

On the sexual reproduction of *Dictyosiphon foeniculaceus* (Phaeophyceae, Dictyosiphonales)

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ABSTRACT: *Dictyosiphon foeniculaceus* from Sweden and Newfoundland was studied in laboratory culture. Zooids from unilocular sporangia developed into dioecious microscopic filamentous gametophytes which produced uniseriate plurilocular gametangia in low temperatures (0 to 8 °C). Zygotes and unfused isogametes gave rise to filamentous protonemata on which parenchymatous macroscopic sporophytes were formed. Isolates from Sweden and Newfoundland were interfertile. Although formed in culture, genetically unisexual sporophytes were not detected in nature. Female gametes of *D. foeniculaceus* produced a sexual pheromone. It was identified as finavarrene, which is also known as the sperm attractant in *Ascophyllum nodosum*.

INTRODUCTION

Dictyosiphon foeniculaceus (Huds.) Greville is a common brown alga of the lower littoral and upper sublittoral of cold and temperate waters of the northern hemisphere (Okamura, 1932). It was the first species of brown algae with a conspicuous alternation of heteromorphic generations in which isogamy was observed (Sauvageau, 1917; Arasaki, 1949). Meiosis occurs in the unilocular sporangium (Abe, 1940). However, a recent culture study of isolates from Greenland did not confirm sexual reproduction (Pedersen, 1984). *D. foeniculaceus* belongs to Dictyosiphonales, an order in which sexual reproduction seems to be suppressed in many species (cf. Wynne & Loiseaux, 1976). Some reports of sexual life histories are uncertain since direct observation of gamete fusions is lacking [*Stictyosiphon adriaticus* Kütz., Caram, 1965; *Litosiphon pusillus* (Carm.) Harvey, Dangeard, 1969; *Asperococcus fistulosus* (Huds.) Hooker, Dangeard, 1969 (as *A. echinatus*)]. Planogamy (fusion of swimming gametes) was reported in *Striaria attenuata* (A.) Grev. (Caram, 1964) and *Soranothera ulvoidea* Post. et Rupr. (Wynne, 1969). Although planogamy is well known in the Chlorophyceae, it does not seem to occur in brown algae. Müller (1967) showed that in *Ectocarpus siliculosus* (Dillw.) Lyngb., "planozygotes" are actually twins of non-separated zooids and are not formed by gamete fusions. Hence, reports of planogamy in brown algae cannot be considered as evidence for sexual reproduction. Neither does cytological evidence for an alternation of nuclear phases prove a sexual cycle in the absence of direct observation of gamete fusions. In *Elachista*

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stellaris Aresch., an asexual cycle was reported which includes a haploid microthallus and a diploid macrothallus (Wanders et al., 1972).

