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Biologia i ekologia glonów naśnieżnych 1. Rozmnażanie płciowe u *Chlamydomonas nivalis* (B a u e r) W i l l e *(Chlorophyta, Volvocales)**

Biology and ecology of snow algae 1. The sexual reproduction of *Chlamydomonas nivalis* (B a u e r) W i l l e *(Chlorophyta, Volvocales)*

Wpłynęło 30 kwietnia 1977 r.

A b s t r a c t — Sexual reproduction of the snow algae *Chlamydomonas nivalis* kept in the meltwater of the snow was observed. Gametes were the motile cells. They had walls, two flagella, and varied distinctly in size and shape ranging from spherica to oblong. Fusion was observed between morphologically similar as well as dissimilar gametes. Three phases of fusion were distinguished: 1. Contact and adhesion of gametes; 2. Formation of a bridge; 3. Fusion of protoplasts to from a zygote in two ways: a) contents of one of the gametes migrated to the other, or b) gametes merged into one cell which consisted of the combined cell walls of both gametes. Zygotes were nonmotile and there were no observable changes in the cell wall sculpture.

Chlamydomonas nivalis, the most common species of snow algae, complete its life cycle within the environment of snowbanks and glaciers (K o l 1968). Its active life is limited to a very narrow temperature range with optimum at perhaps 2°C (H o h a m 1975). Below the freezing point of water the algae go into a resting stage, and above 4°C they die

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(Huber-Pestalozzi 1961).

The life cycle of *Chl. nivalis* is not completely known; the resting stages (akinetes or aplanospores), which are capable of dividing into daughter cells, are usually observed. Rarely a motile stage with two flagella is seen. It is not certain that sexual reproduction occurs; although gametes have not been reported, cells with two wall layers covered by wartlike projections have been taken to be zygotes (Ettl 1976).

This paper reports some observations on the life cycle of *Chl. nivalis*, that show both vegetative and sexual reproduction in samples taken from algal blooms in the Tatra Mountains, and cultured in laboratory.

Material and method

Collections and observations were made during the summer and autumn 1975 and 1976 in the Polish High Tatra Mountains on the upper part of a valley "Za Mnichem" at altitude 2070 m, a.s.l. Patches of snow remain there all year round. The rock is granite, and pH of snow was 5.5 (measured colorimetrically). Air temperature ranged from 5.3—16.5°C, but the temperature of snow was 0.2°C.

