Theoretical and Experimental Dissection of Gravity-Dependent Mechanical Orientation in Gravitactic Microorganisms

YOSHIHIRO MOGAMI,* JUNKO ISHII,† AND SHOJI A. BABA

Department of Biology, Ochanomizu University, Otsuka, Tokyo 112-8610, Japan

Abstract. Mechanisms of gravitactic behaviors of aquatic microorganisms were investigated in terms of their mechanical basis of gravity-dependent orientation. Two mechanical mechanisms have been considered as possible sources of the orientation torque generated on the inert body. One results from the differential density within an organism (the gravity-buoyancy model) and the other from the geometrical asymmetry of an organism (the drag-gravity model). We first introduced a simple theory that distinguishes between these models by measuring sedimentation of immobilized organisms in a medium of higher density than that of the organisms. Ni²⁺-immobilized cells of Paramecium caudatum oriented downwards while floating upwards in the Percoll-containing hyper-density medium but oriented upwards while sinking in the hypo-density control medium. This means that the orientation of Paramecium is mechanically biased by the torque generated mainly due to the anterior location of the reaction center of hydrodynamic stress relative to those of buoyancy and gravity; thus the torque results from the geometrical fore-aft asymmetry and is described by the drag-gravity model. The same mechanical property was demonstrated in gastrula larvae of the sea urchin by observing the orientation during sedimentation of the KCN-immobilized larvae in media of different density: like the paramecia, the gastrulae oriented upwards in hypo-density medium and downwards in hyper-density medium. Immobilized pluteus larvae, however, oriented upwards regardless of the density of the medium. This indicates that the orientation of the pluteus is biased by the torque generated mainly due to the posterior location of the reaction center of gravity relative to those of buoyancy and hydrodynamic stress; thus the torque results from the fore-aft asymmetry of the density distribution and is described by the gravity-buoyancy model. These observations indicate that, during development, sea urchin larvae change the mechanical mechanism for the gravitactic orientation. Evidence presented in the present paper demonstrates a definite relationship between the morphology and the gravitactic behavior of microorganisms.

Introduction

Many swimming microorganisms, including ciliate and flagellate protozoa and the planktonic larvae of some invertebrates, are negatively gravitactic; that is, they tend to swim preferentially upwards in water columns despite being heavier than water. This behavior requires the organism to orient upwards in relation to the gravity vector. Several mechanisms have been postulated for the gravitactic orientation of aquatic microorganisms (Chia et al., 1983; Bean, 1984; Machemer and Bräucker, 1992). From a physical point of view and taking account of the mechanical properties of these microorganisms, it has been postulated that the interaction of gravitational and hydrodynamic forces may cause them to orient with fore end upward. In addition to the mechanical basis, gravitactic orientation might also be explained on the physiological basis of gravity perception. To modulate the propulsive activity, some mechanosensitive devices that sense gravity (for example, statocysts) might be needed. Although functional statocysts have been found in some unicellular organisms (Fenchel and Finlay, 1984, 1986), a line of evidence for gravity-dependent modulation of propulsion has been accumulated for Paramecium (Machemer et al., 1991; Ooya et al., 1992) and Euglena (Machemer-Röhnsch et al., 1999), which have no statocyst-like structure.

The present paper focuses on the mechanical properties of
MECHANICAL BIAS OF MICROBIAL GRAVITAXIS

microorganisms, which, irrespective of propulsion, generate the torque to orient the organisms either upwards or downwards. This mechanical torque should bias the gravitactic orientation, even if the organisms have active physiological mechanisms of gravitaxis. According to Roberts (1970), two mechanical mechanisms have been considered as possible sources of the orientation torque. These are reconsidered, in the present paper, as two mechanical models, the gravity-buoyancy model and the drag-gravity model.

