

# The Influence of Hypergravity on the *Paramecium bursaria-Chlorella* sp. Symbiotic Association

Tomasz Bator\* and Ryszard Pado

Department of Microbiology, Institute of Biology, Pedagogical University of Cracow, Podbrzezie 3, 31-054 Kraków, Poland. E-mail: tbator@ap.krakow.pl

\* Author for correspondence and reprint requests

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The aim of the research was to determine the influence of strong hypergravity on the *Paramecium bursaria-Chlorella* sp. symbiotic association, which is considered to be a model example of symbiosis between a heterotroph and an autotroph. The paramecia cells were exposed to  $1073 \times g$ ,  $4293 \times g$ , and  $9658 \times g$  hypergravity for 15 min. Then they were incubated for 21 d on a standard lettuce medium. The experiments were conducted in parallel under constant white light and in the dark. The changes in the number of paramecia cells during incubation were determined. Measurements of the number of *Chlorella* sp. endosymbionts inside host cells were also made. The results showed that a 15-min exposure to hypergravity attenuates the subsequent growth of *Paramecium bursaria* in the dark, but it may stimulate the growth of paramecia under constant light. Moreover, it causes an increase in the number of algae inside the paramecia cells. Presumably, the influence of hypergravity on the studied symbiotic complex is connected with its effect on the endosymbiotic *Chlorella* sp. cells. This subject requires further research, focused on the influence of hypergravity on the physiology and growth of the *Chlorella* sp. endosymbionts living inside the *Paramecium bursaria* cells.

*Key words:* Endosymbiosis, Hypergravity, *Paramecium*

## Introduction

The ciliate *Paramecium bursaria* is considered as a model example of obligatory endosymbiosis and presents most typical features of symbiotic associations formed by autotrophic and heterotrophic organisms. The main characteristic of *Paramecium bursaria* is the presence of the endosymbiotic algae *Chlorella* sp. inside the protozoan cell. The algae make products of photosynthesis available to the ciliate and take advantage of carbon dioxide and water which are produced as a result of protozoan respiration. The number of *Chlorella* cells is largely determined by light conditions. *Paramecium bursaria* cultivated in the light are characterized by a vivid green taint which is the result of a large number of *Chlorella* cells (up to 1000) being present in one protozoan cell. On the other hand, *Paramecium bursaria* which have been cultivated in the dark are pale, almost colourless and almost completely devoid of algae. This stems from the fact that in the dark photosynthetic processes do not occur. Under these conditions *Chlorella* cells start feeding on organic compounds obtained by paramecia, thus becoming a “trophic burden”. Then the parame-

cia cells reduce the number of endosymbionts (Pado, 1965).

Our studies on this interesting symbiotic association have been conducted for many years in the Department of Microbiology at the Pedagogical University of Cracow, Poland. These studies have resulted in, among other things, obtaining mass cultures of different strains of *Paramecium bursaria*, collected from several sites in Poland (Pado and Bator, 2001). Furthermore, we have demonstrated that the *Paramecium bursaria-Chlorella* sp. association shows highly developed adaptive abilities (Bator, 2005). During our experiments, we have often tried to increase the accumulation of paramecia cells in the cultures by centrifugation. In this case we observed some changes in the behaviour of centrifuged paramecia cells in comparison with non-centrifuged cells. During the centrifugation, the paramecia were exposed to hypergravity, which is the result of the inertial force. For many years the influence of hypergravity on living organisms has been a subject of many tests. Unicellular organisms have been chosen very often as the objects of these experiments (Bräucker *et al.*, 1994; Häder *et al.*, 2005; Kato *et al.*, 2003; Kuźnicki, 1968; Ortiz *et al.*, 2000; Popova,

2003). These studies allowed to determine the influence of hypergravity on the processes occurring in a single cell. However, among many reports on this topic, there are no data about the influence of strong hypergravity on symbiotic complexes, formed by unicellular autotrophs and heterotrophs. That is why an attempt was made to determine the influence of hypergravity on the *Paramecium bursaria*-*Chlorella* sp. association.