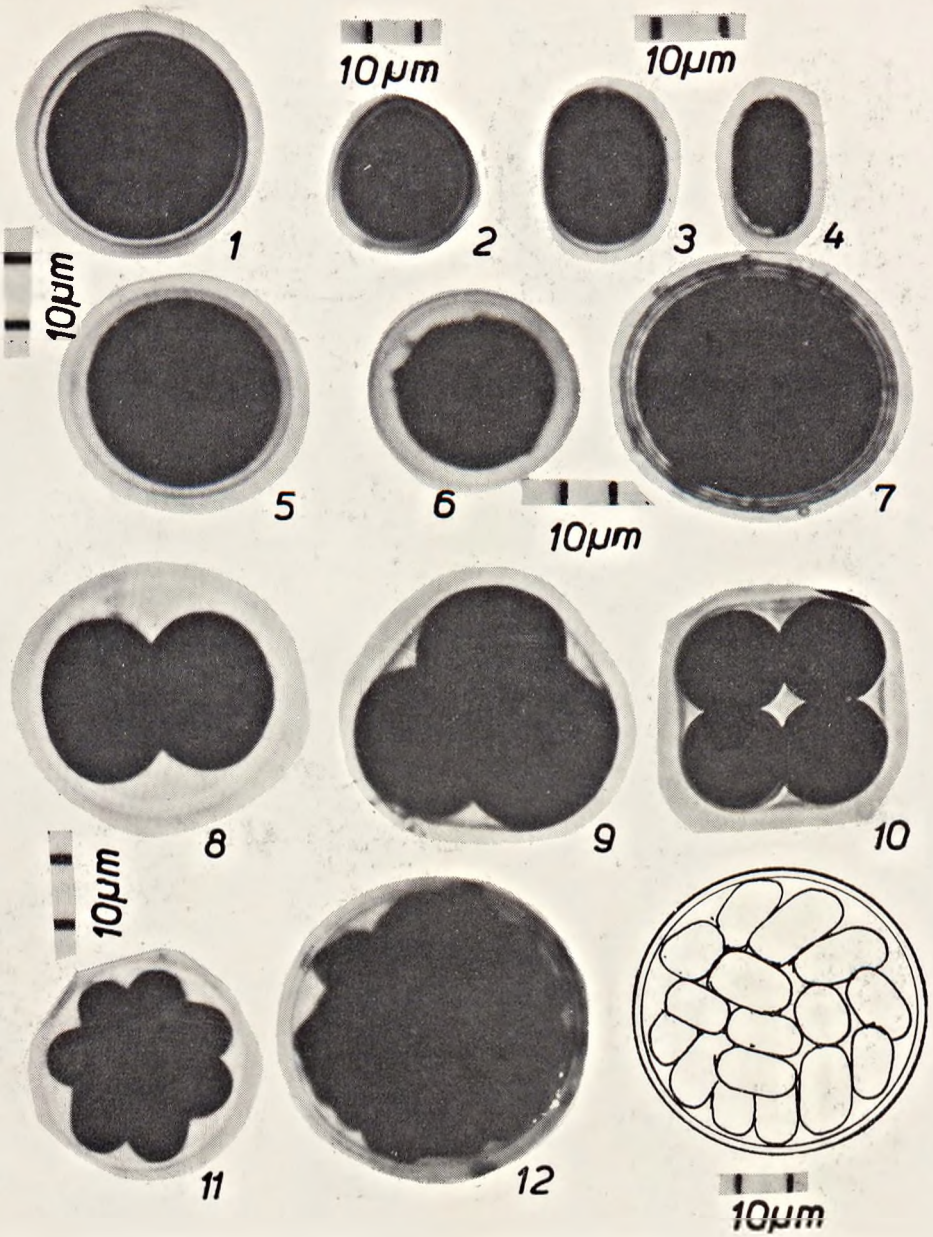
Algae were collected with snow and kept alive in a thermos bottle during transfer to the Laboratory of Water Biology in Kraków. Here the material was divided into flasks (100 ml) which were kept on ice. After some time, snow in the samples melted and the algae settled on the bottom of the flasks. Evaporated water was replaced with distilled water.

Algae were kept in a refrigerator with illumination from fluorescent lamp of 1600 lux. However, during the summer-autumn season the samples were exposed to sunlight for a few hours every day; then the temperature was kept at 0.0—2.0°C.

For observation algae were enclosed in a plexiglass chamber with ice and examined with an inverted microscope (PZO MOD-2) or the normal Zeiss (NU-2).

