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Bioconvective pattern formation of *Tetrahymena* under altered gravity

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Summary

Bioconvection is a result of the negative gravitactic behavior of microorganisms. When the top-heavy density gradient generated by gravitaxis grows sufficiently large, an overturning convection occurs leading to a formation of characteristic patterns, which involve highly concentrated aggregation of cells into extended two-dimensional structures. Although gravity is a crucial factor, few experiments have been done with reference to gravity as an experimental variable. In order to gain an insight into the hydrodynamic as well as biological dependence of the convective motion on gravity, we investigated changes in bioconvective patterns of *Tetrahymena* under altered gravity acceleration generated by a long-arm centrifuge. Bioconvective patterns recorded of three different cell strains (*T. pyriformis*, *T. thermophila* and its behavioral mutant, TNR) were analyzed quantitatively using space–time plot and Fourier analysis. For example, under subcritical conditions, when *T. pyriformis* (1.0×10^6 cells ml^{-1}) was placed in a 2 mm-deep chamber, no spatial pattern was observed at 1 *g*. When the suspension was centrifuged, however, patterns began to appear as acceleration increased over a critical value (1.5 *g*), and then remained steady. The formation was reversible, i.e. the patterns disappeared again as acceleration decreased. Under supracritical conditions, i.e. when a suspension of the same density was placed in a 4 mm-deep chamber, a steady state pattern was formed at 1 *g*. The pattern spacing in the steady state was observed to decrease stepwise in response to step increases in acceleration. Fourier analysis demonstrated that for TNR the mean wave number changed almost simultaneously with step changes in acceleration, whereas the responses were less sharp in the wild-type strains. This may suggest that the locomotor phenotype of the cell, such as its avoiding response ability, has a crucial role in bioconvective pattern formation. These findings are discussed in relation to former theoretical studies.

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Key words: *Tetrahymena*, bioconvection, altered gravity, pattern formation, pattern spacing, behavioral mutant.

Introduction

Bioconvection is a collective behavior of microorganisms. It refers to the spatial patterns that develop in suspensions of swimming microorganisms, including bacteria, ciliate and flagellate protozoa and the planktonic larvae of some invertebrates (Platt, 1961; Levandowsky et al., 1975; Kessler, 1989; Pedley and Kessler, 1992). When viewed from above, the patterns are characterized by the highly concentrated aggregation of microorganisms into two-dimensional structures with a scale much greater than the size of the microorganisms. This phenomenon is related to the swimming behavior of microorganisms, with special reference to gravity. Many aquatic microorganisms swim preferentially upwards: negative gravitaxis. Due to the orientation torque generated on the basis of mechanical (Mogami et al., 2001) as well as physiological mechanisms (Mogami et al., 1988b; Machemer et al., 1991; Ooya et al., 1992), microorganisms tend to propel themselves upwards, irrespective of their mass density being greater than that of water. The suspension of such organisms does not remain homogeneous but forms a layer of organisms accumulated at the top of the water column. This stratification, however, can be unstable when the potential energy released by the downward movement of a lump of the accumulated organisms is sufficient to overcome the associated viscous dissipation. As noted by Plesset and Winet (1974), such instability is therefore the viscous counterpart to the Rayleigh–Taylor instability of a stratified fluid.

There have been many studies on the subject within a theoretical framework. Childress et al. (1975) proposed the first extensive theory for bioconvection of gravitactic microorganisms. In their theory the source of the convective pattern formation was attributed to a hydrodynamic instability analogous to that causing spatial patterns in a horizontal layer of fluid, in which an adverse temperature gradient is created by heating the underside (Rayleigh–Bénard convection;

Chandrasekhar, 1961). Because most of the negative gravitactic microorganisms usually have a higher density than that of the surrounding medium, their upward migration causes the upper region of the suspension to become denser than the lower. When this inverted density gradient grows sufficiently large, an overturning convection occurs. A dimensionless number, the critical Rayleigh number after the Rayleigh–Bénard convection, can specify the critical condition for convection to occur leading to a collective pattern formation.

Kessler (1985, 1986) demonstrated experimentally as well as theoretically that gravitactic algal cells can be reoriented in a shear flow for balance between the viscous torque due to shear stress and the gravitational torque resulting from the asymmetrical mass distribution within the cell body. He argued that this orientation, termed 'gyrotaxis', causes bottom-heavy cells to swim away from regions of upflow toward those of downflow. This results in an accumulation of cells in the regions of downflow, which makes these regions denser than the ambient suspension, and thus increases the rate of downflow. This is an alternative mechanism for inducing a spontaneous growth of density fluctuation even in the absence of a global vertical density gradient. There have been several intensive theoretical analyses of this mechanism, leading to a quantitative model to explain the onset of the gyrotactic convection and also its initial pattern spacing (Kessler, 1986; Pedley et al., 1988; Hill et al., 1989; Pedley and Kessler, 1990).

In contrast to the intensive theoretical works, there have been few quantitative analyses on bioconvection. Bees and Hill (1997) presented a quantitative analysis of observations of bioconvective pattern formation by means of a computer-assisted image analysis. In addition to Fourier analysis conducted by Bees and Hill (1997), Czirok et al. (2000) used a pair-correlation function for the assessment of the pattern formation. These authors investigated quantitatively the relationship between the pattern spacing and either the mean suspension density of organisms or the depth of the suspension as a variable parameter, with others being fixed.

The purpose of the present study is to analyze the temporal as well as spatial characteristics of bioconvective pattern formation observed under altered gravity acceleration. Gravity is one of the essential factors for bioconvection. However, little attention has been focused on gravity acceleration as an experimental variable in experiments on bioconvection. Noever (1991) reported changes in the pattern spacing in bioconvection of *Polytomella parva* and *Tetrahymena pyriformis* under variable gravity performed by the parabolic flight of an airplane: polygonal patterns formed, with either species disappeared under microgravity during the parabolic flight and appeared again at 1 $\boldsymbol{g}$. The paper also reported that in hypergravity (1.8–2 $\boldsymbol{g}$) phases before and after the microgravity, both specimens increased their polygonal pattern spacing (therefore decreased the number of polygons formed in the suspension). These facts indicate that bioconvective pattern formation is highly sensitive to the gravity environment. Although parabolic flight is one of a few available experimental tools with which to simulate microgravity in ground-based experiments, oscillatory changes in gravity, which involve alternating phases of different gravities, each lasting only some 20 s, might affect the consequential pattern forming response, which would continue for up to several tens of seconds (Gittleson and Jahn, 1968; Wille and Ehret, 1968; Childress et al., 1975).

We used a long-arm centrifuge to apply centrifugal acceleration to the suspension of *Tetrahymena* and analyzed the temporal and spatial changes in bioconvection pattern with varying gravity, quantitatively by means of space–time plot, newly introduced in the present study, and also by Fourier analysis. An increase in gravity by centrifugation induced pattern formation in the suspension that had shown no convective pattern when placed under subcritical conditions at normal gravity. The pattern spacing decreased with increase in gravity, opposite to the observations of Noever (1991) mentioned above. Analyses were conducted on different species and a behavioral mutant of *Tetrahymena*, revealing that the gravity-sensitive pattern formation is closely related to the swimming activity of the organisms included in the suspension.

Materials and methods

Tetrahymena

Tetrahymena pyriformis (strain W), *T. thermophila* (strain wild II) and a behavioral mutant of *T. thermophila* (TNR; *Tetrahymena* Non-Reversal, strain TNR B) (Takahashi et al., 1980) were a gift from Dr M. Takahashi, Tsukuba University, Japan. They were grown axenically at 24°C in a culture 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 early stationary phases of density $>1\times10^6$ (typically $1.2–1.6\times10^6$) cells ml^{-1} were used throughout the experiments. For measuring the density, cells in a small volume of the culture were fixed in 5% formaldehyde containing 0.01% Brilliant Green and counted on a Fuchs–Rosenthal counting chamber (Watanabe, 1963).

