# Sexual pheromones and gamete chemotaxis in *Analipus japonicus* (Phaeophyceae)

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*Summary.* Female gametes of the marine brown alga *Analipus japonicus* secrete a complex bouquet of olefinic $C_{11}$-hydrocarbons. The major compound is ectocarpene, while hormosirene and dictyotene are present at levels of 2%. Although a minor constituent of the pheromone bouquet, hormosirene is 100 times more active as a male-attractant than ectocarpene. Thus, sex attraction in *Analipus* is mediated by two compounds, which diverge largely in concentration and biological activity.
*Key words.* Sexual pheromone; chemotaxis; Phaeophyceae; *Analipus*; ectocarpene; hormosirene.

*Analipus japonicus* is a perennial brown alga, which persists as a crust on rocks of the higher intertidal zone on the coast of the Northern Pacific Ocean. From winter to early summer it forms deciduous upright thalli up to 40 cm long. Reproductive structures appear on the entire surface of the thallus. *Analipus* is dioecious and has an alternation of isomorphic generations[1], and three types of plants are found in field populations: sporophytes, and male and female gametophytes.

Both types of gametes are motile. Female gametes are slightly larger in size, indicating a moderate degree of anisogamy. Sexual fusion occurs in the same fashion as in *Ectocarpus*; after a short swimming period female gametes settle on the substratum and become attractive to male gametes.

Sexual pheromones (attractants and sperm-releasing factors) are well known in many species of brown algae. At present, 11 molecules with confirmed pheromone character are known[2, 3]. We report here on pheromone secretions in *Analipus*, their identification and bio-assays with synthetic compounds.

## *Materials and methods*

Fifteen plants of *Analipus japonicus* (Harv.) Wynne of 10–15 cm length were collected in July 1989 at Akkeshi, Hokkaido, Japan, and kept dry and dark in a plastic bag at temperatures between + 5 and + 10 °C. Ten days later, gametophytes released huge masses of gametes when immersed in seawater. The sex of the plants was determined by examining gamete size, motility and crossing behavior.

Suspensions of female gametes were placed in closed-loop extraction vessels of the Grob-Hersch-type[4], with a capacity of 300 ml. Volatile compounds were adsorbed on 2 mg activated carbon and eluted with 30 µl dichloromethane.

Mass spectra were obtained with a Finnigan ITD 800, combined with a Carlo Erba Vega gas chromatograph using an RSL 300 fused silica capillary (25 m × 0.25 mm) and He at 0.5 bar as carrier gas. Elution was programmed from 45 °C (3 min isothermal) at 5 °C/min to 200 °C. Ionization potential: 70 eV; scan range 35–249 Da/s; transfer line at 250 °C. Compounds were identified by gas chromatography/mass spectrometry, and the structures were confirmed by comparison with synthetic references.

The absolute configuration and enantiomeric excess of hormosirene was determined by HPLC on Chiracel OB (35 cm × 0.46 mm, Daicel, Co., Japan) using methanol/water (85:15, v/v) for elution[5]; flow rate: 1.2 ml/min; detection: UV at 247 nm. Synthetic (+)- or (−)-hormosirene[6] was used to establish the relative retention of the two enantiomers (separation factor a = 1.40).

Bio-assays followed the method described previously[5]. Suspensions of freshly released male gametes were exposed to micro-droplets of the fluorocarbon liquid FC-72, which contained known concentrations of synthetic compounds. Attraction was quantified with the quotient by which experimental droplets were preferred against a blank of pure solvent.

## *Results and discussion*

One female plant with a fresh weight of 2.4 g produced an especially rich gamete suspension. During 14 h 107 µg of the main compound was collected, and identified as ectocarpene. In addition, gas chromatography revealed 2 smaller peaks. One, amounting to 2.75% of the ectocarpene, was identified as hormosirene, the other, amounting to 2.13%, as dictyotene (fig. 1). Several additional compounds were found at levels far below 1% (fig. 1, table).

Bio-assays with male gametes gave the following results: Ectocarpene, although dominating the secretion bouquet, has a far lower biological activity than hormosirene. The response curves in figure 2 indicate that the activities differ by a factor of 100. Multifidene had no activity, and dictyotene 20 times less than ectocarpene.

