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Gravity-Dependent Changes in Bioconvection of *Tetrahymena* and *Chlamydomonas* during Parabolic Flight: Increases in Wave Number Induced by Pre- and Post-Parabola Hypergravity

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Bioconvection emerges in a dense suspension of swimming protists as a consequence of their negative-gravitactic upward migration and later settling as a blob of density greater than that of water. Thus, gravity is an important parameter governing bioconvective pattern formation. However, inconsistencies are found in previous studies dealing with the response of bioconvection patterns to increased gravity acceleration (hypergravity); the wave number of the patterns has been reported to decrease during the hypergravity phases of parabolic aircraft flight, while it increases in centrifugal hypergravity. In this paper, we reassess the responses of bioconvection to altered gravity during parabolic flight on the basis of vertical and horizontal observations of the patterns formed by *Tetrahymena thermophila* and *Chlamydomonas reinhardtii*. Spatiotemporal analyses of the horizontal patterns revealed an increase in the pattern wave number in both pre- and post-parabola hypergravity. Vertical pattern analysis was generally in line with the horizontal pattern analysis, and further revealed that hypergravity-induced changes preceded at the top layer of the suspensions while microgravity-induced changes appeared to occur from the bottom part of the settling blobs. The responses to altered gravity were rather different between the two sample species: *T. thermophila* tended to drastically modify its bioconvection patterns in response to changes in gravity level, while the patterns of *C. reinhardtii* responded to a much lesser extent. This difference can be attributed to the distinct physical and physiological properties of the individual organisms, suggesting a significant contribution of the gyrotactic property to the swimming behavior of some protists.

Key words: bioconvection, *Tetrahymena*, *Chlamydomonas*, microgravity, hypergravity, wave number

INTRODUCTION

Bioconvection is a collective phenomenon that arises from interactions between population of microorganisms and terrestrial gravity (Platt, 1961; Wager, 1911). Regular patterns spontaneously emerge in the suspension of some swimming microorganisms, even if initially the suspension is completely homogenous. Protists, particularly the ciliate *Tetrahymena* and the flagellates *Euglena*, *Polytomella*, and *Chlamydomonas*, have been extensively used for experimental studies on bioconvection (Wager, 1911; Robbins, 1952; Loefer and Mefferd, 1952; Gittleson and Jahn, 1968; Levandowsky et al., 1975; Fornshell, 1978; Kessler, 1985a; Yamamoto et al., 1992; Bees and Hill, 1997; Mogami et al., 2004; Akiyama et al., 2005; Nguyen-Quang et al., 2009). Negative gravitaxis, i.e., preferential swimming against the direction of the gravity vector even when the organism's mass density exceeds that of water, is considered one of the major driving forces of bioconvective behavior of such protists.

The pattern formation progresses roughly as follows. First, negative gravitaxis makes the protists accumulate beneath the water surface to form a concentrated layer. Because they are heavier than water, the organisms then locally sink as plumes or "settling blobs," becoming too concentrated to stay there any longer. As a result, two-directional convective flows occur; an upward flow due to negatively-gravitactic swimming, and a downward settling flow due to gravity. This convective motion leads to organization of characteristic three-dimensional patterns, which resemble stripes, dots, or polygons of various sizes when viewed from above, depending on the concentration of the suspension, the depth and the shape of the vessels, the time course, and other physical and physiological parameters (Wager, 1911; Yamamoto et al., 1992; Akiyama et al.,

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2005). Childress et al. (1975) have proposed a hydrodynamic model for the onset of bioconvection based on the above process, which is referred to as "density-instability model" (Mogami et al., 2004), attributing the occurrence of the convective current to the inverted density stratification caused by negative gravitaxis.

A different swimming property of an organism can result in the proposal of alternative theories. Kessler (1985a, 1985b, 1986) reported "gyrotaxis", biased behavior in a shear flow of some swimming algae that have a heterogeneous mass distribution within a cell. Bottom-heavy, unicellular motile algae such as *Chlamydomonas*, *Dunaliella* and *Carteria*, tend to be pulled into the center of the downward stream as a result of the counteraction of hydrodynamic torque due to the spatial variation of the fluid velocity (vorticity) with the orientation torque generated by their bottom-heaviness. He presented a theory, which here we call the "gyrotactic-instability model" (Mogami et al., 2004), that assumes that these swimming characteristics contribute to the onset of bioconvection (Kessler, 1985b, 1986). According to Kessler (1986), the emergence of bioconvection patterns in such bi- or quadriflagellated algae can take place even if the initial density instability is not sufficient to make the organisms fall. The local gradient of flow rate makes the algae swim up the gradient, and, consequently, a plume appears in the collected cells. Meanwhile, ciliates such as *Tetrahymena* do not show clear gyrotactic behavior, suggesting that the gyrotactic instability model does not apply to the case of ciliates (Kessler, 1985a, 1986). In fact, Mogami et al. (2004) experimentally demonstrated that the onset of bioconvection of *Tetrahymena* cannot be explained by the gyrotactic instability model. They also showed that different species and behavioral mutants of the genus *Tetrahymena* form slightly different bioconvective patterns, a piece of experimental evidence that individual swimming behavior might affect the occurring bioconvection.

Although the gyrotactic-instability model claims that the gravitactic accumulation of cells is not essential for bioconvection, in actual experimental systems, the density instability and the gyrotactic instability may cooperate to initiate bioconvection (Kessler, 1985a; Mogami et al., 2004). Gravity allows bioconvection to emerge as it exerts control over the underlying behavior of individuals. Despite this significance of gravity, both experimental and theoretical studies have paid little attention to gravity.