Hypergravity experiments

Before hypergravity experiments, cells were diluted with fresh culture medium to a density of 1×10^6 cells ml^{-1}, and the cell suspension was put into a flat circular glass chamber of inner diameter 110 mm. The cell suspension was transferred into this chamber without an air gap between the top- and bottom-glass plates, which were separated by a 2 or 4 mm thick plastic spacer.

Bioconvection patterns were recorded by a camcorder (TRV20, SONY, Tokyo, Japan) under dark field illumination using a circular fluorescent bulb as a light source. The bulb was operated at high frequency (>20 kHz) to avoid fluctuations of image brightness between frames. Throughout the recordings specimens were illuminated through a heat absorption filter.

A recording setup was placed in a bucket of a long-arm centrifuge (max. arm length=1.5 m; Tomy Seiko, Tokyo). The

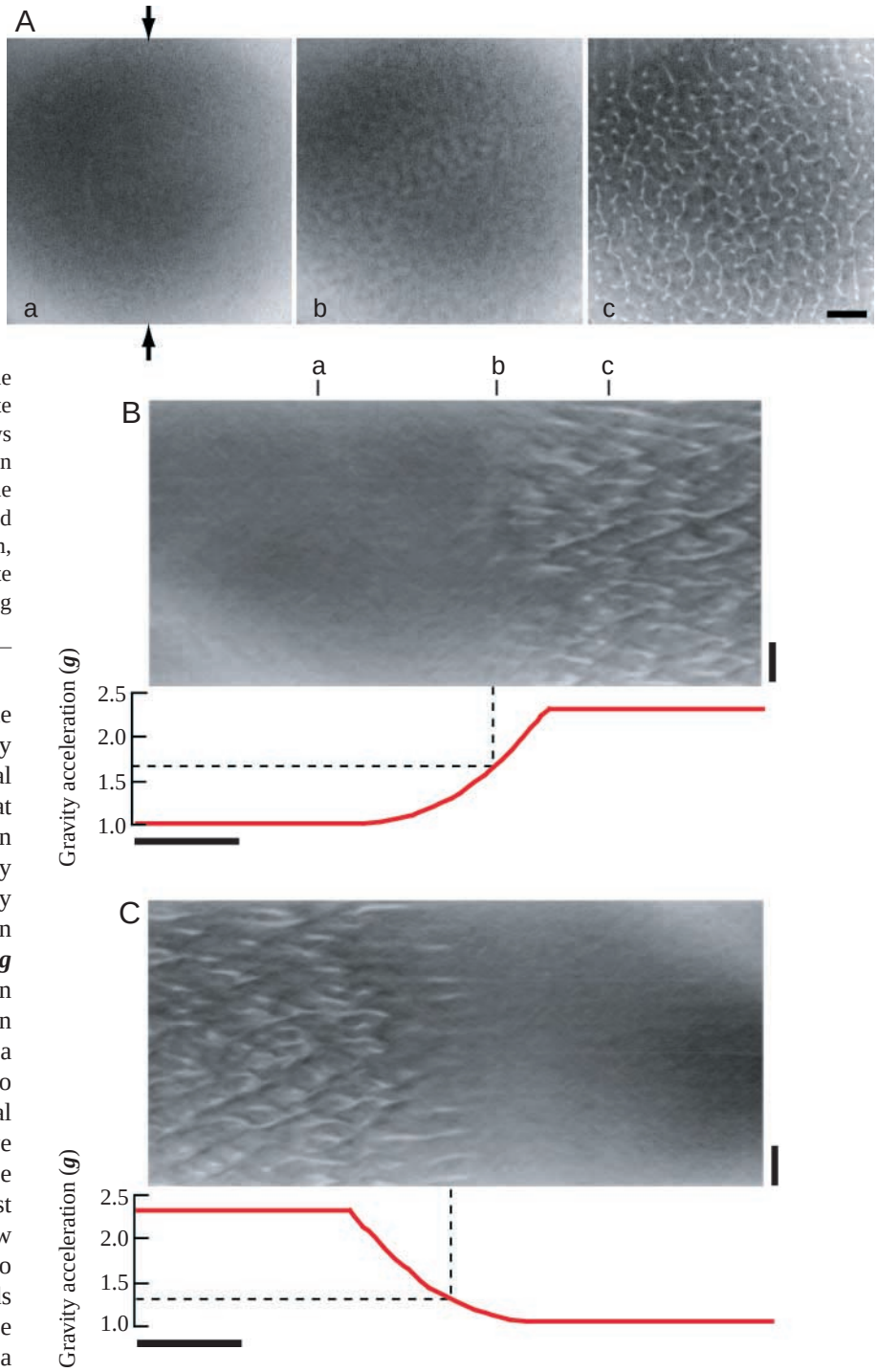

Fig. 1. Gravity-dependent pattern formation of *Tetrahymena pyriformis* enclosed in a circular glass chamber 2 mm deep at a density of 1.0×10^6 cells ml^{-1}. (A) Plan view of the suspension under subcritical (a), threshold (b) and supracritical (c) conditions for pattern formation with increase in gravity. Regions with higher cell densities appear as bright white areas under dark field illumination. A linear region indicated by faced arrows is the portion on which space–time plots in (B) and (C) were made. Bar, 10 mm. (B) Space–time plot of an experiment of increasing gravity and the corresponding gravity profile. Letters a–c indicate the time corresponding to respective plan views a–c shown in A. (C) Space–time plot of an experiment of decreasing gravity and the corresponding gravity profile. Horizontal and vertical bars in B and C are 1 min and 10 mm, respectively. Broken lines in B and C indicate threshold levels for increasing and decreasing gravity, respectively.

bucket swings up freely due to the centrifugal force so that the resultant gravity (a vector sum of gravitational and centrifugal acceleration) is kept perpendicular to the flat surface of the chamber of cell suspension. In the present study the magnitude of gravity was increased in two ways: one continuously up to 2 ***g*** for the analysis of the initiation process, and the other stepwise also up to 2 ***g*** for the analysis of the steady state pattern formation, and in both ways the rotation speed of the centrifuge was increased at a fixed rate of 0.2 revs min^{-1} s^{-1}. In order to minimize the effects of vibrational perturbation at the onset of rotation, we conducted a low-speed centrifugation to be used as a control, i.e. specimens were first spun for a while (usually >5 min) at a low speed (10.5 revs min^{-1} s^{-1}, corresponding to 1.01 ***g***), and then at increased speeds corresponding to the acceleration to be tested. Experiments were carried out at a controlled temperature of 23±1°C.

Analyses

For assessment of the time-dependency of pattern formation in *Tetrahymena* suspension, we constructed a 'space–time plot' from a video recording as follows (Fig. 1). Recorded images to be analyzed were converted to a stack of image files, each of which consisted of 640 pixels × 480 pixels on a 256 gray scale by an image board (DIG98, DITECT, Tokyo, Japan). A linear portion was selected at a given position in each image and the density profile of the portion (a linear array of gray scale data along a line) was calculated and stored in a line buffer provided by the application. The linear data were then displayed side by side in a time sequence to form an image that has space and time dimensions: space–time plot (Fig. 1B,C). The procedure, the so-called digital slit camera method, was done with the assistance of a public domain NIH Image program (developed at the US National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/).

