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# OLEFINIC HYDROCARBONS IN SEA-WATER: SIGNAL MOLECULES FOR SEXUAL REPRODUCTION IN BROWN ALGAE

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There are various ways for a scientist to look at the sea. A chemist may consider it as a huge container offering lots of chemicals to examine separately or in their complex interaction. A biologist will tend to envision the same environment as a biotope housing enormous numbers of organisms of largely diverging organization levels. Science history provides many examples which demonstrate that progress is stimulated when different disciplines begin to share interests. The seas of the world are especially suitable to demonstrate this effect. Chemists and biologists may cooperate in looking for the interaction of organisms with the environment, such as the uptake of nutrients or the excretion of waste products. Other fields are the chemical composition of organisms and the passage of various compounds along the food chains.

In any case, it is clearly evident that natural sea-water contains an immense diversity of organic chemicals, either being excreted as waste products or released during decomposition of dead material. In addition, there are chemicals released by organisms for specific purposes, such as ectoenzymes or protective slimes, enemy-repellents and the like. All these types of chemicals present in the sea can be considered as truly "natural products" insofar as they are connected with the normal activities of undisturbed populations. Much concern has to be expressed, however, when man begins to add to the marine environment chemicals that are not normally found there. Such materials may severely disturb the delicately balanced interaction of organisms.

I am going to present here the story of three organic compounds which have recently been found to be secreted by plant cells to serve a very specific function during reproduction. For more than 120 years, workers studying reproduction in brown seaweeds have noticed that sperm cells or male gametes are influenced by the egg. It is obvious that such an interaction in microscopic dimensions in the range of 1 mm is based upon a chemical message. Various attempts to identify these chemicals failed, until about 10 years ago the methods of analytical chemistry had been refined to such a degree that successful handling of microgram quantities became possible. Intense cooperation between biologists and biochemists resulted in the identification of three messenger molecules that are involed in sexual reproduction of the brown algae Ectocarpus, Cutleria and Fucus. Surprisingly enough, a look into last century's literature revealed that all three cases had been anticipated by admirably precise observations several generations ago (1).

Let us now take a look at the structural formulae of the substances. Their names were derived from the organisms in which they had been found for the

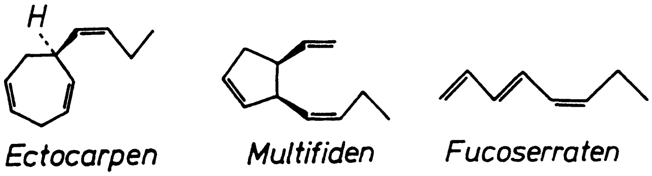

Fig. 1. Structure of the three sex hormones in Phaeophyceae discussed here

first time. All three compounds are unsaturated hydrocarbons. Ectocarpen and multifiden have the same composition, both of them are $C_{11}H_{16}$ cycloolefines (Fig.1). All three molecules have a distinct odor, which can be easily detected by the human nose. Sensing the fragrance over a water sample containing the producing cells in suspension gives the observer the impression that the molecules are literally expelled from the water right after having been secreted. This property of immediately moving out of the water into the gas space made it very easy to collect the compounds free from contaminants. The only serious problem in this phase of the work was to be patient enough in accumulating the necessary amounts of material.

Which are the organisms producing these hydrocarbons and what is their biological function?

1. Ectocarpus siliculosus is a simple filamentous brown alga found as dense mats growing on stones, ropes and other supports in shallow water. The strains that were used for the production of ectocarpen came from the vicinity of the Stazione Zoologica at Napoli. The sexual plants are present only in springtime. Mature plants release into the water small motile cells of about 5 µm in diameter, called gametes. Their function is to fuse with a cell of opposite sex to form a zygote that grows out to a new plant.

It is obvious that a critical stage in this course of events is the moment when male and female cells are swimming in the water in desperate search of a partner cell of matching sex. This problem has been solved by assigning different properties to these gametes. One type, which we classify as female, is only a modest swimmer. It rather prefers to settle down on a substrate and change its strategy. As soon as the cell comes to rest, it starts secreting ectocarpen into the surrounding water. Contrarily, the male cells are very good and permanent swimmers and equipped with a sensory mechanism to detect the presence of ectocarpen and find its source. Normally, lots of males will thus arrive at the site where a female has lodged, and a little later cell fusion is accomplished. Then, the supernumerous males lose their interest because the zygote no longer produces ectocarpen. We may, therefore, classify ectocarpen as a sex attractant as it is used by the female cells to lure their matching male partners.

