

Size of *Paramecium bursaria* individuals under cold and dark conditions

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Abstract: The photobiont-containing ciliate, *Paramecium bursaria*, was cultured for a week under three treatments: 25 °C with light (25 L), 10 °C without light (10 D), and 10 °C with light (10 L). Cells of two strains under the 10 D treatment were enlarged relative to those under the 25 L treatment. This effect was very pronounced in that the length of enlarged cells was on average 130–140% of that of normal cells, and also reversible, as cells showed the opposite pattern within a week after switching treatments. In addition, many achromic drops were observed in cells cultured under the 10 D treatment. These drops are probably lipids produced by the photobiont, *Chlorella variabilis*.

Key words: cell size; *Chlorella variabilis*; lipid; *Paramecium bursaria*; winter

Introduction

Ciliates are an important component of aquatic ecosystems. They are both predators of bacteria, algae and other protozoa, and prey for higher protozoa, invertebrates and small vertebrate larvae (Finlay & Esteban 1998; Sherr & Sherr 2002; Tirok & Gaedke 2006). The biomass of ciliates in an ecosystem varies seasonally, possibly in response to changes in prey biomass. It also decreases dramatically during cold winters (e.g., Mironova et al. 2012), although this relationship is not yet fully understood.

Many ciliate species have small green coccoidal photobionts living within their cells. It is not known whether all of these photobionts provide photosynthates to their host species, but this probably differs between species (Reisser 1984; Reisser & Widowski 1992; Hoshina et al. 2013). The green ciliate *Paramecium bursaria* is one of the best-studied protists because of its observable endosymbiosis. The photobiont species found in *P. bursaria* are almost exclusively either *Chlorella variabilis* or *Micractinium reisseri* (Chlorellaceae, Trebouxiophyceae) (Hoshina et al. 2010; Pröschold et al. 2011, and references therein). When isolated from *P. bursaria* cells in a laboratory, these two algal species excrete at least half of their photosynthate (maltose), as calculated from total carbon fixation (e.g., Reisser 1984; Kessler et al. 1991; Kamako & Imamura 2006). It is assumed that the host absorbs these photosynthates when they are inside *P. bursaria* cells.

One aspect of the biology of these symbiotic ciliates that is yet to be understood is how they survive

cold winter temperatures. Experience shows that the photobiont-containing ciliates, including *P. bursaria*, are hardly collected in winter. It is interesting how such a photobiont-containing ciliate survive during cold winter days. In a set of experiments on *P. bursaria* to be exposed to low temperature, some strange phenomena have been found. The present study introduces the size shift of *P. bursaria* cells and the accumulation of achromic drops under artificial winter conditions.

Material and methods

The three *P. bursaria* strains used were collected from the USA (Florida and Washington) and Japan. Preliminary DNA inspections showed that *C. variabilis* was the photobiont in all three strains (for detailed methodology, see Hoshina et al. 2010). The culture medium was 1.25% fresh lettuce juice in modified Dryl's solution (MDS) (Dryl 1959) (KH₂PO₄ was used instead of Na₂H₂PO₄ · 2H₂O). This was inoculated with a nonpathogenic strain of *Klebsiella pneumoniae* one day before use (Hiwatashi 1968). In ordinary cultures, several hundred cells were inoculated into 2 ml of culture medium. Afterwards 4 ml of fresh culture medium was added every second day. The cultures were maintained at 25 °C under artificial light (1000–1500 lux; 25 L).

Clone cultures were created by isolating single *P. bursaria* cells under a depression slide and maintaining them for four weeks in the same conditions as the ordinary culture. Two clone cultures were created for each strain. Each clone culture was divided into several test tubes and cultured under three treatments: 25 L, 10 °C in the dark (10 D), and 10 °C with light (10 L; Fig. 1). In all cases cultures were fed with *K. pneumoniae* every second day. After seven days, *P. bursaria* cells were retrieved by condensation using a manual centrifuge. The cell-liquid was pipetted onto a micro-