Fourier analysis to estimate an average spacing of

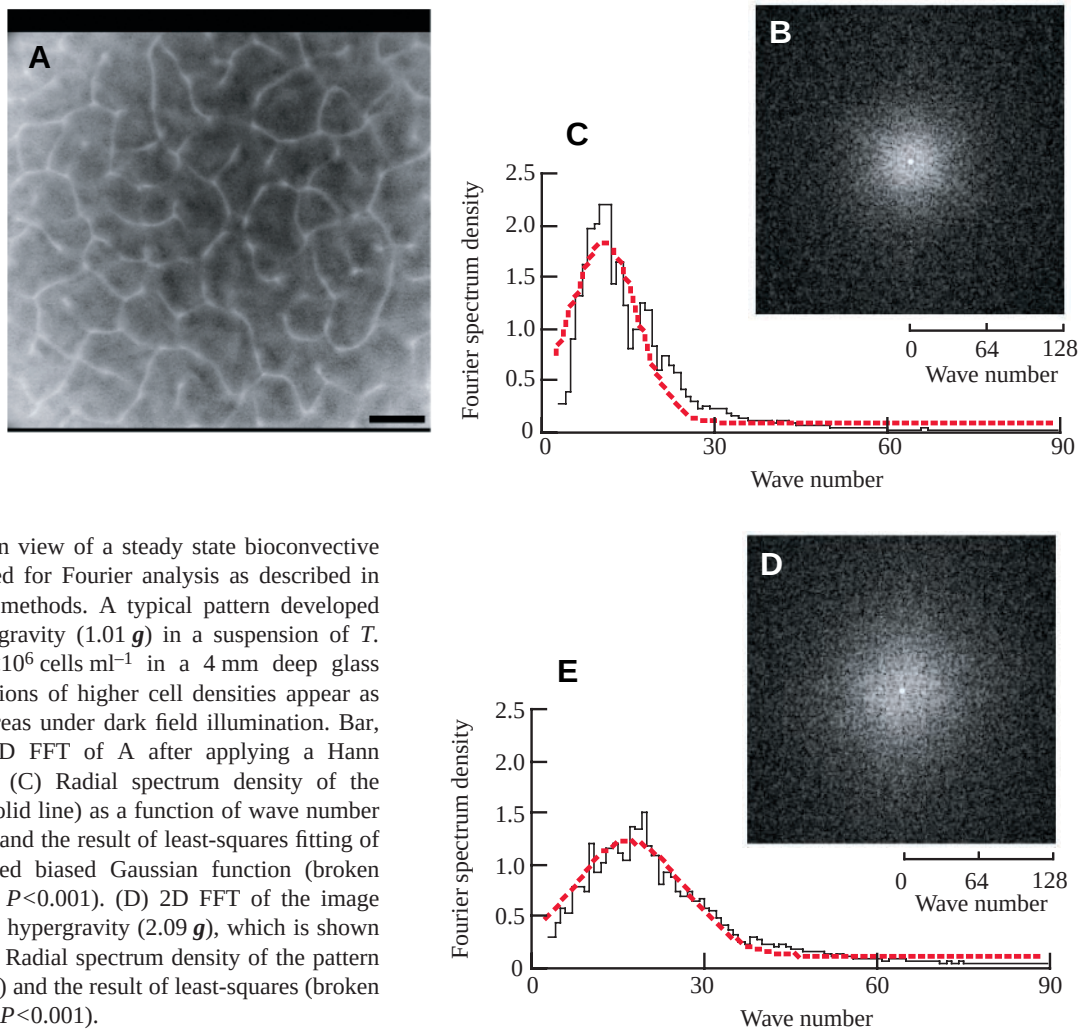

Fig. 2. (A) Plan view of a steady state bioconvective pattern prepared for Fourier analysis as described in Materials and methods. A typical pattern developed under control gravity (1.01 ***g***) in a suspension of *T. pyriformis* (1×10^6 cells ml^{-1} in a 4 mm deep glass chamber). Regions of higher cell densities appear as bright white areas under dark field illumination. Bar, 10 mm. (B) 2D FFT of A after applying a Hann window filter. (C) Radial spectrum density of the pattern in B (solid line) as a function of wave number (per 72.5 mm) and the result of least-squares fitting of an unnormalized biased Gaussian function (broken line) (r^2=0.92, P<0.001). (D) 2D FFT of the image recorded under hypergravity (2.09 ***g***), which is shown in Fig. 3A. (E) Radial spectrum density of the pattern in D (solid line) and the result of least-squares (broken line) (r^2=0.97, P<0.001).

bioconvection patterns of *Tetrahymena* suspension was carried out largely following the method described by Bees and Hill (1997). For the calculation of two-dimensional discrete fast Fourier transform (2D FFT), a rectangular digitized image was converted to a square (512 pixels × 512 pixels) image by cutting off both sides and also by adding null density pixels at the top and the bottom of the image (Fig. 2A). The Hann window filter was applied not only to eliminate the oscillatory errors due to the effects of sharp edges but also to weight the information in the center of the image. 2D FFT was performed using an image processing software package, Image-Pro Plus (Media Cybernetics Inc., Silver Spring, MD, USA). From the resultant amplitude pattern (Fig. 2B), a radial spectrum density as a function of wave number, $P(n)$, was calculated following the equation 3 of Bees and Hill (1997). The mean wave number was obtained as a dominant wave number of the spectrum determined by the least-squares fitting of an unnormalized biased Gaussian function $G(x)=C+A\exp\{-(x-m)^2/D^2\}$ (Fig. 2C), instead of a double Gaussian distribution as used by Bees and Hill (1997). This function was selected to avoid additional errors that may be introduced because of a greater number of parameters in the double Gaussian distribution fitting. In our fitting we always found that the goodness of fit was highly reasonable (coefficient of determination r^2>0.9). In the equation above, m gives the dominant wave number. We did not find any significant qualitative differences between the dominant wave number obtained by the least-squares fitting and that determined by eye. For the calculation of the least-squares fitting, data coming from uneven illumination in the area of low spacial frequencies were removed for accuracy.

Results

Increased gravity induced bioconvective pattern formation in the subcritical suspension

Three strains of *Tetrahymena* used in the present study showed negative gravitaxis, as described in the previous papers (Mogami et al., 1988a; Hirashima et al., 2003). The tactic behavior was not affected by illumination for recordings. They formed, beneath the glass surface at the top of suspension, various spatial patterns, such as dotted and polygonal (a lattice of nodes joined by lines) patterns, depending on the cell density and the inner dimensions of the chamber. Direct measurement

