroalgae; model species; Phaeophyceae

Research into the biology of the brown algae has been stimulated by their global economic and ecological importance. Brown seaweeds represent an important resource, with a wide range of uses in the food, cosmetic, and fertilizer industries, and are attracting increasing attention as a source of active biomolecules

(McHugh 2003). The brown algae are also of interest from a developmental point of view, because they represent one of only five eukaryotic lineages that have independently evolved complex multicellularity (the four others being animals, fungi, green plants, and red algae). Also, the alternation between gametophyte and sporophyte, which involves sequential development of two independent complex multicellular organisms, represents a novel situation compared with the life cycles of model organisms from other groups, such as green plants and animals, in which the gametophyte generation is usually highly reduced or absent. Hence, brown algae also are an excellent model with which to study the genetic basis of the alternation of generations.

Many interesting features of the brown algae stem from their phylogenetic distance from other more intensively studied groups. However, this is also one of the reasons that research in this field has progressed relatively slowly. Research on animals and higher plants has benefited greatly in recent years from the development of model organisms such as *Drosophila melanogaster*, *Caenorhabditis elegans*, and *Arabidopsis thaliana* that combine complete genome sequences with powerful tools for forward and reverse genetics, such as mutagenesis, genetic transformation, and RNA interference (Dawis 2004). However, outside the animal and green plant kingdoms, the usefulness of such models is limited. There is therefore a pressing need in phylogenetically distant groups for similar models that can be used to address questions specific to these lineages. In a recent review, Waaland et al. (2004) proposed the Bangiophyte *Porphyra yezoensis* Ueda as a candidate macroalgal model for genomics. The economic importance of *Porphyra* makes this species the model of choice for red algae. However, a red algal species would be of limited use to groups working on brown algae for the same reasons as described above, that is, the red algae are probably as phylogenetically distant from the brown algae as is, for example, *A. thaliana*.

The aim of this study was to propose a model species for the Phaeophyceae. Among the 10 top

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macroalgal genome candidates identified by Waaland et al. (2004), there were five brown algae: *Macrocystis*, *Laminaria*, *Ectocarpus*, *Dictyota*, and *Fucus*. We examined these and other brown algal species with the aim of identifying a species that is potentially amenable to the combination of genetic and genomic approaches that have been so effective in the animal and green plant model species. Such approaches are greatly facilitated in organisms with small genomes, and hence, genome size is a major factor influencing the usefulness of a model organism. An estimation of the genome sizes of five species of brown algae carried out by Le Gall et al. (1993) indicated large haploid genomes of 500–1550 Mbp, including 560, 640, and 720 Mbp for *Undaria pinnatifida*, *Laminaria digitata*, and *L. saccharina*, respectively. Stache (1993), however, reported a smaller size for *Ectocarpus siliculosus*, with different strains possessing genomes of between 132 and 506 Mbp. To date, genome sizes have not been reported for the Fucales despite the importance of these algae in ecology (Chapman 1995, Worm et al. 2001, Santelices 2002), population biology (Coyer et al. 2003), and cell biology (Brownlee and Wood 1986, Coelho et al. 2002). Using flow cytometry of nuclei isolated from gametes, we determined the genome sizes of *Fucus serratus*, *F. vesiculosus*, and *Ascophyllum nodosum*. We also examined genome sizes for two small brown algae with sexual life histories, *Striaria attenuata* and *Scytosiphon lomentaria*, the latter of interest because of classical studies on the ecology of the heteromorphic life history (Lubchenco and Cubit 1980) and because of recent cell biological work (Nagasato et al. 2000, 2003). Finally, we redetermined the nuclear genome sizes of *Ectocarpus* spp.

Another major factor that has contributed to the usefulness of many model organisms is the facility with which genetic analyses can be carried out. This implies that the organism be easily maintained and manipulated under laboratory conditions and that the life cycle be sufficiently rapid to allow classical genetic analyses to be carried out in a reasonable time span. This criterion excludes species of the Laminariales and Fucales, despite their high commercial, ecological, and scientific importance, because of the difficulty in obtaining mature sporophytes under laboratory conditions. Identification of alternative candidate model brown algae is complicated by the fact that although

the life cycles of many smaller brown algae have revealed sexuality, data concerning the length of the life cycle, particularly under laboratory conditions, are scarce. Also, in numerous species meiosporangia do not form readily in culture, and hence small endophytic species such as *Laminariocolax macrocystis* Peters must be excluded despite their very short generation times of about 2 weeks in asexual cycles (Peters 1991a). We determined generation times in laboratory cultures of the ectocarpalean taxa, *Striaria attenuata*, *Ectocarpus siliculosus*, and *Myriotrichia claviformis*, which have haploid–diploid life histories, dioecious gametophytes, and isogamy and whose life cycles of different complexity (see Results) can be completed in the laboratory (Müller 1967, Peters 1988, 1991b). In nature, *Striaria* is annual, whereas *Ectocarpus* and *Myriotrichia* are referred to as ephemerals because repeated generations of sporophytes can follow each other during the growing season.

We studied a range of features of *Striaria*, *Ectocarpus*, and *Myriotrichia* relevant to their potential as model organisms and compared them with published data for other Phaeophyceae. Based on the criteria considered, we suggest that *Ectocarpus siliculosus* would currently be the best choice as a genetic/genomic model organism for the brown algae.

#### MATERIALS AND METHODS

**Algal material.** Authorities for algal species names are provided in Tables 2 and 3. Algal strains (Table 1) were cultivated in autoclaved seawater enriched with 10 mL Provasoli enrichment  $\cdot L^{-1}$  (enrichment prepared according to Starr and Zeikus 1993), at 4, 10, 14, and 18°C (each  $\pm 1^\circ C$ ). Illumination at 10–18°C was performed by cool-white fluorescent tubes, with 20–30  $\mu mol photons \cdot m^{-2} \cdot s^{-1}$  irradiance. At 4°C the cultures received natural light of varying intensity at a north-facing window. *Striaria* sporophytes were cultivated in 100-mL volumes in glass dishes. Thalli of *Ectocarpus* and *Myriotrichia* and gametophytes of *Striaria* were cultivated in 7- to 10-mL volumes in 5 cm diameter polystyrene Petri dishes.

**Determination of genome sizes.** In previous studies (Le Gall et al. 1993, Ar Gall et al. 1996, Asensi et al. 2001), nuclei of macroalgae were extracted from vegetative thalli. Because this method was not successful for ectocarpalean species, we collected their zooids, released their nuclei by suspension in buffer (see below), and measured their DNA content immediately by flow cytometry. Depending on cell density, each single measurement was performed on 1–20  $\mu L$  of phototactically concentrated zooid suspension released from cultured

TABLE 1. Cultures used.

Species	Sex	Origin	Isolation date
<i>Ectocarpus fasciculatus</i>	Male	Perharidy, Roscoff, France	January 2002
<i>Ectocarpus siliculosus</i>	Female	Roscoff, France	1970
	Male	Naples, Italy	1975
	One female, one male	San Juan, Peru	November 1988
<i>Myriotrichia claviformis</i>	One female, one male	Finavarra, Co. Clare, Ireland	August 1986
<i>Striaria attenuata</i>	Two females, two males	Yaldad, Chiloé, Chile	October 1987
	Three females, three males	Quetalmahue, Chiloé	November 1989

The strains were clonal gametophytes obtained from meiospores of field-collected sporophytes and were free of eukaryotic contamination. They were maintained as stock cultures after isolation.

