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NOTES ON SEXUAL REPRODUCTION IN DESMIDS.

II. EXPERIENCES WITH CONJUGATION EXPERIMENTS IN UNI-ALGAL CULTURES

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SUMMARY

Over 120 randomly selected strains belonging to 16 genera and to more than 80 species of Desmids were brought into uni-algal culture and subjected to conditions provoking conjugation. Only 3 isolates – all homothallic – exhibited sexual reproduction: *Closterium limneticum* Lemn., *Cl. moniliferum* Bory ex Ralfs, and *Micrasterias papillifera* Bréb. Of *Cl. limneticum* no zygospores had previously been known. The, generally speaking, poor result of the experiment supports the theory that it is a lack of sexual potential rather than ecological factors which is responsible for the rare incidence of sexual reproduction in Desmids under natural conditions.

1. INTRODUCTION

As pointed out in the first paper of this series (COESEL 1974), the scarcity or absence of zygospore formation in a relatively large number of desmid taxa in nature can to a large extent be explained by a genetically determined incompatibility. The principal argument pleading in favour of this idea is the general experience that the species which have been found to be capable of producing zygotes under natural conditions are not scattered throughout the whole group of the Desmids but mainly belong to the morphologically less differentiated (more primitive) families and genera. Besides, conjugation experiments with uni-algal cultures have demonstrated the incidence of heterothally in a number of species, sexual reproduction between two individuals of the same clone thus being excluded.

The pioneering studies of STARR (1955) concerning the inducement of a sexual response in uni-algal cultures have been repeated and extended by other workers and they also obtained a fair number of successes. It transpired, among other things, that one species may exhibit both homo- and heterothally.

A comparison of the data reported in the literature shows that experimentally induced zygote formation has mostly been achieved in genera which are known to exhibit conjugation relatively often in nature. In the Culture Collection of Algae at Indiana University (STARR 1964), which contains research material received from experienced workers from all over the world, the fertile strains of Desmids are over-represented among the *Mesotaeniaceae* and the genus *Closterium*. Fertile strains of over 30 species of *Closterium* are present, whereas not a single strain is available of such morphologically much differentiated taxa as *Euastrum*, *Micrasterias*, *Xanthidium*, and *Staurastrum*. This culture collection does of course not reflect all experimental results so far obtained in this field, but is is an established fact that there are preciously few reports of experimentally induced zygospore formation in the more advanced genera mentioned above (see, e.g., BRANDHAM & GODWARD 1963, WINTER & BIEBEL 1967, KIES 1968). Since it is to be expected that mostly only positive results of conjugation experiments are being published, it is not so easy to ascertain whether the scarcity of zygote formation in certain genera is indeed attributable to a reduced sexual potential or to an insufficient number of experiments. In order to obtain a better insight in this question, the present authors have subjected a large number of isolates to culturing conditions reputedly favourable for the stimulation of sexual reproduction.

2. MATERIAL AND METHODS

In the course of a number of successive years over 120 clones belonging to more than 80 species of Desmids from various Dutch localities were isolated and subsequently cultured. The uni-algal strains were represented among the genera as follows: *Spirotaenia* (1), *Cylindrocystis* (1), *Netrium* (1), *Closterium* (10), *Pleurotaenium* (2), *Tetmemorus* (1), *Euastrum* (4), *Micrasterias* (8), *Cosmarium* (26), *Xanthidium* (2), *Staurodesmus* (6), *Staurastrum* (17), *Sphaerozosma* (1), *Hyalotheca* (1), *Desmidium* (2), *Bambusina* (1).*

For the purpose of the experiments test tubes with soil-water medium (with a range of soil types and pH values) were inoculated with the isolates; the medium was prepared according to PRINGSHEIM (see STARR 1964). The tubes were placed in a culture cabinet at 20 °C, illuminated with about 1500 Lux, and subjected to a photoperiodic regime of 16 hrs. of light and 8 hrs. of darkness. In order to stimulate sexual reproduction, the CO_2-enrichment method of STARR (1955) was applied: 3 to 5 week old cultures were transferred to watchglasses, kept at 25 °C and illuminated with about 3500 Lux (the photoregime remained unchanged: 16/8).

3. RESULTS

We only succeeded in inducing zygospore formation in this way in three species to be discussed separately.

