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Critical re-examination of sexual reproduction in *Tinocladia crassa*, *Nemacystus decipiens*, and *Sphaerotrichia divaricata* (Phaeophyceae, Chordariales)*

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Gametophytes of *Tinocladia crassa* and *Nemacystus decipiens* from Japan, and of *Sphaerotrichia divaricata* from the Pacific coast of Canada were studied in laboratory culture. All three species were dioecious, and sexual fusions occurred between isogametes. Settled female gametes were surrounded by numerous motile male gametes prior to plasmogamy indicating sex attraction. Planozygotes as reported by previous authors were not observed in any of the species.

Key Index Words: *Chordariales*; Nemacystus decipiens; *Phaephyceae*; *sexual reproduction*; Sphaerotrichia divaricata; Tinocladia crassa.

Introduction

The edible seaweeds *Tinocladia crassa* (SURINGAR) KYLIN, *Nemacystus decipiens* (SURINGAR) KUCKUCK, and *Sphaerotrichia divaricata* (AG.) KYLIN are placed in the order Chordariales (KYLIN 1940). Sexual reproduction has been documented recently in all three species (MIGITA and YOTSUI 1972, YOTSUI 1978, AJISAKA and UMEZAKI 1978). Reproduction of *Tinocladia* and *Nemacystus* has been studied in detail in connection with aquaculture (YOTSUI and MIGITA 1974, YOTSUI 1975 a, b, 1976, 1977, 1979 a, b 1980, 1982). In spite of these efforts, knowledge on sexual reproduction in the three species is still incomplete. In *Tinocladia* plasmogamy follows the common pattern of isogamous brown algae: a settled "female" cell fuses with a motile "male" gamete (Fig. 1B in YOTSUI 1978). In contrast, plasmogamy in *Nemacystus* (Fig. 3B in MIGITA and YOTSUI 1972) and *Sphaerotrichia* (Fig. 2DE in AJISAKA and UMEZAKI 1978) was reported to occur between motile gametes, resulting in planozygotes. Gametophytes of *Sphaerotrichia* from Japan were considered to be "sometimes monoecious" and "either isogamous or anisogamous" by AJISAKA and UMEZAKI (1978). *Tinocladia* and *Nemacystus* are both isogamous, but it is unkown whether their gametophytes are monoecious or dioecious.

Clonal gametophyte cultures of the three species were studied in detail in order to answer the questions pointed out above.

Materials and Methods

Gametophytes of *Tinocladia* and *Nemacystus* were obtained from unilocular sporangia on sporophytes collected at Nomozaki, Nagasaki, Japan in May 1984. Gametophyte cultures of *Sphaerotrichia* were initiated from mature sporophytes collected at Bamfield, British Columbia, Canada in August 1984. Unispores

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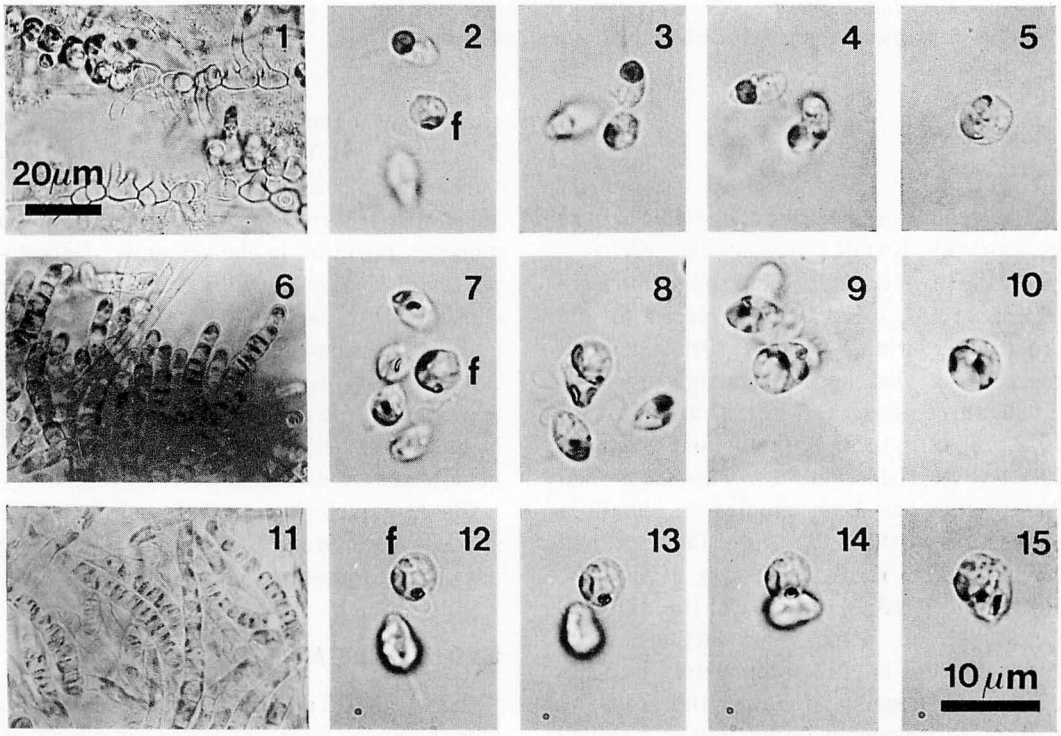
were allowed to settle on fragments of microscopic slides. Clonal cultures were established by isolation of single gametophyte germlings. The algae were cultivated in enriched sea-water (PROVASOLI-ES, after STARR 1978) under daylight-type fluorescent light. Vegetative growth occurred in 17°C with a short-day photoperiod (8 : 16) and a photon-flux density of 10 μmol $m^{-2} s^{-1}$. Gametogenesis was induced by transfer to fresh medium at a 14±2°C long-day photoperiod (16 : 8) and a photon-flux density of 35 μmol $m^{-2} s^{-1}$. Gametophytes of *Sphaerotrichia* were precultivated in 5°C, a long-day photoperiod and a photon-flux density of 5 μmol $m^{-2} s^{-2}$ for at least 8 days before induction of gametogenesis.