The gravity-buoyancy model was first postulated by Verworn (1889, cited in Machemer and Bräucker, 1992) for the negative gravitaxis of Paramecium. This model is based on the differential density within an organism. If the internal density of the organism is not homogeneous, the center of mass (the center of gravity) does not necessarily coincide with the centroid (the center of buoyancy). Posterior accumulation of the mass would result in the upward orientation of the organisms, and anterior accumulation would result in the downward orientation.

The drag-gravity model was postulated by Roberts (1970) on the basis of the low Reynolds number hydrodynamics of the swimming of microorganisms that have a geometrical fore-aft asymmetry. This model is characterized by a dumbbell with two spheres of unequal diameter but homogeneous density, which could mimic the fore-aft asymmetry of the microorganisms. According to Stokes’ drag formula, the larger sphere of the dumbbell can sink faster than the smaller, at the rate of the square of the ratio of diameters. The applicability of this model has been confirmed by scale-model experiments (Roberts, 1970).

Organisms, in general, possess some asymmetry both in internal density and in external geometry. It is therefore possible that these two mechanical models operate independently to generate the gravity-induced orientation torque. Since the mechanical properties for gravitactic orientation are independent of propulsive thrust, we can assess the mechanical influence by measuring the orientation of immobilized organisms sinking under gravity. Both models predict that, when immobilized, an organism orients upwards when sinking in a medium with a density lower than its own.

In the present paper, we show that the above two models can be distinguished by observing what happens to an organism placed in a medium whose density is higher than its own. We show the results of the experiments on the gravitactic orientation of Paramecium and sea urchin larvae, both of which are known to perform typical negative gravitaxis (Mogami et al., 1988; Ooya et al., 1992).

Theory

The external forces acting on the body of an aquatic microorganism due to gravity acceleration are gravitational ($F_G$) and buoyant forces ($F_B$), each of which is generated as the product of the volume and density of the body or of the external fluid. The vector sum of the forces encounters the hydrodynamic force ($F_H$). Since the Reynolds number of an aquatic microorganism in translational motion is significantly less than unity (of the order of $10^{-5}$), $F_H$ is generated in proportion to the velocity (Happel and Brenner, 1973; Vogel, 1994). $F_G$, $F_B$, and $F_H$ act on the center of mass ($G$), the centroid ($B$), and the reaction center of hydrodynamic stress ($H$), respectively. For an immobilized microorganism sinking in the fluid, these three forces are balanced as

$$F_G + F_B + F_H = 0.$$  \hspace{1cm} (1)

Each term in the equation (positive in upward direction) is described as

$$F_G = -V \rho g,$$  \hspace{1cm} (2)

$$F_B = V \rho g,$$  \hspace{1cm} (3)

$$F_H = -K s,$$  \hspace{1cm} (4)

where $V$ and $\rho$ are the total volume and the average density of the organism, $\rho$ and $g$ the density of the external fluid and the acceleration due to gravity, and $K$ and $s$ the coefficient of hydrodynamic drag and the sinking velocity.

We assume in the present paper that a microorganism has a body of rotating symmetry on its fore-aft axis. The simplest case of this approximation is that the body has fore-aft symmetry, such as a prolate spheroid. When a prolate spheroid with uniform density is sinking in the fluid, the three forces act on the same point and therefore do not generate any torque to rotate the body (Fig. 1a).

If, however, the body of a prolate spheroid has a region of higher density in the rear half of the body, as postulated in the gravity-buoyancy model, $G$ is located posterior to $B$ and $H$ (Fig. 1b). This generates the torque ($T_V$; subscript $V$ is after Verworn) which is given by

$$T_V = F_G L_G \sin \theta,$$  \hspace{1cm} (5)

where $L_G$ is the distance between $G$ and $B$ (and/or $H$), and $\theta$ is the orientation angle of the fore-aft axis of the body to the vertical.