3.1. **Closterium moniliferum** Bory ex Ralfs

This species had already been found te be capable of sexual reproduction in culture (see, e.g., FOX 1957, LIPPERT 1966, DUBOIS-TYLSKI 1972). FOX (l.c.) used material from six localities and in this way procured both homo- and hetero-

* A complete list of the culture collection of our laboratory, and living material from this collection can be obtained upon request from the senior author (P. F. M. Coesel, Hugo de Vries-laboratory, Plantage Middenlaan 2A, Amsterdam-A1-004).

thallic strains. He found that conjugation in the heterothallic ones results in the formation of so-called twin zygospores owing to the mitotic division of a paired +cell and −cell previous to their conjugation. In his homothallic strains Fox only observed single zygospores resulting from conjugation between two daughter cells. LIPPERT (1966), on the other hand, who made a comparative analysis of variation in cell structure and in sexual reproduction in *Closterium moniliferum* and *Cl. ehrenbergii* using about 70 isolates, recorded two strains among the homothalic strains of *Cl. moniliferum* which appeared to be capable of producing twin zygospores. DUBOIS-TYLSKI (1972) who made an extensive study of the life-cycle of *Cl. moniliferum* worked exclusively with homothallic and twin-zygospores forming strains. She assumes that the pairing of cells belonging to the same clone is based on a mutual chemotactic attraction directly controlled by the internal mechanism of sexual induction.

In our culture collection *Cl. moniliferum* was represented by two clones. One of these, isolated from a sample from a marshy area in the "Molenpolder", near Utrecht, appeared to be homothallic. This clone very readily started reproducing sexually (zygotes were often encountered already in the test tubes), and in this case also formed twin zygospores *(figs. 1* and *2)*.

3.2. Micrasterias papillifera Breb.

Sexual response in uni-algal culture has only been reported by KIES (1968), who made an exemplary study of the course of the conjugation process and the structure of the zygospore wall.

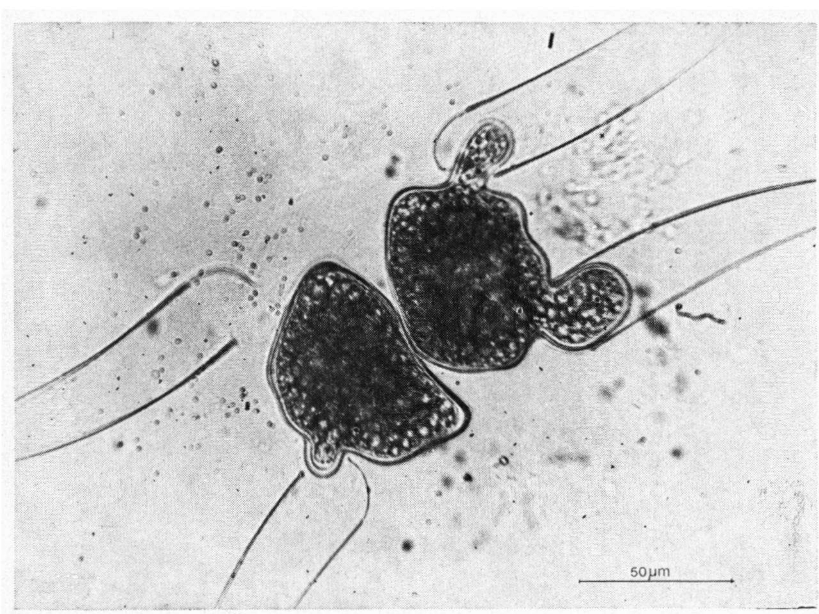


Fig. 1. *Closterium moniliferum*, conjugation.

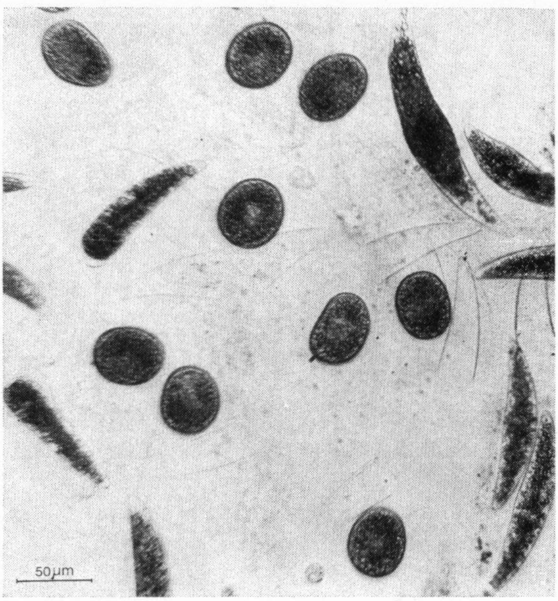


Fig. 2. *Closterium moniliferum*, twin zygospores.