Only a few studies have been published concerning gravity as an experimental parameter, and their results are in conflict. Noever (1991) reported on an experiment of altered gravity using parabolic flight of an aircraft, where the size of polygonal patterns in bioconvection of *Polytomella parva* and *Tetrahymena pyriformis* increased in hypergravity (1.8–2 ***g***) phases. In contrast, the results of two independent hypergravity experiments (Itoh et al., 1999; Mogami et al., 2004), both of which used a centrifuge as a means of obtaining hypergravity and *Tetrahymena* as a material, conflicted with the hypergravity results of Noever (1991). In these recent studies, wavelengths of the steady-state bioconvection patterns became smaller in hypergravity. This disagreement may derive from a difference in the method of increasing the magnitude of gravity; parabolic flight has a rapid profile of gravity changes and each micro- or hypergravity phase lasts for only about 20 seconds, whereas a centrifuge takes minutes to accelerate or decelerate. It may also result from other factors lurking in the experimental procedures or, most likely, from interpretations of the results.

From these points of view, more precise recording, description, and particularly interspecific comparison of the response of bioconvection to altered gravity should be carried out. Samples are preferably representatives of the two theoretical models mentioned above, because such comparison is expected to shed light on biological aspects of bioconvection and also to bridge the gap between the theoretical and experimental studies.

We conducted parabolic flight experiments, using *Tetrahymena thermophila* (representative of the density-instability model) and *Chlamydomonas reinhardtii* (representative of the gyrotactic-instability model) as samples. Two types of observations were made. The first is the top-view, or horizontal pattern observation, which is the same as that used in all of the previous three altered gravity experiments (Noever, 1991; Itoh et al., 1999; Mogami et al., 2004). The other is the side-view, or vertical pattern observation, in which we can directly observe the settling blobs of microorganisms.

Our findings from the horizontal pattern observations are consistent with those from the previous two centrifugal experiments (Itoh et al., 1999; Mogami et al., 2004), but not with those obtained in the parabolic flight-induced hypergravity (Noever, 1991). Findings obtained from the vertical pattern observation support those from the horizontal ones, and reveal some important facts about responses of bioconvection to altered gravity. In both horizontal and vertical pattern observations, *T. thermophila* and *C. reinhardtii* showed rather different responses from each other, suggesting some interspecific differences in responses of the bioconvection patterns probably based on the distinct swimming behavior of the microorganisms.

MATERIALS AND METHODS

Cell culture

Tetrahymena thermophila (strain wild II) was axenically cultivated in a shaker at a rate of ca. 80 rpm at 24°C in a medium containing 2% (w/v) proteose-peptone, 1% yeast extract, and 0.8% glucose as described by Watanabe (1963). Cells at the late-log to the early stationary phases of density $> 1 \times 10^6$ cells·ml^{-1} were used (Mogami et al., 2004). *Chlamydomonas reinhardtii* (wild type, strain 137 c, mt-) was kept aerated in an autoclaved tris-acetate-phosphate (TAP) medium (Gorman and Levine, 1965) under an illumination cycle of 12 h light (5×10^3 lux) and 12 h dark at 24 or 25°C. Cells at the early stationary phases were used (Akiyama et al., 2005).

To calculate population density, cells were fixed in 5% formaldehyde containing 0.01% brilliant green and counted on a Fuchs-Rosenthal hemocytometer (Watanabe, 1963). Before each flight, the suspension was diluted with a fresh culture medium to a density of ~1.0×10^6 cells·ml^{-1} for *T. thermophila*, and ~1.0×10^7 cells·ml^{-1} for *C. reinhardtii* (Mogami et al., 2004; Akiyama et al., 2005). Both species were kept gently aerated until injection into the experimental chamber.

Parabolic flight

Variable gravity was induced using an aircraft, MU-300 or G-II, belonging to the Diamond Air Service Incorporation (Toyoyama, Aichi, Japan; http://www.das.co.jp/new_html_e/index-static.html). When an aircraft flies in a parabolic trajectory, the objects inside the

aircraft are exposed to changes in gravity. One parabola trial includes sequential changes in gravity as follows: (1) pre-parabola hypogravity (ca. 0.5 ***g***), (2) pre-parabola hypergravity (ca. 2 ***g***), (3) microgravity (ca. 0.01 ***g***) and (4) post-parabola hypergravity (ca. 1.5 ***g***); each phase lasts about 20 seconds. A typical one-hour flight includes about 10–15 parabolas.

Three series of flights were conducted in November 2006, March 2008 and March 2009. Each series included four or five flights. Experiments were carried out in cabin air at a controlled temperature of 20–25°C.

Data recordings

For the horizontal pattern observations, we used a plastic circular chamber with an inner diameter of 96 mm. A flat glass lid was placed inside the chamber and fixed with small 4-mm-thick plastic spacers at the periphery of chamber to give a constant depth. The vertical pattern observations were conducted with a narrow chamber made of two sheets of slide glass (76 mm × 26 mm) separated by a silicone rubber sheet spacer (2- or 1-mm-thick for *T. thermophila*, and 1-mm-thick for *C. reinhardtii*), which left a 4-mm-deep narrow well for the formation of bioconvection patterns when placed with the glass surface vertical. Several minutes before the first parabola trial (i.e., under 1 ***g*** conditions), the cell suspension was transferred with a peristaltic pump through a silicone tube into either the horizontal or the vertical chamber. This early transfer allowed the bioconvection to reach a steady state well before the aircraft started to follow a parabolic trajectory (i.e., before the principal changes in gravity started). After several parabola trials, the pattern formation capability in 1 ***g*** somewhat declined as seen in Noever (1991), particularly in *T. thermophila*. Upon this decline, we restarted experiments with new specimens transferred into the same chamber.

For video recordings, we used a high-definition camcorder (HDR-HC1, SONY, Tokyo, Japan), with which we can obtain images of 1920 pixels × 1080 pixels at a rate of 30 fps. For quantitative analyses, the images were converted to a stack of image files, each of which consists of 1920 pixels × 1080 pixels on a 256-level gray scale.