TABLE 2. Haploid nuclear genome sizes of brown algae.

Species	Classification	Haploid genome size (Mbp) $\pm$ SD; <i>n</i>	Source	Comment
<i>Striaria attenuata</i>	Ectocarpales, Chordariaceae	127 $\pm$ 9; 3	This study	Strain SattY5f
<i>Scytosiphon lomentaria</i>	Ectocarpales, Scytosiphonaceae	222 $\pm$ 14; 9	This study	Asexual field thalli
<i>Ectocarpus siliculosus</i>	Ectocarpales, Ectocarpaceae	132–506	Stache (1993)	
<i>Ectocarpus siliculosus</i>	Ectocarpales, Ectocarpaceae	214 $\pm$ 13; 8	This study	Strain Esil 32 m from Peru
<i>Ectocarpus siliculosus</i>	Ectocarpales, Ectocarpaceae	240 $\pm$ 8; 28	This study	Strains from Naples and Roscoff
<i>Ectocarpus fasciculatus</i>	Ectocarpales, Ectocarpaceae	290 $\pm$ 8; 2	This study	
<i>Pylaiella littoralis</i>	Ectocarpales, Acinetosporaceae	500	Le Gall et al. (1993)	An asexual species
<i>Undaria pinnatifida</i> (Harvey) Suringar	Laminariales, Alariaceae	580	Le Gall et al. (1993)	
<i>Laminaria digitata</i> (Hudson) Lamouroux	Laminariales, Laminariaceae	640	Le Gall et al. (1993)	
<i>Laminaria saccharina</i> (Linnaeus) Lamouroux	Laminariales, Laminariaceae	720	Le Gall et al. (1993)	
<i>Fucus serratus</i> Linnaeus	Fucales, Fucaceae	1095 $\pm$ 70; 10	This study	
<i>Fucus vesiculosus</i> Linnaeus	Fucales, Fucaceae	1140 $\pm$ 61; 6	This study	
<i>Ascophyllum nodosum</i> (Linnaeus) Le Jolis	Fucales, Fucaceae	1271 $\pm$ 39; 6	This study	
<i>Sphacelaria</i> sp.	Sphacelariales, Sphacelariaceae	1550	Le Gall et al. (1993)	Life history unknown

For the strains newly studied, SD and number of independent measurements (*n*) are provided.

gametophytes of *Ectocarpus fasciculatus*, *E. siliculosus*, and *Striaria attenuata* and from field thalli of *Scytosiphon lomentaria*, *Fucus serratus*, *F. vesiculosus*, and *Ascophyllum nodosum*. Gametes were used in all cases except *Scytosiphon* (sexuality has not so far been detected in *S. lomentaria* at Roscoff). Zoids were resuspended into 500  $\mu$ L buffer containing 30 mM MgCl<sub>2</sub>, 120 mM trisodium citrate, 120 mM sorbitol, 55 mM HEPES, 5 mM EDTA supplemented with 0.1% (v/v) Triton X-100, and 5 mM sodium bisulfite. The nucleic acid-specific stain SYBR Green I (Molecular Probes Inc., Eugene, OR, USA) was used at a final dilution of 1:10,000. Samples were analyzed using a FACSort flow cytometer (Becton Dickinson, San Jose, CA, USA). Nuclei of field-collected *Chondrus crispus* gametophytes (150 Mbp, extracted by chopping thalli, details in Le Gall et al. 1993) were added as an internal reference for the determination of the genomes of Ectocarpales and gamete nuclei from European strains of *Ectocarpus siliculosus* (240 Mbp, see Results) for the genomes of Fucales.

## RESULTS

**Nuclear genome sizes.** Flow cytograms obtained with nuclei isolated from zoids of brown algae showed single maxima. With 127  $\pm$  9 Mbp, *Striaria attenuata* possessed the smallest haploid nuclear genome, followed by a Peruvian strain of *Ectocarpus siliculosus* (214  $\pm$  13 Mbp), *Scytosiphon lomentaria* (222  $\pm$  14 Mbp), European strains of *Ectocarpus siliculosus* (240  $\pm$  8 Mbp; genome sizes of strains from Naples and Roscoff were not significantly different and thus pooled), and *E. fasciculatus* (290  $\pm$  10 Mbp). In contrast, *Fucus serratus* (1095  $\pm$  70 Mbp), *F. vesiculosus* (1140  $\pm$  61 Mbp), and *Ascophyllum nodosum* (1271  $\pm$  39 Mbp) exhibited significantly larger values (Fig. 1, Table 2).

**Life histories and generation time under laboratory conditions (Fig. 2).** *Striaria attenuata*: Meiospores released from unilocular sporangia developed into microscopic gametophytes that required cultivation at low temperature to induce gametogenesis (5–7

weeks at 4° C to reach full maturity). Gametangia were ectocarpoid and distinguishable from vegetative gametophyte filaments at low magnification using a binocular microscope. Sporophytes were macroscopic and became reproductive after 6–7 weeks when cultivated at 18° C, producing clusters of unilocular sporangia. At lower temperatures growth was slower, and the thalli did not reach reproductive maturity, although it is possible that they may have become reproductive if cultivated for longer. The minimum time required to complete the entire life cycle was 3 months. *Striaria* sporophytes did not show direct reproduction via spores, but sporophytes could be propagated vegetatively by fragmentation because each apex of the profusely branched thallus is capa-

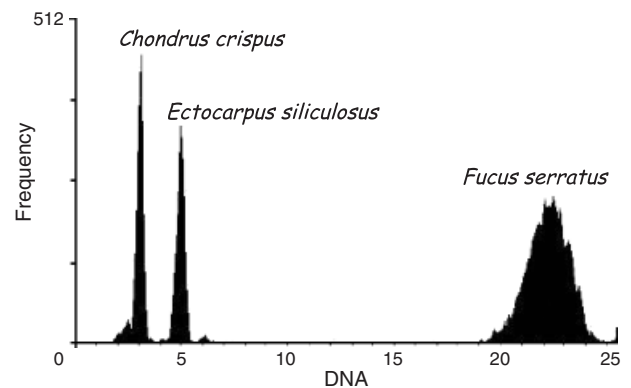


FIG. 1. Measurement of DNA content by flow cytometry of isolated haploid nuclei of *Chondrus crispus* (150 Mbp), a strain of *Ectocarpus siliculosus* from Naples (240 Mbp), and *Fucus serratus* (1095 Mbp). The DNA stain was SYBR Green I. The DNA content per particle is given in an arbitrary linear scale in the *x* axis and the numbers of corresponding particles in the *y* axis. The DNA content of *Fucus* nuclei is about 4.5 times that of *Ectocarpus*.

ble of regenerating a complete thallus. Nonfused gametes developed parthenogenetically. In five male strains tested, all gametes developed into thalli of sporophytic morphology (parthenosporophytes). In five female strains tested, 48%–97% (mean, 72%; SD, 22%) of the nonfertilized gametes also developed into parthenosporophytes and the remaining gametes developed into gametophytes.

