

Anisogamy in the dinoflagellate *Noctiluca*?

E. Schnepf¹ & G. Drebes²

¹Zellenlehre, Universität Heidelberg; Im Neuenheimer Feld 230, D-69120 Heidelberg,
Federal Republic of Germany

²Biologische Anstalt Helgoland, Wattenmeerstation Sylt; D-25898 List, Federal Republic
of Germany

ABSTRACT: We disagree on the generally accepted elements of the description of the sexual life cycle of *Noctiluca* as presented by Zingmark (1970a), namely that (1) the swarmers of *Noctiluca* are isogametes, that (2) the zygote develops directly into a large trophont, and we question that (3) *Noctiluca* is a diplont with meiosis occurring during the formation of the swarmers. We observed a highly distinct attraction between swarmers (microgametes?) and certain large cells (macrogametes?) which resemble adult trophonts in shape and size. *Noctiluca* thus appears to be anisogamous, and the zygote does not need to grow to become a large trophont.

INTRODUCTION

Noctiluca scintillans (Macartney) Kofoid is a dinoflagellate distributed worldwide in marine coastal waters, and is most popular owing to its conspicuous bioluminescence (Ehrenberg, 1834). Extensive long-term investigations on its ecology have been conducted by Uhlig & Sahling (1982, 1990) during the past decades, dealing with population dynamics, circadian rhythms, distributional and red-tide phenomena of *Noctiluca* in the German Bight (North Sea).

A part of the *Noctiluca* life cycle, however, namely sexual reproduction, is still insufficiently known and disputed, as stated recently by Uhlig & Mühlhäusler (1992). Uhlig (1972) questioned the data of Zingmark (1970a) on the existence of isogamy. It is well-known that *Noctiluca* cells occasionally form several hundred to more than a thousand small, unflagellate swarmers by a series of 2^7 – 2^{11} mitoses (Pratje, 1921; Zingmark, 1970a; Uhlig, 1972). Zingmark (1970a) proposed that these swarmers result from a meiosis in a "gametocyte mother cell" and subsequent mitoses, and that they represent isogametes which fuse. The resulting zygote was claimed to give rise directly to a vegetative, diploid trophont cell; but this was not convincingly proven. Isogamy of swarmers had been previously suggested (Hofker, 1930; Gross, 1934). Uhlig (1972) assumed that misdivisions during the last cytokineses might give the appearance of fusion of swarmers. Earlier reports on copulation even between large trophonts were likewise interpreted as abnormalities during the vegetative divisions (Pratje, 1921).

These contradictions, and the fact that the vague and inconclusive observations and descriptions of Zingmark (1970a) have been incorporated in many textbooks (e.g. van den Hoek, 1978; Christensen, 1980; Bold & Wynne, 1985; Pfiester & Anderson, 1987), prompted us to search again for sexual reproduction in *Noctiluca*. We extended our

observations to cover the behaviour of the swarmer – hitherto neglected. Our premise was that a chemotactic attraction between the gametes should precede copulation and that an accumulation of swarmer around another swarmer or a trophont-like cell would be a strong indication of a sexual process.

Our results suggest that the "swarmer" are microgametes and that a microgamete copulates with a large macrogamete which looks like a trophont (vegetative cell). The uninucleate cell which gives rise to the microgametes would thus be a microgamete mother cell. It develops into the multinucleate microgametocyte from which the microgametes bud off. We shall use this terminology in the following text (see also Zingmark, 1970a).

