

## Studies on sexual compatibility between *Ectocarpus siliculosus* (Phaeophyceae) from Chile and the Mediterranean Sea\*

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**ABSTRACT:** Clonal isolates of the brown alga *Ectocarpus siliculosus* (Ectocarpales) originating from Naples (Mediterranean Sea) and southern Chile were compared in laboratory culture studies. The two isolates showed distinctly different morphological characters, but very similar details of life history and sexual reproduction. Gametes are sexually compatible; hybrid zygotes are formed and sporophytes develop, which are fertile on the basis of mitotic spores. However, unilocular sporangia were abortive, indicating segregational sterility caused by chromosomal mismatch during meiosis. Although the biological species concept in a strict sense does not apply, and appreciable morphological variability exists in this cosmopolitan taxon, local populations are considered as representatives of the same species.

### INTRODUCTION

*Ectocarpus siliculosus* is a branched filamentous brown alga. It has a cosmopolitan distribution in cold to warm temperate waters. The reproduction and life history of this species have been studied over many decades since the first reports on fertilization by Berthold (1881). The sexual life history involves two generations, which are connected by fertilization and meiosis: dioecious haploid gametophytes alternate with a diploid sporophyte of slightly different morphology. Sex determination is genotypic. The motile gametes are morphologically identical, but physiologically different, showing either male or female characters. Meiosis occurs in unilocular sporangia on diploid sporophytes. Several accessory reproductive pathways have been described. Unfertilized gametes develop into haploid sporophytes, which show two different types of reproduction: Plurilocular mito-sporangia perpetuate these genetically unisexual sporophytes, while zooids from unilocular sporangia can either re-establish the gametophyte phase, or develop into new unisexual sporophytes (Müller, 1967).

The taxonomic treatment of the genus *Ectocarpus* has varied greatly in the past. Newton (1931) listed 52 taxa for the British flora. More recently, several genera such as *Feldmannia*, *Giffordia* and *Kuckuckia* were introduced in order to separate a number of taxa. Russell (1966), emphasizing the great morphological variability, reduced the genus *Ectocarpus* of the British coast to two species, *E. fasciculatus* and *E. siliculosus*. The very few experimental data available support separation of these two species. They do not hybridize although mutual attraction of gametes does occur (Müller & Gassmann, 1980).

\* Dedicated to Dr. Dr. h. c. Peter Kornmann on the occasion of his eightieth birthday.

In a laboratory study, sexual compatibility among cultures of *E. siliculosus* clones from various geographical areas were compared (Müller, 1979). Except for two localities in North America with partial sexual incompatibility, zygote formation was observed between all isolates. Therefore, all cultures showing plasmogamy were considered to be members of the species *E. siliculosus*. Differences in branching patterns and gametangium morphology were attributed to intraspecific variability.

In 1985, isolates from southern Chile showed zygote formation with clones from the Mediterranean Sea. With these cultures, crossing experiments were expanded to include observations on the viability of zygotes and hybrid sporophytes, as well as the development of unilocular sporangia, which are the site of meiosis in brown algae.

MATERIALS AND METHODS

Origin of cultures

Gametophytes of Mediterranean *Ectocarpus siliculosus* (Dillw.) Lyngb. were collected in the vicinity of the Stazione Zoologica at Naples, Italy, in 1959 and 1965 (Müller, 1967; Fig. 1). A sporophyte of *E. siliculosus* from Chile appeared in a raw culture of