*Ectocarpus siliculosus*: Both generations grew well and became fertile at 14° C, and this temperature was therefore chosen for cultivation of this species. The two generations were macroscopic but morphologically distinguishable. Sporophytes were initially prostrate thalli that formed erect filaments with few branches. Sporophytes reproduced directly via mitospores formed after 4 weeks in plurilocular sporangia borne on both the prostrate and the erect filaments. About 1 week after the appearance of plurilocular sporangia, unilocular sporangia (meiosporangia) developed on the erect filaments. Spores from unilocular sporangia developed into gametophytes that formed branched erect thalli. These thalli bore fertile gametangia after 3 weeks. Regardless of sex, unfertilized gametes developed into parthenosporophytes. In our fastest growing Peruvian strains of *E. siliculosus*, completion of the life history in culture required 2 months.

*Myriotrichia clavaeformis*: Sporophytes cultivated at 18° C produced fertile unilocular sporangia (meiosporangia) after 3 weeks. If cultivated at 10 or 14° C, however, the sporophytes formed plurilocular sporangia (mitosporangia) instead (after 4 and 3 weeks, respectively), initiating an asexual cycle that produced a further generation of sporophytes. Spores from unilocular sporangia developed into gametophytes that required 2 weeks at 10–15° C to become fertile. If cultivated under long-day conditions, gametes were produced, and these were able to

fuse with gametes of the opposite sex to form zygotes and reestablish the sporophyte generation. In contrast, under short-day conditions, the zoids produced were asexual and reproduced the gametophyte generation. Parthenogenesis of unfused gametes mainly resulted in gametophytes, but regardless of sex a small proportion of gametes developed into parthenosporophytes. Under optimum conditions, the sexual cycle was completed in 6 weeks.

#### DISCUSSION

Ideally, a genetic model organism should have sexuality, a small genome size, be easily cultivated, and be able to complete its life cycle under laboratory conditions. The size and the fertility of the organism is also important if large populations are to be created and maintained, as is the length of the life cycle. Other factors that should be taken into consideration include the possibility to perform controlled crosses, to isolate meiosporangia for tetrad analysis, amenability to gene transfer, economic importance, common use of the species in ecological, cell biological or physiological research, temperature requirements, and the availability of axenic cultures.

We conducted a survey of relevant characters to evaluate the potential of brown algal species for the application of genetic and genomic approaches with the aim of selecting a model organism for this group (Table 3). Asexual species were excluded. Genome sizes of Laminariales and Fucales are 580–720 Mbp (Le Gall et al. 1993) and larger than 1 Gbp, respectively (this study). Genetic approaches are difficult in algae of these orders because of their large thallus sizes and long life cycles. Furoid species, which have a diplontic life history, cannot easily be cultivated in the laboratory, and outplanting is required to obtain mature thalli. However, parthenogenesis does not occur, and con-

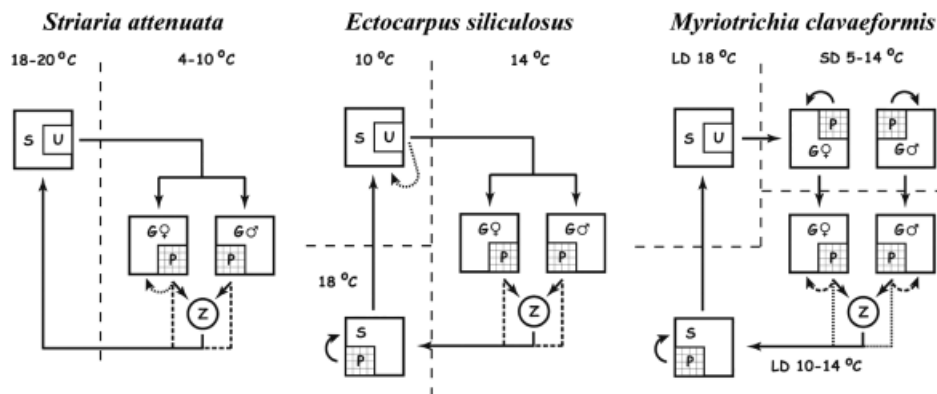


FIG. 2. Life histories of increasing complexity in *Striaria attenuata*, *Ectocarpus siliculosus*, and *Myriotrichia clavaeformis* in culture. Generations (S, sporophyte; G, gametophyte) symbolized by squares. Z, zygote. Arrows originating at reproductive organs (U, unilocular; P, plurilocular zoidangium) indicate production and developmental fate of zoids. Arrows crossing broken lines indicate changes in environmental factors, effected in the laboratory by transfer of cultures to new growth conditions. LD, long day length; SD, short day length. Broken and stippled lines originating at gametangia represent parthenogenetic development of the majority or the minority of gametes, respectively. The stippled line in the *Ectocarpus* schema indicates the development of a minority of meiospores to give sporophytes. After Müller (1963, 1967) and Peters (1988, 1991b).

TABLE 3. Characteristics of selected brown algae relevant for genetic and genomic approaches.