MATERIAL AND METHODS

In October 1992, net samples containing *Noctiluca scintillans* (Macartney) Kofoid (Syn. *N. miliaris* Lamarck) were taken from the Wadden Sea near List/Sylt (German Bight, North Sea). About 25 specimens of the dinoflagellate were isolated and cultivated in Met 44 media (Schöne & Schöne, 1982), together with the green flagellate *Dunaliella tertiolecta* Butcher as food organism, in 10-cm high glass vessels with a volume of 50 ml, at 16°C, a 14:10 h light:dark rhythm, and a light intensity of about 35 $\mu\text{mol m}^{-2} \text{sec}^{-1}$. Similar results were obtained at 13°C and "natural" illumination in a window facing north. For inspection, microgametocytes, together with a few trophont-like cells (among them putative macrogametes), were selected and transferred to Petri dishes with new media. They were observed, photographed and recorded on videotape, in part using Leitz sea-water immersion objectives.

RESULTS

Development of gametes

Under our culture conditions the overwhelming majority of the cells were trophonts, big peach-shaped cells with a diameter of 400–800 μm , a large (30 μm) nucleus with a structure untypical of a dinoflagellate (Zingmark, 1970b), and a prominent tentacle (for detailed descriptions see e. g. Kofoid & Swezy, 1921; Pratje, 1921). As a rule, only very few gametocytes developed within a culture vessel, if at all. Occasionally, the flasks contained a relatively high number (up to about 15 %) of microgametocytes. Uhlig (1972) even noted that sometimes all the cells in his cultures became microgametocytes.

The development of microgametocytes (Figs 1–5) has repeatedly been described (Ishikawa, 1894; Pratje, 1921, 1925; Zingmark, 1970a; Uhlig, 1972). The microgametes are ovoid in dorso-ventral view, measure about 14 \times 18 μm and are flattened but slightly concave on the ventral side where the single longitudinal flagellum is inserted in an indistinct furrow between the larger anterior part (epicone) and the thinner posterior hypocone (Figs 6 and 7) (for further details see Pratje, 1921; Zingmark, 1970a). The nucleus is situated in the hypocone. It is highly condensed so that the chromosomes cannot be recognized under the light microscope, but the true dinokaryon structure has been revealed by Soyer (1969).

A few microgametes were found to have two flagella (Fig. 8). We have never observed a fusion of microgametes in the Petri dish cultures. When microgametocytes

were isolated and up to more than ten of them were put together either in Petri dishes or the 10 cm culture glasses, the liberated microgametes died after about one day. We have not observed zygote-like stages or any intermediate growth stages which would eventually lead to the normal, large trophonts.

Chemotactical attraction

In order to clearly visualize a chemotactical attraction, we placed 10–40 microgametocytes in a 10-ml Petri dish, together with a few trophont-like cells from the same sample. The liberated microgametes swam preferentially on the bottom of the Petri dish. We did not detect any attraction between microgametes but frequently observed many microgametes accumulating near some (but not all) of the trophonts which we believe to be macrogametes.

The attractive macrogametes were more or less immobile and mainly found at the bottom of the Petri dish, with tentacle and cytostome region downwards. They had usually stopped feeding but still contained food vacuoles. Microgametes – in some cases 100–200 of them – accumulated near a “mature” macrogamete (Fig. 9), preferentially in the oral groove (Figs 10 and 11) and in the apical trough, i. e. near the nucleus. If they did not reach that position, where they became more firmly fixed, they attached to the cell surface with their ventral side and remained mobile (Fig. 12). Microgametes gathered also on the bottom of the Petri dish below the stomatal region of the macrogamete. They formed a dense swarm there but soon dispersed after removal of the macrogamete.

Long-term observations suggest that the attractivity of a macrogamete increased gradually and then decreased later, within the range of some hours. The specificity of the attraction is indicated by the fact that almost none of the *Dunaliella* cells collected around a mature macrogamete (Fig. 9). Feeding trophonts “unspecifically” attracted sometimes a few microgametes; but in these cases *Dunaliella* cells likewise gathered around them. The oral region was most attractive also for the green flagellate.