Behaviour of zoids was observed in hanging-drop preparations.

Results

In all three species gametophytes formed gametangia (Figs. 1, 6, 11) as described by various authors (MIGITA and YOTSUI 1972, YOTSUI 1978, AJISAKA and UMEZAKI 1978). Gamete release occurred in the morning. Gametes of *Tinocladia* were negatively, those of *Nemacystus* and *Sphaerotrichia* positively phototactic. In microscopic mounts consisting of one clone only, gametes settled without fusions.

In all three species, zygotes were only formed in hanging drops containing a mixture of gametes from compatible gametophyte clones. Fertilization begins after a female gamete has settled on a solid substrate and withdrawn its flagella. The tip of the



Figs. 1–5. *Tinocladia crassa*. 1. Plurilocular gametangia, consisting of 1 to 3 loculi ("paucilocular"), mostly released. 2–5. Sequence of gamete fusion. Figs. 6–10. *Nemacystus decipiens*. 6. Gametangia. 7–10. Sequence of gamete fusion. Figs. 11–15. *Sphaerotrichia divaricata*. 11. Gametangia 12–15. Sequence of gamete fusion. Figs. 1, 6, 11 : Same magnification. Figs. 2–4, 7–9, 12–14 taken at intervals of about ls, figs. 5, 10, 15 few minutes after plasmogamy. f=female gamete. Figs. 2–5, 7–10, 12–15 : Same magnification, hanging-drop preparations.

anterior flagellum of a male gamete attaches to the surface of the female cell. The bodies of the two cells touch and fuse. Subsequently the posterior flagellum of the male gamete is withdrawn. The zygote is usually irregular in shape, but rounds up within a few minutes. If male gametes are in excess, female cells are approached by several male gametes before a zygote is formed. Occasionally, two male gametes fuse with one female. Serial photomicrographs of gamete fusions in all three species are given in Figs. 2-5, 7-10, 12-15.

Female and male gametes are morphologically identical but physiologically different: (i) No interaction occurs if motile female gametes are combined with settled male gametes; (ii) Female gametes produce a conspicuous sweet fragrance not encountered in male cultures.

Sex distribution among the randomly isolated gametophyte clones was 2:2 in *Tinocladia*, 4 female: 3 male in *Nemacystus*, and 5 female: 6 male in *Sphaerotrichia*. No evidence for monoecism was found in this study.

Discussion

Our study shows that *Tinocladia crassa*, *Nemacystus decipiens*, and *Sphaerotrichia divaricata* are dioecious and isogamous. Plasmogamy takes place in the way described above which is known from brown algae since BERTHOLD's observations on *Ectocarpus siliculosus* (DILLW.) LYNGB. (1881). Sexual fusions between two motile gametes resembling plasmogamy in isogamous green algae have been reported and depicted by several authors for various species of brown algae (e. g. ARASAKI 1943a, b, 1948, LOISEAUX 1964, 1966, 1967, 1970), but have not been documented convincingly in any brown alga to date. In *Sphaerotrichia divaricata* (AJISAKA and UMEZAKI 1978) and *Acrothrix pacifica* OKAMURA et YAMADA (AJISAKA 1979) planozygotes have been reported recently. The photomicrographs in these papers can be more plausibly interpreted as showing unfused gametes that are separated by cell walls (Fig. 3E in AJISAKA and UMEZAKI 1978, Fig. 2J in AJISAKA 1979). From the corresponding descriptions in the text it remains uncertain whether genuine gamete fusions occurred. In *Nemacystus decipiens*, fusion of gametes was documented (Fig. 4C in MIGITA and YOTSUI 1972). In this photomicrograph, only one flagellum (presumably the hind flagellum of the male gamete) is visible during plasmogamy. The zygote does not possess any flagella. Thus, evidence for planogamy is not convincing in *Nemacystus*.

