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CULTURE, FOOD SELECTION AND GROWTH RATE IN THE MYCOPHAGOUS CILIATE *GROSSGLOCKNERIA ACUTA* FOISSNER, 1980: FIRST EVIDENCE OF AUTOCHTHONOUS SOIL CILIATES

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Summary—A culture method with defined medium for the soil ciliate *Grossglockneria acuta* Foissner, 1980 is described. Food selection studies reveal that it fed exclusively on three out of 11 fungi tested (*Mucor mucedo*, Mucoraceae and *Aspergillus* sp.) although all have a chitinous cell wall. The rejected species may synthesize antiprotozoal substances. No growth was obtained with yeast, bacteria, flagellates or the ciliate *Colpoda aspera* Kahl. Under laboratory conditions generation times ranged from 34.25 h (4.5°C) to 3.86 h (30°C). Correspondingly, population growth rate values ranged between $0.036\,h^{-1}$ up to $0.323\,h^{-1}$. A significant increase in population growth rate was observed between 5.5° and 21°C, whereas there was no reproduction at 40°C. The most pronounced acceleration in population growth rate occurred between 10° and 21°C. This is near the highest mean monthly temperature of the natural habitat (16.7°C) of our population. Field observations yielded a higher density and frequency of *G. acuta* in alpine soils than in lowland ones. The experiments suggest that the low annual mean temperature could be responsible for this because highest individual densities develop at 4.5°C. The highly specialized diet and the oral structure—a tentacle which is used in breaking up and sucking out hyphae and spores—are convincing proof that *G. acuta* is autochthonous to soil. This is emphasized by the fact that we could not find any member of the family Grossglockneridae during the investigation of more than 200 running and stagnant waters.

INTRODUCTION

Soil-dwelling ciliates have long been thought to be only common limnetic invaders. In some recent papers, however, Foissner (1981) and Foissner and Peer (1984a) suggested that autochthonous soil ciliates do exist. Major evidence for their assumption comes from the paper by Foissner and Didier (1983). They showed that *Pseudoplatyophrya nana* (Kahl), with the help of its so-called oral trapeze, feeds perhaps exclusively on yeast cells. This special oral structure is used in breaking up the cell wall and sucking out the yeast protoplasm. Thus, the family Grossglockneridae harbours some of the most promising candidates for being considered autochthonous soil ciliates.

To test this hypothesis, we investigated the food requirements of another prominent member of this family, *G. acuta*.

MATERIALS AND METHODS

The investigated population of *G. acuta* originated from the uppermost soil layer (0–5 cm; litter and humus horizon) of a small spruce forest near Aigen-Schlägl, Upper Austria (540 m NN). The fungal species (except yeast), bacteria and flagellates were isolated from the same location. The fungi were grown on agar (3.5% malt extract, 0.5% peptone, 3% agar, tapwater; 3 × 20 min fraction sterilization) in Petri dishes (10 cm dia) held at room temperature. Yeast was obtained from a bakery and *Colpoda aspera* from stock cultures.

Population growth rate and generation time of *G. acuta* were tested with *Mucor mucedo* in at least three replicates at temperatures in the range from 4.5° to 40°C and with *Aspergillus* sp. at 21°C. Cultures were initiated usually with 3–5 individuals suspended in 12 or 15 ml sterile Knop's solution. Stock cultures were maintained as clones with *M. mucedo*.

To measure cell numbers, cultures were mixed to remove density differences and 1 ml of medium was counted on a slide under a dissection microscope at 20× to 30× magnification. After counting, the organisms were transferred back into the culture.

Generation time was assessed by the formulae of Vater-Dobberstein and Hilfrich (1982):

$$g = (\log N_2 - \log N_1)/\log c \quad r = g/t \quad d = 1/r$$

where g = number of divisions; N_1, N_2 = cell numbers at t_1, t_2; c = birth rate per cyst; r = division rate per time unit; t = time between counting $(t_2 - t_1)$; d = generation time. For *G. acuta*, c is 3.47 because in the majority of fissions (73.6%, $n = 106$) 4 individuals emerge per cyst whereas in 26.4% bipartition occurs. This latter proportion increases with culture age.