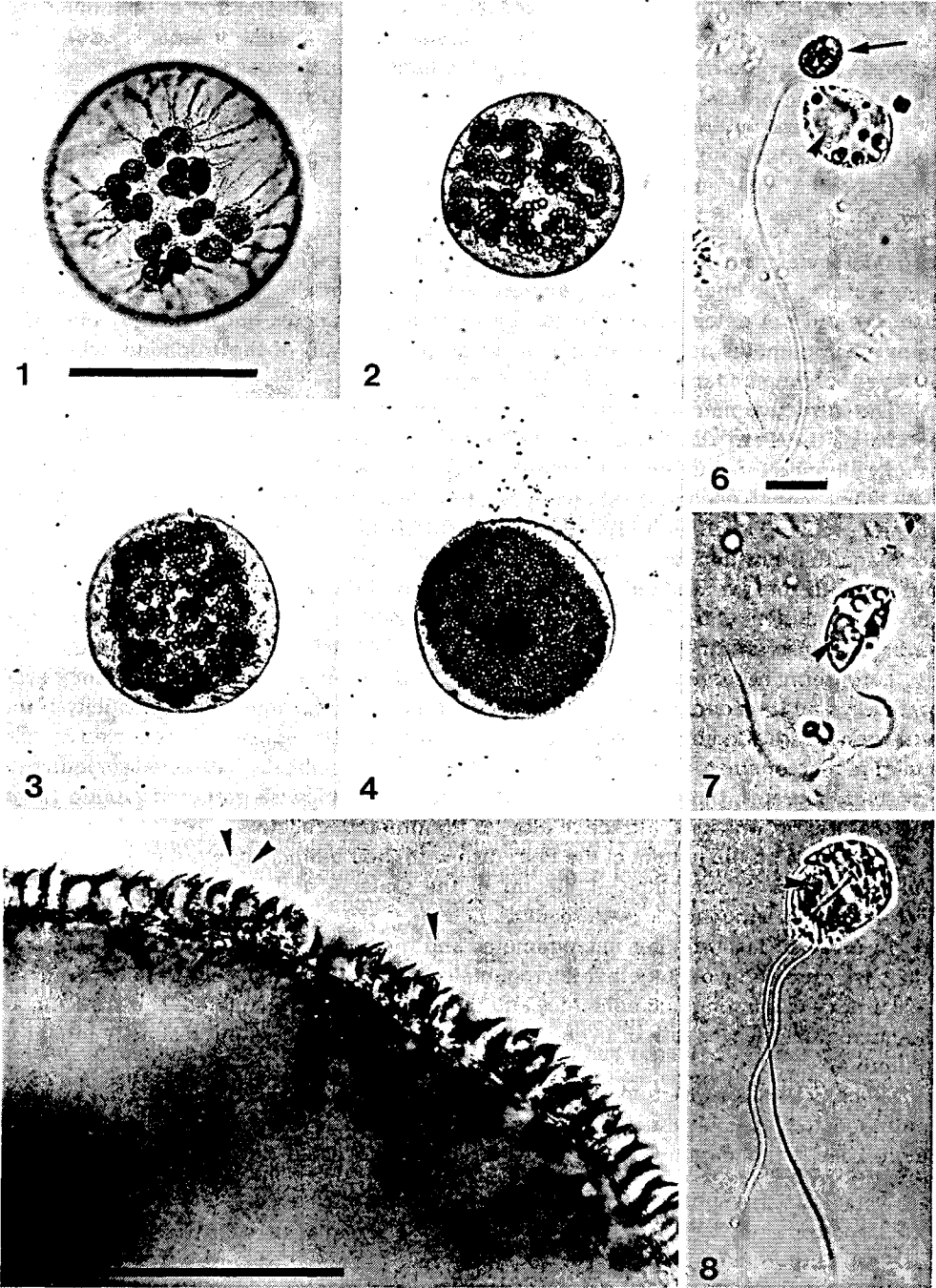
Trophonts on the bottom of the Petri dish with their tentacle upward caught not only *Dunaliella* cells in the slime at the tip of the tentacle and ingested them but also, occasionally, microgametes (senescent gametes?).