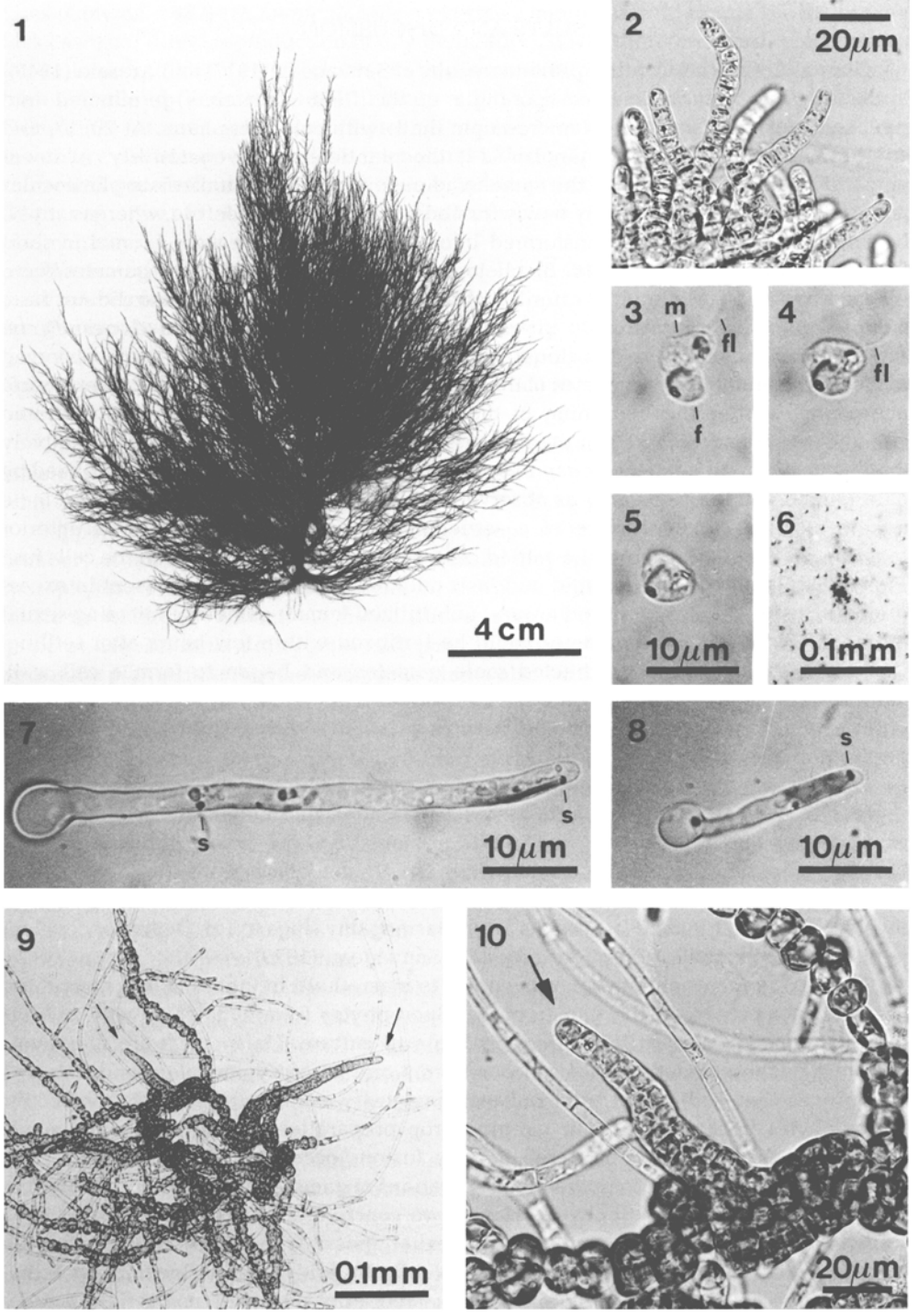
Life histories with sexual reproduction were found recently in three species of Dictyosiphonales. *Corycus lanceolatus* (Kütz.) Skottsberg (Asensi, 1975) and *Adenocystis utricularis* (Bory) Skottsberg (Müller, 1984) are isogamous; *Hummia onusta* (Kütz.) Fiore is anisogamous (Fiore, 1977).

In *Dictyosiphon foeniculaceus*, the original reports of sexual reproduction, made decades ago, leave open several questions: (1) Is this species monoecious as assumed by Sauvageau (1917)? (2) In a second attempt to repeat his original observations Sauvageau did not succeed in inducing gametogenesis (Sauvageau, 1929). Recent studies have revealed the influence of temperature, irradiance, light quality, and photoperiod on gametogenesis in brown algae (Lüning, 1985). What are the conditions that induce gametogenesis in *D. foeniculaceus*? (3) Sauvageau (1917) reported apomictic development of gametes into sporophytes in culture. Is it possible to find in nature sporophytes that did not develop from zygotes? (4) In several brown algae, female gametes attract male gametes by means of low-molecular-weight hydrocarbons (for a recent list of species examined so far, see Maier & Müller, 1986). Do female gametes of *D. foeniculaceus* also produce a sexual pheromone?

MATERIALS AND METHODS

One plant of *Dictyosiphon foeniculaceus* (Fig. 1) was collected near Kristineberg (west coast of Sweden) in July 1983. Thallus fragments with unilocular sporangia were washed in sterile culture medium (Provasoli-ES; after Starr, 1978) and placed in spot-plates. Unialgal clonal cultures of microthalli were obtained as described for *Papenfussiella callitricha* (Rosenv.) Kylin (Peters, 1984). In September 1984, 34 mature specimens of *D. foeniculaceus* were collected in insular Newfoundland (Canada): 20 specimens from an exposed locality on the northern shore of Bay Bulls, 14 specimens from a less exposed site near Portugal Cove, Conception Bay. Clones of microthalli from all specimens were obtained in the same way as in the plant from Sweden. The algae were cultivated under daylight-type fluorescent light. Hydrophobic compounds produced by female gametes were isolated as described for *Spermatochnus paradoxus* (Roth) Kütz. (Müller et al., 1981). They were identified by glass capillary gas chromatography. Synthetic pheromones were used in order to evaluate biological activity of identified compounds in a biological assay described previously (Müller, 1976).