If quantitative representation in the secreted bouquet and biological activity of ectocarpene and hormosirene are compared, the conclusion is evident that gamete attraction in *Analipus* is mediated by both compounds; a

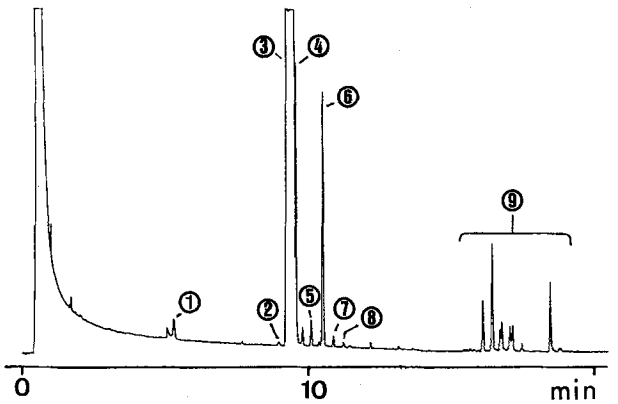



Figure 1. Gas chromatogram of eluate from female gametes of *Analipus*. Peak numbers correspond to the table.

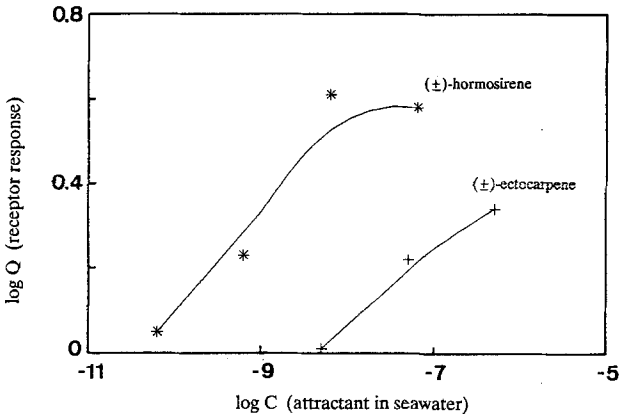
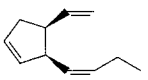
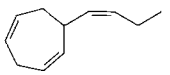
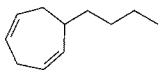
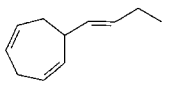
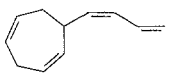



Figure 2. Attractive potential of synthetic racemic ectocarpene and hormosirene. Molar concentrations are corrected for the partition coefficients in the system FC-72/seawater. Q stands for the preference factor of experimental droplets over the solvent blank by male gametes of *Analipus*.

Components and their relative quantities detected in eluates from female gametes of *Analipus*. Peak numbers correspond to fig. 1.

| Peak | Compound name | Structure | % in extract (ectocarpene = 100) |
|---|---|---|---|
| 1 | Multifidene |  | 0.37 |
| 2 | – | $(C_{11}H_{14})$ | 0.19 |
| 3 | Ectocarpene |  | 100.0 |
| 4 | Dictyotene |  | 2.13 |
| 5 | – |  | 0.36 |
| 6 | Hormosirene (+)-(1S, 2S) |  | 2.75 |
| 7 | Desmarestene |  | 0.12 |
| 8 | – | $(C_{11}H_{14})$ | 0.22 |
| 9 | $C_{11}H_{16}O$ and $C_{11}H_{16}O_2$ | | n.d. |

high concentration level of ectocarpene with low biological activity and a low concentration of hormosirene with high biological activity.

Many pheromone secretions in brown algae are found to be bouquets of structurally related molecules[2]. Generally, biological activity can be attributed to one prominent component in the extracts. To date, only one clear case of interference between bouquet constituents has been encountered. (+)-Ectocarpene could be shown to act as an antagonist for multifidene-induced sperm-release in *Chorda tomentosa*[2]. The bouquet character of many brown algal gamete secretions was interpreted until now as being a reflection of limited enzyme specificity in pheromone biosynthesis, with rarely detectable physiological significance.