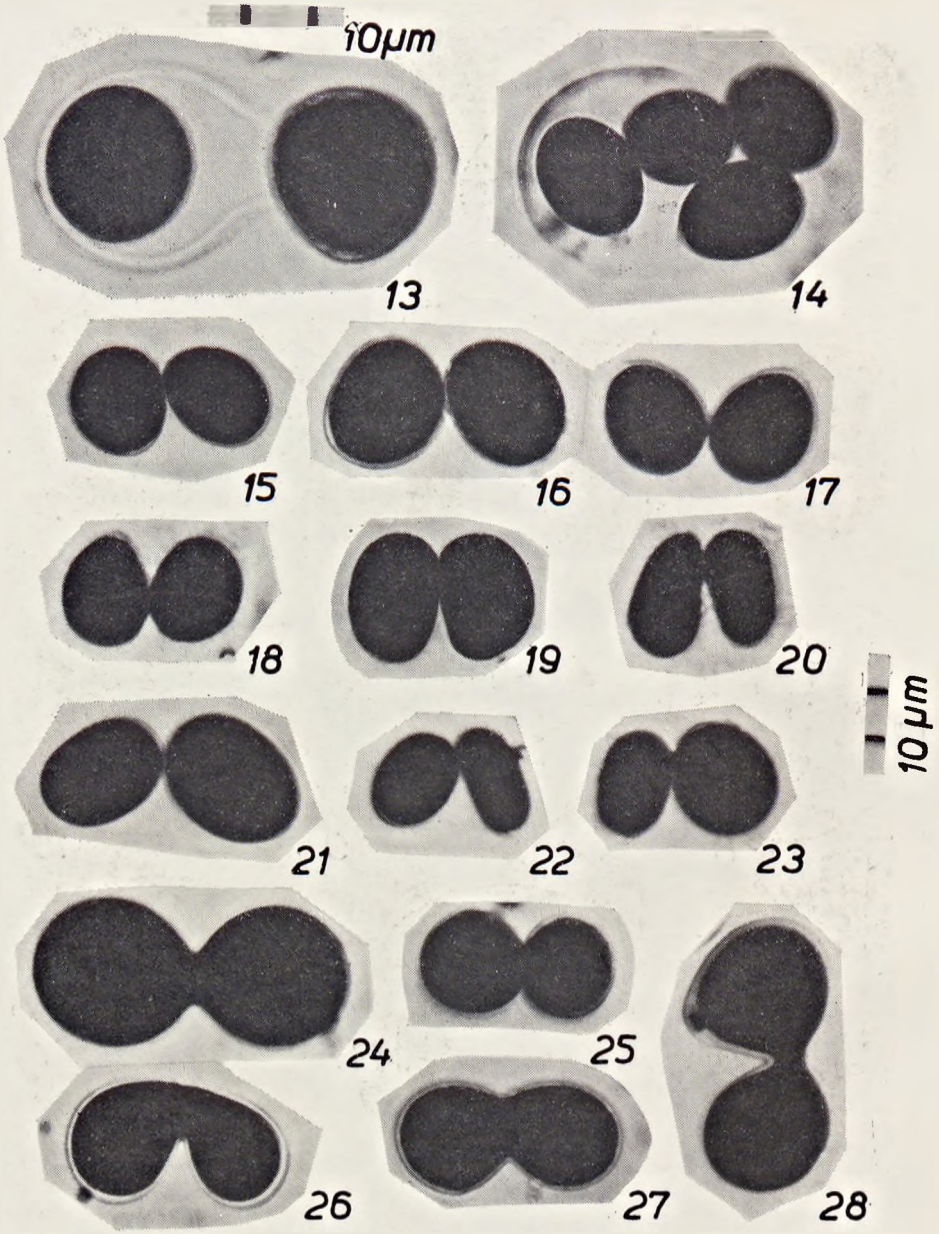
Results

Samples of snow containing *Chlamydomonas nivalis* were collected on 7 September 1975 and the algae were kept alive in the laboratory for approximately 6 months.