Some reports of planozygotes may be due to observation of swarmers with four flagella containing two chloroplasts and eyesports. There is no evidence that these "twins" originate from sexual fusions between gametes. In *Ectocarpus siliculosus* it was shown that such swarmers result from incomplete cell divisions in gametangia or sporangia (MÜLLER 1967, 1975). Actually, such "twin" zoids have not been observed in our study.

Some of our results on *Sphaerotrichia divaricata* from the Pacific coast of Canada differ from the fidings reported for Japanese plants as cited above (AJISAKA and UMEZAKI 1978). They also deviate from the indirect proof of anisogamy, monoecism, and planogamy in plants from Norway given by HYGEN (1934). Hygen did not observe copulations directly, and what he assumed to be male gametes lacking a chloroplast and bearing usually only one flagellum may have been a contaminant. These "male gametes" were not able to germinate apomictically and died, whereas unfused male gametes in our study developed to gametophytes or sporophytes (PETERS unpublished). I-KI-fixed "zygotes" with three flagella as reported by HYGEN do not prove existence of planozygotes convincingly. Since we doubt wether AJISAKA and UMEZAKI report true sexual fusions, their conclusions for anisogamy and monecism are not valid either. Occasionally encountered morphological evidence of anisogamy may

be due to variability in gamete size. Sexual differentiation is defined as anisogamous in cases where persistent differences of gamete size can be established. ARASAKI (1943a) studied the life histories of *Chordaria firma* E.S. GEPP and *Sphaerotrichia japonica* KYLIN, two taxa that were later included in *Sphaerotrichia divaricata* (INAGAKI 1958). ARASAKI described planogamy in both species, isogamy in *S. japonica*, and anisogamy in *C. firma*.

Although our isolates from a Canadian plant are dioecious and isogamous, the possibility that *Sphaerotrichia* is monecious and anisogamous in Japan cannot be excluded. Since sex distribution is important for artificial cultivation and breeding, a re-examination of Japanese *Sphaerotrichia* using clonal gametophyte cultures seems necessary.

Clustering of male gametes around females prior to plasmogamy, and odorous (i.e. volatile) substances produced by female gametes only, indicate sexual attraction. Pheromone systems have been demonstrated so far in several brown algae: *Adenocystis utricularis* (BORY) SKOTTSBERG, *Ascophyllum nodosum* (L.) LE JOLIS, *Chorda tomentosa* LYNGBYE, *Colpomenia peregrina* (SAUV.) HAMEL, *Cutleria multifida* (SMITH) GREV., *Desmarestia aculeata* (L.) LAMOUR., *D. viridis* (D.F. MÜLL.) LAMOUR., *Dictyosiphon foeniculaceus* (HUDS.) GREVILLE, *Dictyota dichotoma* (HUDS.) LAMOUR., *Ectocarpus siliculosus*, *Fucus serratus* L., *F. vesiculosus* L., *Hormosira banksii* (TURN.) DECAISNE, *Scytosiphon lomentaria* (LYNGB.) C. AG., *Sphacelaria rigidula* (KÜTZ.) PRUD'HOMME VAN REINE, some fucalean species from Australia and New Zealand, and several members of the Laminariales (MAIER and MÜLLER 1986).

Within the order Chordariales, only *Spermatochnus paradoxus* (ROTH.) KÜTZING has been examined in respect of gamete secretions so far (MÜLLER *et al.* 1981). Gamete suspensions of this species produced the unsaturated hydrocarbon finavarrene which is also known as sperm attractant of *Ascophyllum nodosum* (Fucales: MÜLLER *et al.* 1982). *Spermatochnus* is monoecious and no biological effect of the gamete secretion could be detected. Isolation of female gamete secretions of *Tinocladia*, *Nemacystus*, and *Sphaerotrichia* are presently attempted.

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ピーターズ A.K.・ミューラー D.G.: フトモズク，モズク，イシモズク（褐藻類，ナガマツモ目）の有性生殖についての再調査

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