Acta Bot. Neerl. 23(4), August 1974, p. 361–368.

NOTES ON SEXUAL REPRODUCTION IN DESMIDS.
I. ZYGOSPORE FORMATION IN NATURE (WITH SPECIAL REFERENCE TO SOME UNUSUAL RECORDS OF ZYGOTES)

P. F. M. COESEL

Hugo de Vries-laboratorium, Universiteit van Amsterdam

SUMMARY

The, upon the whole, rare incidence of sexual reproduction in Desmids, and the possible explanation of this scarcity, are discussed. Records of zygotes of *Cosmarium ochthodes* Nordst var. *amoebum* West, *C. pachydermum* Lund. var. *aethiopicum* W. & G. S. West, *Micrasterias papillifera* Bréb., and *Tetmemorus laevis* Kütz. ex Ralfs are discussed in some detail. The duplicity of the wall of the zygospore of *Tetmemorus laevis*, as originally described by RALFS (1848) but queried by KRIEGER (1937), is confirmed.

INTRODUCTION

It is an established fact that sexual reproduction (by means of conjugation) of the large majority of the Desmids occurs only sporadically in nature or has not been observed at all. According to FRITSCH (1930) there is a general tendency in this group, particularly in the more advanced (i.e. morphologically most differentiated) genera, towards the elimination of sexual reproduction. It is true that zygospore formation takes place much more freely in the *Mesotaeniaceae* as well as in the structurally rather simple desmidiaceous genera (such as *Penium* and *Closterium*) than in representatives of such genera as *Euastrum* and *Micrasterias*. Of many species no zygotes are known, and our information concerning the zygospores of several other ones is based on preciously few records or only a single gathering. It is possible that an incidental record of this kind is based on the observation of an incompletely developed spore, which may account for the relatively large number of discrepancies in the descriptions of the sporal morphology of desmidiaceous taxa. Some examples of such contradictory reports will be mentioned in the present paper. On the 14th and 15th June, 1971, the present author collected samples in some mesotrophic quaking bogs in the State nature reserve "De Weerribben" (province of Overijssel) by squeezing out handfuls of moss growing along the edges of small depressions in the bog. Three samples from sites in close proximity of one another proved to contain zygotes of at least seven species of desmids, viz. of *Penium spinospermum* Josh., *Closterium dianae* Ehr. ex Ralfs, *C. kuetzingii* Bréb., *Tetmemorus laevis* Kütz. ex Ralfs, *Cosmarium ochthodes* Nordst., *C. pachydermum* Lund., and *Micrasterias papillifera* Bréb. In addition, several zygospores were found which could not be identified with a sufficient degree of reliability owing to the absence of associated, empty semi-cells. The species of *Penium* and *Closterium* belong to the group of taxa that relatively often enter into conjugation and whose zygotes are well-known. The zygospores of these species studied by the present author agree in their dimensions and morphology satisfactorily with, e.g., the figures published by KRIEGER (1937) and will, therefore, not be discussed here.

DESCRIPTION OF SOME ZYGOSPORES

1. Tetmemorus laevis Kütz. ex Ralfs

The first description and illustration of the zygospore of this species were given by RALFS (1848). He described the process of conjugation as follows (l.c., p. 146):

"After coupling, the segments of the fronds are separated by the formation of a large, quadrate, central cell, in which all the contents of both fronds are collected, the empty segments being loosely attached to its corners. The endochrome at first fills the cell, large starch globules being scattered throughout the minutely granular substance; but at length it becomes a dense, round homogeneous body of a dark green colour, which finally changes to an olive-brown. In this stage the segments of the original fronds fall off, and leave the quadrate cell inclosing the sporangium". (Compare *fig. 1*.)