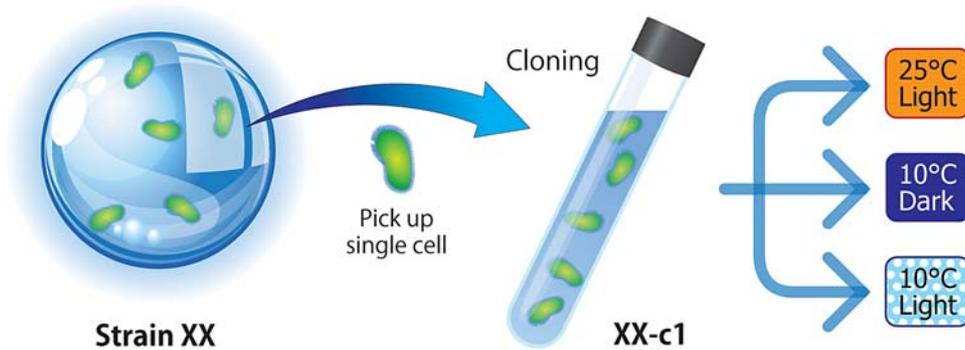


Fig. 1. Schematic diagram of the experiment conducted to investigate sizes of *Paramecium bursaria* cells cultured under different light and temperature conditions. Cells of three strains were cultured at 25°C under artificial light (1000–1500 lux). Single cells were isolated and cultured to produce two clone cultures for each strain. Each clone was separated into three groups, which were subjected to different treatments for seven days. The three treatments were 25°C with light, 10°C without light and 10°C with light.

scope slide and then dried using an air dryer. In this way cell burst and deformation was avoided. Cell lengths of *P. bursaria* were measured using cellSens software (Olympus, Tokyo) with an Olympus BX60 microscope. Cells undergoing division were removed to be measured separately. The variance of cell size was analyzed by One-Way ANOVA via VassarStats (<http://vassarstats.net/index.html/>). This program also performed pair-wise comparisons of sample means via the Tukey's HSD test. All other observations were conducted on cells before they were desiccated.

Results and discussion

The first clone of the Florida strain (FL-c1) under the 25 L treatment had cell lengths of 106–168 μm ($n = 47$, mean 139.8, SD 16.4; Fig. 2). Under the 10 D treatment, however, FL-c1 cells were 142–228 μm long ($n = 78$, mean 190.0, SD 18.2). The second clone of this strain (FL-c2) showed the same pattern. FL-c2 cells cultured under the 25 L treatment had lengths of 94–167 μm ($n = 80$, mean 131.2, SD 17.3), but lengths of 121–241 μm ($n = 83$, mean 186.3, SD 22.8) under the 10 D treatment. There were very pronounced differences between treatments, to the extent that the ranges defined by mean \pm SD did not overlap (Fig. 3). These results did not change if cultures were allowed to remain under the same conditions for a second week (data not shown). Although only cell length was measured, overall proportions of the enlarged cells were similar to those cultured under the 25 L treatment. These changes in size were found to be temporary and reversible; when 25 L and 10 D treatments were switched and cells were measured after one further week, cell size distributions were reversed (data not shown).

Paramecium bursaria multiplies by simple cell division. Predivisional cells are therefore generally larger or longer than normal cells, and newly divided cells are smaller. Cells undergoing division comprised approximately 1–5% of each experimental culture. In the Florida strain, cells under the 25 L treatment divided when they were \sim 150 μm in length. Under the 10 D treatment cells did not divide until they were \sim 200 μm

in length. Cell division timing therefore changed based on ambient conditions.

Cells of the Florida strain were cultured under two additional treatments: 10 L and 25°C without light (25 D; data not shown). FL-c2 cells under the 10 L treatment were slightly larger than those under the 25 L treatment ($n = 59$, mean 154.8, SD 14.4; Fig. 2). Although Tukey's HSD test showed significant differences ($P < 0.01$) between 25 L and 10 L cells (Supplementary file), these distributions overlapped (Fig. 3). Cells under the 25 D treatment were the same size as those under the 25 L treatment. Thus only the combination of cold temperatures and absence of light produced the observed strong effect on cell size.