Taxon	Genome size (Mbp)	Haploid chromosome number <sup>a</sup>	LH type <sup>b</sup>	Minimum generation time in laboratory culture	Controlled crosses possible	Gamete fusion	Isolation of single unilocs for tetrad analysis possible	Economic importance	Of common use in genetic, ecological, or cell biological research	Key references
<i>Ectocarpus siliculosus</i> (Dillwyn)	214–240 <sup>c</sup>	ca. 25	SG <sup>d</sup>	2 mo	Yes	Isogamy	Yes	No <sup>e</sup>	Genetics	Müller 1967; this study
<i>Myriarthra claviformis</i> Lyngbye	ND	10–15	Sg	6 wk	Yes	Isogamy	Difficult	No	No	Peters 1988; this study
<i>Striaria attenuata</i> Greville	127	10	Sg	3 mo	Yes	Isogamy	Difficult	No	No	Peters 1991b; this study
Laminariales	580–720	30–34	Sg	12 mo <sup>2</sup>	No	Oogamy	Difficult	Yes	Ecology	van den Hoek et al. 1995, Reed et al. 2004
<i>Fucus</i> spp.	ca. 1100	32	S	—	Yes	Oogamy	No	Yes	Genetics, ecology, cell biol.	Chapman 1995, Samelices 2002, Coyer et al. 2002, Coelho et al. 2002
<i>Dictyota dichotoma</i> (Hudson) Lamouroux	ND	32	SG	≥12 mo	Yes	Oogamy	Yes	No	Ecology	van den Hoek et al. 1995, Cronin and Hay 1996
<i>Codium multijidat</i> (Smith) Greville	ND	24–25	sG	ND	No	Anisogamy	ND	No	No	Müller 1974
<i>Sphaclaria rigidula</i> Kürzing	1550	25–30	SG <sup>d</sup>	ND	ND	Anisogamy	Yes	No	Cell biol	van den Hoek and Flinterman 1968, Katsaros 1995, Dimitriadis et al. 2001
<i>Anallipis gunji</i> (Yendo) Kogame et Yoshida	ND	ND <sup>f</sup>	SG	ND <sup>g</sup>	Yes	Anisogamy	Difficult	No	No	Kogame et al. 1998
<i>Syringoderma phinneyi</i> Henry et Müller	ND	30–34	Sg	ND	Yes	Isogamy	Yes	No	No	Henry and Müller 1983
<i>Scytothamnus fasciculatus</i> (Hooker et Harvey) Cotton	ND	12–13	Sg	12 mo	Yes	Isogamy	Difficult	No	No	Clayton 1986
<i>Scytothamnus australis</i> (J. Agardh) Hooker et Harvey	ND	8–14	Sg	12 mo	Yes	Isogamy	Difficult	No	No	Clayton 1986
<i>Scytosiphon lomentaria</i> (Lyngbye) Link	222	22–24	sG	5–7 mo	Yes	Isogamy	Yes	No	Ecology, cell biol.	Lubchenco & Cubit 1980, Kogame 1998, Nagasato et al. 2000, 2003
<i>Scytosiphon gracilis</i> Kogame	ND	ND	sG	ND <sup>h</sup>	Yes	Isogamy	Difficult	No	No	Kogame 1998
<i>Scytosiphon tenellus</i> Kogame	ND	ND	sG	5 mo	Yes	Isogamy	Difficult	No	No	Kogame 1998
<i>Scytosiphon canaliculatus</i> (S&G) Kogame	ND	ND	sG	5–6 mo	Yes	Anisogamy	Yes	No	No	Kogame 1996
<i>Petalonia fasciata</i> (O.F. Müller) Kuntze	ND	20–24	sG	7 mo	Yes	Isogamy	Yes	No	Ecology	Lubchenco and Cubit 1980, Kogame 1997
<i>Colpomenia peregrina</i> (Sauvageau) Hamel	ND	ND	sG	4–5 mo	Yes	Anisogamy	Yes	No	No	Kogame and Yamagishi 1997
<i>Chnoospora implexa</i> J. Agardh	ND	ND	sG	ND <sup>i</sup>	Yes	Isogamy	Yes	No	No	Kogame 2001
<i>Dictyosiphon foeniculacens</i> (Hudson) Greville	ND	18	Sg	ND <sup>j</sup>	Yes	Isogamy	Difficult	No	No	Peters 1992; Peters and Müller 1985
<i>Adenocystis utricularis</i> (Bory) Skottsberg	ND	20–26	Sg	3 mo	Yes	Isogamy	Difficult	No	No	Müller 1984

<sup>a</sup>Lewis (1996).

<sup>b</sup>S, sporophyte; G, gametophyte; upper and lowercase represent macro- and microthallus, respectively.

<sup>c</sup>Stache (1993) determined 147 Mbp for *E. siliculosus* from Naples, but we consider our higher values for the same strain more conservative (see Discussion).

<sup>d</sup>Sporophyte and gametophyte morphologically similar but distinguishable.

<sup>e</sup>“High” according to Waaland et al. (2004), but to our knowledge there is no commercial use of *Ectocarpus*. However, as a fouling alga it may be a costly nuisance.

<sup>f</sup>Twenty in *Anallipis japonicus* (Harvey) Wynnec.

<sup>g</sup>Gametogenesis 5–8 months.

<sup>h</sup>Two months for maturity of parthenosporophytes; no data for maturity of gametophytes.

<sup>i</sup>Unilocular sporangia form after 6 weeks.

<sup>j</sup>Gametogenesis requires 4–6 weeks.

trolled crosses can be performed (Coyer et al. 2002). The life history of Laminariales is obligately sexual with an alternation of heteromorphic generations. Parthenogenetic sporophytes may be confused with weakly growing heterozygotic sporophytes (Ar Gall et al. 1996), making controlled crosses difficult. Laboratory cultivation of Laminariales sporophytes requires spacious and expensive tank systems (Gómez and Lüning 2001). The disk method, used to induce precocious sorus formation in *Laminaria* (Mizuta et al. 1997, Buchholz and Lüning 1999), may shorten the generation time (which requires a year in nature), but so far there are no data on the minimum time required to complete the life cycle in the laboratory. We carried out a limited analysis of *Dictyota dichotoma*, a sexual species that exhibits an isomorphic life history and oogamy. However, gametophytes required 1 year in our cultures to become fertile, and a life cycle of this length would not be practical for genetic approaches. Non-fertilized *Dictyota* eggs showed incipient parthenogenetic development, but no viable plants were produced parthenogenetically. We have not determined the size of the *Dictyota* genome. In summary, despite their economic importance and the recent report of a transformation system for *Laminaria japonica* Areschoug (Jiang et al. 2003), we suggest that the Fucales, Laminariales, and Dictyotales would not be optimal choices as models for the development of genetic and genomic approaches because of the large sizes of their genomes and their long life cycles.

We thus concentrated our experiments on Ectocarpales, which contain the majority of small brown algae with known sexual life histories. For three of them, *Striaria attenuata*, *Ectocarpus siliculosus*, and *Myriotrichia claviformis*, cultures were available to us. Despite their permanence as vegetative stock cultures for several decades, the strains we used had retained their sexual competence.

**Genome size.** The genome size of *Striaria* ( $127 \pm 9$  Mbp) is similar to that of *Arabidopsis* (125–157 Mbp; The Arabidopsis Initiative 2000, Bennett et al. 2003). *Ectocarpus siliculosus* ( $214 \pm 13$  and  $240 \pm 8$  Mbp in two different strains) and *E. fasciculatus* ( $290 \pm 10$  Mbp) also possess significantly smaller genomes than Laminariales and Fucales. For reasons possibly based on the different methods used, our value for the European strains of *E. siliculosus* ( $240 \pm 8$  Mbp) deviates from the smaller value obtained by Stache (1993) for five strains from Naples ( $147 \pm 13$  Mbp). She did not measure the Peruvian strain. For isolates of *E. siliculosus* from Chile, New Zealand, North Carolina, and Texas, Stache (1993) found genome sizes of  $208 \pm 6$ ,  $218 \pm 16$ ,  $225 \pm 21$ , and 506 Mbp, respectively. The genome of the asexual species *Pylaiella littoralis*, the only ectocarpalean species for which the mitochondrial genome has been sequenced (Oudot-Le Secq et al. 2001, 2002), is also significantly larger (500 Mbp; Le Gall et al. 1993) than those of *Striaria* and *Ectocarpus*. We were not able to sufficiently concentrate zooids of *Myriotrichia* to obtain an esti-

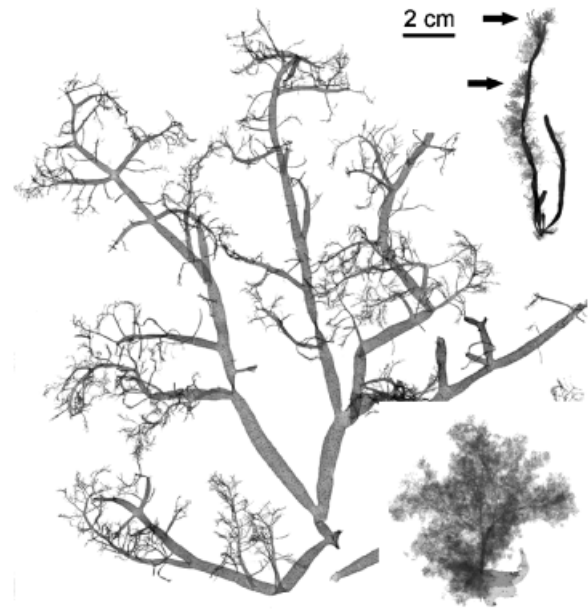


FIG. 3. Field thalli (herbarium specimens) of *Striaria attenuata* (left; forma *fragilis*, Quetalmahue, Chiloé, Chile, 11 November 1989), *Ectocarpus siliculosus* (below right; epiphytic on *Ulva* sp., Roscoff, France, 1 July 2003), and *Myriotrichia claviformis* (above right, arrows, epiphytic on *Scytosiphon lomentaria*, Pardelas, Peninsula Valdés, Argentina, 9 December 1987). All at same scale.

mation of its genome size, but the small size of the approximately 12 chromosomes counted in this species (Peters 1988) also suggests a small genome.