Afterwards, at least as far as could be ascertained, only three original illustrations of zygospores of the species under discussion have been published, viz., by WEST (1912, t. 1, f. 10), by HOMFELD (1929, t. 3, f. 33) and (of the var. *tropicus* Krieger) by KRIEGER (1937, t. 54, f. 15). They all depict the spore case as four-angled and horned, but their figures do not show the rounded zygote envelope which is formed inside it according to RALFS. RALFS' illustration was reproduced in the flora of WEST & WEST (Vol. I, 1904, t. 32, fs. 15 & 16), but in the more recent monographs (KRIEGER 1937, t. 54, f. 12; RŮŽIČKA 1952, t. 1, f. 9; KOSSINSKAJA 1960, t. 40, f. 13) the figure of HOMFELD (see *fig. 2*) has been borrowed. This is most surprising, considering that RALFS could study the conjugation process in great detail in his rich material collected in the course of three successive years, whereas HOMFELD's description appears to have been based on a find of a single zygote which – as he reports himself (p. 28) – had died off prematurely. Although we may safely assume that RALFS' figure is more accurate than HOMFELD's, the lack of any confirmation of the presence of a subglobose inner spore envelope seems to have prevented the acceptance of RALFS' description (compare, e.g., KRIEGER 1937, p. 456). Observations of zygotes of this species by the present author confirm RALFS' description beyond doubt, however (see *fig. 3*).

Tetmemorus laevis var. *laevis* is common in the area studied by the present author where it occurs in *Sphagnum* vegetation (consisting mainly of *S. squarrosum* and *S. plumulosum*) along the edges of shallow depressions. A few zygotes were found on June 15th, 1971. A sample collected at the same site

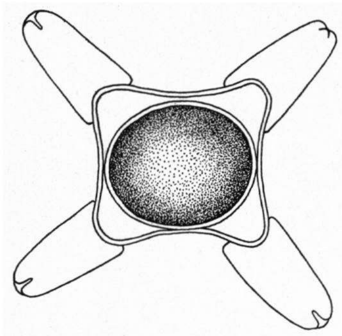

Fig. 1

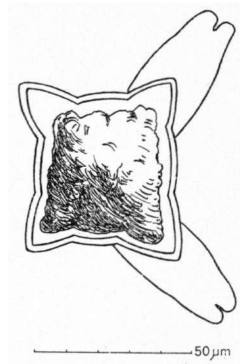

Fig. 2

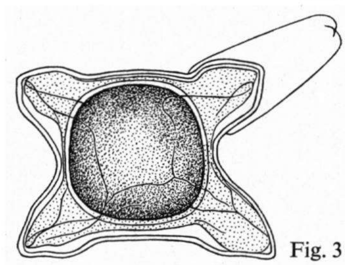

Fig. 3

Fig. 1. Zygospore *Tetmemorus laevis*, after RALFS (1848, t. 24, f. 3e).
Fig. 2. Zygospore *Tetmemorus laevis*, after HOMFELD (1929, t. 3, f. 33).
Fig. 3. Zygospore *Tetmemorus laevis*, specimen from N.W. Overijssel.

later in the year (Aug. 12th, 1971) was much richer in zygotes. Among the numerous zygospores examined (after fixation) a few specimens were found which resemble HOMFELD's illustration. Such spores, in which the cytoplasm is absent or forms a small lump irregular in outline, indeed strongly suggest to represent cases of arrested development. In all other spores the zygote proper is surrounded by a globose to ovoid inner envelope. When viewed at higher magnifications (× 1000), this about 3 µm thick wall proves to be built up of five layers among which a brown-coloured mesoporium. The outer sporecover, with its four angles attenuated into horns, also exhibits a lamellate structure, which is not so conspicuous, however. In an appreciable number of specimens the innermost layers of this outer spore cover (inclusive of the brownish mesosporium) had become detached from the outer layer to a smaller or a larger extent to form a separate wrinkled membrane between the inner and outer spore cover (compare *fig. 3*). The size of the outer sporal envelope is 76–96 × 54–64 µm, and the diameter of the inner one 44–56 µm.