The Washington strain showed a similar tendency to the Florida strain. Although these cells were smaller, there was also a clear difference between treatments. Cells cultured under the 25 L treatment had lengths of 90–120 μm and 90–110 μm for each clone, and those under the 10 D treatment had lengths of 130–160 μm and 120–150 μm respectively (Figs 2, 3). Results from the 10 L treatment were also similar to those for the Florida strain, i.e., HSD test showed a significant difference ($P < 0.01$, Supplementary file), but they overlapped with those from the 25 L treatment (Fig. 3).

The Japanese strain, however, did not show the same clear separation. Cells under the 10 D treatment were only slightly longer than those under the 25 L treatment (Fig. 2). Although the variances kept HSD difference barely alive ($P < 0.01$, Supplementary file), the two size distributions overlapped to a large extent (Fig. 3). It appears therefore that the phenomenon of *P. bursaria* cells growing larger under cold, dark conditions varies between strains and/or regions. There also remains much uncertainty concerning the function of this phenomenon and the mechanisms by which it operates.

For the description of ciliate species, the body length is usually an important element. In some cases, the body length becomes a key component of species identification. For example, two similarly-shaped *Paramecium* species, *P. caudatum* and *P. aurelia* are sympatric in eutrophic water bodies. Textbooks

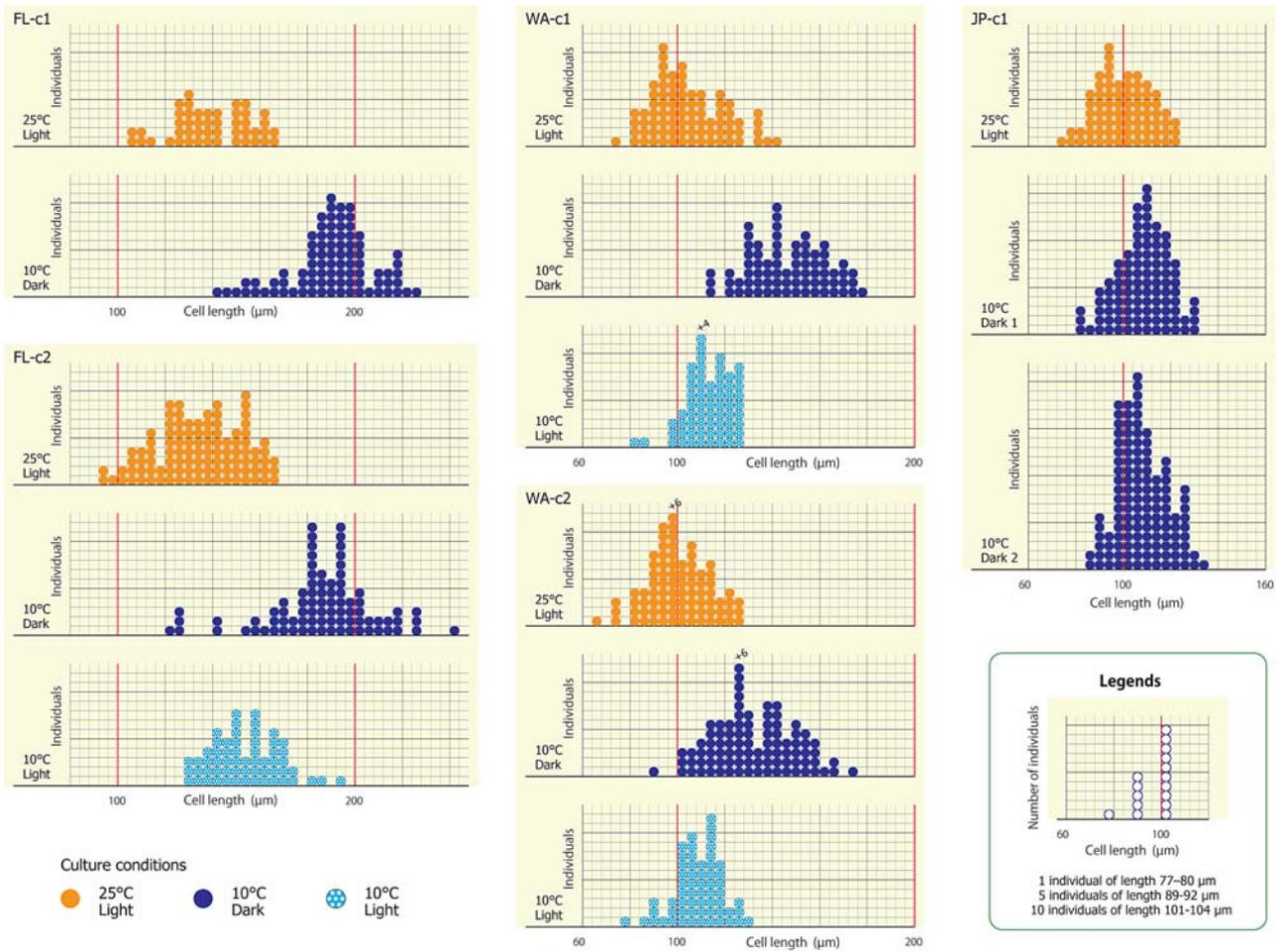


Fig. 2. Size distribution of *Paramecium bursaria* individuals after a week under one of three treatments: 25°C with light, 10°C without light and 10°C with light (see text for details). Each set of graphs presents results for one of two clone cultures (c1 or c2) of one of three strains (Florida – FL, Washington – WA, Japan – JA), as indicated in the top left corner of each set. Horizontal axes indicate the length of the major axis of *P. bursaria* cells. Longitudinal axes show the number of individuals.

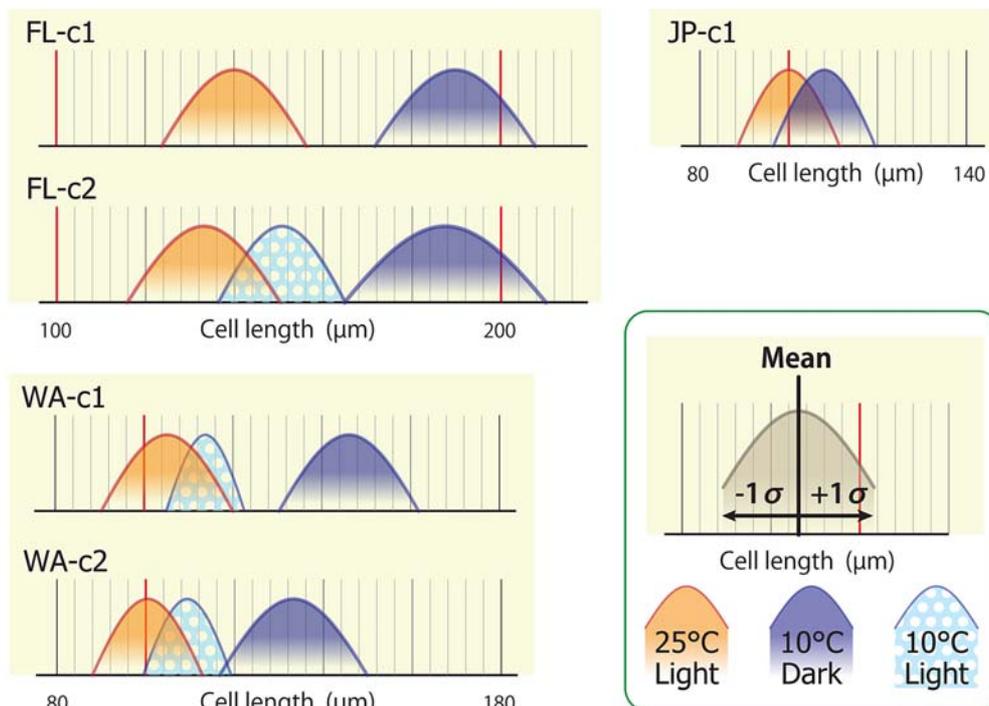
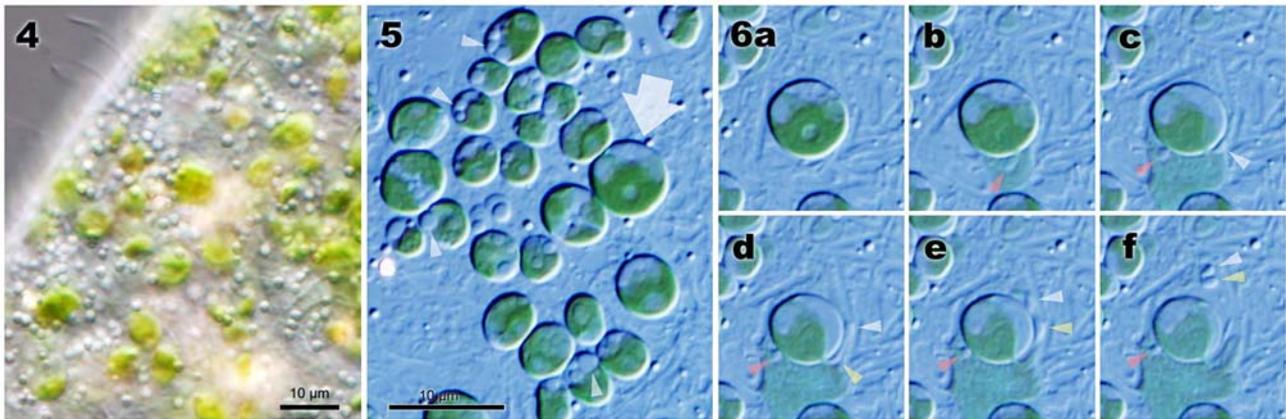