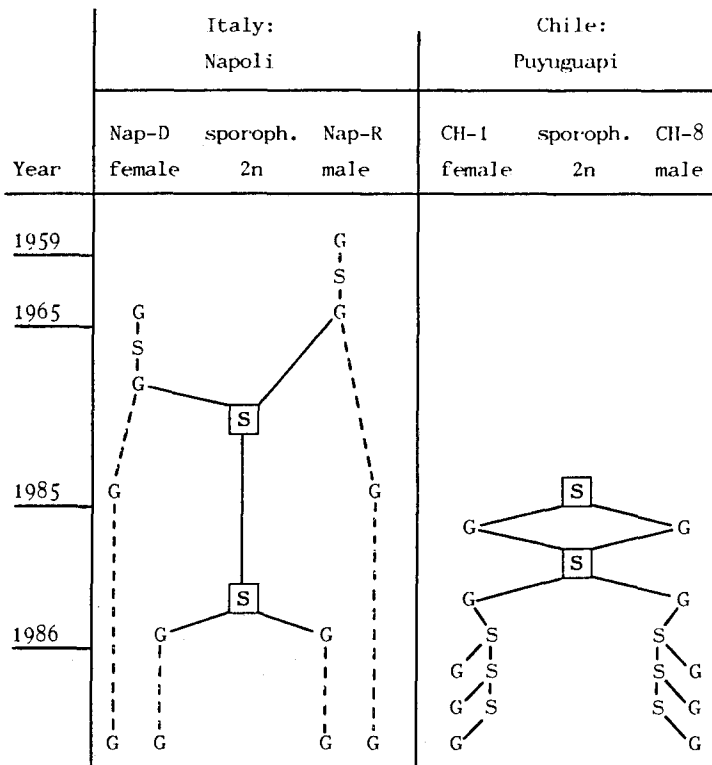


Fig. 1. *Ectocarpus siliculosus* from Naples, Italy and Puyuguapi, Chile. Pedigree of gametophytes used for the crossing experiments. G: Gametophyte; S: Zygotic or parthenogenetic sporophytes. Solid lines: Reproduction by zoids; broken lines: Propagation by fragmentation of filaments

*Adenocystis utricularis* (Bory) Skottsb., which was collected in January 1985 at Puerto Puyuguapi, XI Region (72°36' W; 44°24' S). Spores from unilocular sporangia developed into male and female gametophytes.

### Maintenance of cultures

Gametophytes of the Italian isolates were propagated by cutting filaments into few-celled fragments, which regenerated to new gametophytes. An additional pair of gametophytes was restored from a heterozygous sporophyte of the same isolates (Fig. 1). The Chilean clones could not be successfully propagated by fragmentation, because gametophyte filament fragments showed limited or no growth. Therefore, successive crops of gametophytes were generated from unilocular sporangia of unisexual partheno-sporophytes (Fig. 1). Culture medium was natural North Sea water (German Bight, salinity 28‰), enriched according to Provasoli (Starr, 1978). Cultures were kept at  $12 \pm 1^\circ\text{C}$  under illumination with daylight-type white fluorescent light at a photon fluence rate of  $12 \mu \cdot \text{m}^2 \cdot \text{s}^{-1}$  for 14 h per day. Culture medium was changed at weekly intervals.

### Crossing experiments

Small, mm-size fragments of gametophytes of opposite sex with mature maetangia were mounted between a microscope slide and cover-glass, using a 0.5 mm thick plastic ring or vaseline as spacer. In these mounts gamete fusions could be observed directly under the microscope. Zygotes were easily identified by their size and double organelle content. In order to follow their development, individual zygotes were marked on the outside of the cover-glass with a small ink-mark. Then the cover-glass was removed and placed upside-down on the bottom of a plastic petri dish filled with culture medium. It was thus possible to follow the development of individual zygotes until the germlings were of sufficient size to be pipetted and isolated into a separate petri dish.

## RESULTS

### Development and life history

The life history of *Ectocarpus siliculosus* from Naples, Italy has been studied and described in detail (Müller, 1967). The isolates from Chile showed essentially the same characteristics:

(1) Gametophytes (Figs 2–3 and 6–8) are morphologically different from zygotic and partheno-sporophytes (Figs 10–11 and 12–14).

(2) Unfertilized gametes develop to genetically unisexual partheno-sporophytes (Figs 4–5 and 9), which can regenerate gametophytes of the original sex through spores from unilocular sporangia.

(3) Zygotes develop to diploid heterozygous sporophytes, the meiospores of which develop to equal numbers of male and female gametophytes.