2. **Cosmarium ochthodes** Nordst.
The only illustration of a zygospore of *C. ochthodes* is the one in the flora of WEST & WEST (see *fig. 4*). HOMFELD (1929) recorded zygotes of this species (incl. of its var. *amoebum* West) from only one locality. He remarked that the

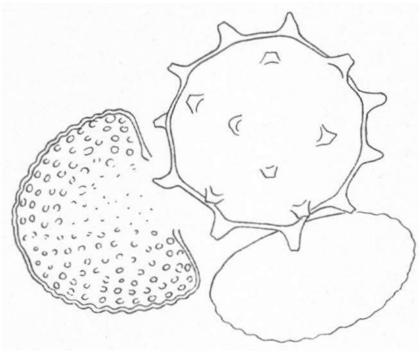

Fig. 4

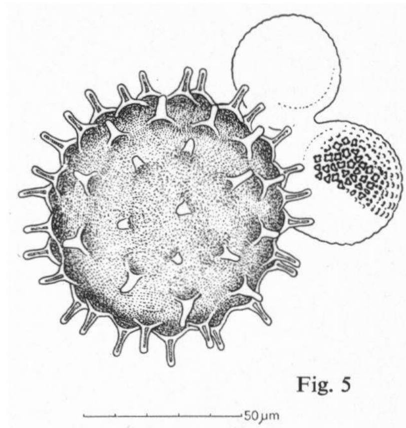

Fig. 5

Fig. 4. Zygospore *Cosmarium ochthodes*, after WEST & WEST (1912, t. 98, f. 3).
Fig. 5. Zygospore *Cosmarium ochthodes* var. *amoebum*, specimen from N.W. Overijssel.

spines of both varieties are regularly conical and more closely set than in the figure in WEST & WEST, but he did nog give an illustration.

The single zygospore of *C. ochthodes* found by the author in a squeezing of the moss *Acrocladium cuspidatum* (see *fig.* 5) is also more spinose than the figure of WEST & WEST. The spines are also longer and of a different shape. It is somewhat doubtful whether their figure indeed represents *C. ochthodes* if we accept RŮŽIČKA's (1952) arguments to regard the figures of *C. ochthodes* 'typicum' sensu W. & G.S. West (WEST & WEST 1912, Vol. 4, t. 98, fs. 2 & 3) as atypical and possibly combining features of two different species. The wall of the empty semi-cells of the zygospore studied by the present author shows superficial, lobed wall thickenings with a characterisetic 'amoeboid' pattern, which establishes the identity of the spore as that of *C. ochthodes*, more particularly of the var. *amoebum* West. According to RŮŽIČKA (1952), this variety distinguishes itself from *C. ochthodes* 'typicum' only by its more trapezium-wise rounded semi-cells with a broader 'cap'.

3. **Cosmarium pachydermum** Lund.

KRIEGER & GERLOFF (1962) reported that zygotes of this species are of rare occurrence. The description and illustration of the spore in their monograph of the genus *Cosmarium* are borrowed from HOMFELD (1929, p. 48, t. 6, f. 67) who collected but a single specimen. Apart from the figure of a 'naked' spore or the forma *minor* distinguished by RICH (1936, p. 135, fig. 12 f.) no other figures could be traced.

Zygospores of *C. pachydermum* were rather frequently encountered by the present author in the same water sample in which the zygote of *C. ochthodes* was found. These spores agree rather closely with HOMFELD's figures, although my material is referable to the var. *aethiopicum* W. & G.S. WEST (see *fig. 6*). The spore diam. without the spines is 70–80 μm, with the spines 100–115 μm.