Population growth rate (intrinsic rate of natural increase) was calculated by the formula:

$$r_m = (\ln N_2 - \ln N_1)/dt.$$

Cells were stained by the silver impregnation methods of Fernandez-Galiano (1976) or Chatton–Lwoff as modified by Corliss (1953).

Fungi were determined according to von Arx (1967), Bessey (1950), Gäumann (1964) and Lindau (1922).

RESULTS

Short description of G. acuta

G. acuta is a small (40–60 × 15–35 μm, $n = 30$) colpodid ciliate with about 10–12 ($n = 29$) somatic kineties (Fig. 1). Common to the whole family Grossglockneridae is the oral trapeze inserted near the anterior end of the body. Under low magnification, well-fed living animals appear nearly black due to the presence of numerous food vacuoles.

The silverline system (Fig. 2)—shown here for the first time as prepared by the Chatton–Lwoff technique—is very similar to that of the genus *Colpoda* (cf. Foissner, 1980). It is a silver-accumulating structure located tightly underneath the pellicle and one of the more important taxonomic features.

A detailed description of *G. acuta* and related species is given by Foissner (1980).

Culture

Field observations indicated that *G. acuta* might be a fungal feeder. Thus, we offered hyphae and spores of different fungi suspended in soil extract medium to this organism but the resulting growth of the ciliate was very poor. Probably it did not like dying or non-growing hyphae. Therefore, we cultured the fungi on agar plates in Petri dishes. After the culture medium for *G. acuta* was added, the fungi ceased to grow under these submerged conditions. Finally, we grew the fungi on agar slants in glass Petri dishes (10 cm dia). Only 1/3 to 1/4 of the Petri dish was covered by the nutrient agar. While the agar is solidifying, some hyphae from a pure culture were inoculated onto the agar. After sufficient development of the fungus, a few individuals of *G. acuta* suspended in 12 or 15 ml Knop's solution were added. In pilot investigations we used soil extract diluted with distilled water, tapwater or spring water as culture medium. Later we switched to the better defined Knop's solution which yielded even better growth (Knop's solution: KNO_3–1.0 g, $Ca(NO_3)_2$–0.1 g, K_2HPO_4–0.2 g, $MgSO_4 \cdot 7H_2O$–0.1 g, $FeCl_3$–1 mg, distilled water–1000 ml; pH 6.3).

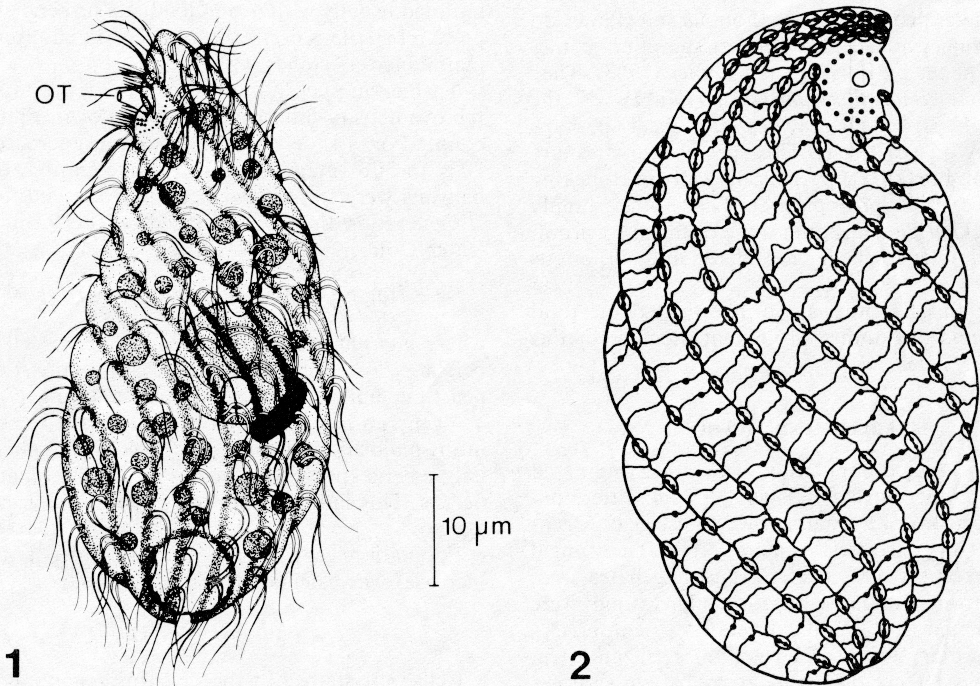
It is crucial that many hyphae are immersed in the solution but sufficient are free of the solution so that the fungus remains alive (Fig. 3). Thereby, masses of spores fall into the medium. It is necessary to change the medium every 7–14 days and to provide fresh food.