(4) Zygotic and partheno-sporophytes form plurilocular sporangia as accessory reproductive organs. Differences between isolates from Italy and Chile concern morphology and growth characteristics of gametophytes and sporophytes. Gametangia of the



Figs 2-9. *Ectocarpus siliculosus*. Gametophytes and parthenosporophytes  
 Figs 2-3. Female gametophyte from Naples. 2 Habit (scale bar 0.5 mm); 3 Gametangia (scale bar 100  $\mu$ m)  
 Figs 4-5. Female partheno-sporophyte from Naples. 4 Habit (scale bar 0.5 mm). Unilocular sporangium (scale bar 50  $\mu$ m)  
 Figs 6-8. Female gametophyte from Chile. 6 Habit (scale bar 0.5 mm). 7 Gametangia (scale bar 100  $\mu$ m); 8 Gametangium (scale bar 50  $\mu$ m) Fig. 9. Male parthenosporophyte (scale bar 0.5 mm)

Chilean gametophytes are more irregular in shape and often appressed to the supporting filament (Figs 7–8). Gametophytes of the Mediterranean isolates can be propagated indefinitely by regeneration of vegetative fragments, while this was found to be impossible with the Chilean strains. In contrast, mature sporophytes of the Mediterranean clones cease growth and exhaust themselves in sporangia formation, while sporophytes of the Chilean cultures can be maintained as filamentous mats over long periods of time.