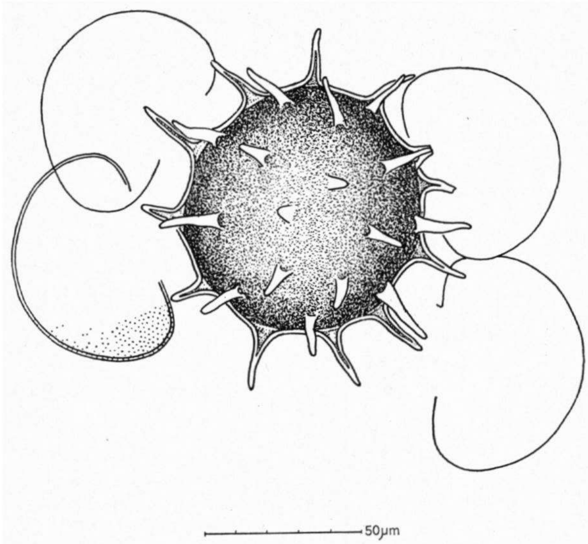

Fig. 6. Zygospore *Cosmarium pachydermum* var. *aethiopicum*, specimen from N.W. Overijssel.

4. Micrasterias papillifera Bréb.

Records and descriptions of zygotes of this species can be found in RALFS (1848, p. 73, t. 9, fig. 1, f, g), WEST, WEST & CARTER (1923, p. 266, t. 167, f. 11), CUSHMAN (1905, p. 225, t. 7, f. 7), and HOMFELD (1929, p. 35, t. 5, f. 44). HOMFELD drew attention to the considerable variation in the shape of the spines of the zygospores. In his material he found predominantly unbranched spines, but not unfrequently also once or twice bifurcated ones. RALFS depicted only twice bifurcate ones, CUSHMAN both unbranched and bifurcate ones, and CARTER (in WEST, WEST & CARTER), apart from simple and bifurcate spines, also trifurcate ones.

KIES (1968) succeeded in inducing conjugation in *M. papillifera* experimentally and discovered that the shape of the zygote spines is determined by the turgor of the cytoplasmic contents of the spore. When he placed young and still smooth-walled zygospores in distilled water and left them in this medium for a day, the majority of the spines became twice bifurcate as in RALFS' figure. According as the osmotic value of the medium to which young spores are transferred increases, the degree of branching of the spine diminishes until ultimately the spine formation becomes inhibited altogether. The different degrees of spinosity reported in the descriptions of the same species by different authors must, according to KIES, be ascribed to the fact that in this group of algae zygote formation in nature takes place along the almost horizontal edges of desiccating waters where a local increase of the osmotic value of the medium surrounding the spores can readily take place (e.g., by evaporation), so that the spine formation is suppressed to a smaller or larger extent. The present author found a single zygospore of *M. papillifera* in the same sample which contained the zygotes of *Tetmemorus laevis*. This zygospore possessed only once bi- or trifurcate spines.

DISCUSSION

The experience of the present author that in a single sample zygospores of several species may be found, whereas a large number of samples yield none at all, agrees with the findings of HOMFELD (1929, p. 11) that often zygotes "*nesterweise von verschiedenen Desmidiaceenarten gleichzeitig gebildet werden*". This raises the question which ecological factors play a role in the stimulation of conjugation processes under natural conditions, and in how far the absence of such factors, or what other special conditions, may explain the (relative) rarity of sexual reproduction. HOMFELD (1929), who studied Desmids for years on end in N.W. Germany and recorded the zygotes of 152 species, a 'harvest' never equalled by any other worker, had to admit that he did not have any tangible indication of the nature of these factors or conditions. The only conclusion he could draw was that zygotes are sooner found in small waters such as *Viehtränke* (horse- and cattle-ponds) and peat pits than in larger bodies of stagnant water.