In order to test whether microgametes and macrogametes differentiate simultaneously, we repeatedly put together microgametes and trophont-like cells from two sources, i. e. cultures containing microgametes and those without microgametes. The attraction of the microgametes was rare or even lacking in the latter samples but distinctly higher in the former.

Noctiluca cells surrounded by microgametes, and thus expected to become zygotes, continued to develop normally, i. e. as trophonts with feeding and cell division. In a single case, a microgametocyte was differentiated. We did not observe any cyst-like stages.

DISCUSSION

We agree with Zingmark (1970a) and most other authors that the swarmer, formed occasionally by *Noctiluca*, are actually involved in sexual reproduction and that resting stages do not occur in the life cycle (see the scheme of Pfiester & Anderson, 1987). We



disagree with Zingmark's proposal that the small *Noctiluca* swimmers are isogametes. We suggest that they are, on the contrary, only microgametes (males), and that *Noctiluca* is anisogamous.

The life cycle established by Zingmark (1970a) is not well-enough proven:

(1) A copulation of isogametes, described likewise by Hofker (1930) and Gross (1934) (see also Pratje, 1921, 1925) has not been documented convincingly. Most probably, products of misdivisions, not uncommon in dinoflagellates, have erroneously been recognized as fusion stages (Uhlig, 1972).

(2) More important is the fact that, after the supposed fusion of small isogametes, the further development of the zygote into the big trophont has never been observed, either in culture or in nature, in spite of numerous microscopical investigations of the plankton. This zygote must have a mean diameter of about 15 μm (seen as a globule). Very small trophont cells measure about 300 μm in diameter. Neither the existence of direct intermediate stages nor that of cysts which later develop into trophonts have ever been shown (with a single, questionable exception; Zingmark, 1970a).

(3) It is highly improbable that these intermediate stages exist but have been overlooked consistently. If this were the case they certainly would have to undergo a kind of metamorphosis. A zygote with a diameter of 15 μm has to increase by a factor of 20 in diameter, or by a factor of 8000 in volume to become a trophont with a diameter of 300 μm . The nucleus of the trophont (30 μm) is even bigger than the whole "zygote". It is therefore impossible for this increase in size to be realized by a simple vacuolization, as assumed by Zingmark (1970a). Alternatively, one can assume that the intermediate stage between zygote and trophont takes up food in order to grow. If it were a phagotrophic cell, the ingestion apparatus would have to be quite different from that of the adult trophont, merely because of the size of the tentacle of the latter. If it were an "osmotrophic" cell, the feeding apparatus of the adult trophont could develop during the differentiation of the zygote into the trophont. These latter alternatives are likewise pure speculation and unsupported by observation.

(4) A sexual attraction between isogametes has never been described for *Noctiluca*. It is to be expected, but could be less pronounced or even overlooked, especially if the sex ratio is 1:1.

(5) The assumption of Zingmark (1970a) that the first divisions of the gamete mother cell nucleus are meiotic, is speculation. There are no data which support this idea. It would imply diploidy in *Noctiluca*, which contrasts with our knowledge of other dinoflagellates, described as haplonts (Pfiester & Anderson, 1987). The occurrence of a "nuclear tetrad" alone is not a convincing argument. In a series of free nuclear divisions, a four-nucleus stage is necessarily included.

Our alternative suggestion, namely that *Noctiluca* reproduces sexually by anisogamy (nearly oogamy, considering the size of the gametes), that the "swimmers" represent

Figs 1-4. *Noctiluca*. Microgametocytes, different stages of microgamete development. Scale bar 500 μm . Fig. 5. *Noctiluca*. Nearly mature microgametes budding off from the microgametocyte, with developing flagella (arrowheads). Scale bar 100 μm . Fig. 6. Microgamete (arrowhead: nucleus) in dorso-ventral view. Arrow: *Dunaliella* cell. Scale bar 10 μm . Fig. 7. Microgamete in lateral view (arrowhead: nucleus). For scale bar see Fig. 6. Fig. 8. Microgamete with two flagella (arrowhead: nucleus). For scale bar see Fig. 6.