The patterns of *T. thermophila* were recorded under dark-field illumination. For the horizontal pattern observation, a circular fluorescent bulb with a heat absorption filter was provided. For the vertical pattern observation, two parallel-arranged cold cathode fluorescent lamps were used. The bulbs were operated at > 20 kHz in order to avoid fluctuations of image brightness between frames (Mogami et al., 2004).

The patterns of *C. reinhardtii* were recorded under bright-field illumination. The horizontal patterns were recorded using a flat light viewer (MedaLight LP-100, Minato Shokai Co. Ltd., Yokohama, Japan) as a light source, which was placed under the chamber to give homogeneous illumination of constant intensity (Akiyama et al., 2005). For the vertical observations, a smaller flat light viewer was used (Hakuba Handy-viewer M35, Hakuba Photo Industry Co. Ltd., Tokyo, Japan), which was placed on the backside of the chamber. Specimens were illuminated with red light through sharp-cut filters (Fuji Photo Film Co. Ltd., Tokyo, Japan), SC 62 (λ > 620 nm) for horizontal pattern observations and SC 64 (λ > 640 nm) for vertical pattern observations, as *C. reinhardtii* is known to show no behavioral response to that red light (Matsuda et al., 1998).

Three components of the acceleration vector, fore-aft (x), lateral (y), and vertical (z), were monitored during flight by an onboard sensor on the aircraft. Changes in the z component (i.e. gravity acceleration) were converted to the light intensity of an LED, which was superimposed in the recording frame. The horizontal chambers were placed flat in the x-y plane and the patterns formed in the chamber were recorded as viewed from above (top view). The vertical chambers were placed in the x-z or y-z plane, and the patterns formed in the chamber were recorded as viewed from the side (side view). No difference in pattern formation was observed between the samples placed in the x-z and the y-z plane, and therefore we will treat them as data obtained under the same conditions.

Image analyses

For each image of the horizontal observations, two-dimensional discrete Fast Fourier Transform (2D-FFT) was carried out for the quantitative evaluation of an average spacing of the bioconvection pattern, following the method described by Mogami et al. (2004). The 2D-FFT analysis was conducted for an area of either 512 × 512 or 1024 × 1024 pixels in a given part of the recorded images. The dominant wave number, i.e., the reciprocal of a mean spacing of the pattern, was obtained by fitting a Gaussian distribution function to the radial spectral density calculated (Bees and Hill, 1997; Mogami et al., 2004). In essence, the greater the spacing, the smaller the dominant wave number. In the following, "wave number" refers to this dominant wave number calculated by the above procedure.

Space-time plots were made by the "digital slit camera method" (Mogami et al., 2004), in which a thin rectangle area was cut out of images at a fixed position forming a single image which has space and time dimensions when reassembled by sequential placement.

RESULTS

Horizontal pattern analysis

Both *Tetrahymena thermophila* and *Chlamydomonas reinhardtii* formed bioconvection patterns within several minutes after transfer to the recording chambers. The patterns developed to the steady state in the 1 ***g*** environment, and then the aircraft started parabolic flight. The time course and the morphology of the steady-state patterns were similar to those observed in the laboratory. As described by Akiyama et al. (2005), the patterns of *T. thermophila* showed horizontal movement, while those of *C. reinhardtii* appeared rather static.

Figs. 1 and 2 show the plan views obtained from the horizontal pattern observation; a typical response of bioconvection patterns of *T. thermophila* and *C. reinhardtii*, respectively, to altered gravity in a parabola trial. The high-density regions of cells accumulating and moving downwards as settling blobs are seen as bright (Fig. 1) and dark (Fig. 2) patches under dark- and bright-field illuminations, respectively. Under 1 ***g*** conditions, *T. thermophila* formed either dotted or polygonal steady-state patterns (Fig. 1A). The patterns became finer in hypergravity (ca. 2 ***g***; Fig. 1B), vanished in microgravity (Fig. 1C), and recovered their initial morphology in 1 ***g*** after the parabola (Fig. 1D). *C. reinhardtii* formed regular polygonal patterns (Akiyama et al., 2005) at the steady state in 1 ***g*** (Fig. 2A). In marked contrast to *T. thermophila*, the *Chlamydomonas* patterns barely changed their wave number in response to changes in gravity. The bioconvection pattern became sharp-edged in hypergravity (Fig. 2B) and obscured in microgravity (Fig. 2C). After the parabola, they recovered their original morphology, as in *T. thermophila* (Fig. 2D).

The dominant wave number determined by the two-dimensional Fast Fourier Transform (2D-FFT) analysis of the bioconvection pattern of *T. thermophila* quantifies the changes in wavelength mentioned above. As shown in Fig. 3, the wave number increased in hypergravity and decreased rapidly, becoming undefined (i.e., no pattern could be detected) in microgravity. It increased again in hypergravity during the pull-up flight to escape from the parabolic trajectory. A comparison of these two classes of hypergravity

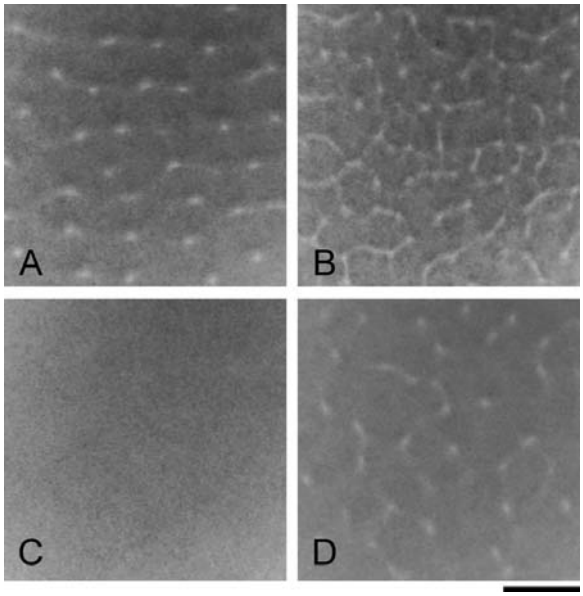

Fig. 1. Plan views of the horizontal bioconvection pattern of *T. thermophila* in altered gravity during parabolic flight (top-view). Patterns in 1 ***g*** before a parabola trial **(A)**, in pre-parabola hypergravity **(B)**, in microgravity **(C)**, and in 1 ***g*** after the parabola **(D)** are shown. Times of A to D are indicated by arrows at the top in Fig. 3. Scale bar: 10 mm.