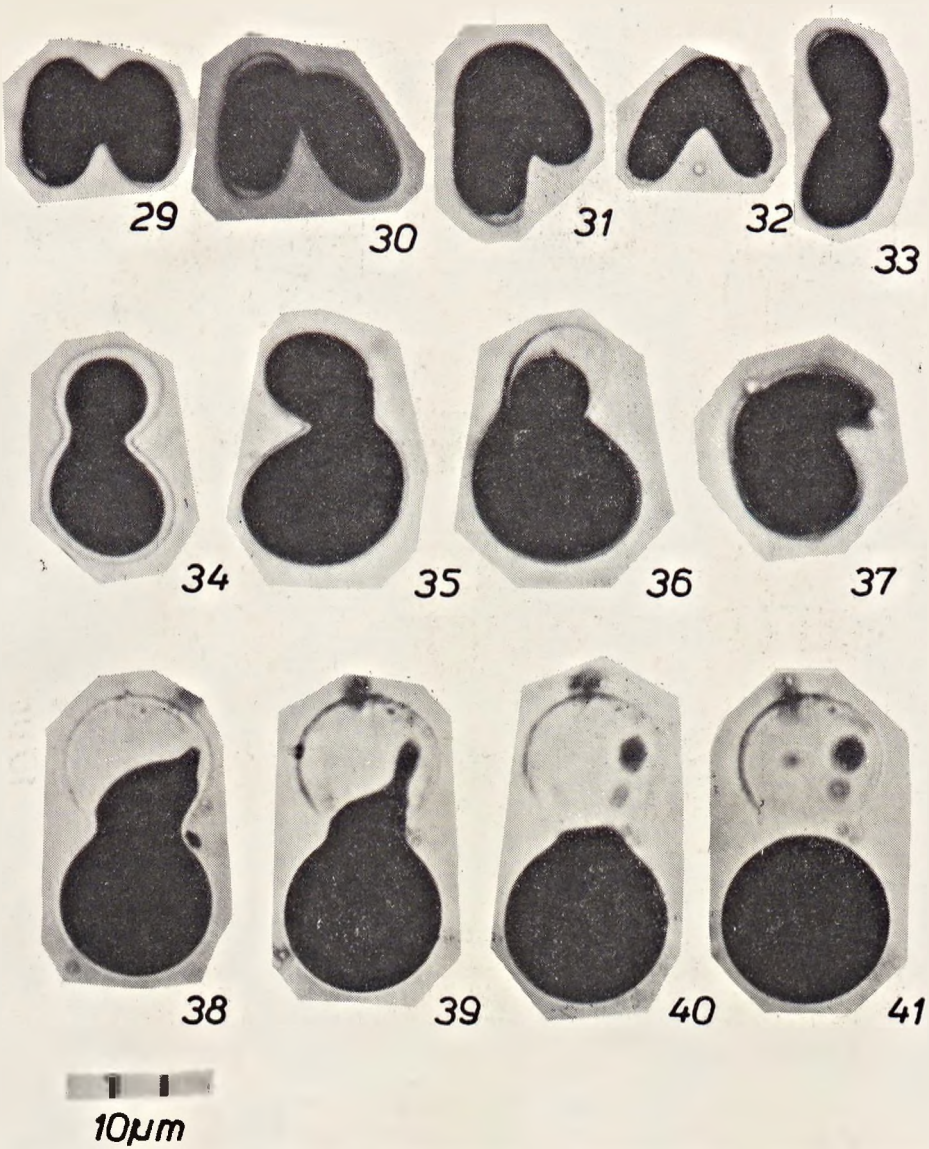
Ryc. 1—12. *Chlamydomonas nivalis*. 1—4 — komórki ruchome; 5—7 — komórki nieruchome; 8—12 — formowanie się komórek potomnych na drodze wegetatywnej

Figs. 1—12. *Chlamydomonas nivalis*. 1—4 — motile cells; 5—7 — nonmotile cells; 8—12 — vegetative formation of daughter cells