Food selection

With the above procedure we tested 12 species of fungi as food in 5–10 parallel experiments. Only three of them, *Mucor mucedo*, Mucoraceae and *Aspergillus* sp., enabled reproduction of the ciliate. In separate experiments it was shown that *G. acuta* was able to feed exclusively on the contents of hyphae or spores. No growth occurred with three species of *Penicillium*, *Verticillium* sp., *Botrytis* sp., *Sclerotium* sp., Gymnoascaceae, Aspergillaceae, yeast, and with different kinds of soil bacteria, soil flagellates and the ciliate, *Colpoda aspera*.

Population growth rate and generation time

We always refer to the shortest generation time found. Correspondingly, population growth rate stands for the highest one calculated.



Figs 1 and 2. *Grossglockneria acuta*: (1) Left lateral view from life; (2) Infraciliature and silverline system of the right side after Chatton–Lwoff silver impregnation. OT = Oral trapeze.

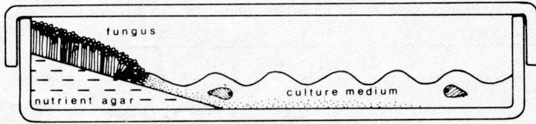


Fig. 3. Culture equipment for *G. acuta*.

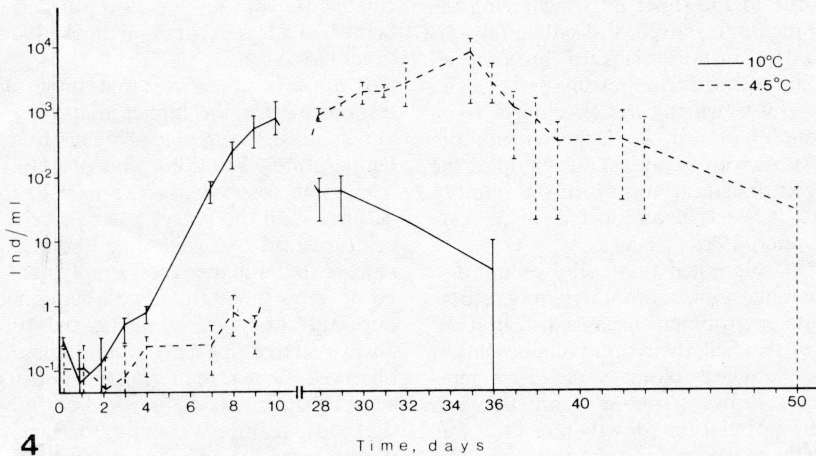
There was a significant shortening of mean generation time between 4.5° and 10°C, but it was much less pronounced than between 5.5° and 21°C (Table 1). The least significant difference method (LSD-method) of Sokal and Rohlf (1981) even showed a significant difference in mean generation time between 10° and 21°C ($\alpha = 0.1$). At higher temperatures there were only slight but insignificant alterations (Table 1). At 10°C the fastest population growth took place on day 8 of culture whereas at 21°C it took place on day 2.