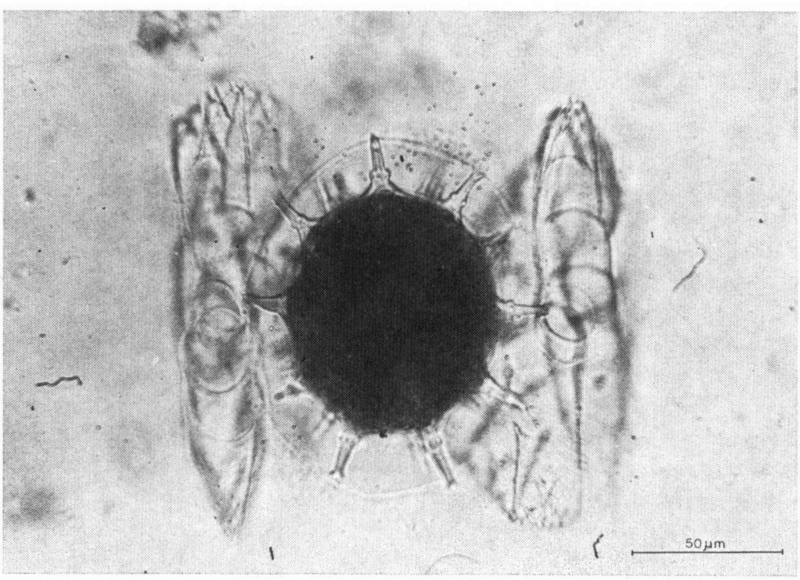


Fig. 3. *Micrasterias papillifera*, zygospore.

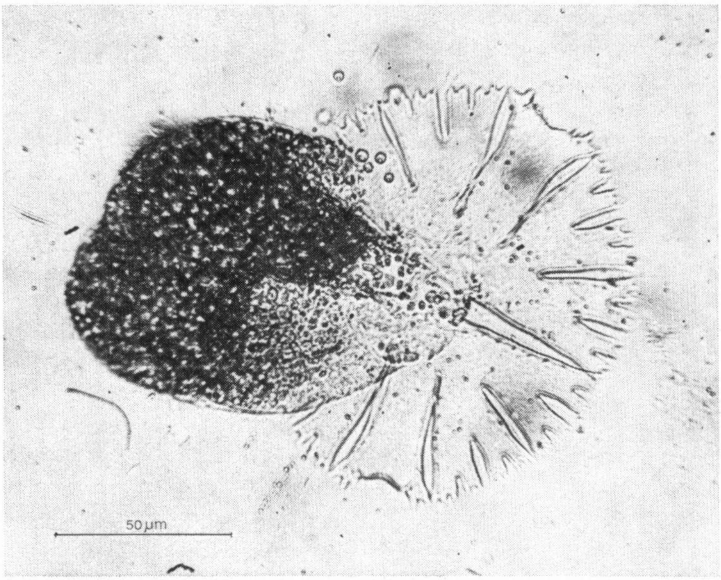
The two isolates we had at our disposal one (from a moor fen near Staverden, prov. of Gelderland) appeared to be homothallic like the strain isolated by

Kies. Two days after transfer of the culture to a watch glass under strong illumination the first cell pairings were noted. After 7 days (when about 10 per cent of the total number of cells were involved in sexual reproduction) the number of conjugations did not increase any further. The formation of the spines on the wall of the zygospore *(fig. 3)* was partly suppressed, only once – instead of twice – bifurcate spines being formed. This is according to Kies attributable to too high an osmotic value of the medium. In exceptional cases the formation of a parthenospore was observed: the contents of a single cell protruded from the wall, rounded itself off into a smooth, globose cell which did not form spines *(fig. 4a, b)*. This phenomenon was not mentioned by Kies, although the occurrence of parthenospores in conjugating cultures has been reported in other species (see, e.g., BRANDHAM 1965, LING & TYLER 1972).

3.3. **Closterium limneticum** Lemm. var. **fallax** Růžička

As pointed out by RŮŽIČKA (1962) in a well-documented paper, the species *Closterium limneticum* described by Lemmermann in 1899 has been misinterpreted for a long time. The principal reason is presumably that WEST & WEST (1900, *fide* RŮŽIČKA) erroneously included it in *Cl. gracile* Bréb, as a synonym, an interpretation subsequently adopted by nearly all authors. Růžička described the morphological features of three varieties of *Cl. limneticum* (and their variability) in great detail, and indicated the differences with *Cl. gracile* and with a number of different species which have been confused at one time or another, such as *Cl. acutum*, *Cl. pronum*, and *Cl. strigosum*. Zygospores of *Cl. limneticum* were hitherto unknown, but this is understandable because many workers did not distinguish it from *Cl. gracile*. However, the ecology of this species may also explain why conjugation was never observed in nature. RŮŽIČKA (1962, p. 186) describes *Cl. limneticum* as a typically planktonic form occurring in moderately alkaline, eutrophic and beta-mesosaprobic waters. It is especially a planktonic way of life that offers very little scope for sexual reproduction (see COESEL 1974).