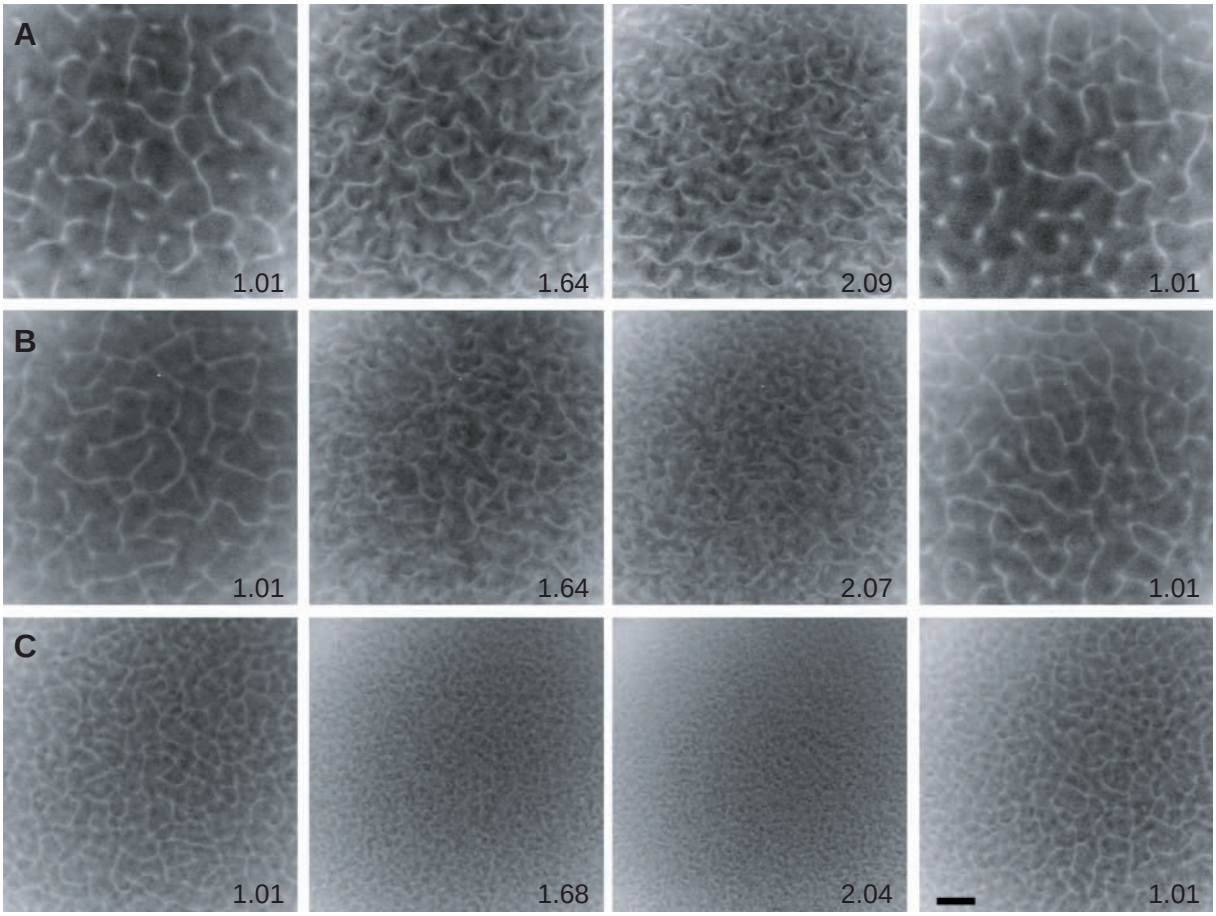

Fig. 3. Plan views of steady state bioconvective patterns developed under various gravities and recorded with suspensions of *T. pyriformis* (A), *T. thermophila* (B) and TNR (C). These suspensions were placed in a 4 mm deep glass chamber at a density of 1×10^6 cells ml^{-1}. Numbers indicate the magnitude of applied gravity in ***g***. The mean wave numbers obtained by Fourier analysis from these pictures are shown by filled symbols in Fig. 4. Bar, 10 mm.

of the temperature of the top and bottom glasses of the chamber demonstrated that the patterns were formed in the absence of any temperature gradient, as indicated by Platt (1961). In the case of polygonal pattern formation, cells swam up inside the polygon and down at the boundary between adjoining polygons, as observed in Bénard cells formed by thermal convection (Chandrasekhar, 1961). These steady state patterns were observed to shift horizontally at almost a constant speed (several tens of micrometers per second). The 'vigorous' horizontal movement of patterns (sideways transition of patterns) was characteristic to suspensions enclosed by glass plates (termed rigid surfaces, by Childress et al., 1975), but not to suspensions with an air–water interface (free surface) at the top.

Several observations on *Tetrahymena* suspensions have demonstrated that bioconvective patterns appear when either the depth of suspension or the organism number density exceeds a critical value (Childress et al., 1975; Levandowsky et al., 1975). When the suspension of *T. pyriformis* of a density of 1.0×10^6 cells ml^{-1} was placed in a chamber 2 mm deep, no clear patterns were found, while patterns emerged in a suspension of the same density but placed in a chamber 3 mm deep. That is, the critical value for depth is between 2 and 3 mm. A similar critical depth was obtained with *T. thermophila*, whereas TNR formed bioconvective patterns even in a chamber 2 mm deep, suggesting a lower critical depth.

Suspensions prepared under subcritical conditions remained stably homogenous for at least 1 h without pattern formation. Patterns appeared, however, when such a suspension was spun to increase gravity. Fig. 1A,B shows the time course of pattern formation in this suspension with increase in gravity. As summarized in a space–time plot in Fig. 1B, this suspension did not form patterns as long as gravity was below a threshold level (Fig. 1Aa), and then began to form patterns over the whole area at nearly the same time as gravity increased beyond the threshold. The threshold was 1.5 ± 0.12 ***g*** (N=8). At the threshold small vague accumulations appeared at an almost regular spacing (Fig. 1Ab). These were observed to be condensed and to form a polygonal pattern at about 2 ***g*** (Fig. 1Ac) and to move horizontally, as observed under normal gravity in deeper suspension. Patterns induced under

hypergravity disappeared with decrease in gravity (Fig. 1C). Before their complete disappearance, polygonal patterns lost their connecting lines between nodes and the resultant dot pattern remained for a while. The level at which the gravity-induced patterns disappeared was 1.2±0.12 ***g*** (*N*=8).

Gravity dependent pattern formation is shown in Movies 1 and 2 (supplementary material), each of which corresponds to the space-time plot in Fig. 1B (increasing gravity) and that in Fig. 1C (decreasing gravity), respectively.

Decrease in the pattern spacing with increase in gravity

For the analysis of steady state patterns, cell suspensions of a density of 1.0×10^6 cells ml^{-1} were placed in a 4 mm deep chamber. Under these conditions suspensions of each of three strains formed a steady state pattern several tens of seconds after transfer and stirring to ensure uniform distribution. Fig. 2A shows an example of steady state patterns of *T. pyriformis* recorded under normal gravity, from which Fourier spectrum density was calculated (Fig. 2B,C). In order to assess the effect of gravity on the steady state pattern formation, we changed the gravity in stepwise increments and measured the mean wave number (the reciprocal of mean pattern spacing) of the pattern formed during the maintained gravity steps.

Plan views of bioconvective patterns shown in Fig. 3 demonstrate that the mean pattern spacing decreased with increase in gravity. This tendency was the same among the three different strains tested, although there were significant differences in the mean spacing of the pattern formed under normal gravity. Changes in the pattern spacing were clearly dependent of gravity, as confirmed by Fourier analysis (Fig. 2D,E). Fig. 4 shows the profiles of changes in the mean wave number with stepwise changes in gravity. It is clear that the mean wave number of steady state pattern is closely related to changes in gravity. The relationship was closest in the pattern formed by the suspension of TNR, the non-reversal mutant of *T. thermophila*, which changed mean wave number almost simultaneously with the step changes in gravity, showing a sharp transition of plots corresponding to the step changes (Fig. 4C), whereas the observed transitions were less sharp or delayed in the plots obtained from wild-type strains (Fig. 4A,B). As shown in Fig. 5, the mean wave number increased almost linearly with increased gravity. An increase in gravity from 1 to 2 ***g*** decreased the average pattern spacing from 6.5 to 4.9 mm for *T. pyriformis*, 6.1 to 4.1 mm for *T. thermophila* and 3.5 to 1.8 mm for TNR. Interestingly we found two types of responses in *T. thermophila* to gravity, i.e. several batches showed higher and the others lower responsiveness, as demonstrated by the split of plots at higher accelerations (two out of five sequences of filled squares in Fig. 5). The differences among cell strains in their sensitivity to gravity indicate that bioconvective patterns are formed collectively on the basis of the motile activity expressed by the individual cells. Especially, the fact that characteristics of the pattern formation in TNR are substantially different from those in wild-type strains suggests that their avoiding reaction ability (radical changes of swimming direction accompanied with backward swimming due to ciliary reversal) has some crucial roles in the formation of bioconvective patterns by *Tetrahymena*.

The steady state pattern formations under altered gravity are also shown in Movie 3 (supplementary material; *T. pyriformis*, corresponding to Figs 3A, 4A), Movie 4 (supplementary material; *T. thermophila*, corresponding to Figs 3B, 4B) and Movie 5 (supplementary material; TNR, corresponding to Figs 3C, 4C).