**Thallus size and morphological complexity.** In *Ectocarpus*, field thalli may be up to 30 cm high but may already become fertile at a size of a few millimeters (Cardinal 1964). Both generations are filamentous and macroscopic but distinguishable morphologically (Müller 1964). *Striaria* and *Myriotrichia* have macroscopic parenchymatous sporophytes and microscopic filamentous gametophytes. Field sporophytes of *Striaria* may reach 2–3 dm in length (Peters 1991b), whereas those of *Myriotrichia* reach only up to 40 mm (Fig. 3; Fletcher 1987).

**Life cycles.** The life histories of the three ectocarpalean species studied here show an increasing degree of complexity, with that of *Striaria* being the simplest and the *Myriotrichia* cycle being the most complex (Fig. 2). All species were able to complete their life cycles in the laboratory. The minimum time required for the entire sexual cycle was shorter for the ephemeral species *Myriotrichia* (6 weeks) and *Ectocarpus* (2 months) than for the annual *Striaria* (3 months). Other annual algae with microscopic gametophytes, such as *Dictyosiphon foniculaceus*, which has basically the same life history as *Striaria* (Peters and Müller 1985), and *Adenocystis utricularis* (Müller 1984) complete their life cycle in culture in about the same time as *Striaria* (Table 3). *Scytothamnus* spp. require up to a year for completion of their life histories in culture. In species of the Scytosiphonaceae, which have a

microscopic or crustose sporophyte and macroscopic gametophytes, minimum times for the completion of their life histories are between 4 and 7 months (Table 3). The life cycles of *Myriotrichia* and *Ectocarpus* are among the shortest observed for brown algae that are able to complete their sexual cycle in culture.

All species studied here were highly fertile, producing large numbers of progeny at each stage of the life cycle. Thus, large populations can easily be produced for genetic analysis. An interesting characteristic of our *Myriotrichia* isolate is that both the gametophyte and the sporophyte can be induced to produce either sexual or asexual reproductive organs (gametangia vs. mitosporangia or meiosporangia vs. mitosporangia, respectively) by varying the culture conditions (day length for the gametophyte and temperature for the sporophyte). In *Ectocarpus*, only the sporophyte is amenable to asexual direct reproduction. However, as in both generations of *Striaria*, gametophytes of *Ectocarpus* may be vegetatively propagated by fragmentation. Gametophytes of *Striaria* and *Myriotrichia* may be maintained vegetative by cultivation at elevated temperatures and short day lengths, respectively. In *Ectocarpus*, gametogenesis may be prevented by transferring settled meiospores into low-light conditions.

The sexual pheromone of *Striaria* and *Myriotrichia* has not been identified. However, its intense odor (Peters 1988, 1991b) suggests the main component of the bouquet released by female gametes in both species is finavarrene, which was identified as pheromone in other taxa of this lineage (Müller et al. 1981, Peters and Müller 1985, Peters 1987). The presence of this conspicuous odor in fertile female cultures facilitates the identification of the sex of isolated gametophyte clones. The pheromone of *Ectocarpus* (Müller et al. 1971, Pohnert and Boland 2002) has a less intense odor and only occasionally is it sufficiently concentrated in Petri dish cultures to be noticed.

*Availability of axenic cultures.* We obtained axenic cultures of all three ectocarpalean species studied here by incubation in a mixture of penicillin, streptomycin, and chloramphenicol according to Hoshaw and Rosowski (1973). It was also possible to maintain axenic cultures on agar plates for at least a year. All three species tolerated exposure during 12 h to 25° C, a characteristic that facilitates laboratory handling of strains.

*Genetic analysis.* The potential of an organism for the application of classical genetic approaches, such as the creation and screening of mutant populations and mapping of mutated genes, is an important consideration in the choice of a model organism. Genetic crosses have been successfully carried out under laboratory conditions with all three species studied here. All three species are isogamous, with gametes of approximately 5 µm in length. Zygotes can be identified by microscopy, selected, and isolated (see Müller 1967, Peters et al. 2004 for *Ectocarpus*, for example). In contrast, in strongly anisogamous (e.g. *Cutleria multifida*) or oogamous species (such as Laminariales),

parthenogenetic development of nonfertilized female gametes can lead to them being mistaken for germinating zygotes. Female gametes of *Myriotrichia* have a strong tendency to settle in clusters that renders the raising of single zygotes more difficult than in *Striaria* and *Ectocarpus*. One important advantage of *Ectocarpus* compared with *Striaria* and *Myriotrichia*, in terms of genetic analysis, is that unilocular sporangia do not form clusters and, hence, individual sporangia can be isolated easily. This character can be exploited to analyze the products of individual meioses (tetrad analysis) because each unilocular sporangium contains up to 100 or more meiospores that are produced by a single meiosis followed by several synchronous mitotic divisions (Müller 1991). In other species considered in Table 3, such as *Scytothamnus*, *Dictyosiphon*, and *Adenocystis*, meiosporangia are surrounded by cortical tissue and are more difficult to isolate.

Another essential feature of any genetic model is the availability of a protocol for the introduction of transgene constructs by transformation. To date, transformation protocols have not been reported for any ectocarpalean species, but a recent report of *Laminaria* transformation (Jiang et al. 2003) indicates that the development of transformation protocols for the brown algae is feasible.

*Research history of potential models.* The availability of a published body of knowledge concerning a potential model species greatly increases its interest. Of the three species compared here, the literature describing *Ectocarpus* is by far the most extensive. Importantly, *Ectocarpus* spp. are the only brown algae for which detailed genetic studies have been described, validating the potential of this organism as a genetic model. These investigations have included studies of the inheritance of sex factors (Müller 1967, 1975, 1976), Mendelian inheritance of a biochemical character (Müller and Eichenberger 1995), and non-Mendelian inheritance of organelles (Peters et al. 2004). Other characteristics of *Ectocarpus* studied include a detailed description of its life cycle (Müller 1967), the sexual pheromone (Maier 1995), ultrastructure (Maier 1997a, b), physiology (Schmidt and Dring 1993, Busch and Schmid 2001, Hillrichs and Schmid 2001, Schmid and Hillrichs 2001), ecophysiological variation (Bolton 1983), and phylogeography (Stache-Crain et al. 1997). The virus EsV-1, which infects *Ectocarpus siliculosus*, has been studied in considerable detail, and the complete sequence of its 336 kbp genome has been determined (Müller et al. 1990, 2000, Müller 1991, Kuhlenskamp and Müller 1994, Bräutigam et al. 1995, Sengco et al. 1996, Del Campo et al. 1997, Delaroque et al. 1999, 2001). The EsV-1 genome is potentially an interesting source of tools such as promoter regions for the development of molecular approaches in *Ectocarpus*. *Myriotrichia* is also infected by a specific virus (Müller et al. 1996, Wolf et al. 2000) and also may be infected by a virus isolated from *Ectocarpus fasciculatus* (Maier

et al. 1997), but we have not tested whether the strains we used are susceptible. No virus has so far been described for *Striaria*.