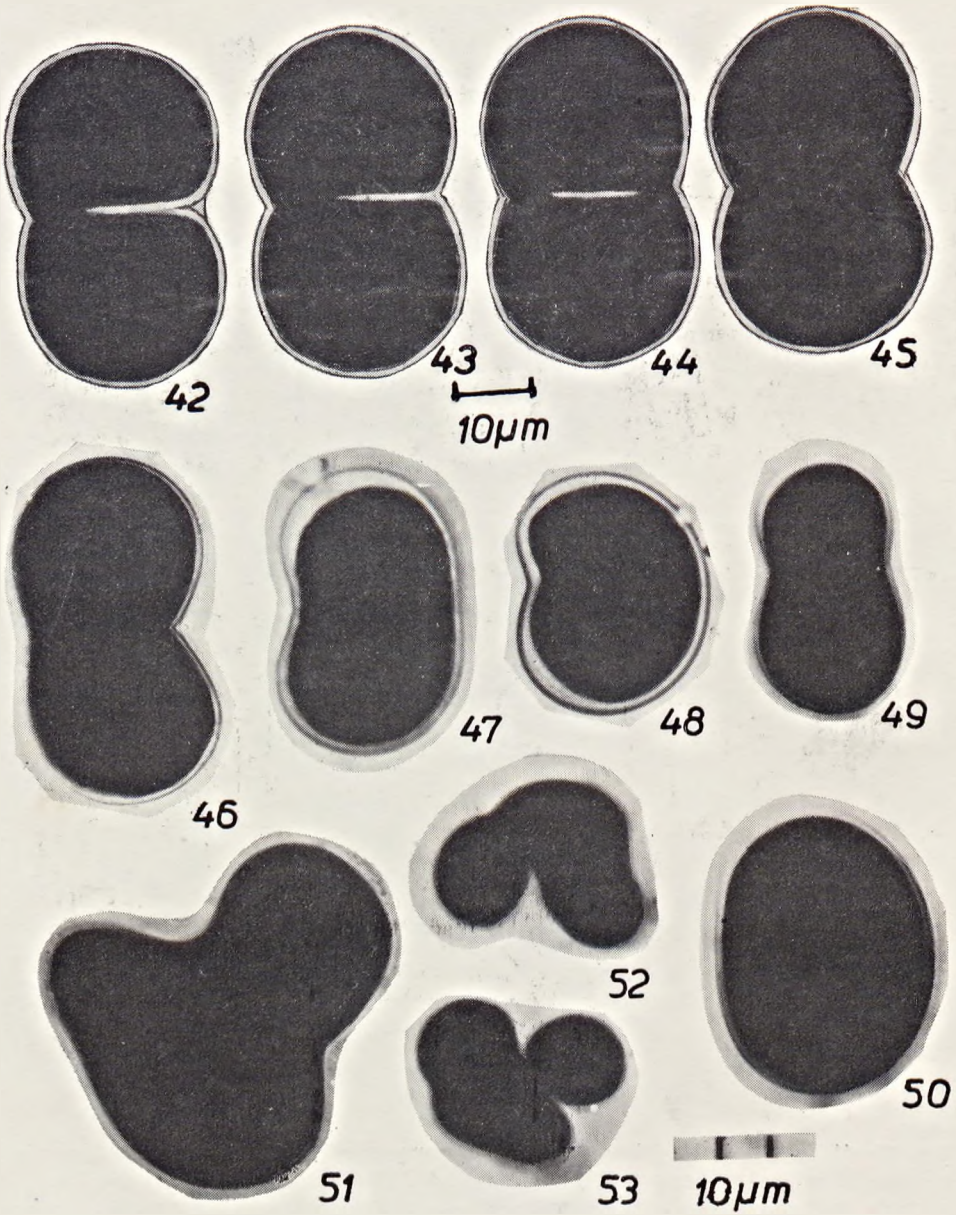
Ryc. 13—28. *Chlamydomonas nivalis.* 13—14 — formowanie komórek potomnych na drodze wegetatywnej; 15—20 — rozmnażanie płciowe: faza wstępnego łączenia się jednakowych gamet; 21—23 — rozmnażanie płciowe: faza wstępnego łączenia się niejednakowych gamet; 24—28 — rozmnażanie płciowe: faza formowania się kanału pomiędzy jednakowymi gametami

Figs. 13—28. *Chlamydomonas nivalis.* 13—14 — vegetative formation of daughter cells; 15—20 — sexual reproduction: adhesion phase of fusion of identical gametes; 21—23 — sexual reproduction: adhesion phase of fusion of dissimilar gametes; 24—28 — sexual reproduction: bridge formation phase of fusion of identical gametes



Ryc. 29—41. Rozmnażanie płciowe u *Chlamydomonas nivalis*. 29—33 — faza formowania się kanału pomiędzy jednakowymi gametami; 34—37 — faza formowania się kanału pomiędzy niejednakowymi gametami; 38—41 — faza formowania się zygoty. Seria zdjęć przedstawia tę samą parę gamet w różnych etapach przelewania się protoplastu z jednej gamety do drugiej

Figs. 29—41. Sexual reproduction of *Chlamydomonas nivalis*. 29—33 — bridge formation phase of fusion of identical gametes; 34—37 — bridge formation phase of fusion of dissimilar gametes; 38—41 — zygote formation phase. Series of figures show one pair of gametes in different stages of migration of protoplast from one cell to the other