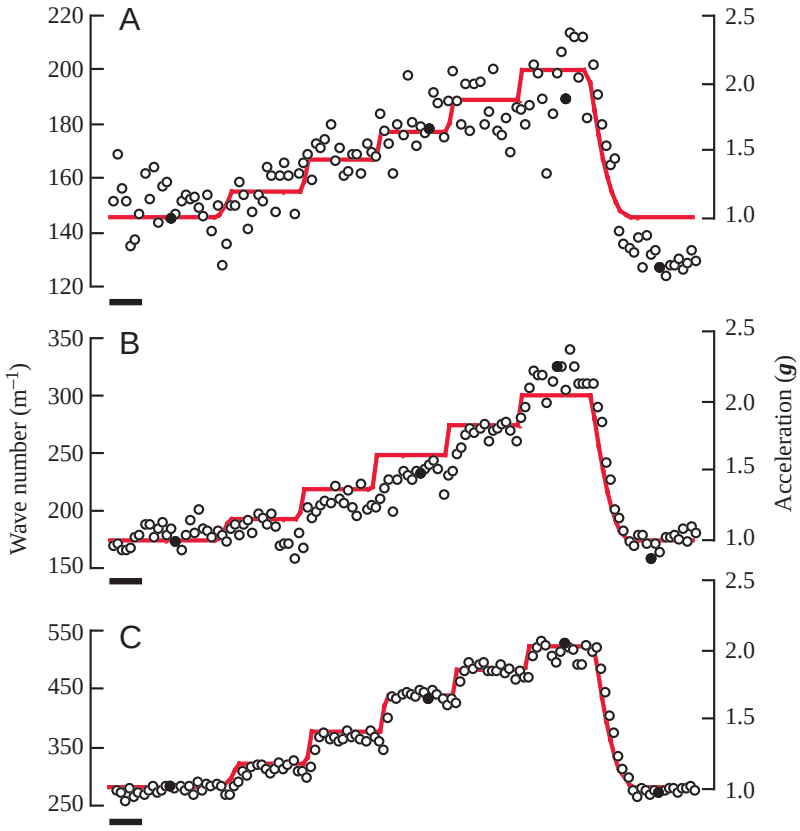

Fig. 4. Profiles of changes in the mean wave number with stepwise changes in gravity obtained from the suspension of *T. pyriformis* (A), *T. thermophila* (B) and TNR (C). The mean wave number (circles) and the corresponding profile of altered gravity (solid lines) are shown as a function of time. The mean wave number (left ordinate) has been scaled in relation to the magnitude of changes in gravity (right ordinate). Filled symbols correspond to the wave numbers of the patterns shown in Fig. 3. Bars, 1 min.

Discussion

In the present paper we describe the bioconvective pattern formation of *Tetrahymena* under altered gravity, applied using a long-arm

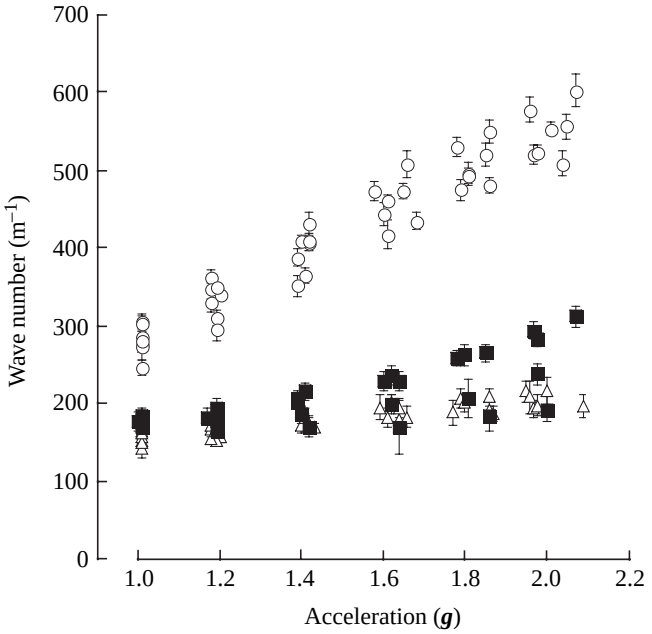

Fig. 5. Mean wave number of bioconvective patterns as a function of the magnitude of applied gravity step. The average of the mean wave numbers measured within each gravity step from *T. pyriformis* (triangles), *T. thermophila* (filled squares) and TNR (circles) are shown ± S.D.

centrifuge. The temporal and spatial characteristics of the pattern formation were analyzed quantitatively by means of space–time plot and Fourier analysis. We demonstrate that an increase in gravity induces bioconvection in the suspension that had been under subcritical conditions at normal gravity, and that the spacing of the steady state pattern decreases with increase in gravity. We also demonstrate that the sensitivity to gravity in pattern formation is closely related to the swimming activity of the cells included in the suspension.

For the initial instability of bioconvective pattern formation, Levandowsky et al. (1975) and Childress et al. (1975) introduced a dimensionless number analogous to the Rayleigh number in the theory of thermal convection. We will refer to this number as density-instability Rayleigh number, R_d, because it originated from the theoretical model for the instability of top-heavy density stratification (also referred to as density-instability model), and distinguish it from a similar number defined for the gyrotactic instability, R_g, which originated from the theoretical model for the instability of the suspension of gyrotactic microorganisms (referred to as gyrotactic-instability model).

For the suspension of microorganisms of the density ρ_o and the volume V_o with average number concentration of the microorganisms N_{av} and suspension depth H, R_d has been defined as:

$$R_d = \left(\frac{\boldsymbol{g}}{\nu}\right) \alpha V_o \times \left(\frac{N_{av}\lambda}{1-\exp(-\lambda)}\right) \times \left(\frac{\kappa_v^2}{U_v^3}\right), \qquad (1)$$

where $\boldsymbol{g}$ is gravity or acceleration, ν the kinematic viscosity (the ratio of the viscosity μ_m to the density ρ_m of the surrounding medium), $\alpha=(\rho_o-\rho_m)/\rho_m$, κ_v the coefficient for vertical component of an anisotropic diffusive movement of the organisms, U_v a vertical drift (the speed of gravitactic swimming), and $\lambda=HU_v/\kappa_v$ (rewritten from Childress et al., 1975; Levandowsky et al., 1975). We take for the medium $\nu=0.01$ cm^2 s^{-1} and $V_o=2.2\times10^{-8}$ cm^3 from the calculation of the volume as a rotating spheroid with long axis of 63±6.4 μm (N=26) and radius of 26±2.9 μm. For *T. pyriformis* we found in the literature $U_v=5.6\times10^{-2}$ cm s^{-1} (Kowalewski et al., 1998) and $\rho_m=1.035$ g cm^{-3} (Kowalewski et al., 1998; Machemer-Roehnisch et al., 1999), which gives $\alpha=3.5\times10^{-2}$. These values were compatible with those obtained by the measurement of swimming velocity (presented in the text below) and by the preliminary sedimentation experiment in the Percoll density gradient. κ_v can be evaluated as hU_v, where h is a characteristic depth and approximately equal to the depth of subsurface layer (Childress et al., 1975; Levandowsky et al., 1975), by which λ can be expressed as H/h. We assume h=1 mm, as these authors did on the basis of horizontal microscope observation of the suspension of *T. pyriformis* (Plesset et al., 1975). This gives $\kappa_v=5.6\times10^{-3}$ cm^2 s^{-1}. From Equation 1, R_d of our subcritical conditions ($N_{av}=1\times10^6$ cells ml^{-1} and H=2 mm) is now calculated to be 31. The value of R_d at the critical gravity, 1.5 $\boldsymbol{g}$, is also calculated to be 47, keeping κ_v and U_v constant irrespective of $\boldsymbol{g}$. Childress et al. (1975) computed the critical Rayleigh number R_c as a function of λ and found $R_c(\lambda)/\delta=121.3$ for $\lambda=2.0$ and with two rigid surfaces (our case), where δ is a parameter of anisotropic diffusion, i.e. the ratio of horizontal diffusive component κ_h to κ_v. Our value of 47 described above for R_c implies $\delta=0.39$, a value smaller than a unity, which is not unreasonable as discussed in Childress et al. (1975).