### Material and Methods

The ciliates *Paramecium bursaria* from a laboratory culture kept for many years in the Department of Microbiology, Pedagogical University of Cracow, Poland (Pado and Bator, 2001) were used as the research material. The hypergravity conditions were obtained in a laboratory centrifuge (MPW-210). In the initial phase of the experiments the survival of the ciliates exposed to hypergravity was determined. In order to do this, 100 cells of *Paramecium bursaria* were taken from the stock culture and transferred with a glass micropipette into the 5-ml centrifugal tube. The number of paramecia cells was determined under a stereomicroscope. Then a 15-min centrifugation was applied. The experiments were conducted at different values of rotational speed, corresponding to different values of hypergravity: 4000 RPM (rotations per min)  $\hat{=} 1073 \times g$  ( $g = 9.81 \text{ m/s}^2$ , earth's gravitational acceleration); 8000 RPM  $\hat{=} 4293 \times g$ ; 12,000 RPM  $\hat{=} 9658 \times g$ . After centrifugation, the paramecia cells were transferred into a watch glass and the number of living, non-damaged cells was determined under a stereomicroscope. This procedure was repeated 5 times for each rotational speed, and the mean number of living cells with standard deviation was calculated. The results were expressed as the percentage of the initial value.

Next, the capability of subsequent growth of paramecia cells previously exposed to hypergravity was examined. Three cells of *Paramecium bursaria* were taken from each centrifuged sample and transferred with a glass micropipette into a watch glass containing 4 ml of a newly prepared lettuce infusion inoculated with a bacteria strain isolated from the natural habitat of *Paramecium bursaria* (Pado and Bator, 2001). The watch glasses were placed in Petri dishes, lined with wet filter papers in order to minimize the evaporation of water. The experimental cultures

prepared in this way were incubated for 21 d. Two cultures from each centrifuged sample were made. One of them was incubated under constant white fluorescent light ( $1.02 \text{ W/m}^2$ ) and the other was incubated in the dark. The control cultures, incubated simultaneously under the same conditions as the experimental cultures, were prepared from the paramecia cells derived from the stock culture, not exposed to hypergravity. In all cultures paramecia cells were counted under a stereomicroscope on consecutive days of incubation. Next, the cell division coefficient for each day of incubation was calculated by dividing the number of paramecia cells noted on a given day by 3 (the initial number of paramecia cells). After completion of incubation the mean value of cell division coefficient was calculated on the basis of the values collected during the incubation period. In this way, the average cell division coefficient was obtained. This is the parameter used to describe the intensity of the paramecia culture growth during 21 d of incubation. The values of the average cell division coefficient obtained from the experimental cultures were compared with the results from the control cultures (*t* test) and expressed as the percentage of the control sample. The described procedure was repeated 5 times for each value of hypergravity.

Additionally, during the last repetition of the experiment in the cultures incubated under light, the number of *Chlorella* sp. endosymbionts inside the *Paramecium bursaria* cells was measured on days 11 and 21 of incubation. In order to do this, several cells of paramecia from the watch glass cultures were placed on microscope slides and covered with cover slips. Then, the cell membrane of paramecia cells was broken as a result of the cover slip pressure and the algae were released from the host cell. The number of *Chlorella* sp. was determined using a light microscope with  $400\times$  magnification. The measurements were made for the cultures of paramecia cells exposed to  $1073 \times g$  hypergravity and for the control cultures. For each culture, the number of algae was determined in 5 *Paramecium bursaria* cells, and the mean value was calculated. In order to statistically compare the results obtained for the cells exposed to hypergravity and for the control cultures, the *t* test was applied.

## Results

Our experiments showed a marked influence of hypergravity on the survival of *Paramecium bursaria* cells (Fig. 1). It was found that the number of living paramecia cells in the studied population decreased with an increase in hypergravity. At  $1073 \times g$  the survival of *Paramecium bursaria* (expressed as a percentage of the initial number of living paramecia cells) was 83.0%, at  $4293 \times g$  it was 70.8%, and at the highest applied hypergravity ( $9658 \times g$ ) it was 57.8%. A distinct change in shape was observed in paramecia cells exposed to hypergravity. This change consisted in the accumulation of endosymbiont cells at one pole of the *Paramecium bursaria* cells.

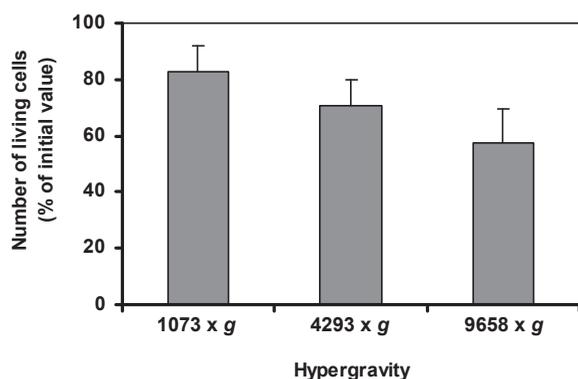


Fig. 1. Survival of *Paramecium bursaria* exposed for 15 min to hypergravity; mean with standard deviation.