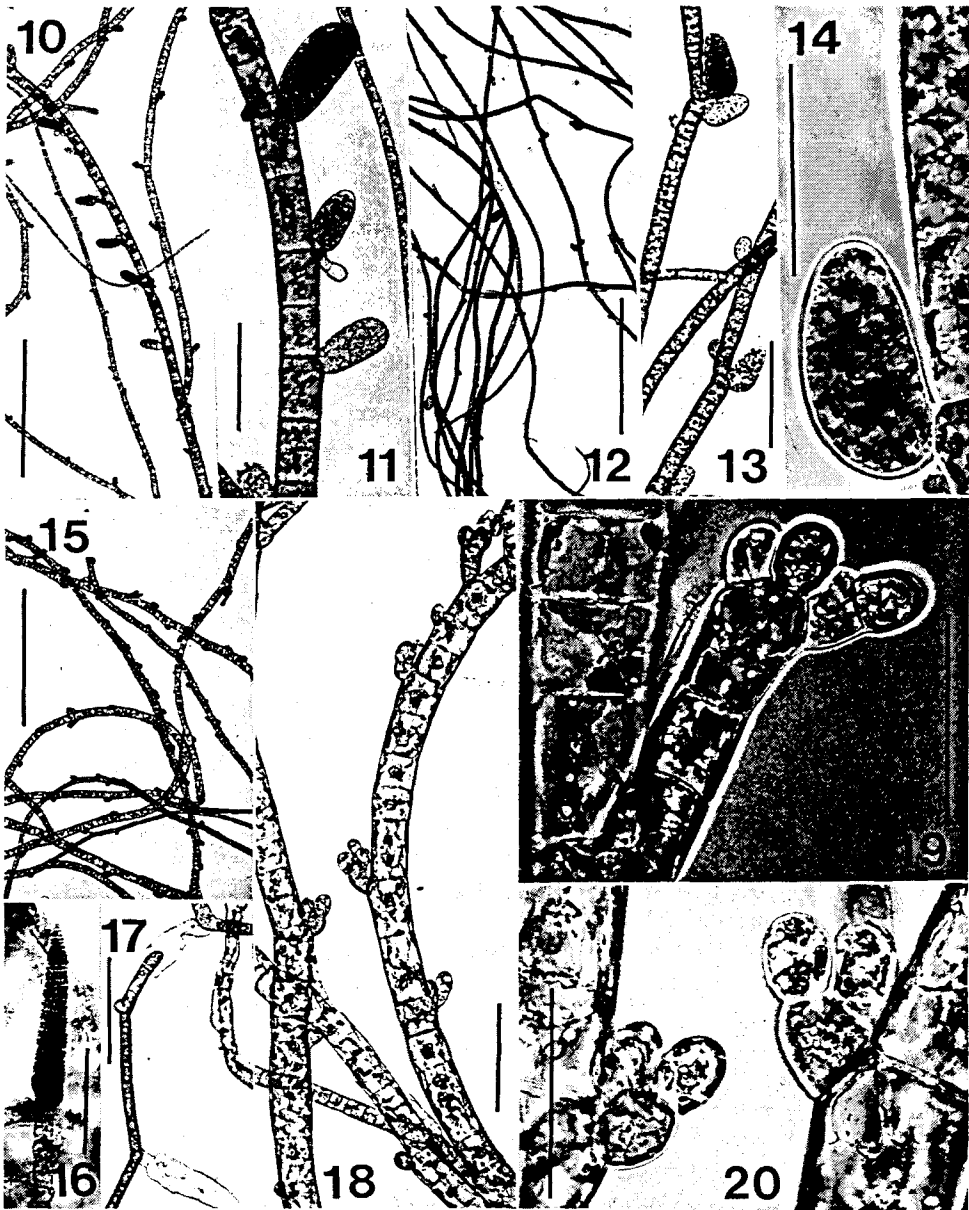
### Crossing experiments

Combination of female gametes from Naples with male gametes from Chile as well as the reciprocal crossing showed the typical pattern of "isogamy" in brown algae: the female gametes settle down on the substratum and incorporate their flagella. Then male gametes are attracted and attach with the tip of their front flagellum to the female gamete. Eventually one male gamete, including both of its flagella fuses with the female gamete. The zygotes resulting from crossings of Naples female  $\times$  Chile male developed into plants with typical sporophyte habit (Fig. 15). At the age of about 4 weeks numerous unilocular sporangium initials appeared, which did not increase in size and showed no further development (Figs 18–20), in contrast to normal sporangia on haploid (Fig. 5) or diploid sporophytes (Fig. 14). Careful screening of all hybrid plants did not reveal normal unilocular sporangia, but functional plurilocular mito-sporangia on older plants (Figs 16–17). Their zooids reestablished new sporophytes with the same phenomenon of abortive unilocular sporangium initials. Hybrids from reciprocal crosses (Chile female  $\times$  Naples male) developed more slowly, but eventually showed the same characteristics.

### DISCUSSION

The work reported above deals with sexual compatibility of geographically separated populations. If properly done, it should include a reasonable number of individuals, representing the whole genetic range of the populations studied. However, the large amount of laboratory culture work required precludes this approach in a preliminary study. Moreover, *Ectocarpus siliculosus* from Chile was not obviously present in its natural habitat at the time of collection, but appeared in culture as a single epiphytic specimen on fragments of *Adenocystis*. A systematic study of 176 individual plants taken from various natural habitats at Naples revealed 94 female and 82 male functional gametophytes. In addition, 5 plants with gametophyte habit were found, the zooids of which were apparently non-sexual. (Müller, 1976). Thus, it can be assumed with reasonable certainty, that any pair of sexually compatible gametophytes from a specific locality can be taken as representative of the local population for its reproductive characteristics.

*E. siliculosus* from the continental coast of southern Chile is sexually compatible with plants from the Mediterranean Sea. This observation confirms and expands earlier findings: Gametes of *E. siliculosus* from both sides of the North Atlantic, the Mediterranean Sea and Australia were found to interact sexually and to form hybrid zygotes (Müller, 1979). Since the present study adds to this pattern isolates from the Pacific coast of South America, it seems possible that sexual compatibility of gametes applies to *E. siliculosus* on a world-wide scale. The partial sexual incompatibility found in two populations of the North American Atlantic coast (Müller, 1979) has been interpreted as



Figs 10–20. *Ectocarpus siliculosus*. Sporophytes originating from zygotes

Figs 10–11. Isolate from Naples. 10 Habit (scale bar 0.5 mm); 11 Unilocular sporangia (scale bar 100 μm)

Figs 12–14. Isolate from Chile. 12 Habit (scale bar 0.5 mm); Figs 13 (scale bar 100 μm) and 14 (scale bar 50 μm); unilocular sporangia