Since the randomness of swimming has been successfully approximated in terms of diffusion in a number of theoretical works, other estimations of κ_v independent of the depth of the subsurface layer are possible. Kessler (1986) estimated the coefficient of diffusivity on the basis of random walk theory. According to his estimation, we can take $\kappa_v=LU_v/3$, where L is the mean free path or the average distance covered by swimming cells between radical changes in direction. Kessler (1986) took, from direct observation of swimming, $L\approx100a$, where a is the average radius of the cell. L is also given by the product of the swimming speed and the mean free time of swimming, during which cells swim straight between changes in direction due to the spontaneous avoiding reaction (Pedley and Kessler, 1990). The measurement of Kim et al. (1999) gives a value of several seconds to the mean free time of swimming of *Tetrahymena*, which is compatible with the reported values for other ciliates (Machemer, 1989). These considerations indicate that $L/3$ is of the order of 1 mm, supporting the assumption that h=1 mm. If the collision dominated the change of direction instead of avoiding reaction, on the other hand, κ_v would be estimated as $U_v/12\pi a^2N$ based on the collision free path, where N is 2–3 times N_{av} (Kesseler,

1986). If we take $a=(3V_o/4\pi)^{1/3}=18$ μm for *T. pyriformis* and $N=2.5N_{av}$, $\kappa_v=1.8\times10^{-5}$ cm^2 s^{-1}, which gives $h=0.033$ mm and $R_d=0.036$ at $\lambda=60$. These values indicate that δ is of the order of 10^{-2}, implying that the horizontal diffusivity is unrealistically low as compared with vertical. Our observation of *T. pyriformis* swimming in dense suspensions revealed that cells do not necessarily change the swimming direction on collision, whereas they always change it at spontaneous avoiding reactions. This may also indicate that κ_v should be estimated depending on the avoiding reaction rather than simple collision, and also suggest that the value estimated from the sublayer depth would not be unreasonable.

Some effects of collision, however, may be anticipated in highly condensed regions brought as a result of bioconvective pattern formation. Collisions at much higher cell density may reduce the diffusivity of the cell more effectively than those at the density before the onset of pattern formation. The lowered diffusivity prevents the cells from dispersing out of the once condensed regions. This may cause the dotted pattern to be retained for a while during deceleration (Fig. 1C), and hence explains a lower threshold (lower critical Rayleigh number) for decreasing gravity than for increasing gravity.

In a quantitative model for the onset of the gyrotactic bioconvection, Pedley et al. (1988) indicated that a gyrotactic instability induces the pattern formation in the absence of vertical density gradient when R_g exceeds the critical value. R_g is defined differently from R_d but using several common parameters to those in R_d as follows:

$$R_g = \left(\frac{\boldsymbol{g}}{\nu}\right)\alpha V_o \times N_{av}B \times \left(\frac{UH^2}{\kappa}\right), \qquad (2)$$

where κ is a parameter of the diffusive motion, U the swimming speed, and B the gyrotactic orientation parameter. Throughout their theoretical researches on gyrotactic pattern formation, these authors considered the upward orientation of negative gravitactic microorganisms as a consequence of their inhomogeneous mass distribution. The posterior location of the center of mass to that of buoyancy generates a torque to orient the organism upwards. For such bottom-heavy organisms, it is inferred that $B=\alpha_\perp\mu_m/2l\rho_o\boldsymbol{g}$, where $\alpha_\perp$ is a dimensionless constant relating the viscous torque to the relative angular velocity of the organism and l is the displacement of the center of gravity from that of buoyancy of the organism. As $\boldsymbol{g}$ can be eliminated from Equation 2 by substitution of B in this equation, R_g will be independent of $\boldsymbol{g}$, unless U changes depending on $\boldsymbol{g}$. The gyrotactic-instability model cannot, therefore, explain explicitly our findings of the threshold gravity.

Although the bottom-heavy assumption might be suitable for spheriodal unicellular algae such as *Chlamydomonas* and *Dunaliella*, which have been found to perform gyrotaxis, other orientation mechanisms have been proposed for *Tetrahymena* (Kessler, 1985). Mogami et al. (2001) demonstrated that Ni^{2+}-immobilized cells of *Paramecium caudatum* orient downwards while floating upwards in a Percoll-containing hyperdensity ($\rho_o<\rho_m$) medium but orient upwards while sinking in a hypodensity ($\rho_o>\rho_m$) control medium. These findings indicate that the gravitactic orientation of *Paramecium* is primarily due to the torque generated by the morphological fore–aft asymmetry of the cell, which has been termed the drag-gravity model by Roberts (1970). In our preliminary experiments *T. pyriformis* as well as *P. caudatum* showed a positive gravitactic migration in hyperdensity media immediately after they were allowed to swim freely in the vertical direction, whereas they showed an ordinary negative gravitaxis in hypodensity media (Hirashima et al., 2003). These findings indicate that the morphology-dependent mechanical property functions well as an actual mechanical bias for gravity-dependent orientation in swimming ciliates rather than the bottom-heavy mechanical property assumed in spheroidal unicellular algae. In addition, the fact that *T. pyriformis* failed to form a beam of cells focused on the axis of downwardly directed Poiseuille flow in which *Chlamydomonas* formed a sharp beam (Kessler, 1985) does not mean that the gyrotactic-instability model is straightforwardly applicable to the bioconvective pattern formation of *T. pyriformis*. The presence of gyrotaxis, however, can surely modify the results obtained from a pure Rayleigh–Taylor instability model (Hill et al., 1989). As noted by these authors, there might in fact exist two instability mechanisms: negative gravitactic migration, which leads to the top-heavy density instability, and gyrotactic behavior, which leads to the instability growing from the uniform basic state. The two mechanisms seem to cooperate with each other. The density-instability model has been put forward on the assumption that cells swim on average upwards independently of the flow driven by bioconvection. The flow in fact must exert a torque tending to orient the front end of the cell away from the vertical. We would expect the torque due to the drag on the body to be proportional to the sedimentation speed of the cells, which in turn is proportional to the effective gravitational acceleration, $\boldsymbol{g}$, because the motion of the cell occurs at very low Reynolds numbers. However, if this is the case, then B is still proportional to $1/\boldsymbol{g}$ and R_g is still independent of $\boldsymbol{g}$.

Although the linear stability analyses on the density-instability model (Childress et al., 1975) and the gyrotactic-instability model (Pedley et al., 1988) only predict the onset of patterns for suspensions for which the Rayleigh numbers are just above critical, the nonlinear numerical analyses on the basis of the same models showed the formation of steady state patterns well above critical values (Harashima et al., 1988; Ghorai and Hill, 2000). In the density-instability model, the wave number of the pattern arising at the onset of the convection is hypothesized to increase with increasing R_d/σ, where σ is a Schmidt number and given by $\sigma=\nu/\kappa_v$ (Childress et al., 1975). Numerical experiments by Harashima et al. (1988) on the basis of this model demonstrated that the wave number at the onset of the convection increased monotonically with R_d/σ, especially with σ fixed. If the steady state patterns are the results of the monotonic development of the patterns formed at the onset of the convection, the hypergravity-

dependent increase in the wave number of bioconvection patterns (Figs 3–5) may apparently be explained by increased R_d in proportion to gravity acceleration (Equation 1). Bees and Hill (1997), however, showed that the wave number of the bioconvective pattern of *Chlamydomonas nivalis* did not always change monotonically from the onset to the steady state of the pattern formation. They also found that the wave number at the onset of the bioconvection decreased with suspension depth, whereas those at the steady state increased with cell density. These findings might therefore indicate that R_d does not function as a measure of the wave number (pattern spacing) in the steady state of bioconvection.