This simplistic view must now be reconsidered. At present 4 genera of brown algae are known to secrete ectocarpene as the dominating product in their pheromone bouquets. In *Ectocarpus*[8], *Sphacelaria*, and *Adenocystis*[9] ectocarpene was found to be active enough to account for the observed effects of gamete attraction. In two species of *Saccorhiza* ectocarpene, as the main secretion product, did not have biological activity[2]. In 1971, when ectocarpene was first identified as the sexual pheromone of *Ectocarpus siliculosus*[8], analytical methods could hardly detect the presence of, and could certainly not identify, trace compounds in the eluates of female gametes. The *Ectocarpus* pheromone bouquet was re-examined in 1988[4]. Improved analytical techniques showed the presence of about 4% hormosirene and minor quantities of multifidene and dictyotene. These *Ectocarpus* extracts are strikingly similar to those now found in *Analipus*. Re-evaluation of these ectocarpene-systems is now necessary. Enantiomeric excess of ectocarpene and hormosirene should be determined in the respective extracts, and the physiological activities of enantiomers studied in bio-assays. It will also be important to look for synergistic or antagonistic interference effects in hormosirene-ectocarpene mixtures.

Hormosirene has been confirmed as the gamete-attractant in several brown algal genera: *Hormosira* and *Xiphophora* (Fucales), *Durvillaea* (Durvillaeales), *Scytosiphon* and *Colpomenia* (Scytosiphonales)[10]. In all these cases, the gamete secretions contained both the (+) and (−) enantiomers in various ratios[5]. Hormosirene from *Analipus* in our study seems to be exclusively pres-

ent as the (+) enantiomer. The (−) enantiomer was below the detection level, which indicates at least 90% enantiomeric excess. Our bio-assays with *Analipus* were carried out with racemic hormosirene, and a possible enantiomer-selectivity could not be evaluated. Likewise, the enantiomer specificity of ectocarpene secretion and activity is not known at present.

The results reported here on the sexual pheromone system of *Analipus* reveal a degree of elaboration not known before in algae. Although the compounds involved are relatively simple olefinic hydrocarbons, the chemo-communication system is complicated by the involvement of two compounds in a complementary manner; the high concentration of a weakly active agent is combined with a highly active molecule in low concentration. Our knowledge of the details of brown algal pheromone systems is still limited, and it seems premature to comment on the ontogenetic or phylogenetic significance of a two-component system such as that described here for *Analipus*.

The taxonomic position of *Analipus* within the brown algal system is still uncertain. Some authors have placed it in the order Chordariales, referring to anatomical similarities[11]. Nakamura[12] suggested transferring it to the newly established order Ralfsiales. Still other authors[13] dispute the value of the order Ralfsiales and refer *Analipus* to the Ectocarpales, where it occupies a rather isolated position.

The results of this study show that progress in analytical methods will make it necessary to re-evaluate earlier work, since at least some of the brown algal pheromone systems may be more complicated than was previously believed in respect of bouquet composition, relative biological activities, and enantiomer composition.

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## Chemical analysis of the pheromone blends produced by males and females of the neotropical moth, *Mocis megas* (Guénée) (Lepidoptera, Noctuidae, Catocalinae)

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*Summary.* Pheromonal secretions produced by females and males of the noctuid moth, *Mocis megas* (Guénée) have been analyzed by gas chromatography and mass spectrometry (EI (electron impact) and CI (chemical ionization)). The female sex pheromone was a blend of (Z,Z,Z) 3,6,9 heneicosatriene (55%) and (Z,Z) 3,6-cis-9S,10R-epoxyheneicosadiene (45%). Male secretion produced at the level of a prothoracic organ was a blend of two unsaturated major hydrocarbons: (Z,Z) 6,9 heneicosadiene (64%) and (Z,Z,Z) 3,6,9 heneicosatriene (24%) and $C_{19}$, $C_{20}$ and $C_{22}$ homologues (total ratio 12%), as minor components. The trienic hydrocarbon was present in both sexes. The behavioral role of this male secretion has not yet been elucidated.
*Key words.* Sex pheromone; male secretion; chemical analysis; (Z,Z) 6,9 heneicosadiene; (Z,Z,Z) 3,6,9 heneicosatriene; (Z,Z)-3,6-cis-9S,10R-epoxyheneicosadiene; *Mocis megas*.

Noctuid moths of the genus *Mocis* (sub-family Catocalinae) are endemic in tropical areas. About 20 species are found from the southern United States to Brazil, including the Caribbean arc. *Mocis megas* (Guénée) was described for the first time from St. Thomas island, and is common from Puerto Rico to Dominica. The species has