Ryc. 42—53. Rozmnażanie płciowe u *Chlamydomonas nivalis*. 42—45 — faza formowania się zygoty. Seria rycin przedstawia tę samą parę gamet w różnych etapach zlewania się ich w jedną komórkę; 46—50 — formowanie się zygoty; 51—53 — fuzja kilku gamet

Figs. 42—53. Sexual reproduction of *Chlamydomonas nivalis*. 42—45 — zygote formation phase. Serie of figures shows one pair of gametes in different stages of mergence of both gametes into one cell; 46—50 — zygotes formation; 51—53 — the fusion of a few gametes

1. Morphological differentiation of cells of *Chlamydomonas nivalis*

The population of algae consisted of motile and nonmotile cells. Motile cells had two flagella emerging from the apical pole; these cells oscillated of spinned while remaining in place or moved about actively. Motile cells taken from the culture soon lost their flagella; papilla marked the location on the apex where they had been. Motile cells had a thin wall. They were variable in size and shape ranging from spherica cells or spherica with a little oblate protrusion at the apical poles; diameter 20—32.5 μm (figs 1, 2) to oval and bean shaped cells of length 20—25 μm and 10—20 μm width (figs 3, 4).

Nonmotile cells were covered by a thick wall, often having two layers (figs 5—7) and had diameter of 20—50 μm.

2. Reproduction of *Chlamydomonas nivalis*

a) Vegetative reproduction

Chlamydomonas nivalis multipled by cell division within mother cells (figs 8—12). Groups of 2, 3, or 4 round or oval cells as well 2, 4, 8, or 16 bean cells were observed within a membrane of autosporangium. The autosporangium with 16 cells reached a diameter 57.5—60 μm. Cells were liberated by rupture of the cell wall (figs 13, 14).

b) Sexual reproduction

Motile cells could act as gametes. The gametes had a wall, and varied distinctly in size and form. Similar gametes (figs 15—20, 24—33) as well as morphologically different ones (figs 21—23, 34—37), fused.

Three phases in the fusion of gametes were distinguished: adhesion, bridge formation, and zygote formation. 1. Gametes made contact along the sides near the apexes, near apex and base, or near bases (figs 15—23). 2. Gametes became joined by a bridge which developed between the apical poles of both gametes, between the apical pole of one cell and basal pole of the second cell (figs 24—28, 30—37) or rarer between both sides of two cells (fig. 29). 3. Fusion into a zygote. This occurred in one of two ways. In the first case the entire contents of one cell passed into the other cell, and the zygote was nonmotile (figs 38—41).

This process occurred rapidly taking approximately 3—5 minutes. In the second case gametes merged into one cell with the membrane of the zygote formed within the cell walls of both gametes. In this case the cell

walls of both gametes gradually dissolved around the bridge, and one cell filled by the two protoplasts was formed (figs 42—45). Formation of this zygote required about two hours. The process was not observed to the and, because it was not possible to maintain suitable conditions long enough for activity of the cells within the plexiglass chamber. However, on the basis of our observations it is possible to suggest, that the cells of different shapes often found in the culture (figs 46—50) represent zygote formation phase resulting from a joining gametes within one cell.

The fusion of more than two gametes was also observed (figs 51—53).

Zygotes were nonmotile and they possessed no external distinguishing features.

3. Field observations

During September 1976 blooms of *Chl. nivalis* occurred explosively in the field site. All patches of snow were red. In places where melted water accumulated the bloom was blood-red. Some of these algae were collected and observed a few hours later at the laboratory in Kraków. The algae were very active with many motile cells moving about. All stages in the fusion of gametes seen previously in laboratory culture were also observed in this field collection.

Discussion

Observations of *Chlamydomonas nivalis* have been made for many years e.g. Chodat (1896), Kol (1928), Siemińska (1951), Garric (1965), Stein (1967), Kol (1968), Kol, Eurola (1974), Kol (1975), but there are no reports of sexual reproduction.