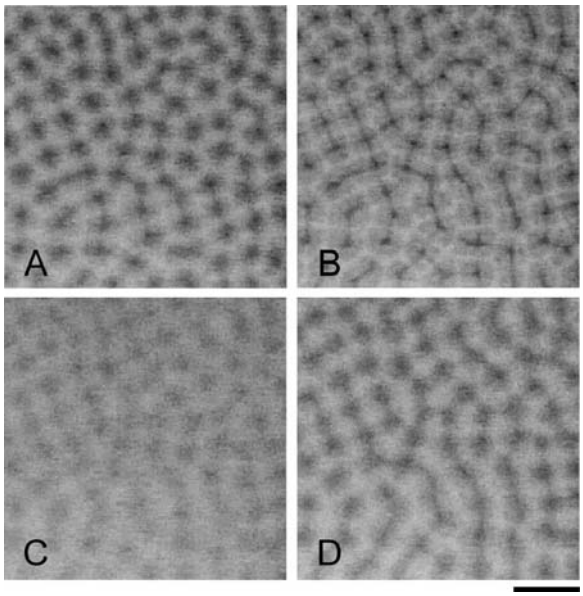

Fig. 2. Plan views of the horizontal bioconvection pattern of *C. reinhardtii* in altered gravity during parabolic flight (top-view). Patterns in 1 ***g*** before a parabola trial **(A)**, in pre-parabola hypergravity **(B)**, in microgravity **(C)**, and in 1 ***g*** after the parabola **(D)** are shown. Times of A to D are indicated by arrows at the top in Fig. 4. Scale bar: 10 mm.

phases revealed that, on average, the wave number was smaller in the post-parabola hypergravity phase than in the pre-parabola hypergravity phase. In addition, the 2D-FFT analysis demonstrated a slow, small decrease in wave number in hypogravity (ca. 0.7 ***g***) just prior to a parabola.

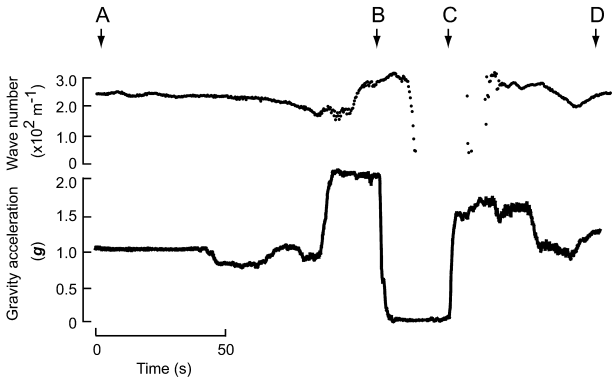

Fig. 3. 2D-FFT of the response of the horizontal bioconvection pattern of *T. thermophila* to altered gravity during parabolic flight. Dominant wave number (upper part) is shown with the profile of the change of gravity acceleration (lower part), with the common time axis shown at the bottom of the figure. Arrows A to D represent the time of the plan views shown in Fig. 1.

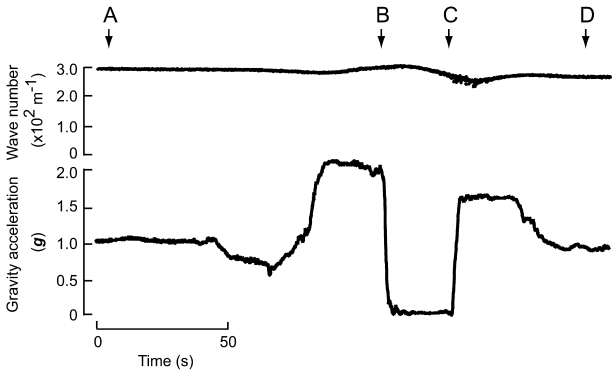

Fig. 4. 2D-FFT of the response of the horizontal bioconvection pattern of *C. reinhardtii* to altered gravity during parabolic flight. Dominant wave number (upper part) is shown with the profile of the change of gravity acceleration (lower part), with the common time axis shown at the bottom of the figure. Arrows A to D represent the time of the plan views shown in Fig. 2.

The 2D-FFT analysis of the bioconvection pattern of *C. reinhardtii* revealed that the wave number increased slightly with increases in gravity, and decreased in micro- and pre-parabola hypogravity (Fig. 4). As the gravity level rose, the wave number increased slightly. This change implies that bioconvection patterns of *C. reinhardtii* responded to altered gravity in a similar way to *T. thermophila*, although to a much smaller extent.

It should be noted that the gravity-dependent changes of the patterns, and thus changes of the wave number, occurred with a clear delay of 10 to 20 s both in *T. thermophila* and in *C. reinhardtii* (Figs. 3 and 4). Fig. 5 shows the mean values of the delayed response of the wave number to altered gravity. The data clearly demonstrate that the wave number of the bioconvection patterns of *T. thermophila* increases, and thus the wavelength decreases with increasing gravity and vice versa, and that a similar but less marked tendency is seen in *C. reinhardtii* as well.