2. Cutleria multifida is a more complex thallus. Sexual plants are found in springtime at the Mediterranean coast. Sexual reproduction is similar to that in Ectocarpus. There is, however, one difference which is considered to indicate an evolutionary progress: the female cells are substantially bigger and, therefore, able to contribute a larger supply of nutrients to the embryo.

3. Fucus serratus is a coarse seaweed of the north European coasts. It is believed to belong to the most advanced brown algae, which is reflected in the fact that the female cells are veritable eggs of considerable size. These eggs produce an attractant which causes male gametes or spermatozoa to become excited and approach the egg to form immense clouds.

In comparing the sexual systems of these three brown algae, one additional feature should be mentioned here: male and female cells in Ectocarpus may germinate and develop into a new plant without sexual fusion. In Cutleria, such parthenogenetic development is possible in female cells only, and in Fucus neither spermatozoids nor unfertilized eggs are able to germinate. Thus, the most advanced type, Fucus, is fully dependent on successful fertilization of its eggs.

So far, we have discussed the attractant molecules and their normal biological function. During the years of cooperation with chemists, I saw that immediately after an interesting molecule had been discovered they tried to synthesize it. Thus, synthetic attractants were made available very soon in reasonable quantities, which in turn stimulated the biologist to use them for physiological experiments. One of the most urgent questions concerned the threshold concentrations necessary to excite the male gametes. Another problem was to estimate the degree of specificity between the chemical structure of the compounds and the reaction of the male cells. Both types of experiments called for the development of a quantitative assay method. It proved to be quite difficult to apply defined amounts of highly volatile and hydrophobic substances to living cells swimming in seawater. The technique finally adopted makes use of a liquid fluorochemical that is manufactured for technical purposes. This product is insoluble in water, biologically inert and dissolves the attractants. When placed at

