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Scenedesmus obliquus Sexuality

Abstract. *The flagellated cells of two mixed strains of* Scenedesmus obliquus *can initiate a sexual cycle when grown at 15°C in a defined medium lacking a nitrogen source. Clumps of five to ten cells, paired gametes, plasmogamy, and quadriflagellated zygotes were observed.*

Reproduction in *Scenedesmus*, particularly *S. obliquus*, a widely distributed fresh-water alga, was thought to occur by the development of nonmotile spores, which become arranged in a definte pattern within the parent cell before release as a colony. However, motility was observed in cultures of *S. obliquus* and *S. dimorphus* (*1*). Although we tried different media and conditions of growth, we were not able to keep the motile cells alive. Because of this difficulty, as well as the fact that the motile cells were produced under conditions which stimulate a sexual cycle in other algae (*2*), we thought it possible that the motile cells were obligate gametes.

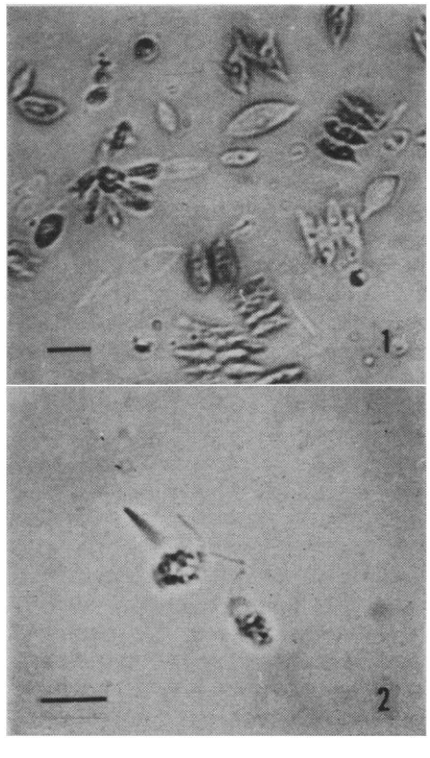
In culture studies dealing with pleomorphism in *Scenedesmus*, forms resembling *Dactylococcus*, *Chlorella*, *Oocystis*, and *Ankistrodesmus* are commonly observed (*3*). Among the spine-bearing species, certain pleomorphic strains can produce several different coenobial types that resemble other stable species, which in turn can produce just one coenobial type in culture (*4*). Although variability may result from a nutritional deficiency (*5*), existence of sexuality in *Scenedesmus* would provide a basis for other explanations.

Numerous clones of *S. obliquus* which produce motile cells have been isolated into axenic culture. To induce motility, a dense suspension of an actively growing culture in glucose-supplemented medium was transferred to a medium lacking a nitrogen source. A 3-ml sample in a sterile flask was incubated under continuous illumination at 15°C for 48 hours. When motile cells of one of our clones (*6*) were mixed with the WH-50 strain of *S. obliquus* (*7*), there was immediate clumping of gametes (Fig. 1). Usually there were five to ten cells per clump, and the clumps were numerous. When pairs broke away from the clumps, the flagella tips were joined (Fig. 2). The pairs swam actively for several minutes, but plasmogamy soon followed. Within 30 seconds the gametes had fused laterally, and a quadriflagellated zygote resulted (Fig. 3). Zygotes remained motile for many hours, but showed neither a phototactic response nor a tendency to become quiescent near the edge of a hanging-drop preparation. It was not possible to determine their fate.

Previously we could not follow motile cells through a division after quiescence; presumably they all died (*1*). During the present experiments many of the unpaired gametes also disintegrated. Cells that had recently conjugated might live after transfer to a medium containing a nitrogen source, for example, our complete medium (*5*). Or, if gametogenesis occurs in a medium providing some nitrogen, the cycle might be completed.

Fritsch (*8*) placed *Scenedesmus* in the order Chlorococcales and family Coelastraceae, a family in which no member has a motile stage or sexual cycle. Among the strictly colonial or coenobial algae in this order, those with zoospores or sexuality are placed in the Hydrodictyaceae. With the demonstration of sexual reproduction in *Scenedesmus*, we now agree with Fritsch that at least this member of the Coelastraceae could be transferred to the Hydrodictyaceae without any great alteration of the diagnosis. Furthermore, he might indeed be correct in questioning the feasibility of maintaining these two families.

Numerous strains of *Scenedesmus* have been used in many laboratories in photosynthetic and nutritional studies, not only because they can be easily grown and manipulated, but also because one avoids some of the complications which might result with any organism possessing a sexual cycle. If sexuality is found to be common in the



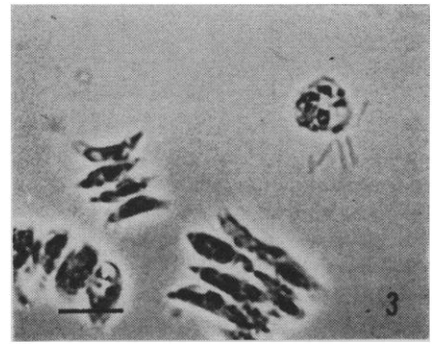


Fig. 1. A clump of approximately eight gametes. Some colonies visible. Living specimens. Fig. 2. Tips of flagella of two gametes joined. Material killed with iodine potassium iodide. Fig. 3. Quadriflagellated zygote and colonies. Material killed with iodine potassium iodide. The scale on all figures is 10 microns.

genus and if both homothallic and heterothallic species exist, then one might question the choice of such organisms for experimentation. These considerations must also be kept in mind during investigations of the problem of morphological variability.