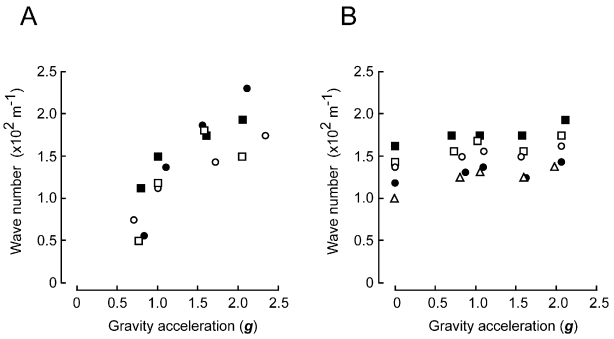

Fig. 5. Plots of the mean values of the wave number in the responses of *T. thermophila* **(A)** and *C. reinhardtii* **(B)** to altered gravity obtained from the horizontal pattern analysis. Wave numbers found after a delay of 10 to 20 s are plotted against the corresponding gravity acceleration. Individual sets of different symbols are from measurements taken during respective parabolic flights.

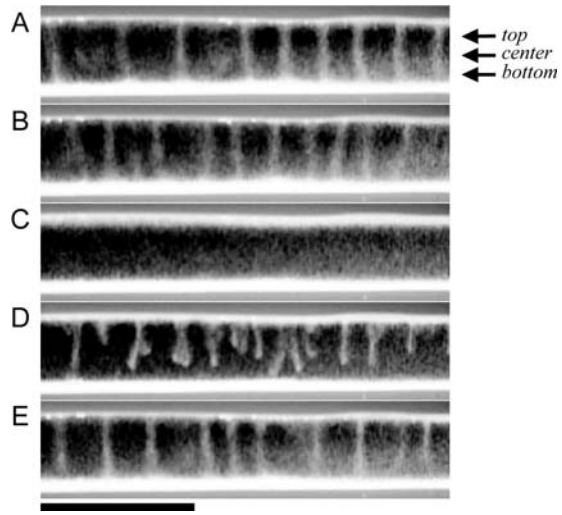

Fig. 6. Sequential images of the vertical bioconvection pattern of *T. thermophila* in altered gravity during parabolic flight. Patterns in 1 ***g*** before a parabola trial **(A)**, in pre-parabola hypergravity **(B)**, in microgravity **(C)**, in post-parabola hypergravity **(D)**, and in 1 ***g*** after the parabola trial **(E)** are shown. Times of A to E are indicated by arrows at the side in Fig. 7. Arrows beside A correspond the positions (*top*, *center*, and *bottom*) for the space-time plot analysis shown in Fig. 7. Scale bar: 10 mm.

Vertical pattern analysis

When viewed from the side, the bioconvection is seen as columns or "settling blobs" of cells that have once accumulated densely at the top of the chamber by negative-gravitactic swimming. As shown in Figs. 6 and 8, the settling blobs of *T. thermophila* and *C. reinhardtii* are seen as bright and dark accumulations of cells under dark- and bright-field illuminations, respectively.

Fig. 6 shows a typical response of the vertical patterns of *T. thermophila* to altered gravity during a parabola. Settling blobs were formed steadily at 1 ***g*** (Fig. 6A). They increased in number in hypergravity (ca. 2 ***g***; Fig. 6B), disappeared in microgravity (Fig. 6C) and recovered their initial appearance after the parabola (Fig. 6E). The disappearance of blobs in microgravity initially occurred near the bottom of the chamber and gradually propagated to the top. This was followed by an increase in the width of the bright region at the top of the chamber (Fig. 6C), which may indicate the accumulation of the cells swimming out of the blobs. After the parabola, blobs reappeared from the top, descending toward the bottom; i.e., the accumulation of cells again settled downwards. Fig. 7 quantifies the changes of the settling blobs.

In the upper part of Fig. 7A, temporal changes in the settling blobs are shown by the space-time plots made at different positions of the chamber (*top*, *center*, and *bottom*; indicated by arrows in Fig. 6A). The bright vertical stripes in the space-time plots correspond to the settling blobs. They disappeared first at *bottom*, then at *center* and finally at *top*. The stripes reappeared in the reverse order. Changes in the number of the settling blobs (Fig. 7, middle) delayed for 10–20 s from the changes in gravity. The disappearance of blobs in microgravity had delays longer than the reappearance in hypergravity.

Fig. 8 shows a typical response of the vertical patterns of *C. reinhardtii* to altered gravity during a parabola. In 1 ***g***, the vertical patterns of *C. reinhardtii* seldom changed their shape and position after being fully developed (Fig. 8A). These settling blobs became bottle-shaped in pre-parabola hypergravity (ca. 2 ***g***; Fig. 8B), and obscured in microgravity (Fig. 8C). Under microgravity conditions, some of the blobs vanished almost completely (Fig. 8C left); others remained vague (Fig. 8C right). After the parabola, the remaining blobs maintained their position, while the traces of the vanished ones were not always resurrected (Fig. 8D, E). Narrower necks of the "bottles" in hypergravity may correspond to the sharpened pattern in the horizontal recordings. Fig. 9 shows the space-time plots taken from three different positions (arrows in Fig. 8). In the pre-microgravity hypergravity phase, the dark vertical stripes become narrower at *top* and at *center*; and wider and denser at *bottom*. After the microgravity phase started, all of the stripes gradually became obscure.

