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Hiroshi Kawai, Wilhelm Boland and Dieter G. Müller: Sexual reproduction and sexual pheromones in *Myelophycus simplex* (Harvey) Papenfuss (Phaeophyceae)

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Myelophycus simplex (Harvey) Papenfuss is an annual brown alga which occurs on temperate coasts of the north-western Pacific. Unilocular and plurilocular sporangia are formed on separate thalli, but their reproductive character has not been clarified since the life history of the species is still unknown. Tanaka and Chihara (1984) cultured unispores of *M. simplex* released from unilocular sporangia and obtained protonemata which directly developed into erect thalli resembling field plants. However, they did not observe further development of these erect thalli. They also cultured the unispores of their new taxon *Myelophycus cavum* Tanaka et Chihara, which showed a very similar development as *M. simplex*. Wynne (1969) cultured *Melanosiphon intestinale* (Saunders) Wynne, a closely related taxon and reported the occurrence of erect thalli bearing unilocular and plurilocular sporangia, but sexual reproduction was not detected.

In the present paper we report the occurrence of sexual reproduction in *Myeophycus simplex* from Shimoda, Izu Peninsula, including identification of sexual pheromones.

Sexual reproduction: Collections of specimens were made at Nabeta and Suzaki, Shimoda, Izu Peninsula on May 14, 1991, January 21 and April 1, 11 and 22, 1992. The macroscopic erect thalli of *Myelophycus simplex* are terete, gregarious, growing on rocks in the upper intertidal zone (Fig. 1). The plants bearing unilocular sporangia are somewhat larger and more numerous than those bearing plurilocular ones. Plurilocular sporangia are formed on the entire surface of the thallus (Fig. 2), and it was rather hard to distinguish between unilocular and plurilocular sporangial thalli from the surface. Therefore, mature thalli with plurilocular sporangia were selected from apparently mature thalli by examining thin sections of mature thalli under low magnification, and each individual was stored separately in a plastic petridish overnight in the refrigerator. Roughly half of the mature plurilocular sporangial thalli secreted a specific odor resembling that produced by sexual plants of *Scytosiphon* and, therefore, were presumed to be female gametophytes. Plurispores from plants without odor were presumed to be male gametes. Judging from the occurrence of specific odor and the results of mating experiments as described below, among 14 gametophytes 8 were male and 6 were female in the collection of 11 April, 1992. We found no morphological differences between male and female gametophytes.

Sexual fusions were observed in combinations with presumptive male (Fig. 3) and female (Fig. 4) gametes (plurispores). They were almost identical in size, teardrop-shaped, containing a single chloroplast with a stigma. A longer anterior and a shorter posterior flagellum were inserted laterally. Female gametes tended to settle faster than male gametes and both of the flagella coalesced to the cell body (Fig. 5). When