The dependence of the pattern spacing upon gravity we report is the opposite to that reported by Noever (1991), where the polygonal pattern size in bioconvection of *Polytomella parva* and *Tetrahymena pyriformis* increased in the hypergravity (1.8–2 $\boldsymbol{g}$) phases during parabolic flights of an airplane. It might be possible that the discrepancy between the results of the two experiments is due to differences in the methods of increasing gravity, since a tendency of decreasing pattern spacing similar to ours was reported in a separate centrifuge experiment (Itoh et al., 1999). As noted by Noever (1991), hypergravity during flight experiments occurred and continued for 20 s before and after the short-term (25 s) microgravity, so that the changes in the pattern size were transitional during the limited period of hypergravity and also occurred with the oscillatory changes in gravity (normal – hyper- – micro- – hyper- – normal) within a few minutes. It might be possible that the rapid oscillatory changes in gravity affect the pattern-forming response of organisms, which occurs over a time period of several tens of seconds (Gittleson and Jahn, 1968; Wille and Ehret, 1968; Childress et al., 1975). In addition, it is possible that the direction of gravity vector changed with respect to the suspension chamber under hypergravity performed by pulling up and down the trajectory of the airplane in the parabolic flight maneuvers. A deviation of gravity vector of some angles from normal to the chamber floor may result in increasing the suspension depth by more than the distance between the top and bottom planes of the closed chamber, as used by Noever (1991), which was considered to be the same as the ordinary depth under normal gravity. This unexpected increase in the suspension depth may lead to a decrease in the pattern spacing, as observed in *Tetrahymena* (Wille and Ehret, 1968). In the centrifuge experiment, on the other hand, hypergravity was applied for a longer period with smaller fluctuations than those in the flight experiment, and the direction of the gravity vector remained almost at right angles to the chamber floor.

Three strains of *Tetrahymena* used in the present study have different locomotor properties which might be reflected in the pattern formation in response to altered gravity: velocities of horizontal swimming were 0.56±0.05 mm s^{-1} (mean ± S.D. from N=12 measurements, each of which included 100–150 cells), 0.37±0.02 (N=14) and 0.46±0.04 (N=10) *for T. pyriformis*, *T. thermophila* and TNR, respectively, and TNR lacks genetically the avoiding reaction ability. We found little difference in either critical depth or pattern spacing between the two wild-type strains, irrespective of a large difference in swimming velocity. On the other hand, we found that $R_d > R_c$ in TNR under normal gravity when $R_d < R_c$ in wild-type strains and that the wave number was larger in TNR than in wild-type strains under otherwise similar conditions. We also found that TNR changed the pattern spacing as soon as gravity changed, whereas the changes were less sharp in the wild-type strains (Fig. 4). These facts indicate that the avoiding reaction rather than swimming speed is more crucial for the bioconvective pattern formation. TNR has a genetic defect in membrane excitability responsible for ciliary reversal, which causes a total loss of spontaneous avoiding reaction. Therefore the pattern formation characteristic to TNR can be explained in terms of changes in the diffusivity of the cell.

Bioconvection has been treated as a physical problem in which microorganisms are considered as moving particles with no characters other than the tendency of upward migration. This was in line with the theory of gravitaxis, so far explained largely in terms of the physical properties of microorganisms that are not assumed to have any mechanisms of gravity sensation. However, the physical theory of gravitaxis is disputed by proposals of feasible physiological mechanisms (Machemer and Brauecker, 1992; Hemmersbach et al., 1999). Gravity-induced sensory input and the subsequent modulation of locomotor activity in *Paramecium* was suggested from precise measurements of the difference in swimming velocity between galvanotactically fixed cells in upwards and downwards orientations (Machemer et al., 1991) and by analyses of swimming velocity as a function of swimming direction with respect to the gravity vector under natural and hypergravity (Ooya et al., 1992). As a result of gravireception, *Paramecium* appears to modulate its propulsive effort depending on the swimming direction by increasing the propulsive speed in upward and decreasing it in downward directions. This gravity-induced change in propulsion, i.e. ‘gravikinesis’, introduced by Machemer et al. (1991), has also been reported in *Terahymena* (Kowalewski et al., 1998). Gravikinesis is explained on the basis of cellular mechanosensitivity in combination with close coupling between the membrane potential and ciliary locomotor activity (Machemer, 1990). As shown in *Paramecium*, depolarizing mechanosensitive channels are located mainly at the anterior end of the cell membrane and hyperpolarizing mechanosensitive channels mainly at the posterior end (Ogura and Machemer, 1980). This arrangement of channels may lead to bidirectional changes in the membrane potential due to the selective deformation of the anterior and posterior cell membrane responding to the orientation of the cell with respect to the gravity vector: hyperpolarization or depolarization in upward or downward orientation, respectively. In response to the membrane potential shift, ciliary beating changes to increase the propulsive thrust in upward swimming and decrease it in downward swimming (Machemer et al., 1991; Ooya et al., 1992; Machemer-Roehnisch et al., 1999). In addition to the gravikinesis, Ooya et al. (1992) postulated a

physiological model of gravitaxis, in which the gravity-dependent membrane potential shift causes changes in the pitch angle of helical swimming trajectories as a result of the changes in ciliary motility strongly coupled to the membrane potential. Using electrophysiological data on ciliary electromotor coupling, computer simulation of the model demonstrated that cells swim preferentially upward along the super-helical trajectories without taking account of any mechanical properties for upward orientation (Mogami and Baba, 1998). If this is the case, *Tetrahymena* could change the propulsive thrust and orientation rate as a result of the physiological responses to the gravity stimulus increased by hypergravity. TNR may respond to hypergravity differently from the wild-type strains due to a defect in membrane excitability, which affects the coupling between gravity-dependent membrane-potential shift and ciliary motility. This may lead to the different sensitivity to gravity in TNR pattern formation.

We found firstly that bioconvective pattern formation in the suspension of *Tetrahymena* is highly sensitive to gravity. The sensitivity could be explained partly on the basis of the density-instability theory for the onset of the instability. Briefly, our experiments on a critical Rayleigh number verified this theory. Our second finding that the pattern spacing decreases with increasing gravity should aid the advancement of theoretical works. Our third finding of a clear difference in the sensitivity to hypergravity between cell strains with a different genetic background of motility suggests that the locomotor characteristics of individual cells would strongly affect the pattern formation. Gravity affects the locomotor characteristics of the cell through sedimentation and mechanical orientation. In addition, it may also do so through cellular gravireception by changing the propulsive thrust and orientation rate. Hypergravity might enhance both the physical and the physiological effects on the cell and cause the gravity-dependent behavior of bioconvection demonstrated above. It should therefore be required in the further analysis of bioconvection that the locomotor characteristics derived from the physical as well as physiological features of the individual cells are incorporated into the experimental as well as theoretical frameworks. Hypergravity experiments will still reveal a variety of characteristics, which have crucial roles in bioconvection.

List of symbols

a	average radius of microorganism
B	gyrotactic orientation parameter
$\boldsymbol{g}$	acceleration due to gravity
G	unnormalized biased Gaussian function
h	depth of subsurface layer
H	suspension depth
l	displacement of the center of gravity from that of buoyancy of the organism
L	mean free path of swimming microorganism
m	dominant wave number determined by least squares fitting of G
N_{av}	average concentration of microorganisms
$P(n)$	radial spectrum density as a function of wave number
r^2	coefficient of determination
R_c	critical Rayleigh number
R_d	density-instability Rayleigh number,
R_g	gyrotactic-instability Rayleigh number
U	swimming speed
U_v	vertical drift (the speed of gravitactic swimming)
V_o	volume of a microorganism
α	ratio of the increase in medium density per single microorganism
$\alpha_\perp$	dimensionless constant relating the viscous torque to the relative angular velocity of microorganism
δ	a parameter of anisotropic diffusion (ratio of κ_h to κ_v)
κ_h	coefficient for horizontal component of an anisotropic diffusive movement
κ_v	coefficient for vertical component of an anisotropic diffusive movement
λ	ratio of H to h
μ_m	viscosity of medium
ν	kinematic viscosity
ρ_m	density of medium
ρ_o	density of microorganism
σ	Schmidt number

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References

Bees, M. A. and Hill, N. A. (1997). Wavelength of bioconvection patterns. *J. Exp. Biol.* **200**, 1515-1526.