DISCUSSION

In the present study, we demonstrated gravity-dependent responses of bioconvection of *Tetrahymena thermophila* and *Chlamydomonas reinhardtii* to altered gravity experienced in the course of entering, maintaining, and leaving parabolic flight. The responses were analyzed on the basis of horizontal and vertical pattern observations. During both the pre- and the post-parabola hypergravity phases, the horizontal patterns of *T. thermophila* increased in wave number and thus decreased in wavelength, while *C. reinhardtii* was observed to sharpen the polygonal pattern with a subtle change in wave number. In microgravity, the patterns of *T. thermophila* entirely vanished, while those of *C. reinhardtii* only became obscured or diffused, leaving some traces of patterns visible, also with a little change in wave number. In both species, post-parabola hypergravity induced a rapid, but slightly delayed, recovery of the bioconvection patterns. The vertical pattern analysis generally supported the horizontal analysis. It revealed further that hypergravity-induced changes preceded at the top layer while microgravity-induced changes appeared to occur from the bottom part of

the settling blobs.

Bioconvection is a result of the collective motion of aquatic microorganisms upon gathering at the top layer of a water column as a result of negative gravitaxis. When the heterogeneity due to this accumulation increases up to a certain extent, the spatial patterns of density difference emerge in the suspension through collective interaction between the organisms under the influence of terrestrial gravity. Although gravity is a crucial factor for bioconvection pattern formation, the effect of gravity on the spatial characteristics of the bioconvection patterns had remained to be established. Conflicting experimental results have been reported on the pattern wave number in hypergravity. Hypergravity induced by centrifugation caused an increase in wave number of the horizontal patterns (Itoh et al., 1999; Mogami et al., 2004). In constrast, the patterns were observed to decrease in wave number during the hypergravity phases of parabolic flight (Noever, 1991).

The results of the present parabolic flight experiments are consistent with the results of the centrifuge experiments; i.e. patterns became greater in wave number in hypergravity. Since the specimens used in the present study (particularly *Tetrahymena*) were prepared by the same procedures as those in the centrifuge experiments of Mogami et al. (2004), it is evident that the increase in the wave number is induced by hypergravity irrespective of the method of increasing gravity (i.e., centrifuge *vs*. parabolic flight). Therefore, the discrepancy between the previous hypergravity experiments described above cannot be explained solely by differences in the methods of generating hypergravity. Although we have no direct evidence that can resolve the discrepancy between the results of Noever's experiments and ours, it should be noted that changes in pattern wave number always occur with a substantial delay from alteration of the gravity acceleration. This latency of the response may lead to misinterpretation of the relationship between the phases of altered gravity and the responses of the bioconvection.

We found that the responses of the bioconvection to altered gravity were different between species of microorganisms. The patterns of *T. thermophila* were more sensitive to the changes in gravity than those of *C. reinhardtii*. This interspecific difference in sensitivity to altered gravity may reflect the stability of the patterns of *C. reinhardtii* in 1 ***g***. In the ground experiments, the horizontal patterns of *T. thermophila* are observed to fluctuate in position, while those of *C. reinhardtii*