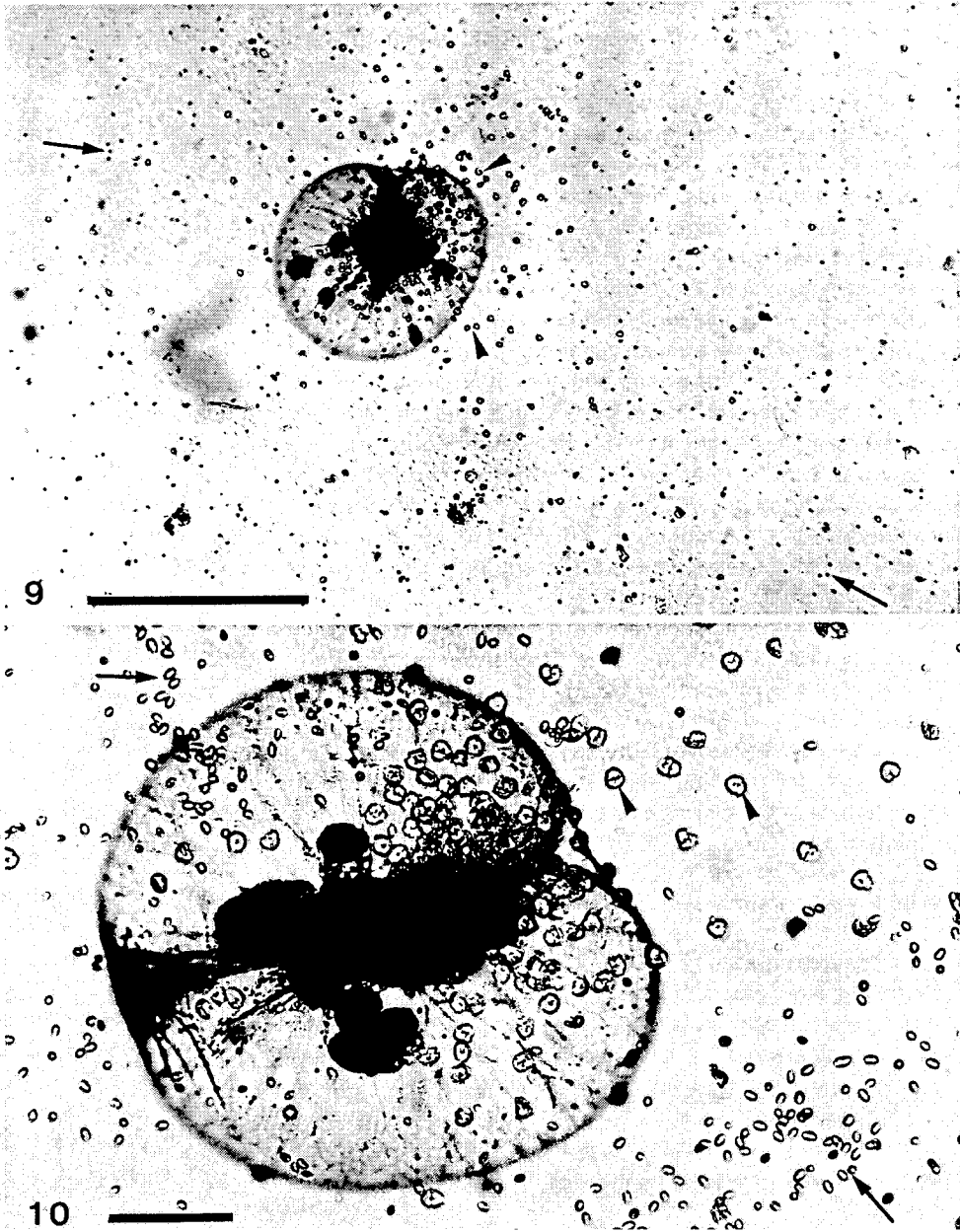


Fig. 9. *Noctiluca*. Chemotactical attraction of microgametes (arrowheads) which accumulate on or near a macrogamete. The *Dunaliella* cells (arrows) are more equally distributed. Scale bar 500 μ m.
 Fig. 10. Similar cell as in Fig. 9, with many microgametes clustering in the nuclear region. Arrow: *Dunaliella* cells. Scale bar 100 μ m