The morphological characteristics of a strain of *Cl. limneticum* isolated by us from the eutrophic river Amstel near Amsterdam agree in every respect with those of Růžička's diagnosis of var. *fallax* (1962, p. 188): length 127-289 μm, breadth 7.6–11.5 μm, apex width 1.7–2 μm (see our *fig. 5)*. When subjected to conditions promoting conjugation the strain proved to be homothallic, albeit that the number of cells that started zygote formation did not exceed about 5 per cent of the total number of cells. The zygospores are smooth, globose to subellipsoid, with a diam. of 28–33 μm *(figs. 6* and *7)*. On account of its zygote morphology, among other things, it may be concluded that *Cl. limneticum* Lemm. is not identical with *Cl. lundellii* Lagerh. which it resembles rather closely in its cell shape (compare RŮŽIČKA 1962, p. 185, *f.* 32).



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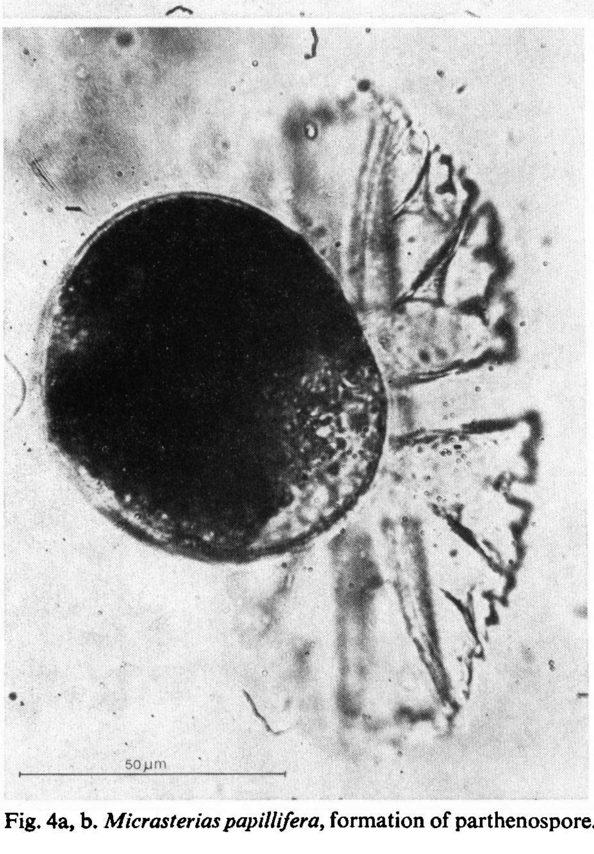

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Fig. 4a, b. *Micrasterias papillifera*, formation of parthenospore.

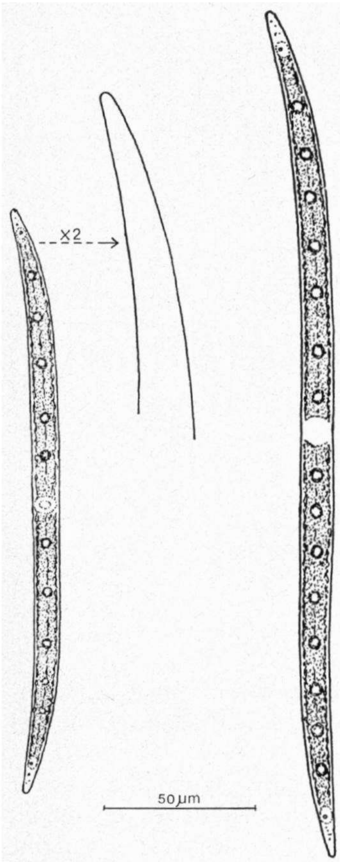

Fig. 5. *Closterium limneticum* var. *fallax*, vegetative cells isolated from river "Amstel".

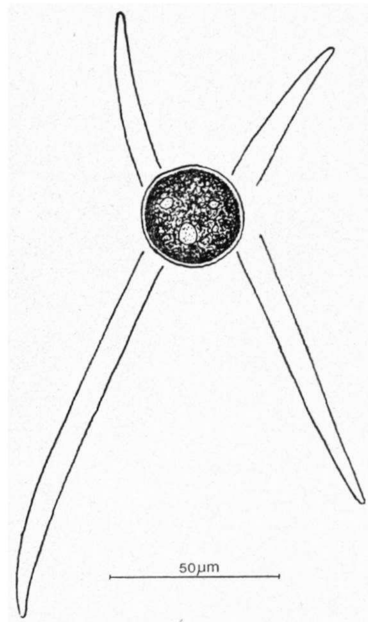

Fig. 6. *Closterium limneticum* var. *fallax*, zygospore.