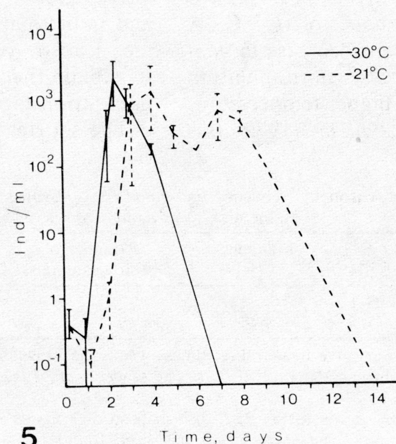
Corresponding results were obtained with *Aspergillus* sp. The values for 21°C are $d = 4.85$ h, $r_m = 0.257$ h^{-1}.

As is shown in Fig. 4, *G. acuta* reached very high individual densities at 4.5°C (max. 12,874 cells ml^{-1}). In repetitions of this experiment we obtained two kinds of results. In three cases we got normal densities as at the other temperatures. Three other trials yielded similarly high densities as the first experiment (8000–10,000 cells ml^{-1}). Probably small temperature variations (about 1°C) were responsible for this different behavior.

DISCUSSION

The three fungi which *G. acuta* feeds on are common and widespread saprophytes in soil (Lindau, 1922). Of the rejected fungal species, the majority belong to the Fungi Imperfecti. Križková *et al.* (1979) demonstrated that up to 78% of the investigated members of this class synthesize antiprotozoal substances. Coûteaux and Dévaux (1983) and Heal and Felton (1969) observed antagonistic effects between soil amoeba and fungi. This is emphasized by our findings. Repeated inoculation of *G. acuta* into cultures of *Verticillium* sp. and *Penicillium* sp. caused rapid encystment or death. Furthermore, if *Penicillium* hyphae contaminate cultures, e.g. *M. mucedo*, growth of *G. acuta* is considerably depressed. It is unlikely that the ciliate cannot break up the cell walls





Figs 4 and 5. Population growth curves at various temperatures with *Mucor mucedo*: (4) 4.5°C, 10°C; (5) 21°C, 30°C.

Table 1. Generation time and population growth rate of *Grossglockneria acuta* at different temperatures with *Mucor mucedo*

Temperature (°C)	4.5	5.5	10	21	24	30	40
Shortest generation time d(h)	34.25	26.12	18.20	4.86	4.62	3.86	NG[a]
Mean generation time d(h)[b]	48.8	29.1	20.9	6.1	4.8	4.0	NG
Multiple comparisons of mean generation time d(h)[c]	———	———					
		———	———				
			———	———	———	———	
Highest population growth rate r_m(h^{-1})	0.036	0.048	0.068	0.256	0.269	0.323	NG
Max individual density (ml^{-1})	12,874	2845[d]	1487[d]	3189	5765	3901	NG

[a]NG = no growth, maybe due to deterioration of the fungus.
[b]Mean generation time of the three shortest values at each temperature.
[c]Multiple comparisons were made with the T-Method of Sokal and Rohlf (1981) and Rohlf and Sokal (1981). Values underscored by the same line are not significantly different from each other ($\alpha = 0.05$).
[d]Possibly submaximal numbers, but the condition of the cultures suggested that the actual values were not much higher.

of these fungi because they consist of chitin (Bessey, 1950; Braune *et al.*, 1976), like that of the fungi which it feeds on. On the other hand Yakimova *et al.* (1978) reported a considerable acceleration of the fission rate in the freshwater species *Paramecium caudatum* by metabolites of *Actinomyces subflavus* 434, *Gliocladium roseum* 19 and strain 31 of genus *Fusarium*.

The population growth curves (Figs 4 and 5) at all investigated temperatures show a slight decrease in active individual numbers 1–3 days after inoculation of the cultures, prior to the beginning of the log phase. These individuals do not die, but encyst. Perhaps this is due to the shock of transferring the animals to fresh medium. Indeed, if disadvantageous conditions arise (for instance lack of food or an ageing medium), *G. acuta* forms resting cysts (mean dia 23.4 μm, $n = 80$), which are enveloped by a thicker membrane than are the larger (mean dia 37.2 μm, $n = 18$) division cysts. This enables the ciliate to endure complete drying up of the habitat. The capability to encyst is also reported for *P. nana* (Foissner and Didier, 1983).