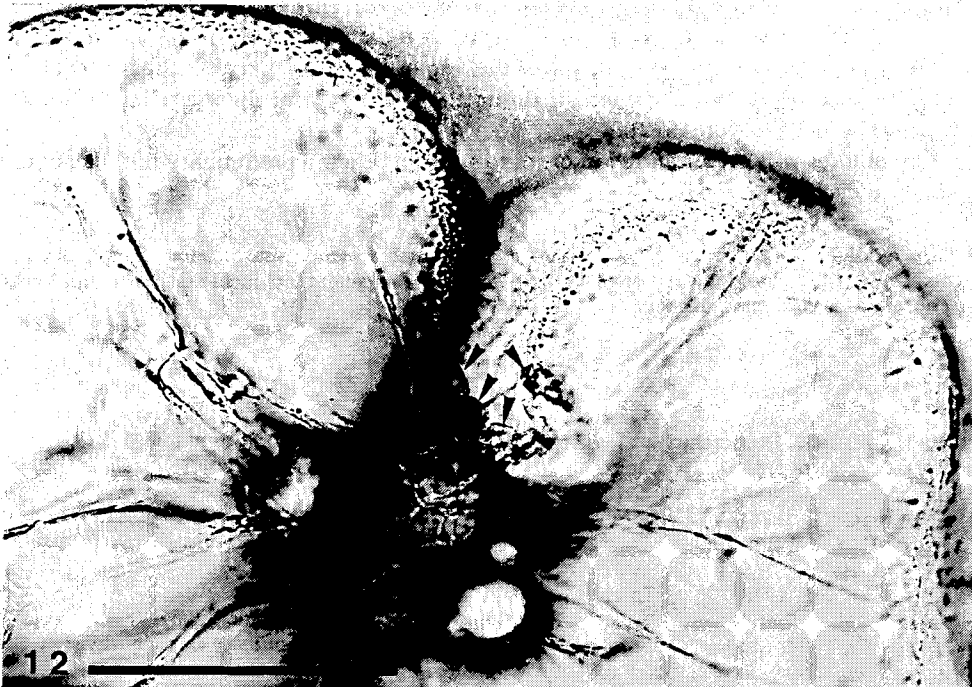
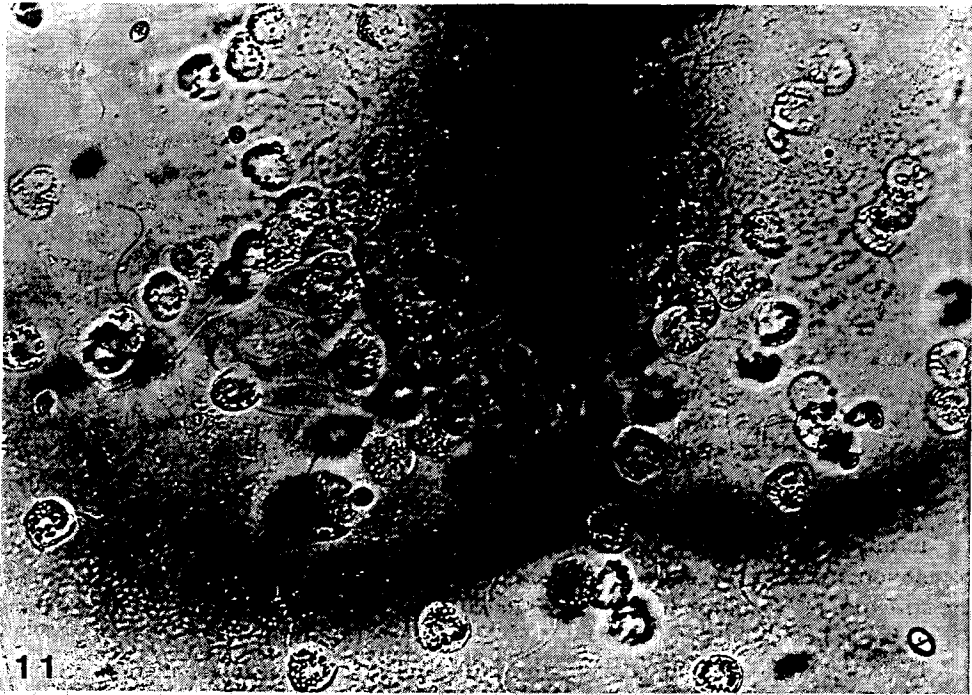