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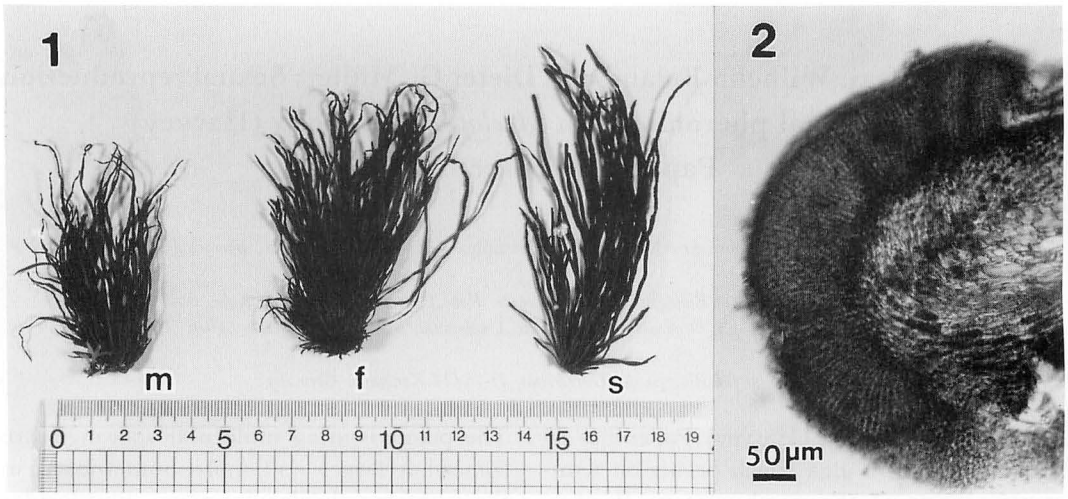
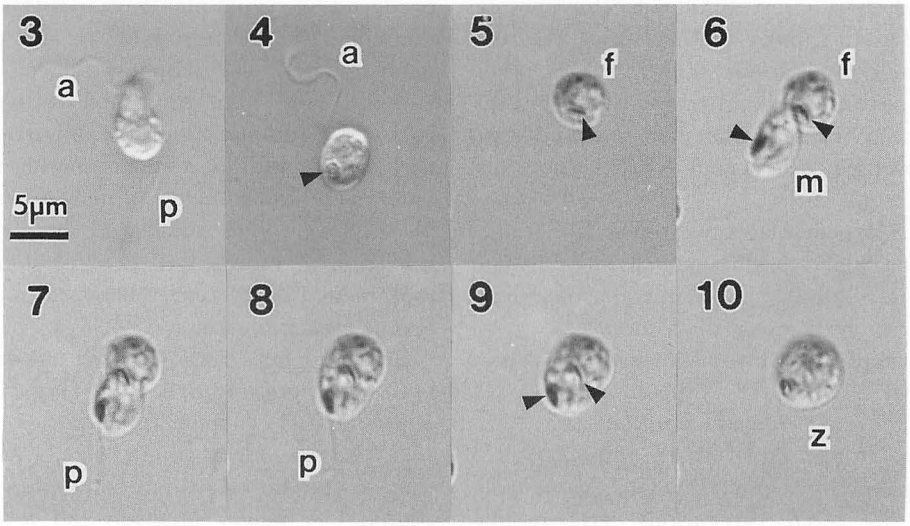


Figs. 1–2. *Myelophycus simplex* (Harvey) Papenfuss from Shimoda. Fig. 1. Habit of field-collected plants. m, male plurilocular gametophyte; f, female plurilocular gametophyte; s, sporophyte (unilocular sporangial). Fig. 2. Cross section of mature male gametophyte bearing plurilocular gametangia.

male gametes were added to settled female gametes, one to several male gametes were attracted by a female gamete. The male gamete first anchored the distal part of its anterior flagellum to the female cell (Fig. 6). Then the male gamete approached to the female cell and cell fusion occurred, resulting in the zygote (Fig. 7). The bending motion of the male posterior flagellum could still be seen one to two seconds after the cell fusion (Fig. 8). Eventually, it also coalesced with the zygote (Fig. 9), which gradually attained a spherical shape (Fig. 10). Two stigmata were easily recognized in each zygote (Fig. 9). The details of sexual reproduction in *M. simplex* described here agree well with those reported for other isogamous brown algae (Müller 1984, Peters 1987). Sexual fusion



Figs. 3–10. Gamete morphology and gamete fusion in *Myelophycus simplex* (Harvey) Papenfuss from Shimoda. Figs. 3, 4 fixed by glutaraldehyde vapor. Fig. 3. Male gamete. Fig. 4. Female gamete. Figs. 5–10. Consecutive series of gamete fusion. Fig. 5. Settled female gamete. Fig. 6. Male gamete anchored to settled female gamete. Figs. 7–9. Gamete cell fusion. Note that posterior flagellum of male gamete is still visible in Fig. 8 but already coalesced to male cell boty in Fig. 9. Fig. 10, Rounded zygote.

was not observed when specimens were kept for several days in the laboratory even though female gametes secreted their odor and both male and female gametes were still fully motile.

The findings reported here suggest that *Myelophycus simplex* has an isomorphic life history alternating between dioecious gametophytes and sporophytes.

Sexual Pheromones: For pheromone isolation, female gametophytes recognized by the odor as described above were immersed in sterilized seawater where gametes were released. These were collected using their photoaccumulation response and the resulting gamete suspension were placed in a Grob-Hersch-type pheromone extractor with a capacity of 180 m*l*. Volatile substances secreted from settled gametes were collected in a bed of 2 mg activated carbon, and extracted with 30 μl dichloromethane. Extraction of volatile substances was performed within 2-3 hours after spore release. Samples were analyzed by gas chromatography combined with mass spectrometry. Two different injection temperatures (i.e. 120°C and 250°C) were applied in the gas chromatography.