Figs 1–10. *Dictyosiphon foeniculaceus*. 1. Herbarium specimen of *D. foeniculaceus* from Kristineberg, Sweden. 2. Filaments of gametophyte entirely transformed into gametangia. 3–5. Sequence of gamete fusion. Hanging-drop preparation. All the same magnification. Time difference between 3 and 4: 0.5 s, between 4 and 5: 3 s. m: male; f: female gamete; fl: hind flagellum of male gamete. 6. Sex attraction. Male gametes of *D. foeniculaceus* attracted by a female gamete of *Papenfussiella callitricha* which acts as source of the sexual hormone, finavarrene. 7. 5-day-old germling of zygote, with two stigmata (s) still visible. 8. 5-day-old germling of unfused gamete, possessing only one stigma (s). 9. Protonemal filaments with young macrothalli (arrows). 10. Section of Fig. 9 showing parenchymatous organization of tip of macrothallus (arrow)



RESULTS AND DISCUSSION

Our culture studies confirm previous results of Sauvageau (1917) and Arasaki (1949). Zoids released from unilocular sporangia of the field specimens germinated into branched prostrate filamentous microscopic thalli with colourless hairs. At 20, 17, and 12 °C (photon-flux density $25 \mu\text{mol m}^{-2} \text{s}^{-1}$) the plantlets grew vegetatively. At lower temperatures (1, 5, 8 °C) under the same irradiance they formed uniseriate plurilocular gametangia (Fig. 2). At 8 °C only newly formed branches became fertile, whereas at 1 °C the entire microthalli were transformed into gametes. Gametes were formed in short (8:16) as well as long day (16:8) photoperiods. At 5 and 8 °C, first gametes were released after 4 weeks, at 1 °C after 2 months. Gametes within one clone did not fuse, and plasmogamy occurred only in combinations of male and female clones: *Dictyosiphon foeniculaceus* is dioecious. No morphological differences were observed between gametophytes or gametes of the two sexes. Gametes were released from mature gametophytes after the beginning of the light period, and the number of liberated gametes was enhanced by a simultaneous rise in temperature. Gametes were negatively phototactic. Female gametes began to settle 3–4 h after release. They were fertilized by male gametes in the same way as observed in other isogamous brown algae: the male gamete attaches to the surface of a settled female cell with the tip of its anterior flagellum, it is pulled towards the settled cell, their membranes touch, and the cells fuse (Figs 3–5). Gamete fusion is rapid and lasts only few seconds. When present in excess numbers, male gametes clustered around unfertilized female gametes indicating sexual attraction (Fig. 6). Female gametes could be fertilized within few hours after settling. Thereafter, they no longer attracted male gametes and began to form a cell wall. Unfused male gametes settled after several hours. They were not fertilized by motile male or female gametes. Like unfertilized female cells, they formed a cell wall, and germinated after 1–2 days (Fig. 8).

Zygotes were easily distinguished from unfused gametes by their two eyespots and larger size. They germinated within few hours after plasmogamy, i.e. earlier than unfused gametes. A germ tube was formed at one side of the zygote. It gave rise to an initially unbranched prostrate filament (Fig. 7). No zoidangia were produced on this protonema. At 12 °C, the macroscopic parenchymatous thallus of *Dictyosiphon* developed on this filament 2–3 weeks after plasmogamy (Figs 9, 10). Details of development of the macrothallus were described by Sauvageau (1929).

Unfused gametes of both sexes gave rise to macrothalli in the same way as zygotes. They did not propagate the gametophyte. Sporophytes from zygotes as well as from unfused gametes formed unilocular sporangia in culture. Unisporoes were negatively phototactic. The development of zoospores from one heterozygous plant and from two sporophytes that had grown from unfused gametes was followed until the respective gametophytes became fertile. In hanging-drop preparations containing gametophyte offspring of the heterozygous plant, gamete fusions occurred. No gamete fusions or zygotes were observed in comparative preparations of gametophytes developed from the unisporoes of apomictic sporophytes. Hence, we conclude that sex determination in *D. foeniculaceus* is genotypic, and that unisexual (possibly haploid) sporophytes can become mature and reproduce the plant asexually by zoids from unilocular sporangia.