*Selection of a candidate model organism for the brown algae.* The three species studied in detail here showed similar potential for genetic and genomic analysis. All completed their life cycles rapidly under laboratory conditions, could be used in genetic crosses, and were highly fertile, producing large numbers of zooids. All three could also be maintained as unialgal or axenic cultures and were not sensitive to exposures to room temperature. The sizes of the genomes of *Striaria* and *Ectocarpus* (and probably also of *Myriotrichia*) are small compared with the larger brown algae. Finally, it should be noted that the order to which the three species studied here belong, the Ectocarpales, is closely related to the Fucales and Laminariales (Draisma et al. 2003). The three species are therefore relevant as model organisms to address problems pertaining to these economically and ecologically important groups.

Each of the three species analyzed here has particular advantages. *Myriotrichia* showed the shortest life cycle in culture and exhibited more developmental complexity than the other two species. *Striaria* has a particularly small genome size and the most morphologically complex sporophyte. The well-separated unilocular sporangia of *Ectocarpus*, which allow the analysis of individual meiotic events, are particularly interesting for genetic analyses. However, these differences are relatively minor, and the most important factor distinguishing the three species is the body of literature available for *Ectocarpus* that provides essential supporting information about the biology of this species. Based on these considerations, we propose *Ectocarpus siliculosus* as a model organism for genetic and genomic analysis of brown algal biology. We hope the potential of this species will be increased in near future by large-scale sequencing of its nuclear genome and by the development of modern genetic tools, such as genetic transformation, expression of recombinant proteins, expression of *Ectocarpus* genes in heterologous systems, and knock-out of genes by RNA interference.

A project to sequence the genome of *Ectocarpus siliculosus* has been initiated by the French sequencing center GENOSCOPE.

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- Ar Gall, E., Asensi, A., Marie, D. & Kloareg, B. 1996. Parthenogenesis and apospory in the Laminariales: a flow cytometry analysis. *Eur. J. Phycol.* 31:369–80.
- Asensi, A., Ar Gall, E., Marie, D., Billot, C., Dion, P. & Kloareg, B. 2001. Clonal propagation of *Laminaria digitata* (Phaeophyceae) sporophytes through a diploid cell-filament suspension. *J. Phycol.* 37:411–7.

- Bolton, J. J. 1983. Ecocline variation in *Ectocarpus siliculosus* (Phaeophyceae) with respect to temperature growth optima and survival limits. *Mar. Biol.* 73:131–8.
- Bräutigam, M., Klein, M., Knippers, R. & Müller, D. G. 1995. Inheritance and meiotic elimination of a virus genome in the host *Ectocarpus siliculosus* (Phaeophyceae). *J. Phycol.* 31:823–7.
- Brownlee, C. & Wood, J. W. 1986. A gradient of cytosolic free calcium in growing rhizoid cells of *Fucus serratus*. *Nature* 320: 624–6.
- Buchholz, C. & Lüning, K. 1999. Isolated, distal blade discs of the brown alga *Laminaria digitata* form sorus, but not discs, near to the meristematic transition zone. *J. Appl. Phycol.* 16:579–84.
- Busch, S. & Schmid, R. 2001. Enzymes associated with beta-carboxylation in *Ectocarpus siliculosus* (Phaeophyceae): are they involved in net carbon acquisition? *Eur. J. Phycol.* 36:61–70.
- Cardinal, A. 1964. Étude sur les Ectocarpacées de la Manche. *Nova Hedw. Beih.* 15:1–86, 41 plates.
- Chapman, A. R. O. 1995. Functional ecology of fucooid algae: twenty-three years of progress (Phycological Reviews 14). *Phycologia* 34:1–32.
- Clayton, M. N. 1986. Culture studies on the life history of *Scytothamnus australis* and *Scytothamnus fasciculatus* (Phaeophyta) with electron microscope observations on sporogenesis and gametogenesis. *Br. Phycol. J.* 21:371–86.
- Coelho, S. M., Taylor, A. R., Ryan, K. P., Sousa-Pinto, I., Brown, M. T. & Brownlee, C. 2002. Spatiotemporal patterning of reactive oxygen production and Ca<sup>2+</sup> wave propagation in *Fucus* rhizoid cells. *Plant Cell* 14:2369–81.
- Coyer, J. A., Peters, A. F., Hoarau, G., Stam, W. T. & Olsen, J. L. 2002. Inheritance patterns of ITS1, chloroplasts and mitochondria in artificial hybrids of the seaweeds *Fucus serratus* and *F. evanescens* (Phaeophyceae). *Eur. J. Phycol.* 37:173–8.
- Coyer, J. A., Peters, A. F., Stam, W. T. & Olsen, J. L. 2003. Post-ice age recolonization and differentiation of *Fucus serratus* L. (Phaeophyceae; Fucales) populations in Northern Europe. *Mol. Ecol.* 12:1817–29.
- Cronin, G. & Hay, M. E. 1996. Induction of seaweed chemical defenses by amphipod grazing. *Ecology* 77:2287–301.
- Davis, R. H. 2004. The age of model organisms. *Nat. Rev. Genet.* 5:69–75.
- Delaroque, N., Maier, I., Knippers, R. & Müller, D. G. 1999. Persistent virus integration into the genome of its algal host, *Ectocarpus siliculosus*. *J. Gen. Virol.* 80:1367–70.
- Delaroque, N., Müller, D. G., Bothe, G., Pohl, T., Knippers, R. & Boland, W. 2001. The complete DNA sequence of the *Ectocarpus siliculosus* virus EsV-1 genome. *Virology* 287:112–32.
- Del Campo, E., Ramazanov, Z., Garcia-Reina, G. & Müller, D. G. 1997. Photosynthetic responses and growth performance of virus-infected and uninfected *Ectocarpus siliculosus* (Phaeophyceae). *Phycologia* 36:186–9.
- Dimitriadis, I., Katsaros, C. & Galatis, B. 2001. The effect of taxol on centrosome function and microtubule organization in apical cells of *Sphacelaria rigidula* (Phaeophyceae). *Phycol. Res.* 49: 23–34.
- Draisma, S. G. A., Peters, A. F. & Fletcher, R. L. 2003. Evolution and taxonomy in the Phaeophyceae: effects of the molecular age on brown algal systematics. In Norton, T. A. [Ed.] *Out of the Past. Collected Reviews to Celebrate the Jubilee of the British Phycological Society*. The British Phycological Society, Belfast, pp. 87–102.
- Fletcher, R. L. 1987. *Seaweeds of the British Isles, volume 3, Fucophyceae (Phaeophyceae), Part 1*. British Museum (Natural History), London, 359 pp.
- Gómez, I. & Lüning, K. 2001. Constant short-day treatment of outdoor-cultivated *Laminaria digitata* prevents summer drop in growth rate. *Eur. J. Phycol.* 36:391–5.
- Henry, E. C. & Müller, D. G. 1983. Studies on the life history of *Syringoderma phinneyi* sp. nov. (Phaeophyceae). *Phycologia* 22: 387–93.
- Hillrichs, S. & Schmid, R. 2001. Activation by blue light of inorganic carbon acquisition for photosynthesis in *Ectocarpus siliculosus*: organic acid pools and short-term carbon fixation. *Eur. J. Phycol.* 36:71–9.