Conjugation experiments by means of uni-algal cultures (e.g., by STARR 1955) have shown that conjugation is strongly favoured, among other things, by the exhaustion of the culture medium, and also when the illumination, the temperature and the CO_2 tension are at an optimum for mitotic activity. STARR developed a special CO_2 enrichment method after it had transpired that at normal (atmospheric) CO_2 concentrations conjugation only takes place at the surface of the culture medium. One may expect, in the light of such experiments, that in natural habitats the chances of incidence of conjugations are greatest in very shallow waters which can absorb appreciable quantities of CO_2 from the air or from the sapropelium. Such a condition prevails in, e.g., the thin films of water in moss vegetation growing alongside shallow depressions in bogs (gatherings of zygotes by the present author!), in ephemerous, small bodies of water (the conjugating population of *Tetmemorus laevis* observed by RALFS, formed a thin gelatinous cover on damp soil), and along the flat edges of shallow pools with a changing water level. Especially in the latter case the formation of zygospores can functionally be explained as a means of survival when desiccation threatens. Although there are examples of isolation of sexually active strains of Desmids from dry soil samples taken in such sites (COOK, 1963), so that the presence of zygospores in such samples is highly probable, there are no direct indications of the common occurrence of conjugations in such situations. HOMFELD (1929, p. 11) did not notice any correlation between zygospore formation and an increase or decrease of the volume of water. A pool with an abundant Desmid flora may dry up fast or much more slowly without the visible incidence of conjugations taking place. The present author had the same experience with moor fens near Staverden (prov. of Gelderland) which dry up periodically. As already suggested by HOMFELD (l.c.), desmids are capable of surviving vegetatively during dry periods. EVANS (1959) demonstrated this experimentally by subjecting cultures of a number of species to a prolonged and thorough process of desiccation. The chances of survival of the vegetative cells are sometimes considerably favoured by the deposition of reserve metabolites in the cell (in the form of oil droplets) and by the secretion of a thick gelatinous sheath,which renders the cell functionally comparable to a spore.

The fundamental cause of the rarity of zygospore formation in nature must indubitably also, or primarily, be sought in a genetically determined lack of sexual potential. An indication to that effect is already to be found in the experience, relevated in the introduction, that the frequency of sporogenesis varies widely among the various species. Experiments with cultures, as carried out by, e.g., COOK (1963) confirm that certain taxa can much more readily be induced to start conjugating than other ones. However, the apparent failure of clones to reproduce sexually does not imply an absolute sterility of the corresponding taxon as a whole. STARR (1955) who carried out conjugation experiments with *Cosmarium turpinii* (originally but erroneously referred by this worker to *C. botrytis* var. *tumidum*) was only successful after he mixed clones of different origin.

Later on, a fair amount of cases of heterothally in Desmids have been reported. Also because more than two different mating types have been recorded in the same species (BRANDHAM & GODWARD 1963), it does not seem to be altogether improbable that the scarcity of zygotes of many species in nature can, at least in part, be attributed to local clone formation of only one mating type. However, this still does not explain the enigmatic phenomenon that a given sampling site yields a number of zygospores in several taxa in one season but only vegetative cells of these forms in the preceding or the following years, although the environmental conditions have not changed as far as can be ascertained.

BEIJERINCK (1926), who studied the distribution and periodicity of fresh-water algae in the Dutch province of Drenthe, remarked that when a copious fructification of representatives of different algal taxa (*Desmidiaceae*, *Zygnemataceae*, *Oedogoniaceae*) occurs in a certain site, the same phenomenon can be observed in different and often far removed localities of a different ecological character at the same, or practically the same, time. By comparing the number of sexually reproducing species with the relative amounts of daylight during the 24 half-months of the year 1925 (p. 155, f. 3), he noticed that although the highest incidence of fructification falls in the second half of April and in the first half of May after a period of markedly increasing light intensities (and average temperatures), striking increases in the rates of sexual reproduction in autumn, sometimes even as late as November cannot, or only partly, be correlated with a (preceding) light and temperature regime. The obvious conclusion is that even though sexual reproduction in Desmids in nature is, generally speaking, clearly activated by climatic conditions, the connection is not unequivocal, undoubtedly owing to the interference of local ecological conditions.

ACKNOWLEDGEMENTS

The author thanks Professor A. D. J. Meeuse for a critical perusal of the manuscript and for his assistance with the English rendering.

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