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Nitrous Acid Mutation of Transforming DNA: Consideration of Mode of Action

Abstract. *Apparently nitrous acid produces genetic alterations, expressed as antibiotic-resistant markers, directly on heat-denatured transforming DNA of* Haemophilus influenzae, *rather than producing DNA which acts as a nonspecific mutagen. The markers which arise as a result of treatment with nitrous acid behave similarly to naturally occurring antibiotic markers. In addition, data comparing the expression and replication of induced markers to natural markers suggest that the nitrous acid–induced markers express and multiply in the same fashion as do "normal" markers. Therefore, mutations which require additional time to produce a functional DNA by a base-pair substitution, or by replication of the introduced DNA, are not responsible for the mutants observed.*

Nitrous acid is a well-known mutagenic agent and has been employed successfully in a variety of systems (*1*). Horn and Herriott (*2*) showed that nitrous acid was mutagenic for transforming DNA obtained from *Haemophilus influenzae* when the DNA was heat-denatured prior to treatment and renatured after treatment. In that work (*2*) it was not clear whether nitrous acid produced new genetic information in the DNA or whether the treated DNA itself acted as a mutagenic agent in the host's genome.

We have attempted to decide between these alternative hypotheses and have investigated the mechanism of mutagenesis by nitrous acid treatment in the transforming system of *Haemophilus*. The occurrence of interspecific transformations between *H. influenzae* and *H. parainfluenzae* provided us with a useful tool (*3*). Since the interspecific transformation frequencies of certain markers were already known (*3*), we compared these with the transformation frequencies yielded by DNA treated with nitrous acid.

The competent cultures of *H. influenzae* (Rd) were prepared by the Cameron modification of the method of Goodgal and Herriott (*4*); this strain was obtained originally from Alexander and Leidy (*5*).

The *H. parainfluenzae* wild type was originally obtained from Leidy and was made competent by the procedure of Nickel and Goodgal (*3*). At times a 4-hour anaerobic incubation was used in place of an 8-hour period.

Transforming DNA's were prepared and purified according to the procedure of Marmur (*6*). Thermal denaturation, nitrous acid treatment, and transformation of *H. influenzae* have been described (*2*). Assays of the transformation of *H. parainfluenzae* have been reported (*3*).

Horn and Herriott (*2*) found that nitrous acid did not produce mutations in "native" DNA of *H. influenzae*. However, denatured DNA was modified by nitrous acid so that after renaturation and subsequent uptake of DNA by recipient cells, mutations were observed. Nitrous acid was not mutagenic for "native" *H. parainfluenzae* DNA. If the parainfluenzae DNA was heat-denatured before treatment and then renatured, there was new transforming activity upon introduction of this DNA into competent *H. parainfluenzae* cultures. Thus, *H. parainfluenzae* DNA behaves like *H. influenzae* DNA in its mutagenic response to nitrous acid.

The frequency of interspecific transfer of genetic markers between *H. influenzae* and *H. parainfluenzae* depends upon the markers transferred and the direction of transfer (*3*), but the amount of DNA transferred interspecifically does not differ from the amount transferred intraspecifically (*7*). It was, therefore, possible to test whether or not the mutagenicity of DNA treated with nitrous acid was a function of the amount of treated DNA taken up by a population of bacteria or a function of the specific marker integrated by a population undergoing transformation.

In Table 1 are shown the results of a typical experiment in which the recipient is the *H. influenzae* and treated DNA from either species is the donor. With *H. influenzae* DNA, that is homologous DNA, as the donor, there is a significant rise of streptomycin- and erythromycin-resistant colonies with the duration of nitrous acid treatment (Table 1). When the donor is *H. parainfluenzae* DNA treated with nitrous acid, however, there are no transformations arising from this DNA; the number of colonies remains essentially the same, representing only spontaneously occurring mutations.

If nonspecific mutagenic DNA were responsible for the observed mutations, heterospecific transformations should yield similar frequencies of mu-

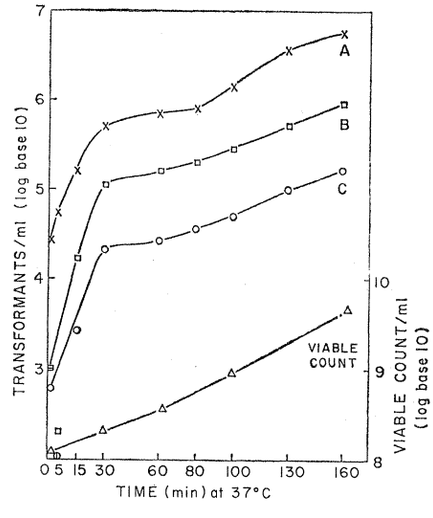

Fig. 1. Comparison of the expression of nitrous acid–induced transformants and "normal" transformants. *Haemophilus influenzae* competent cells (1×10^8/ml) were exposed to (*A*) 0.2 μg of DNA per milliliter, from streptomycin-resistant cells, (*B*) 0.5 μg/ml of denatured and renatured DNA from streptomycin-resistant cells, and (*C*) 0.5 μg/ml of nitrous acid–treated DNA from streptomycin-sensitive cells. Samples were taken, diluted, and plated immediately with 8 μg/ml of streptomycin agar. Background has been subtracted.