Results reported here show that sexual reproduction occurs in *Chl. nivalis* kept in the laboratory in the meltwater of the snow with which they were collected, as well as in the field environment during a very intensive algal bloom in 1976. This bloom coincided with the occurrence of a large accumulation of meltwater within the snowbanks. Siein (1967) suggested that vegetative and sexual reproduction of *Chl. nivalis* occurs when snowbanks contain meltwater for a period of at least 24 hours. Results reported here are in agreement with the hypothesis that snow algae require liquid medium for reproduction. Local weather condition that produce melted water accumulations in the snowbanks do not seem to be common in the climate of the Tatra mountains.

This may explain the fact that *Chl. nivalis* is usually found in the resting stages.

The gametes of *Chl. nivalis* vary in size and form, and they fuse in pairs of all combination. This suggests that both izo- and heterogamy occurs. However, it is uncertain whether or not the sexual differentiation is connected with different morphology of gametes. A similar kind of fusion, called "atactogamy' has been described for the order of *Chlamydomonas* by Korschikoff (in Pascher, 1927). In the case of atactogamy all the gametes are morphologically similar but different in size.

It is interesting that in the sexual reproduction of *Chl. nivalis* two modes of zygote formation exist: 1) the protoplast of one of the gametes passes into the other gamete and 2) gametes merge and the cell walls of both gametes form a single cell. Moevus (1933) found similar alternatives to zygote formation in *Chl. eugametos*. The protoplasts of identical gametes fused in the central part of cell and the new cell wall was formed from the cell walls of both gametes. When the gametes were not identical, the contents of the smaller gamete passed to the cell of the bigger one.

Protoplasts pass between gametes rapidly, the process requiring only approximately 3—5 minutes. On the other hand a longer time, probably a few hours, is needed for gametes to merge into one cell. Goroschankin (1890) observed in *Chl. Braunii* an accelerated and lag process of the fusion of gametes that took from a few minutes to a few hours.

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STRESZCZENIE

Chlamydomonas nivalis jest szeroko rozprzestrzenionym glonem naśnieżnym. Cały cykl życiowy przechodzi on w środowisku kryobiotopu, przy czym jego aktywne życie ogranicza się do bardzo wąskiego zakresu temperatur (0—4°C). Cykl życiowy *Ch. nivalis* nie jest w pełni poznany. Organizm występuje najczęściej w stadiach przetrwalnych, rzadziej spotyka się dwuwiciowe komórki w ruchu. Komórki ze zgrubiałą błoną regularnie brodawkowaną uważa się za zygoty, jednakże przebieg rozmnażania płciowego glonu nie był dotąd obserwowany.

W niniejszym opracowaniu obiektem badań był *Chl. nivalis* rozwijający się w lecie i jesieni na płatach śniegu leżących w dolinie Za Mnichem (Tatry Wysokie) na wysokości 2070 m.

Populacja *Chl. nivalis* składała się z komórek nieruchomych, często otoczonych grubą dwuwarstwową błoną, oraz komórek ruchomych posiadających dwie wici. Ko-

morki ruchome były obłonione, zróżnicowane w wielkości i kształcie, od kulistych do owalnych. W procesie rozmnażania płciowego odgrywały rolę gamet.

W przebiegu rozmnażania płciowego wyróżniono trzy fazy. W pierwszej fazie gamety ustawiały się obok siebie i lekko sklejały w miejscu kontaktu. Bieguny apikalne obu komórek były skierowane w tę samą stronę lub przeciwnie, wskutek czego biegun apikalny jednej komórki sąsiadował z bazalnym biegunem drugiej komórki. W drugiej fazie wytwarzał się kanał pomiędzy gametami, najczęściej biegunowo, rzadziej bocznie. W trzeciej fazie następowało formowanie się zygoty dwoma sposobami: przez przelewanie protoplastu z jednej gamety do drugiej lub przez łączenie gamet w jedną komórkę poprzez zrastanie błon. W pierwszym wypadku proces trwał kilka minut, w drugim kilka godzin. Zygoty były nieruchome, bez dodatkowej skulptury błony komórkowej.

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