Fig. 3. *Paramecium bursaria* cell length distributions for one or two clone cultures (c1 or c2) of each of three strains (Florida – FL, Washington – WA, Japan – JA). Each curve shows the mean ± SD.



Figs 4–6. 4: *Paramecium bursaria* cell from the second clone culture of the Florida strain, cultured under the 10°C without light treatment. Many achromic drops are visible. 5: Photobiont cells from a disrupted *P. bursaria* cell. A few achromic drops were found in each cell (indicated by arrowheads). The cell indicated by the large arrow was photographed as it leaked drops (Fig. 6). 6: Images captured from a video showing drop leakage from the disrupted photobiont cell. Achromic drops are indicated by arrow heads.

indicate that longer cells would be *P. caudatum*, and shorter cells would be *P. aurelia* (e.g., 170 µm as the border, Shiga Study Committee for Science Teaching Materials 2008). Furthermore, some species of *Spirostomum* (heterotrichous ciliate) can only be distinguished by cell lengths (Kudo 1966). The phenomenon shown in this study, however, indicates that the body length varies considerably from situation to situation, and the risk of easily relying on body length to identify the species.

Another phenomenon that emerged was that cells cultured under the 10 D treatment generally contained large numbers of achromic drops (Fig. 4). I attempted to stain these cells with Nile Red, which fluoresces yellow in a neutral lipid environment (Cooksey et al. 1987; Kimura et al. 1987). Although the drops appeared yellowish under a microscope, they could not be photographed because of the excessive intrinsic fluorescence of the chloroplast. Although the origin of these drops was not obvious, similar drops were observed inside photobiont cells (Fig. 5). When a photobiont cell was disrupted, these achromic drops exuded from the damaged cell wall, initially amorphous and then immediately becoming spherical (Fig. 6). In other words, these drops appeared to be liquid rather than granular. *Chlorella* species (including the photobiont, *C. variabilis*) generally produce starch to store photosynthates (Reisser 1987, 1988; Luo et al. 2010). However, recent studies show that *Chlorella* algae also produce lipids or oils prolifically under certain conditions (Přibyl et al. 2012; Chen et al. 2013; Menon et al. 2013). It is therefore likely that the achromic drops observed in the present study were lipids and that photobionts may produce large quantities of lipids under cold, dark conditions. The host *P. bursaria* cells were not in a starvation state, and the drops were observed in both enlarged and unchanged *P. bursaria* groups. This effect therefore appears to be linked to external conditions rather than the condition of the host cells.

Acknowledgements

This work was supported by The Showa Seitoku Memorial Foundation, The Institute for Fermentation, Osaka, and JSPS KAKENHI (25840141).

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Received September 20, 2013

Accepted May 14, 2014