Chandrasekhar, S. (1961). *Hydrodynamic and Hydromagnetic Stability*. New York: Dover Publication Inc.

Childress, W. S. Levandowsky, M. and Spiegel, E. A. (1975). Pattern formation in a suspension of swimming microorganisms: equations and stability theory. *J. Fluid Mech.* **63**, 591-613.

Czirok, A., Janosi, I. M. and Kessler, J. O. (2000). Bioconvective dynamics: Dependence on organism behaviour. *J. Exp. Biol.* **203**, 3345-3354.

Ghorai, S. and Hill, N. A. (2000). Wavelength of gyrotactic plumes in bioconvection. *Bull. Math. Biol.* **62**, 429-450.

Gittleson, S. M. and Jahn, T. L. (1968). Pattern swimming by *Polytomella agilis*. *Am. Nat.* **120**, 413-425.

Harashima, A., Watanabe, M. and Fujishiro, I. (1988). Evolution of bioconvection patterns in a culture of motile flagellates. *Phys. Fluids* **31**, 764-775.

Hemmersbach, R., Volkmann, D. and Haeder, D.-P. (1999). Graviorientation in protists and plants. *J. Plant Physiol.* **154**, 1-15.

Hill, N. A., Pedley, T. J. and Kessler, J. O. (1989). The growth of bioconvection patterns in a suspension of gyrotactic micro-organisms in a layer of finite depth. *J. Fluid Mech.* **208**, 509-543.

Hirashima, M., Moriwaki, A., Mogami, Y. and Baba, S. A. (2003). Morphology-dependent mechanisms of gravitactic behavior in ciliates. *Space Util. Res.* **19**, 33-35 (in Japanese with English abstract).

Itoh, A., Amagai, K., Arai, M. and Mifune, H. (1999). The effect of rotational gravitational field on bioconvection pattern. *Trans. Japan. Soc. Mech. Eng. B* **63/630**, 698-705 (in Japanese with English abstract).

Kessler, J. O. (1985). Hydrodynamic focusing of motile algal cells. *Nature* **313**, 218-220.

Kessler, J. O. (1986). Individual and collective dynamics of swimming cells. *J. Fluid Mech.* **173**, 191-205.

Kessler, J. O. (1989). Path and pattern – the mutual dynamics of swimming cells and their environment. *Comments Theor. Biol.* **1**, 85-108.

Kim, M. Y., Kuruvilla, H. G., Raghu, S. and Hennessey, T. M. (1999). ATP reception and chemosensory adaptation in *Tetrahymena thermophila*. *J. Exp. Biol.* **202**, 407-416.

Kowalewski, U., Braeucker, R. and Machemer, H. (1998). Responses of *Tetrahymena pyriformis* to the natural gravity vector. *Microgravity Sci. Tech.* **6**, 167-172.

Levandowsky, M., Childress, W. S., Spiegel, A. E. and Hunter, S. H. (1975). A mathematical model of pattern formation by swimming microorganisms. *J. Protozool.* **22**, 296-306.

Machemer, H. (1989). Cellular behaviour modulated by ions: electrophysiological implications. *J. Protozool.* **36**, 463-487.

Machemer, H. (1990). Bioelectric control of the ciliary cycle. *Lecture Notes Biomath.* **89**, 169-183.

Machemer, H. and Brauecker, R. (1992). Gravireception and graviresponses in ciliates, *Acta. Protozool.* **31**, 185-214.

Machemer, H., Machemer-Roehnisch, S., Braeucker, R. and Takahashi, K. (1991). Gravikinesis in *Paramecium*: theory and isolation of a physiological response to the natural gravity vector. *J. Comp. Physiol. A* **168**, 1-12.

Machemer-Roehnisch, S., Nagel, U. and Machemer, H. (1999). A gravity-induced regulation of swimming speed in *Euglena gracilis*. *J. Comp. Physiol. A* **185**, 517-527.

Mogami, Y. and Baba, S. A. (1998). Super-helix model: a physiological model for gravitaxis of *Paramecium*. *Adv. Space Res.* **21**,1291-300.

Mogami, Y., Ishii, J. and Baba, S. A. (2001). Theoretical and experimental dissection of gravity-dependent mechanical orientation in gravitactic microorganisms. *Biol. Bull.* **201**, 26-33.

Mogami, Y., Kimura, T., Okuno, M., Yamashita, M. and Baba, S. A. (1988a). Free fall experiments on swimming behavior of ciliates. *Proc. 16th Int. Symp. Space Technol. Sci.* **16**, 2351-2354.

Mogami, Y., Oobayashi, C., Yamaguchi, T., Ogiso, Y. and Baba, S. A. (1988b). Negative geotaxis in sea urchin larvae: a possible role of mechanoreception in the late stages of development. *J. Exp. Biol.* **137**, 141-156.

Noever, D. A. (1991). Evolution of bioconvecive patterns in variable gravity. *Phys. Rev. A* **44**, 5279-5291.

Ogura, A. and Machemer, H. (1980). Distribution of mechanoreceptor channels in the Paramecium surface membrane. *J. Comp. Physiol. A* **135**, 233-242.

Ooya, M., Mogami, Y., Izumi-Kurotani, A. and Baba, S. A. (1992). Gravity-induced changes in propulsion of *Paramecium caudatum*: a possible role of gravireception in protozoan behaviour. *J. Exp. Biol.* **163**, 153-167.

Pedley, T. J., Hill, N. A. and Kessler, J. O. (1988). The growth of bioconvection patterns in a uniform suspension of gyrotactic micro-organisms. *J. Fluid Mech.* **195**, 223-237.

Pedley, T. J. and Kessler, J. O. (1990). A new continuum model for suspensions of gyrotactic micro-organisms. *J. Fluid Mech.* **212**, 155-182.

Pedley, T. J. and Kessler, J. O. (1992). Hydrodynamic phenomena in suspensions of swimming microorganisms. *Ann. Rev. Fluid Mech.* **24**, 313-358.

Platt, J. R. (1961). 'Bioconvection patterns' in cultures of free-swimming organisms. *Science* **133**, 1766-1767.

Plesset, M. S. and Winet, H. (1974). Bioconvection patterns in swimming microorganism cultures as an example of Rayleigh-Taylor instability. *Nature* **248**, 441-443.

Plesset, M. S., Whippele, C. G. and Winet, H. (1975). Analysis of the steady state of the bioconvection of swimming micro-organisms. In *Swimming and Flying in Nature*, vol. 1 (ed. T. T.-Y. Wu, C. J. Brokaw and C. Brennen), pp. 339-360. New York: Plenum Press.

Roberts, A. M. (1970). Geotaxis in motile micro-organisms. *J. Exp. Biol.* **53**, 687-699.

Takahashi, M., Onimaru, H. and Naitoh, Y. (1980). A mutant of Tetrahymena with non-excitable membrane. *Proc. Japan Acad.* **56 Ser B**, 586-590.

Watanabe, Y. (1963). Some factors necessary to produce division condition in *Tetrahymena pyriformis*. *Jap. J. M. Sci. Biol.* **16**, 107-124.

Wille, J. J. and Ehret, C. F. (1968). Circadian rhythm of pattern formation in populations of free-swimming organism, *Tetrahymena*. *J. Protozool.* **15**, 789-792.