Fig. 11. *Noctiluca*. Microgametes clustering in the nuclear region of a macrogamete. For scale bar see Fig. 12. Fig. 12. A macrogamete with only few microgametes. Microgametes firmly attached in the nuclear region (arrowheads). Scale bar 100 μm .

microgametes (males), and that the macrogametes (females) are trophont-like in structure but not in behaviour, avoids various difficulties confronting us in the model proposed by Zingmark (1970a). Anisogamy is not uncommon in dinoflagellates (von Stosch, 1972; Pfiester & Anderson, 1987). In the anisogamous *Ceratium horridum* (Cleve) Gran, von Stosch (1964) observed differences in the structure of the gamete nuclei which are less extremely conspicuous than in *Noctiluca*.

The *Noctiluca* zygote is a planozygote, ready to take up food in the "normal" way. In *Ceratium horridum* the zygote likewise resembles a "normal" vegetative cell (von Stosch, 1964). Our suggestion is supported by what appears to be a chemotactical attraction between what we believe to be macro- and microgametes. It is very improbable, but cannot be completely excluded, that the attraction is "unspecific" (asexual). True trophonts occasionally attract microgametes also, but in this case *Dunaliella*, too. A chemotactical accumulation of many microgametes by gamones around one macrogamete is typical of extreme anisogamy and the logical consequence of the differences in number and size between the two cell types. The attractivity of the macrogametes was perhaps enhanced by their transfer into a new medium, a medium which did not contain gamones of previous sexual partners.

It must, however, be stated explicitly, that the final proof of an anisogamous copulation, i. e. the observation of gamete fusion and karyogamy, has yet to come. Due to the structure of *Noctiluca*, it will be extremely difficult, if not impossible, to follow these processes in live cells.

Further open questions concern the induction of gamete differentiation, the true position of meiosis in the life cycle and the possible occurrence of a nuclear cyclosis. This process, typical of the meiotic prophase of dinoflagellates (Biecheler, 1952; von Stosch, 1972; Pfiester & Anderson, 1987), might be lacking in *Noctiluca*, because the nuclei of the trophonts (also of the macrogametes?) do not have the typical dinoflagellate structure (Zingmark, 1970b; Soyer, 1972).

Our studies will be continued in order to substantiate the preliminary findings of the sexual life cycle of *Noctiluca*.

Acknowledgements. We thank Dr. G. Uhlig for reading the manuscript, and for his critical comments, Dr. M. Elbrächter for discussions, Ms H. Halliger for excellent technical assistance and Ms G. Deichgräber for correcting the text.