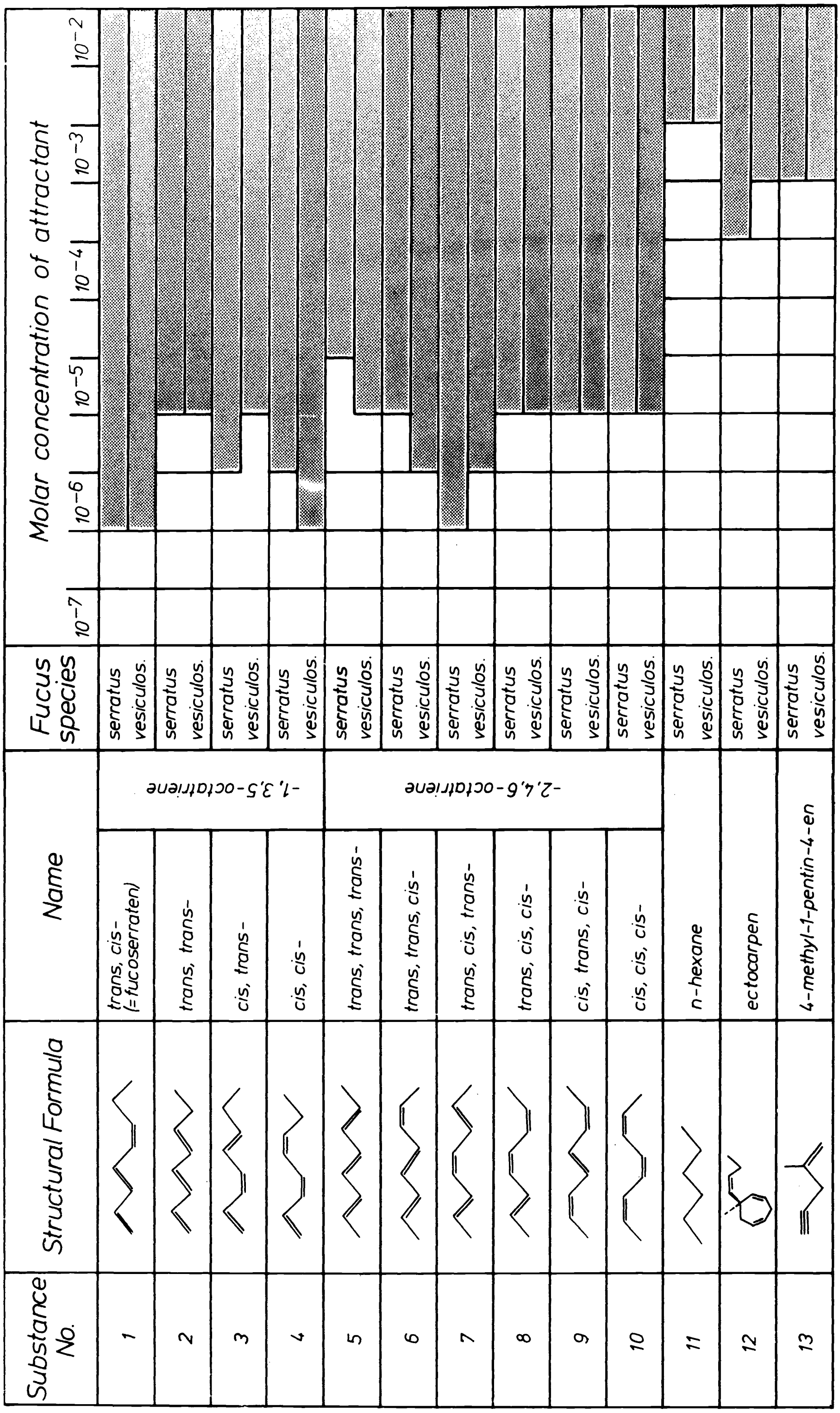

Fig. 2. Chemotactic response of male gametes in Fucus serratus and F. vesiculosus. Fucoserraten and structural isomers were offered using the fluorocarbon FC-78 as a solvent. Concentrations with attractive potential towards spermatozoids are shadowed. Intermediate dilution steps between full decimals have a factor of 3.1.

the bottom of small plastic dishes filled with sea-water, small droplets of the fluorochemical spread out to a flat lense-shaped body. When attractants are present in the solvent, they diffuse out into the water phase where they can be detected by the male cells. The distribution pattern of cells on experimental fields and a blank reference field is recorded by a dark-field flashlight microphotograph. This information can then be quantitized and subjected to statistical treatment (2).

Threshold concentrations were determined in all three sexual systems mentioned above and found to be approximately in the same range. For instance, spermatozoids of Fucus serratus respond to fucoserraten concentrations in the solvent phase as low as $10^{-6}$ M. The volume of the solvent droplets is about 0.1 $\mu$l. This gives an absolute amount of $10^{-11}$ g per droplet which can be detected by Fucus spermatozoids.

Fucoserraten is a conjugated octatriene, and playful chemist colleagues synthesized all possible cis-trans isomers. In turn, the biologist used this collection for a study to find out how well Fucus spermatozoids can distinguish between these closely related molecules. It was revealed that the spermatozoids can quite well distinguish between their specific attractant and isomers with different cis-trans arrangements. The biologist's urge to play expressed itself at this stage by expanding the experiments to a closely related species, Fucus vesiculosus, which grows at the same places and at the same time as F. serratus. Surprisingly, spermatozoids of both species reacted equally sensitive to fucoserraten (Fig.2). In addition, maximum sensitivity to one additional isomer in each species was found. This result was puzzling because it seemed unlikely that both species should use the same attractant. So, I tended to believe that compound Nr. 4 might be the vesiculosus attractant (3). Nevertheless, this was purely speculative reasoning. Further, there still was the old claim proposed as late as 1970, that n-hexane were the attractant of F. vesiculosus (4). Therefore, I decided in cooperation with chemist colleages to try and solve the problem of the Fucus vesiculosus attractant. Viable eggs were collected at Helgoland in winter 1977/78 and volatiles secreted by them stripped from the sea-water and collected. Capillary gas chromatography demonstrated with unique clarity that the attractant from eggs of F. vesiculosus is fucoserraten, the same as that from F. serratus (5). This is at a first glance an unexpected situation, but we have to keep in mind that the two species do not hybridize in the field. Recent results of British workers indicate that components in the cell membrane of the egg are responsible for the recognition of species specificity and effectively exclude sperm from other species (6). We have to accept as a fact that the process of phylogenetic diversification did not require the introduction of a new attractant when the two species separated in the past. Perhaps even a positive selection effect is connected with the conservation of the original attractant. Purposefully miguiding the competitor-species' spermatozoids may belong to the survival strategies in Fucus. If this should be true, any plant using a modified molecule would voluntarily give away this weapon. Strange as it may appear, we have to keep in mind that this consideration is purely speculative at the present state of knowledge. Nevertheless, fouling competitor-species' sexual chemotaxis systems may belong to the arsenal in biological competition. Let us take a brief look at Ectocarpus and Cutleria. There are places at the Mediterranean coast where sexual plants of both species mature simultaneously. One may safely assume that gametes of both species are swimming in the sea at the same time, and male gametes will find their female counterparts. In addition, however, male gametes of Ectocarpus will be trapped by females of Cutleria and vice versa. This effect can be very clearly demonstrated in the laboratory (2). At the moment, we are not able to evaluate whether such inter-specific interaction in Fucus, Cutleria and Ectocarpus is of biological significance in the natural habitat.