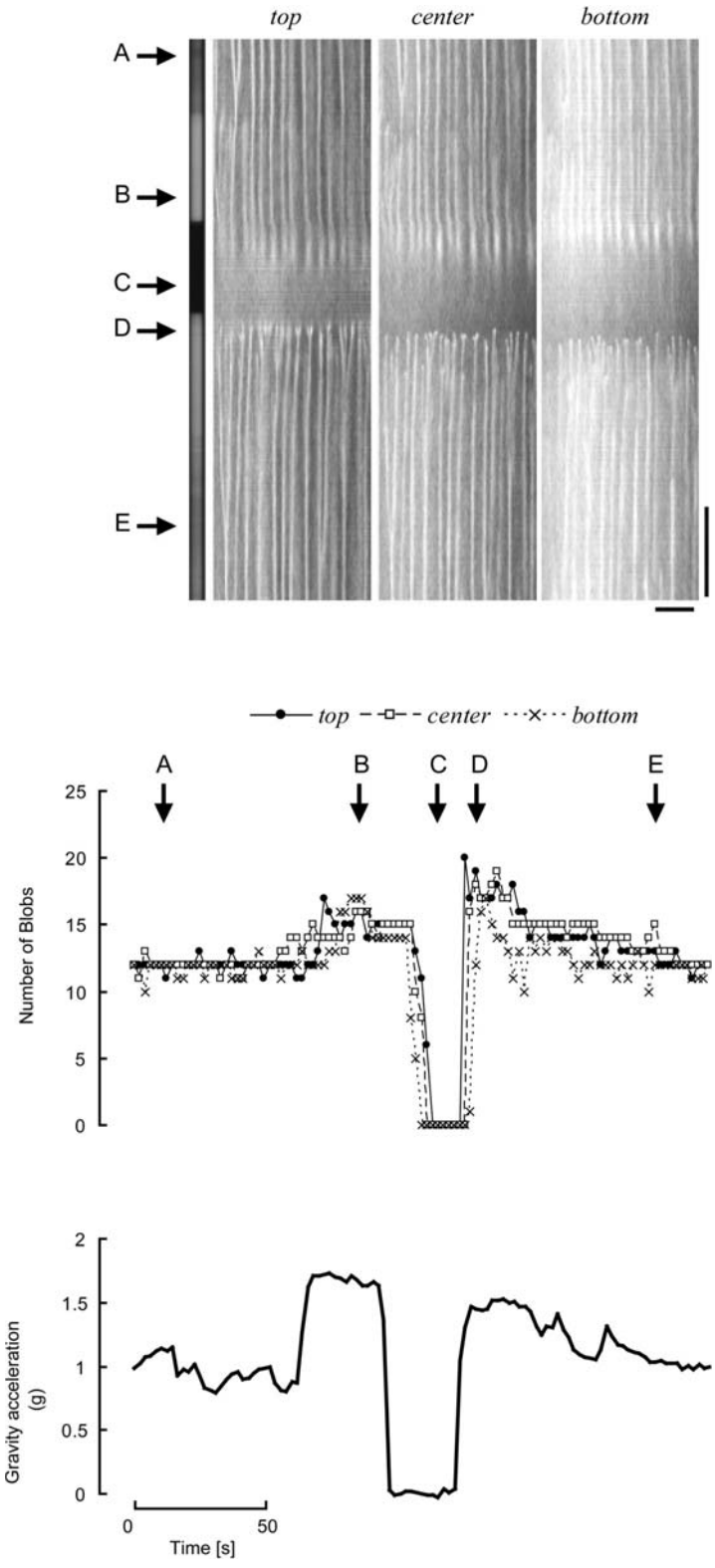

Fig. 7. Top: Space-time analysis of the vertical bioconvection pattern of *T. thermophila* recorded in altered gravity during parabolic flight. Space-time plot obtained three separate positions (*top*, *center*, and *bottom*) indicated in Fig. 6 are shown with time progressing from top to bottom. The narrow bar on the left side of the space-time plots shows the corresponding ***g***-level monitored by a superimposed LED, the brightness of which was roughly proportional to the gravity level (the brighter the LED, the larger the ***g***-level). Arrows A to E indicate the time corresponding to the images shown in Fig. 6. Horizontal and vertical bars: 10 mm and 20 s, respectively. Middle: Quantitative analysis of the vertical bioconvection pattern of *T. thermophila* recorded in altered gravity during parabolic flight. The number of settling blobs counted at three separate positions (*top*, *center,* and *bottom*) indicated in Fig. 6 are shown by respective symbols against time. Bottom: Gravity acceleration plotted against time.

remain highly stable before and after they suddenly become unstable and break down to reconstruct patterns of larger wave number (Akiyama et al., 2005).

Bioconvection of *Tetrahymena* has been explained primarily by a mechanism based on the density-instability model, which considers that the initiation of the pattern formation can be explained by the unstable, top-heavy stratification occurring from negative-gravitactic migration of the cell population, and subsequent settling of the blob of cells (Levandowsky et al., 1975; Childress et al., 1975). The pattern formation of *Chlamydomonas*, on the other hand, has been treated on the basis of the gyrotactic-instability model. In that model, the pattern is likely to be initiated by the migration of the cell population toward the center of the downward flow, which results from the balance between the hydrodynamic torque due to the spatial variation of the fluid velocity (vorticity) and the torque due to the separation of the center of gravity and the center of buoyancy within a bottom-heavy cell body (Kessler, 1985a, 1986). The gyrotactic property is also likely to be involved in the steady state pattern formation in the suspension of *C. reinhardtii*, as shown in a numerical simulation based on the gyrotactic behavior (Ghorai and Hill, 2000). Due to gyrotaxis, *Chlamydomonas* cells focus on the axis of downwardly directed Poiseuille flow and diverge from upward ones (Kessler, 1985a). In steady state bioconvection, this property may function to fix the position of settling blobs, so that the patterns of *C. reinhardtii* rarely change in position and in wave number throughout a succession of changes in gravity. *Tetrahymena* cells, on the other hand, fail to form a column of accumulated cells in the downward flow, possibly due to the absence of gyrotaxis (Kessler, 1985a).

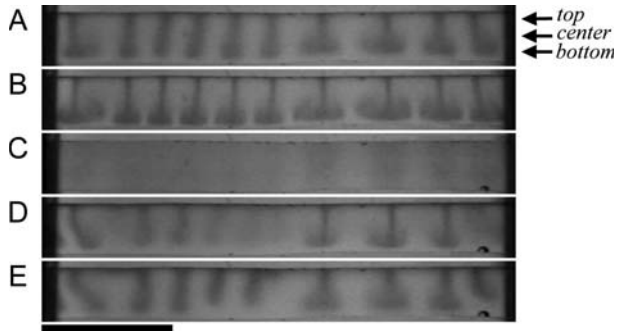

Fig. 8. Sequential images of the vertical bioconvection pattern of *C. reinhardtii* in altered gravity during parabolic flight. Patterns in 1 ***g*** before a parabola trial **(A)**, in pre-parabola hypergravity **(B)**, in microgravity **(C)**, in post-parabola hypergravity **(D)**, and in 1 ***g*** after the parabola trial **(E)** are shown. Times of A to E are indicated by arrows at the side in Fig. 9. Arrows beside A correspond to the positions (*top*, *center,* and *bottom*) for the space-time plot analysis shown in Fig. 9. Scale bar: 10 mm.

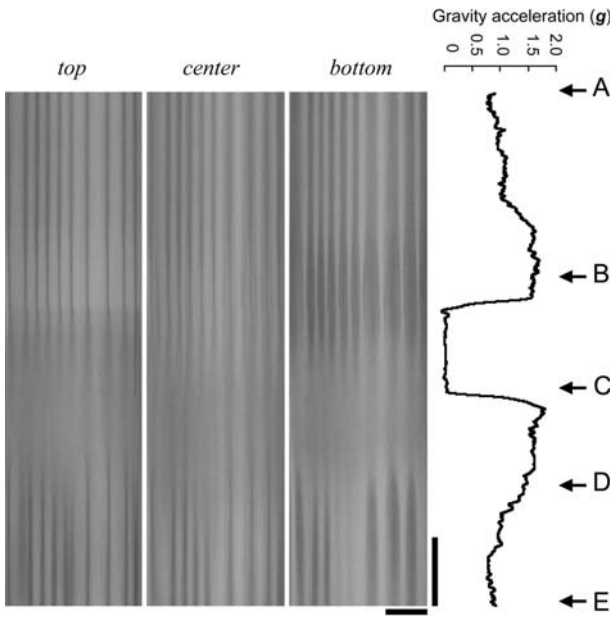

Fig. 9. Left: space-time analysis of the vertical bioconvection pattern of *C. reinhardtii* in altered gravity during parabolic flight. Space-time plot obtained from three separate positions (*top*, *center*, and *bottom*) indicated in Fig. 8 are shown with time progressing from top to bottom. Right: Gravity acceleration at the time of analysis is shown. Arrows A to E indicate the time corresponding to the images shown in Fig. 8. Horizontal and vertical bars: 10 mm and 20 s, respectively.