LITERATURE CITED

- Biecheler, B., 1952. Recherches sur les Péridiniens. – Bull. biol. Fr. Belg. (Suppl.) 36, 1–149.
 Bold, H. C. & Wynne, M. J., 1985. Introduction to the algae. Prentice Hall, Englewood Cliffs, 720 pp.
 Christensen, T., 1980. Algae. Fasc. 1. AiO Tryk, Odense, 228 pp.
 Ehrenberg, C. G., 1834. Das Leuchten des Meeres. – Abh. preuss. Akad. Wiss. 1834, 411–575.
 Gross, F., 1934. Zur Biologie und Entwicklungsgeschichte von *Noctiluca miliaris*. – Arch. Protistenk. 83, 178–196.
 Hoek, C. van den, 1978. Algen. Thieme, Stuttgart, 481 pp.
 Hofker, J., 1930. Über *Noctiluca scintillans* (Macartney). – Arch. Protistenk. 71, 57–78.
 Ishikawa, C., 1894. Studies of reproductive elements. II. *Noctiluca miliaris* Sur., its division and spore-formation. – J. Coll. Sci. imp. Univ. Tokyo 6, 297–334.
 Kofoid, C. A. & Swezy, O., 1921. The free-living unarmored Dinoflagellata. – Mem. Univ. Calif. 5, 1–562.

- Pfiester, L. A. & Anderson, D. M., 1987. Dinoflagellate reproduction. In: The biology of dinoflagellates. Ed. by F. J. R. Taylor. Blackwell, Oxford, 611–648.
- Pratje, A., 1921. *Noctiluca miliaris* Suriray. Beiträge zur Morphologie, Physiologie und Cytologie. I. Morphologie und Physiologie (Beobachtungen an der lebenden Zelle). – Arch. Protistenk. 42, 1–98.
- Pratje, A., 1925. *Noctiluca*. – Tierw. Nord-Ostsee 2(d), 1–12.
- Schöne, H. K. & Schöne, A., 1982. A weakly enriched sea water medium for ecological studies on marine plankton algae, some examples of its application. – Botanica mar. 25, 117–122.
- Soyer, M.-O., 1969. L'enveloppe nucléaire chez *Noctiluca miliaris* Suriray (Dinoflagellata). I-Quelques données sur son ultrastructure et son évolution au cours de la sporogénèse. – J. Microsc., Fr. 8, 569–580.
- Soyer, M.-O., 1972. Les ultrastructures nucléaires de la Noctiluque (Dinoflagellé libre) au cours de la sporogénèse. – Chromosoma 39, 419–441.
- Stosch, H. A. von, 1964. Zum Problem der sexuellen Differenzierung in der Peridineengattung *Ceratium*. – Helgoländer wiss. Meeresunters. 10, 140–152.
- Stosch, H. A. von, 1972. La signification cytologique de la 'cyclose nucléaire' dans le cycle de vie des Dinoflagellés. – Mém. Soc. bot. Fr. 1972, 201–212.
- Uhlig, G., 1972. Entwicklung von *Noctiluca miliaris*. Wiss. Film C 897/1965. Begleitveröff. Inst. Wiss. Film Göttingen, 15 pp.
- Uhlig, G. & Mühlhäusler, A., 1992. *Noctiluca scintillans*: Meeresleuchten. In: Meeresbiologische Exkursion. Hrsg. von P. Emschermann, O. Hoffrichter, H. Körner & D. Zissler. Fischer, Stuttgart, 194–197.
- Uhlig, G. & Sahling, G., 1982. Rhythms and distributional phenomena in *Noctiluca miliaris*. – Annl. Inst. océanogr. 58, 277–284.
- Uhlig, G. & Sahling, G., 1990. Long-term studies on *Noctiluca scintillans* in the German Bight. Population dynamics and red tide phenomena 1968–1988. – Neth. J. Sea Res. 25, 101–112.
- Zingmark, R. G., 1970a. Sexual reproduction in the dinoflagellate *Noctiluca miliaris* Suriray. – J. Phycol. 6, 122–126.
- Zingmark, R. G., 1970b. Ultrastructural studies on two kinds of mesocaryotic dinoflagellate nuclei. – Am. J. Bot. 57, 586–592.