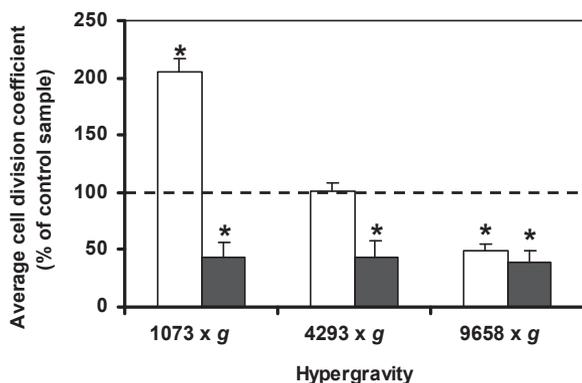


Fig. 2. Intensity of *Paramecium bursaria* growth during 21 days of incubation after previous application of hypergravity for 15 min; white columns, incubation under constant light; black columns, incubation in the dark; mean with standard deviation; asterisks indicate a significant difference between experimental and control samples ( $P < 0.05$ ;  $t$  test).

A 15-min exposure to  $1073 \times g$ ,  $4293 \times g$ , and  $9658 \times g$  hypergravity attenuated the subsequent growth of *Paramecium bursaria* in the dark (Fig. 2). The average cell division coefficients (expressed as the percentage of the control sample) obtained during 21 d of incubation in the dark were: 43.8%, 43.9%, and 39.5%, respectively. Under constant light, similar results were obtained only in the paramecia cells exposed to the maximum value of hypergravity ( $9658 \times g$ –48.9%). There was no influence of  $4293 \times g$  hypergravity on the growth of the paramecia cells which were incubated under light (101.2%), but application of  $1073 \times g$  hypergravity caused marked stimulation of subsequent growth of *Paramecium bursaria* under constant light (204.5%).

The measurements of the number of endosymbiotic *Chlorella* inside the paramecia cells conducted during the last experimental culture (Fig. 3) showed a significant ( $P < 0.05$ ;  $t$  test) increase in the number of algae in paramecia cells exposed to  $1073 \times g$  hypergravity and later incubated under constant light. In these paramecia cells the average number of algae was 346 on the 11th day of incubation and 262 on the 21st day of incubation. In parallel control cultures (not exposed to hypergravity), the average number of algae in one paramecium cell was 280 and 218, respectively.

On the basis of the obtained results, it can be concluded that hypergravity can affect the sub-

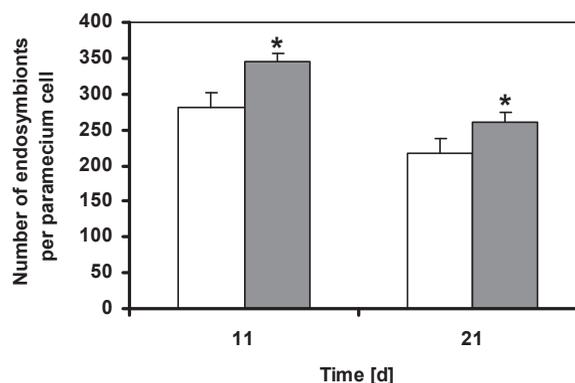


Fig. 3. Number of endosymbionts inside the *Paramecium bursaria* cells after 11 and 21 days of incubation under constant light; white columns, control culture; grey columns, paramecia previously exposed to  $1073 \times g$  hypergravity; mean with standard deviation; asterisks indicate a significant difference between experimental and control cultures ( $P < 0.05$ ;  $t$  test).

sequent growth of the *Paramecium bursaria*-*Chlorella* sp. symbiotic complex, depending on light conditions. In the dark, the paramecia cells which were exposed to hypergravity grow more weakly; however, the application of hypergravity may stimulate the growth of *Paramecium bursaria*

under constant light. This effect probably occurs through the influence of hypergravity on the endosymbiont cells, because hypergravity causes an increase in the number of symbiotic algae cells inside *Paramecium bursaria* cells incubated under light.

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