- Hoshaw, R. W. & Rosowski, J. R. 1973. Isolation and purification. 3. Antibiotic treatment. In Stein, J. R. [Ed.] *Handbook of Physiological Methods. Culture Methods and Growth Measurements*. Cambridge University Press, Cambridge, p. 62.
- Jiang, P., Qin, S. & Tseng, C. K. 2003. Expression of the *lacZ* reporter gene in sporophytes of the seaweed *Laminaria japonica* (Phaeophyceae) by gametophyte-targeted transformation. *Plant Cell Rep.* 21:1211–6.
- Katsaros, C. I. 1995. Apical cells of brown algae with particular reference to Sphacelariales, Dictyotales and Fucales. *Phycol. Res.* 43:43–59.
- Kogame, K. 1996. Morphology and life history of *Scytosiphon canaliculatus* comb. nov. (Scytosiphonales, Phaeophyceae) from Japan. *Phycol. Res.* 44:85–94.
- Kogame, K. 1997. Sexual reproduction and life history of *Petalonia fasciata* (Scytosiphonales, Phaeophyceae). *Phycologia* 36:389–94.
- Kogame, K. 1998. A taxonomic study of Japanese *Scytosiphon* (Scytosiphonales, Phaeophyceae), including two new species. *Phycol. Res.* 46:39–56.
- Kogame, K. 2001. Life history of *Chnoospora implexa* (Chnoosporaceae, Phaeophyceae) in culture. *Phycol. Res.* 49:123–8.
- Kogame, K., Horiguchi, H., Yoshida, T. & Masuda, M. 1998. Morphology, phenology and culture of *Analiplus gunjii* (Ralfsiales, Phaeophyceae). *Bot. Mar.* 41:339–44.
- Kogame, K. & Yamagishi, Y. 1997. The life history and phenology of *Colpomenia peregrina* (Scytosiphonales, Phaeophyceae) from Japan. *Phycologia* 36:337–44.
- Kuhlenkamp, R. & Müller, D. G. 1994. Isolation and regeneration of protoplasts from healthy and virus-infected gametophytes of *Ectocarpus siliculosus* (Phaeophyceae). *Bot. Mar.* 37:525–30.
- Le Gall, Y., Brown, S., Marie, D., Mejjad, M. & Kloareg, B. 1993. Quantification of nuclear DNA and G-C content in marine macroalgae by flow cytometry of isolated nuclei. *Protoplasma* 173:123–32.
- Lewis, R. J. 1996. Chromosomes of the brown algae (Phycological Reviews 16). *Phycologia* 35:19–40.
- Lubchenco, J. & Cubitt, J. 1980. Heteromorphic life histories of certain marine algae as adaptations to variations in herbivory. *Ecology* 61:676–87.
- McHugh, D. J. 2003. *A Guide to the Seaweed Industry*. FAO Fisheries Technical Paper No. 441. FAO, Rome, 105 pp.
- Maier, I. 1995. Brown algal pheromones. In Round, F. E. & Chapman, D. J. [Eds.] *Progress in Physiological Research*. Vol. 11. Biopress Ltd., Bristol, pp. 51–102.
- Maier, I. 1997a. The fine structure of the male gamete of *Ectocarpus siliculosus* (Ectocarpales, Phaeophyceae). I. General structure of the cell. *Eur. J. Phycol.* 32:241–53.
- Maier, I. 1997b. The fine structure of the male gamete of *Ectocarpus siliculosus* (Ectocarpales, Phaeophyceae). II. The flagellar apparatus. *Eur. J. Phycol.* 32:255–66.
- Maier, I., Rometsch, E., Wolf, S., Kapp, M., Müller, D. G. & Kawai, K. 1997. Passage of a marine brown algal DNA virus from *Ectocarpus fasciculatus* (Ectocarpales, Phaeophyceae) to *Myriotrichia clavaeformis* (Dictyosiphonales, Phaeophyceae): infection symptoms and recovery. *J. Phycol.* 33:838–44.
- Mizuta, H., Torii, K. & Yamamoto, H. 1997. The relationship between nitrogen and carbon contents in the sporophytes of *Laminaria japonica* (Phaeophyceae). *Fish. Sci.* 63:553–6.
- Müller, D. G. 1963. Die Temperaturabhängigkeit der Sporangienbildung bei *Ectocarpus siliculosus* von verschiedenen Standorten. *Pubbl. Sta. Zool. Napoli* 33:310–4.
- Müller, D. G. 1964. Life-cycle of *Ectocarpus siliculosus* from Naples, Italy. *Nature* 203:1402.
- Müller, D. G. 1967. Generationswechsel, Kernphasenwechsel und Sexualität der Braunalge *Ectocarpus siliculosus* im Kulturversuch. *Planta Berl.* 75:39–54.
- Müller, D. G. 1974. Sexual reproduction and isolation of a sex attractant in *Cutleria multifida* (Smith) Grev. (Phaeophyta). *Biochem. Physiol. Pflanzen* 165:212–5.
- Müller, D. G. 1975. Sex expression in aneuploid gametophytes of the brown alga *Ectocarpus siliculosus* (Dillw.) Lyngb. *Arch. Protistenk.* 117:297–302.
- Müller, D. G. 1976. Relative sexuality in *Ectocarpus siliculosus*. A scientific error. *Arch. Microbiol.* 109:89–94.
- Müller, D. G. 1984. Culture studies on the life history of *Adenocystis utricularis* (Phaeophyceae, Dictyosiphonales). *Phycologia* 23:87–94.
- Müller, D. G. 1991. Mendelian segregation of a virus genome during host meiosis in the marine brown alga *Ectocarpus siliculosus*. *J. Plant. Physiol.* 137:739–43.
- Müller, D. G. & Eichenberger, W. 1995. Crossing experiments, lipid composition, and the species concept in *Ectocarpus siliculosus* and *E. fasciculatus* (Phaeophyceae, Ectocarpales). *J. Phycol.* 31:173–6.
- Müller, D. G., Jaenicke, L., Donike, M. & Akintobi, T. 1971. Sex attractant in a brown alga: chemical structure. *Science* 171:815–7.
- Müller, D. G., Kawai, H., Stache, B. & Lanka, S. 1990. A virus infection in the marine brown alga *Ectocarpus siliculosus* (Phaeophyceae). *Bot. Acta* 103:72–82.
- Müller, D. G., Marner, F. J., Boland, W., Jaenicke, L. & Gassmann, G. 1981. Identification of a volatile gamete secretion in *Spermatochmus paradoxus*. *Naturwissenschaften*. 67:478–9.
- Müller, D. G., Westermeier, R., Morales, J., Garcia-Reina, G., del Campo, E., Correa, J. A. & Rometsch, E. 2000. Massive prevalence of viral DNA in *Ectocarpus* (Phaeophyceae, Ectocarpales) from two habitats in the North Atlantic and South Pacific. *Bot. Mar.* 43:157–9.
- Müller, D. G., Wolf, S. & Parodi, E. R. 1996. A virus infection in *Myriotrichia clavaeformis* (Dictyosiphonales, Phaeophyceae) from Argentina. *Protoplasma* 193:58–62.
- Nagasato, C., Motomura, T. & Ichimura, T. 2000. Spindle formation in karyogamy-blocked zygotes of the isogamous brown alga *Scytosiphon lomentaria* (Scytosiphonales, Phaeophyceae). *Eur. J. Phycol.* 35:339–47.
- Nagasato, C., Yoshikawa, S., Yamashita, M., Kawai, H. & Motomura, T. 2003. Pyrenoid formation associated with the cell cycle in the brown alga, *Scytosiphon lomentaria* (Scytosiphonales, Phaeophyceae). *J. Phycol.* 39:1172–80.
- Oudot-Le Secq, M. P., Fontaine, J. M., Rousvoal, S., Kloareg, B. & Loiseaux-de Goër, S. 2001. The complete sequence of a brown algal mitochondrial genome, the Ectocarpale *Pylaiella littoralis* (L.) Kjellm. *J. Mol. Evol.* 53:80–8.
- Oudot-Le Secq, M. P., Kloareg, B. & Loiseaux-de Goër, S. 2002. The mitochondrial genome of the brown alga *Laminaria digitata*: a comparative analysis. *Eur. J. Phycol.* 37:163–72.
- Peters, A. F. 1987. Reproduction and sexuality in the Chordariales (Phaeophyceae). A review of culture studies. In Round, F. E. & Chapman, D. J. [Eds.] *Progress in Physiological Research*. Vol. 5. Biopress Ltd., Bristol, pp. 223–63.
- Peters, A. F. 1988. Culture studies of a sexual life history in *Myriotrichia clavaeformis* (Phaeophyceae, Dictyosiphonales). *Br. Phycol. J.* 23:299–306.
- Peters, A. F. 1991a. Field and culture studies of *Streblonema macrocystis* sp. nov. (Ectocarpales, Phaeophyceae) from Chile, a sexual endophyte of giant kelp. *Phycologia* 30:365–77.
- Peters, A. F. 1991b. Primer registro de *Striaria attenuata* (Phaeophyceae, Dictyosiphonales) en Sudamérica, y su ciclo de vida en cultivos de laboratorio. *Revta. Chilena Hist. Nat.* 64:261–9.
- Peters, A. 1992. Culture studies on the life history of *Dictyosiphon hirsutus* (Dictyosiphonales, Phaeophyceae) from South America. *Br. Phycol. J.* 27:177–83.
- Peters, A. F. & Müller, D. G. 1985. On the sexual reproduction of *Dictyosiphon foeniculaceus* (Phaeophyceae, Dictyosiphonales). *Helgol. Meeresunt.* 39:441–7.
- Peters, A. F., Scornet, D., Müller, D. G., Kloareg, B. & Cock, J. M. 2004. Inheritance of organelles in artificial hybrids of the isogamous multicellular chromist alga *Ectocarpus siliculosus*. *Eur. J. Phycol.* 39:235–42.
- Pohnert, G. & Boland, W. 2002. The oxylipin chemistry of attraction and defense in brown algae and diatoms. *Nat. Prod. Rep.* 19:108–22.
- Reed, D. C., Schroeter, S. C. & Raimondi, P. T. 2004. Spore supply and habitat availability as sources of recruitment limitation in