For the bioassay of sexual attraction of male gametes by hormosirene, known concentrations of synthetic hormosirene were used. Droplets of fluorocarbon liquid (Fluorinert FC-72, Sumitomo-3M, Tokyo) containing various concentrations of hormosirene (10^{-5}-10^{-9}M) and pure FC-72 as control were placed on the bottom of a small plastic petri-dish filled with sterilized seawater. Freshly released swimming male gametes were added to each petri-dish, and the arrangement was placed under darkness on the stage of a microscope. After 2 minutes the distribution of male gametes near the droplet surface was recorded by a darkfield image with a high speed film.

The extract of volatile substances released from settled female gametes contained several compounds known as brown algal sexual pheromones, i.e. dictyopterene A, ectocarpene, hormosirene and dictyotene (Fig. 11, Table 1). When the injection temperature was kept at 120°C (Fig. 11a) the major substance was hormosirene (88.5%). However, at an injection temperature at 250°C (Fig. 11b), most of hormosirene rearranged to ectocarpene. The lowest concentration of hormosirene in which the attraction of male gametes was recognizable in the bioassay was 4×10^{-7} M. This threshold concentration is considerably higher than that reported for other taxa (e.g. 10^{-8}-10^{-9} M in *Analipus japonicus*, Müller *et al.* 1990). This could be attributed to the fact that our bioassay system in this study was severely limited because of the lack of stroboscopic photography. Nevertheless, it appears feasible to conclude that hormosirene acts as a major sexual pheromone in *M. simplex*, although the possibility that male gametes of *M. simplex* also respond to some other minor substances in the pheromone extracts still exists.

Table 1. Components and their relative quantitites detected in extracted volatile substances from female gametes of *Myelophycus simplex*. Peak numbers correspond to Fig. 11.

Peak no.	Compound name	% in total pheromone
1	Dictyopterene A	2.6
2	Ectocarpene	5.5
3	Hormosirene	88.5
4	Dictyotene	3

The kind of sexual pheromones in the Phaeophyceae is considered to reflect phylogenetic relationship (Maier and Müller 1986). Hormosirene has been identified as the sexual pheromone in members of Scytosiphonales (e.g. *Scytosiphon, Colpomenia*), *Analipus* and some taxa from southern hemisphere such as *Hormosira* and *Xiphophora* (Fucales) and *Durvillaea* (Durvillaeales) (Maier and Müller 1986, Müller *et al.* 1990).

Taxonomically, *Myelophycus* is currently placed in Dictyosiphonales (Tanaka and Chihara 1984). However, in respect of chloroplast morphology, *Myelophycus* and *Melanosiphon* (single chloroplast with pyrenoid, Wynne 1969) do not fit with the order Dictyosiphonales (several to many chloroplasts with pyrenoids), but compare well with mem-