In a very early phase of my work on Ectocarpus chemotaxis I found that a great variety of low-molecular organic chemicals and complex mixtures may mimic sexual chemotaxis (Table 1). One of these molecules, n-hexane, was included in my later studies on threshold concentrations. It was found that the male gametes respond between 300 and 3000 times less sensitive to n-hexane than to their species-specific attractant (2,3). Nevertheless, as modern man routinely releases immense amounts of chemicals including hydrocarbons into the marine environment, the question arises whether some of these waste chemicals may interfere with the chemical signals used by marine organisms.

TABLE 1. Substances found to mimic sexual chemotaxis in male gametes of Ectocarpus siliculosus (7)

| | |
|---|---|
| n-hexane | gasoline b.p. under 90 °C |
| cyclohexane | pine needle oil |
| limonen | orange oil |
| ß-pinen | mandarin oil |
| ocimen | lemon oil |
| n-octanal | |
| n-decanal | |
| citronellal | |
| nonylacetate | |

Male gametes of Ectocarpus were used to study the effect of the chronic presence of active substances on their locomotive behaviour. The cells normally move freely through the water in straight lines. Upon contact with the surface, they like to slide along the surface in wide loop-like movements. As a result of chronic stimulation by active substances in the water, the thigmic reaction is enforced and the movement pattern changes to circles

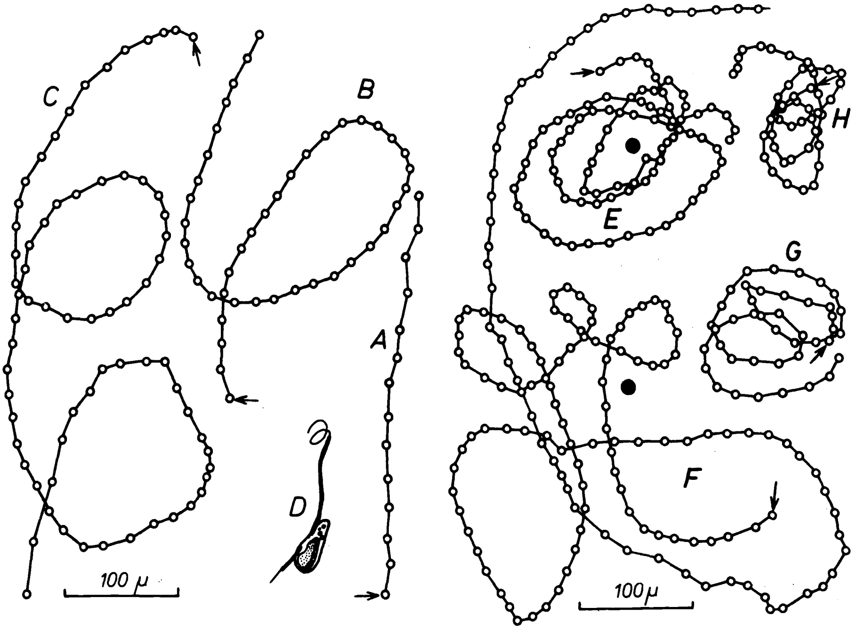

Fig. 3. Ectocarpus siliculosus. Tracks of male gametes, reconstructed by recording their position at 1/10 sec intervals. A, cell swimming in culture medium without contact to a surface; B,C, cells moving in contact with surfaces: B with cover-glass, C with bottom of preparation; note different sense of rotation; D, male gamete with highly asymmetric structure; E,F, tracks around a point source of the attractant (female cell of a non-compatible strain); G,H, behaviour of cells under chronic stimulation by ectocarpen, G: $10^{-4}$ M, H: $10^{-3}$ M. The beginning of each sequence is indicated by an arrow.