Figs 15–20. Sporophytic plants derived from hybrid zygotes Naples female × Chile male. 15 Habit (scale bar 0.5 mm); 16–17 Mature (16) and empty (17) plurilocular sporangia (scale bars 100 μm); 18–20 Multiple initials of abortive unilocular sporangia (scale bars: Fig. 18 100 μm, Figs 19–20 50 μm)

first steps towards speciation through sexual isolation. The results reported here show clearly, that plasmogamy does not necessarily imply interbreeding between geographically separated populations. Although hybrid sporophytes were found to be viable and to reproduce by mitospores, meiosporangium initials do not continue development, and no meiospores were formed. Since unilocular sporangium initials on diploid sporophytes are the site of meiosis in brown algae, this finding is not unexpected for hybrids between parents which differ enough to cause severe problems in chromosome pairing (segregational sterility; Dobzhansky et al., 1977). Thus, the results of this study are explained most plausibly in the following way: *E. siliculosus* from the Mediterranean Sea and the Pacific coast of Chile are similar enough genetically to permit plasmogamy and the development of viable and fertile hybrids on the basis of mitosis. On the other hand, plants from these two locations are different enough to exclude functional chromosome pairing in meiosis, prohibiting gene exchange via sexual reproduction. Even though hybrids are formed, the two populations cannot interbreed sexually, and thus do not fully meet the biological species definition. Nevertheless, for practical reasons it seems permissible to use plasmogamy as a useful indicator to justify the inclusion of all sexual populations studied so far in the species *E. siliculosus*. This concept seems acceptable, since the closely related species *E. faciculatus* does not show plasmogamy with *E. siliculosus* (Müller & Gassmann, 1980) and thus is fully separated.

It should be possible to evaluate experimentally the above interpretation for the observed hybrid sterility. Fertility in such hybrids can often be restored by polyploidization. Like in many domestic higher plants doubling of the hybrid's chromosome set to an allotetraploid state can be expected to reestablish successful meiotic chromosome pairing. Although spontaneous tetraploid individuals have been observed in cultures of *E. siliculosus* (Müller, 1967), high chromosome numbers corresponding to this level are not normally observed in brown algae.

As indicated above, *E. siliculosus* populations on many, and perhaps all cold- and warm-temperate coasts of the globe are genetically close enough to perform zygote formation. This raises the question about the age of the species in geological terms, its origin and its distribution. *E. siliculosus* is an opportunistic species, easily growing on substrates such as ship hulls or drifting material. Theoretically, it cannot be excluded that it has spread over the world with historic and modern ship traffic. This possibility, however, seems unlikely for several reasons:

(1) Bolton (1983) used the cultures of the compatibility study (Müller, 1979) to examine their ecophysiological characteristics. He showed that local populations of *E. siliculosus* are genetically adapted to the temperature conditions of their habitats and therefore represent well-defined ecotypes. Furthermore, local populations show genetically fixed distinct morphological differences (Müller, 1979).

(2) Comparing the geographically most distant representatives from the North Atlantic, Pacific Chile and Australia, the morphological differentiation is quite substantial and the geographic distribution indicates that *E. siliculosus* does not cross the tropical belt by natural means under present climatic conditions.

(3) Recent findings on reproductive physiology of brown algae suggest that some characters are extremely conservative in evolution. All 26 species studied from 3 families in the order Laminariales (Laminariaceae, Alariaceae and Lessoniaceae) were found to use the sexual pheromone lamoxirene (Maier & Müller, 1986). In addition, physiological

and structural details of sperm release and chemotaxis are almost indistinguishable within this group of families. This means that fundamental details of reproductive physiology in the Laminariales have survived unchanged the divergence of families, genera and species. These arguments support the conclusion that *E. siliculosus* is a very old species. It may have crossed the equator belt during the cold periods of the Pleistocene jointly with other cold-water forms like *Macrocystis* (Lüning, 1985). Possibly, it may even have inhabited the coasts of Pangaea, and subsequently followed the drifting continents to their present position. These ideas are admittedly speculative at the present time. More experimental evidence with populations from other parts of the world, supplemented by biochemical data could help to evaluate the age of the species *E. siliculosus*.

*Acknowledgements.* Thanks are due to Dr. R. Westermeier, Valdivia, for organizing a collection expedition to southern Chile, and to the Deutsche Forschungsgemeinschaft for travel funds.

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