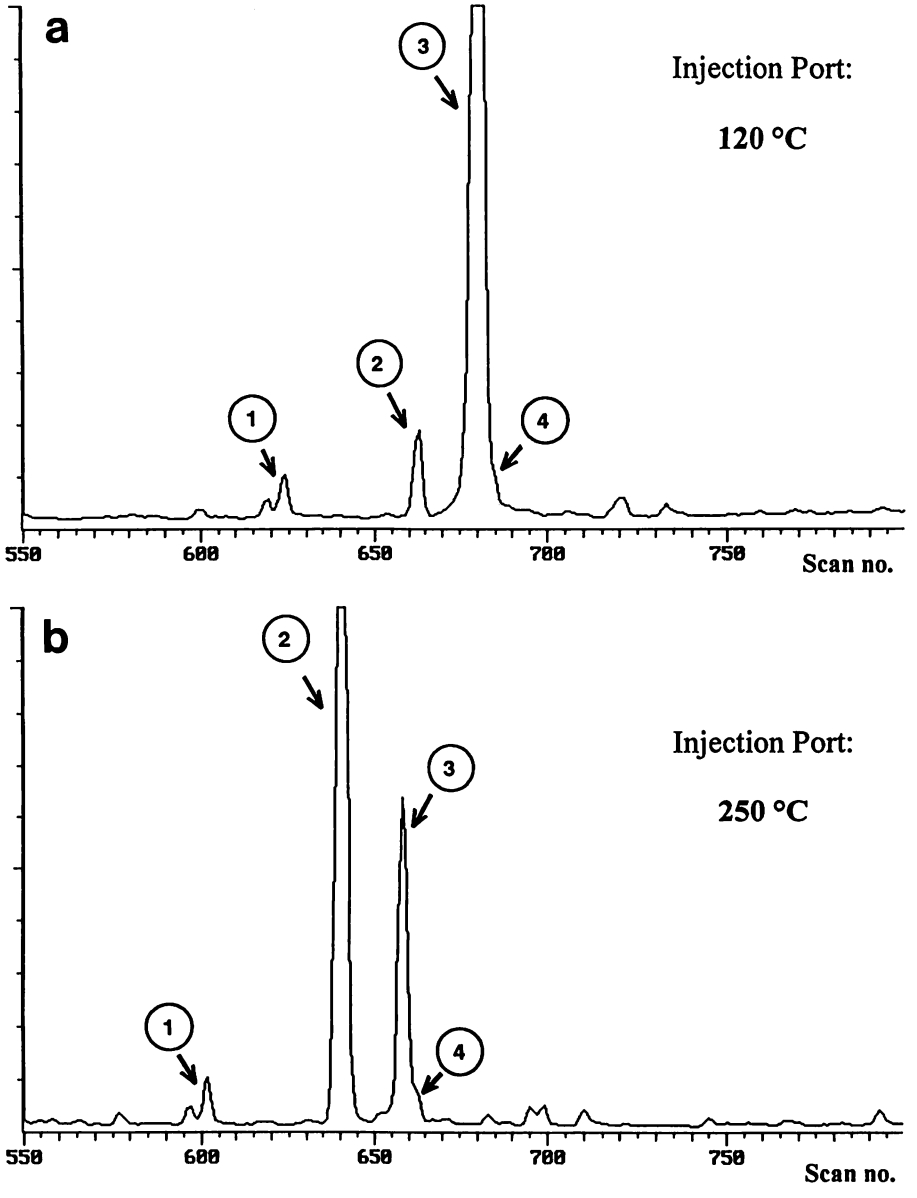


Fig. 11. Gas chromatogram of volatile substances from female gametes of *Myelophycus simplex* at two different injection port temperatures: a, 120°C; b, 250°C. Peak numbers correspond to Table 1.

bers of Scytosiphonales (Kawai 1992). The systematic position of *Analipus* is not satisfactorily clear, and it is placed in Chordariales (Abbott and Hollenberg 1976), Ralfsiales (Nakamura 1972, Yoshida *et al.* 1990) or Ectocarpales (Fritsch 1945, Bold and Wynne 1985) according to different authors. However, *Analipus* also shows an isomorphic alternation of life history between terete erect thalli like *Myelophycus*, and is isogamous. Therefore, the occurrence of the same sexual pheromone among *Myelophycus*, members of Scytosiphonales and possibly also *Analipus* may suggest a closer systematic relationship among them than formerly estimated. In contrast, *Hormosira, Xiphophora* and *Durvillaea* are apparently more distant from those three taxa, considering their more elaborated thal-

lus anatomy and differences in the life history patterns (Fritsch 1945, Clayton 1984, Bold and Wynne 1985, Clayton *et al.* 1985), although they share the same sexual pheromone.

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川井浩史* ・ Wilhelm Boland ・ Dieter G. Müller*** ：褐藻イワヒゲ *Myelophycus simplex* (Harvey) Papenfuss の有性生殖と性フェロモン**

日本産の褐藻イワヒゲにおいて有性生殖を観察するとともに雌性配偶子から放出される性フェロモンを同定した。その結果，複子嚢を生じる大型の藻体が雌雄異株の配偶体であることが明らかになった。雌雄の配偶子嚢と配偶子は形態上はほぼ同じであったが，遊泳後雌性の配偶子は雄性のものよりも早く基物に付着し，雄性の配偶子が先に付着した雌性の配偶子に誘引され接合がおこった。付着した雌性配偶子に由来する揮発性物質を抽出・分析した結果，主要な成分はホルモシレンであり，その雄性配偶子の誘引活性を合成ホルモシレンを用いた生物検定により確認した。(*657 神戸市灘区六甲台町1-1 神戸大学理学部生物学科，**Institut für Organische Chemie der Universtiät, Richard Willstätter Allee, D-76128 Karlsruhe, Germany, ***Fakultät für Biologie der Universität, D-78434 Konstanz, Germany)

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