Little is known about the significance of laboratory observations for field popula-

tions (Clayton, 1982). *D. foeniculaceus* possesses a comparatively simple life history that lacks asexual direct reproduction of any generation. It was therefore considered suitable for an investigation of this problem. Lack of sexuality in an isolate of *D. foeniculaceus* from Greenland (Pedersen, 1984) indicated that unisexual plants may occur in nature. Occurrence of unisexual sporophytes in nature would indicate that findings from laboratory culture are also valid in the natural habitat. The frequency of unisexual plants in natural populations could also be expected to provide information about the actual frequency of sexual fusions (i.e. of the effectiveness of the mechanisms that lead to plasmogamy) in the natural habitat.

We examined the gametophytes that developed from zooids of 34 specimens of *D. foeniculaceus* collected randomly at two localities in Newfoundland. Without exception, the gametophyte cultures (not clonal, but randomly grown from unispores) produced gametes of both genders: zygotes were found in all preparations of fertile gametophytes. Gametophyte clones from two plants (one from each locality) showed that the species is dioecious also in Newfoundland.



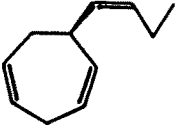
In addition, plants from Newfoundland and Sweden were interfertile. Gamete fusions were observed in combinations of female and male clones from Newfoundland with compatible clones from Sweden.

Apparently, unfused gametes did not develop into mature sporophytes in significant numbers at the examined localities. One reason may be reduced viability of apomictic sporophytes. This may not be crucial in cultures, where competition for space and nutrients is largely absent. As noted above, zygotes germinate earlier than unfused gametes. Hence, in nature zygotes may gain by occupation of suitable substrates before apomictic plants. Possibly, heterozygous plants also exhibit higher growth rates. On the other hand, our results indicate that gamete fusions may occur very frequently in nature. Obviously, diverse mechanisms which enhance the probability of gamete encounter, like synchronous gamete release, phototaxis, and attraction of male gametes, are effective.

A sweet fragrance was noticed emanating from cultures of fertile female gametophytes of *D. foeniculaceus*. Gas chromatographic analysis of extracts from such living cultures revealed 3 compounds. They were olefinic, partly unsaturated hydrocarbons which had been identified previously as gamete secretions of other species of brown algae. The major compound was finavarrene (1), the sperm attractant of *Ascophyllum nodosum* (L.) Le Jol. (Fucales, Müller et al., 1982). Two minor fractions were cystophorene (2), the sperm attractant of *Cystophora siliquosa* J. Ag. (Fucales, Müller et al., 1985a) and ectocarpene (3), the sexual pheromone of *Ectocarpus siliculosus*, *Sphacelaria rigidula* (Kütz.) Prud'homme van Reine, and *Adenocystis utricularis* (Müller et al., 1985b). (1) and (2) were produced in a ratio of 32.5 : 1; (3) occurred only in trace amounts. Threshold concentrations for chemotaxis of male gametes of *D. foeniculaceus* toward synthetic (1), (2), and (3) are given in Table 1. They confirm that finavarrene is the sexual pheromone of *D. foeniculaceus*.

As indicated above, finavarrene is also produced by eggs of *Ascophyllum nodosum* (Müller et al., 1982). Mutual attractance of male gametes between the species is nevertheless unlikely since sexual reproduction of *Ascophyllum* happens mainly during summer (South & Hooper, 1980), whereas the gametes of *D. foeniculaceus* are presumably released in winter or spring, as concluded from the low temperatures essential for gametogenesis. However, male gametes of *D. foeniculaceus* may occasionally be lured

Table 1. Threshold concentrations for chemotaxis of male gametes of *Dictyosiphon foeniculaceus*

Substance		c (Molar in sea-water)
	(1)	1.2×10^{-10}
	(2)	1.8×10^{-8}
	(3)	$> 2.9 \times 10^{-7}$

by other organisms that produce finavarrene in winter, such as female gametes of *Papenfussiella callitricha* and *Sphaerotrichia divaricata* (Ag.) Kylin and asexual swarmerers of *Acrothrix gracilis* Kylin (Fig. 6, and Peters, unpubl.). These three species occur in Newfoundland and inhabit regions where *Dictyosiphon* is also found.

A drop of medium from cultures of fertile female gametophytes of *D. foeniculaceus*, containing the sexual pheromone, when added to fertile male gametophytes, did not cause release of male gametes. Thus, *D. foeniculaceus* does not possess the mechanism of pheromone-triggered (male) gamete release which is known from the Laminariales (Lüning & Müller, 1978), Desmarestiales (Müller & Lütke, 1981), and Sporochnales (Müller et al., 1985c).

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