4. DISCUSSION

For the meagre result of only 3 out of over 100 strains which exhibited conjugation in the circumstances described, three possible explanations can be suggested, viz. (1) suboptimal experimental conditions, (2) heterothally, and (3) complete sterility. Undoubtedly factor (1) has played a role. It is, in the first place, rather obvious (and it has also been established: see STARR 1959, p. 161) that the required experimental conditions for sexual induction vary from species to species. In our set-up of the testing all isolates were subjected to a uniform treatment. Moreover, in a number of species the vegetative reproduc-

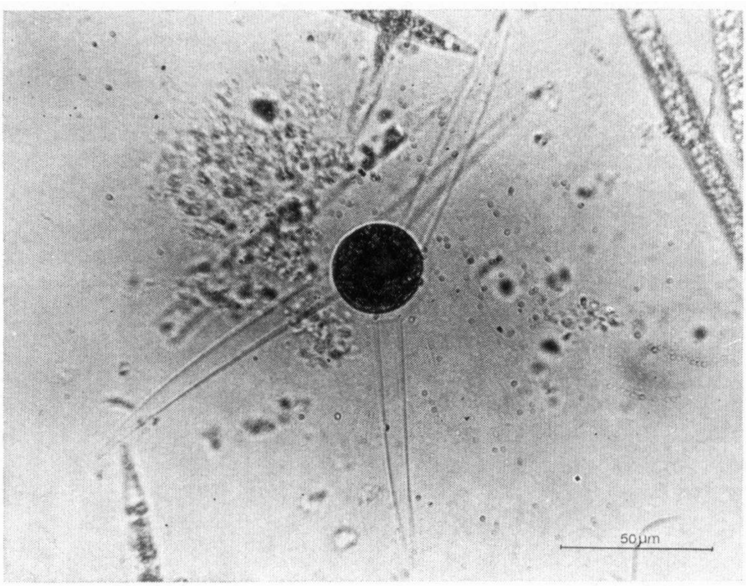

Fig. 7. *Closterium limneticum* var. *fallax*, zygospore.

tion was not very satisfactory in the soil-water media used, which may be indicative of a reduced vitality and an associated greatly reduced chance of sexual induction and conjugation.

Upon the whole it is not very probable that the principal cause of the negative results must be sought in external factors, however. The majority of the isolates exhibited a satisfactory rate of growth with few teratological malformations, if any. As stated before, more than one strain of *Micrasterias papillifera* and of *Closterium moniliferum* was available, but the compatible isolates of these two species certainly did not give the impression of having a higher vitality than those in which sexual reproduction did not occur. A homothallic fertile strain of *Cylindrocystis brebissonii* received from the Indiana University Collection and subjected to the same treatment as a sort of control, consequently started upon wholesale conjugation under the experimental conditions used. Conceivably, in most of the strains subjected to the test genetic factors must be responsible for the failure to reproduce sexually. As long as there is no sexual response after the mixing of such strains with conspecific ones, it cannot be ascertained whether heterothally or absolute sterility is responsible. The present study did not show any heterothally, although of more than 20 species several isolates (from often remote localities) were available. If heterothally occurs at all in the species isolated, the negative results point at any rate to a small chance of one strain meeting another, compatible one. This strengthens our opinion that the explanation of the rare incidence of sexual reproduction in Desmids under natural conditions has to be sought primarily in genetical barriers of one kind or another. This point of view is opposed to that of STARR (1959, p. 161) who believes that the artificial incitement of a sexual response by the simulation of environmental conditions supposed to be favourable for zygospore formation is clearly indicative of unsuitable ecological conditions rather than a lack of sexual potential as limiting factors in nature. However, this author does not seem to take into account that the strains he investigated were all isolated from natural populations in which there was already evidence of sexual reproduction, because he knew that randomly isolated strains cannot easily be induced to start conjugating (STARR 1955, p. 261). The success of his experiments is to a large extent attributable to the fact that his test material had previously already been selected for its sexual potential! A random selection of strains – as once more demonstrated by our experiments – only offers a small chance of success, however. For the time being, a lack of sexual potential surely appears to provide the best explanation of the rare occurrence of zygospore formation in Desmids under natural evironmental conditions.

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