The bottle-shaped settling blobs of *C. reinhardtii* in hypergravity may also emerge as a consequence of gyrotactic behavior. Numerical simulation by Ghorai and Hill (2000) was able to generate downward plumes that appeared very similar to those observed in the present study (Fig. 8). We observed that, in hypergravity, the “neck” of the bottle-shaped downward plumes became narrower and the “bottom” of the plumes became broader (Fig. 8). An increase in the gravitational acceleration induces greater vorticity as a result of an increase in the speed of falling blobs, which enhances the accumulation of cells to the center of the plume. This and our observation also suggest that gyrotactic behavior plays a major role in bioconvective pattern formation of *Chlamydomonas*, and that gyrotaxis may allow us to explain the interspecific differences in sensitivity of bioconvection to altered gravity.

In addition to the hydrodynamic properties described above, the differences in sensitivity to altered gravity could be considered in terms of the physiological properties of the organisms. Gravikinesis; i.e. changes in the propulsive speed with respect to the gravity vector, has been reported in many protists (*Paramecium*: Machemer et al., 1991; Ooya et al., 1992; *Tetrahymena*: Kowalewski et al., 1998; *Bursaria*: Krause and Bräucker, 2008; *Stylonychia*: Krause et al., 2010; *Euglena*: Häder et al., 1995; Machemer-Rhönisch et al., 1999). For ciliates, gravikinetic response is explained on the basis of cellular mechanosensitivity in combination with the close coupling between the membrane potential and ciliary locomotor activity (Machemer, 1990). The heterogeneous arrangement of mechanosensitive channels, i.e. depolarizing mechanosensitive channels located mainly at the anterior cell membrane and hyperpolarizing mechanosensitive channels located mainly at the posterior membrane (*Paramecium*: Ogura and Machemer, 1980; *Stylonychia*: de Peyer and Machemer, 1978; *Bursaria*: Krause and Bräucker, 2008), leads to bidirectional changes in the membrane potential due to the selective deformation

of the anterior and the posterior cell membrane, responding to the orientation of the cell with respect to the gravity vector; i.e., hyperpolarization or depolarization in upward or downward orientation, respectively. Changes in the membrane potential resulting from the gravity-dependent mechanotransduction were directly measured in *Paramecium* (Gebauer et al., 1999) and *Stylonychia* (Krause et al., 2010). Indirect measurement of the membrane potential of *Euglena gracilis* using a voltage sensitive dye revealed acceleration-dependent changes in potential during parabolic flight (Richter et al., 2006). Taken together, these findings indicate that gravity-dependent behaviors of the ciliates are based on a common cellular mechanism referred to as "statocyst hypothesis" (Machemer et al., 1991), where the entire mass of a cell functions as a statolith imposing mechanical stresses on the mechanosensitive channels in the cell membrane.

Although little evidence has been found for the gravikinetic regulation of the swimming behavior of *Chlamydomonas*, mechanosensitive channels were found to exist in the cell body membrane (Yoshimura, 1998) as well as in the flagellar membrane (Yoshimura, 1996) of *C. reinhardtii*. In addition, the presence of the TRP-channel gene homologs in the genome of *Chlamydomonas* (Martinac et al., 2008) suggests that the statocyst hypothesis might be applicable to gravitaxis of *Chlamydomonas*. Possible roles of physiological regulation in gravitaxis of *Chlamydomonas* can also be inferred by the fact that a few mutants deficient in gravitaxis displayed normal motility and had cell bodies of normal physical characteristics (Yoshimura et al., 2003).

If the gravity-induced mechnotransduction commonly plays a role in the gravitactic behavior of both *Tetrahymena* and *Chlamydomonas*, the difference in sensitivity of the transduction system may contribute to the observed difference in sensitivity to altered gravity in formation of their bioconvection pattern. Sensitivity of the gravity sensor is determined by two factors: (1) mass of the statolith, and (2) the ability of the transducer to convert the mechanical deformation due to gravity to the effective cellular signals, such as changes in the membrane potential. The smaller size of the *Chlamydomonas* cell body is likely to reduce its mechanical impact as the functional statolith. This inference seems to be supported by the fact that a giant ciliate, *Bursaria*, shows a greater magnitude of gravikinetic response than do other ciliates of smaller sizes (Krause and Bräucker, 2008). Furthermore, the presence of cell wall in *Chlamydomonas* in close contact with the deformable plasma membrane may increase the rigidity of the transducer, and, therefore, further reduce the overall sensitivity of this hypothetical gravity sensor through less efficient conversion. These lines of evidence imply that the difference in the sensitivity to altered gravity could be explained by the physiological properties as well as the hydrodynamic properties.

Bioconvection patterns are generated by microorganisms swimming in close proximity. In this situation, behavioral characteristics of individual organisms are reflected in the patterns through mutual interactions. The strain-dependent difference in the response of the steady-state patterns to a step increase in centrifugal acceleration (Mogami et al., 2004) suggests that, through collective interactions, even a small difference in behavioral activity can develop into a large spatial and temporal difference in bioconvection pattern.

In order to understand the action of gravity on bioconvection pattern formation, it is therefore important to clarify its effect on the swimming activity, behavior, and interaction of individual organisms in terms of both physiology and hydrodynamics. Further analyses of bioconvection should be required, incorporating the swimming and collective characteristics of individual cells into both experimental and theoretical research.

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