Buitkamp (1979) suggested that soil-dwelling ciliates adjust to the highest mean monthly temperatures of their immediate environment because he observed that these ciliates reached their optimal population growth and their highest biomass at those temperatures. *G. acuta* shows a similar trend: Between 10° and 21°C the population growth rate increases just as dramatically as generation time shortens. The highest mean monthly temperature of the natural habitat of our population amounts to 16.7°C. A further but statistically insignificant decrease in generation time and thus an increase in population growth can be observed towards higher temperatures (Table 1). According to Baldock *et al.* (1980) this appears to be a characteristic of microbial populations, at least of freshwater microorganisms. Field observations in this study showed that *G. acuta* occurs more regularly and in higher abundance in alpine soils than in lowland soils (Table 2); in other words, under lower soil temperatures and in acid environments. The higher individual densities found in the cultures incubated at 4.5°C [a temperature, incidentally, only slightly below the mean temperature of 6.6°C during the vegetation period (June to October) (Weiss, 1978)] also indicate a preference by *G. acuta* for soils with lower temperatures. The ciliate's fungal prey have been found in higher numbers in acid soils than in neutral or alkaline soils (Beck, 1968).

It appears, therefore, that three factors could be responsible for the higher frequency of *G. acuta* in alpine soils: lower pH; a higher food supply; lower temperatures. We think that pH can be ruled out as a main factor because the near-neutral pH in our cultures had no effect. Very likely, the ciliate is acidotolerant. Considering the second factor, it seemed that a higher food supply in alpine soils could be of importance to ciliate abundance. However, in our laboratory experiments, the fungal food was always offered in excess and no apparent effects were observed. Given, then, that extraordinarily high densities of the ciliate were observed only at 4.5°C, and that those cultures kept at 4.5°C survived the longest, it seems that temperature may be the main factor. Clarke (1979) reported that reduced metabolic rates and individual energy demands at low temperatures may lead to a higher standing crop. This could help to explain the increased generation time and reduced population growth rate of *G. acuta* at 4.5°C.

The shorter life time of the populations at higher

Table 2. Comparison of frequency and numbers of *Grossglockneria acuta* in 24 alpine and 70 lowland samples[a]

Sites	pH	Frequency[b] (%)	Number of positive samples[c]	Numbers of individuals[d]
Alpine	3–5	83	12	28.0
Lowland	7–8	44	6	6.4

[a]8 alpine sites were investigated three times a year. The 7 lowland sites were analyzed 10 times during a period of 3 yrs (Foissner *et al.*, 1982; Foissner and Peer, 1984a, b).
[b]Determined according to the Petri dish method of Foissner *et al.* (1982).
[c]"Positive samples" means *G. acuta* has been found in countable numbers by Buitkamp's method (1979).
[d]Numbers are given as means ($\bar{X}$) of individuals g^{-1} dry wt of soil and were estimated by the Buitkamp method (1979).

temperatures (21° and 30°C) may be explained by a faster accumulation of toxic metabolites either from the ciliate or from the fungus. In addition, the fungal prey did not grow so well at 30° than at 21°C.

There are reports (Beers and Sherwood, 1966) of associations between aquatic phycomycetes and freshwater ciliates, but not between soil fungi and soil ciliates. Obviously, *G. acuta* must be an autochthonous soil ciliate because its prey are restricted to the soil and do not grow under submerged conditions. Its fungal diet has led to a specialized adaptation, the oral trapeze, which is lacking from *Woodruffia sporophaga* described by Beers and Sherwood (1966). This unique equipment enables the ciliate to conquer new food niches in soil previously unused by other soil-dwelling ciliates. Further evidence comes from our unsuccessful search for members of the Grossglockneridae during extensive investigations of running and stagnant waters, whereas they occur regularly in soils from different regions throughout the world, e.g. Austria, Germany, Poland and Nepal (Foissner, unpublished).

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