with diameters inversely proportional to the attractant concentration (8). If this crude pattern is transferred to the more refined situation of a point source such as a female cell, the response of the male cells inevitably leads them to the source (Fig.3). The unnatural chronic presence of active compounds in the marine environment thus may, indeed, deprive the male gametes of the ability to perform the reactions which are necessary to find the egg. Such failure of fertilization is not fatal in the cases of Ectocarpus and Cutleria, because at least the female may propagate parthenogenetically without sexual fusion. However, disabling the male cells is fatal for species such as Fucus, where failure of fertilization will result in the death of the population because no offspring will be generated.

The thoughts expressed here are admittedly speculative. Usually, bio-assays performed to examine the damage to organisms by waste products are designed to demonstrate direct poisoning of the vegetative parts. No experiments have been done as yet to examine the disturbance of much more delicate systems, such as the chemical signal transfer involved in sexual reproduction. However, there is one report in the literature where such an effect may have unknowingly been observed.

Steele (9) examined the effect of oil pollution on the reproduction of Fucus edentatus in Rhode Island. He soaked the fertile plants in various concentrations of crude oil products dissolved in sea-water prior to the release of eggs and sperm. In another experiment, he grew zygotes in such pollutant concentrations. In both cases, the toxicity level was found in the range of 10 ppm. In a third experiment, incubation with oil products was restricted to that phase of the procedure when eggs and sperm suspensions were put together. Clean medium was used before and afterwards. Steele describes the result of this experiment as dramatic: no growth occurred at all, even at his lowest concentration of 0.2 ppb. This means that the biological toxicity is at least 10 000 times higher during the phase of fertilization. Steele apparently was not aware of the phenomenon of sexual chemotaxis, and he calls this dramatic increase in toxicity paradoxical. His finding might nevertheless indicate that sperm chemotaxis is an especially sensitive phase of development as far as disturbing chemicals in the environment are concerned.

Finally, I would like to present some remarks on a sexual system in another group of brown algae which is currently being studied and which has some fascinating features.

It is found in the order Laminariales that contributes most of the commercially interesting macrokelps such as the Macrocystis beds of California or the Ecklonias and Undarias which are cultivated in Japan. As in the order Fucales, fertilization of the egg is obligatory. Chemical interaction of egg and sperm is more complicated in the Laminariales. The sexual stages in this group are of microscopic size. Male plantlets form their

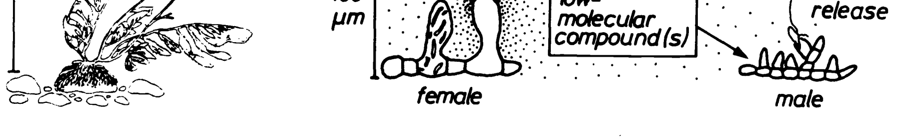

Fig. 4. Chemical interaction in sexual reproduction in the order Laminariales showing different dimensions of sporophytic and gametophytic generations.

spermatozoids in specialized cells, but do not release them spontaneously. In contrast, the male cells sit in their housings up to the moment when the chemical signal emitted by a nearby egg arrives. Then, they literally pop out and start searching for the egg (Fig.4). The substance involved in this interaction is still unknown, but we do know that it is also a highly volatile lipophilic molecule (10).

A brief reminder of the pollution problem mentioned above might indicate that the Laminaria sexual system is still more sensitive to the disturbing action of chemicals in the environment. It is not only the chemotaxis that may be fouled, but the male gametes may be set free at the wrong time when there are no eggs available.

The data presented here indicate that it is highly desirable to design refined experiments that allow to study the influence of pollutants on delicate biological processes.

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