- the giant kelp *Macrocystis pyrifera* (Phaeophyceae). *J. Phycol.* 40: 275–84.
- Santelices, B. 2002. Recent advances in fertilization ecology of macroalgae. *J. Phycol.* 38:4–10.
- Schmid, R. & Dring, M. J. 1993. Rapid, blue-light-induced acidifications at the surface of *Ectocarpus* and other marine macroalgae. *Plant Physiol.* 101:907–13.
- Schmid, R. & Hillrichs, S. 2001. Uptake and accumulation of inorganic carbon in *Ectocarpus siliculosus* and its relation to blue light stimulation of photosynthesis. *Eur. J. Phycol.* 36:257–64.
- Sengco, M. R., Bräutigam, M., Kapp, M. & Müller, D. G. 1996. Detection of virus DNA in *Ectocarpus siliculosus* and *E. fasciculatus* (Phaeophyceae) from various geographic areas. *Eur. J. Phycol.* 31:73–8.
- Stache, B. 1993. *Kreuzungsexperimente bei Braunalgen. Vergleich von Lokalpopulationen des Kosmopoliten Ectocarpus siliculosus*. Ph.D. Thesis. Hartung-Gorre, Konstanz, 107 pp.
- Stache-Crain, B., Müller, D. G. & Goff, L. J. 1997. Molecular systematics of *Ectocarpus* and *Kuckuckia* (Ectocarpales, Phaeophyceae) inferred from phylogenetic analysis of nuclear and plastid-encoded DNA sequences. *J. Phycol.* 33:152–68.
- Starr, R. C. & Zeikus, J. A. 1993. UTEX—The culture collection of algae at the University of Texas at Austin. *J. Phycol.* 29(suppl.): 1–106.
- The Arabidopsis Initiative, 2000. Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature* 408: 796–815.
- van den Hoek, C. & Flinterman, A. 1968. The life-history of *Sphacelaria furcigera* Kütz. (Phaeophyceae). *Blumea* 16:193–242.
- van den Hoek, C., Mann, D. G. & Jahns, H. M. 1995. *Algae. An Introduction to Phycology*. Cambridge University Press, Cambridge, xiv + 623 pp.
- Waaland, J. R., Stiller, J. W. & Cheney, D. P. 2004. Macroalgal candidates for genomics. *J. Phycol.* 40:26–33.
- Wolf, S., Müller, D. G. & Maier, I. 2000. Assembly of a large icosahedral DNA virus, MclV-1, in the marine alga *Myriotrichia claviformis* (Dictyosiphonales, Phaeophyceae). *Eur. J. Phycol.* 35:163–71.
- Worm, B., Lotze, H. K. & Sommer, U. 2001. Algal propagule banks modify competition, consumer and resource control on Baltic rocky shores. *Oecologia* 128:281–93.

Insert reference between Asensi and Bolton (see p.6):

Bennett, M. D., Leitch, I. J., Price, H. J. & Johnston, J. S. 2003. Comparisons with *Caenorhabditis* (~100 Mb) and *Drosophila* (~175 Mb) using flow cytometry show genome size in *Arabidopsis* to be ~157 Mb and thus ca 25% larger than the Arabidopsis Genome Initiative estimate of ~125 Mb. *Ann. Bot.* 91:547–57.