The fore-aft asymmetry of the external geometry, as postulated in the drag-gravity model, also separates the reaction centers of the forces. If a microorganism of homogeneous density has a larger radius of revolution around the fore-aft axis in the posterior part (Fig. 1c), $H$ is located anterior to $B$ and $G$, according to the analogy of a fore-aft asymmetrical dumbbell of homogeneous density (Happel and Brenner, 1973). The torque ($T_K$; subscript $R$ is after Roberts) by the anterior shift of the center of hydrodynamic force is given by

$$T_K = -F_H L_H \sin \theta = (F_G + F_B) L_H \sin \theta.$$  \hspace{1cm} (6)

where $L_H$ is the distance between $H$ and $G$ (and/or $B$).

Provided that the Reynolds number of rotational motion is sufficiently small, all torques should be proportional to
the first power of rotational velocity \( (d\theta/dt) \). In such cases equations of rotational motion are given by

\[
-R\eta \frac{d\theta}{dt} = T_v \text{ or } T_r, \tag{7}
\]

where \( R \) is the coefficient of resistance for rotational motion and \( \eta \) is the viscosity of the external fluid. From these equations the rotational velocity of each model is given as a common form of

\[
\frac{d\theta}{dt} = \beta \sin \theta, \tag{8}
\]

where the proportional factor is the instantaneous rate at \( \theta = 90 \) degrees, and given by

\[
\beta = \beta_v = \frac{V\rho gL_G}{R\eta} \quad \text{and} \quad \beta = \beta_R = \frac{V(\rho_i - \rho)gL_H}{R\eta}, \tag{9}
\]

for the gravity-buoyancy and drag-gravity models, respectively.

Equations 9 and 10 indicate that \( \beta_v \) is insensitive to changes in the density of the external medium (\( \rho \)), whereas \( \beta_R \) reverses the sign as \( \rho \) exceeds the density of organisms (\( \rho_i \)). This means that the two models can be distinguished by increasing \( \rho \) greater than \( \rho_i \). When immobilized organisms are immersed in the hyper-density medium (\( \rho > \rho_i \)), they would orient upwards during floating upwards if they obeyed the gravity-buoyancy model, whereas they would orient downwards if they obeyed the drag-gravity model.

The gravity-buoyancy and drag-gravity models are the two extremes of these conditions that can generate the orientation torque depending on the different physical mechanisms. Passive orientation of the organisms (Eq. 8), in fact, would be explained as a result of combining the two models, because none of three forces would necessarily have a common reaction center. In order to extract the origin of the mechanical bias of the orientation, Equation 8 should be examined by measuring \( \beta \) by the sedimentation experiment using media of different \( \rho \). If \( \beta \) is constant independent of \( \rho \), the gravity-buoyancy model is the only mechanism for generating the orientation torque. Otherwise, the drag-gravity model may play a part in the generation of the torque. A negative value of \( \beta \) in the hyper-density medium indicates

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**Figure 1.** Schematic drawings illustrating the mechanical (physical) basis for the generation of gravity-dependent orientation torque. Gravity (\( F_G \)), buoyancy (\( F_B \)), and hydrodynamic force (\( F_H \)) are balanced in sinking microorganisms; these forces act at the center of mass (\( G \)), the centroid (\( B \)), and the reaction center of hydrodynamic stress (\( H \)), respectively. (a) Three forces act at the same point in the body of prolate spheroid with uniform density. (b) The center of mass is deviated to the rear end of the body of prolate spheroid, which generates the torque in proportion to \( F_G \) and the sine of the orientation angle to the gravity vector (\( \theta \)). (c) The reaction center of hydrodynamic stress is deviated to the front end of the body with fore-aft asymmetry but with uniform density, which generates the torque in proportion to the vector sum of \( F_G \) and \( F_B \) and the sine of the orientation angle.
that the drag-gravity model is the major mechanism in passive gravitactic orientation.

**Materials and Methods**

**Microorganisms and experimental solutions**

*Paramecium caudatum* was grown at 24 °C in a hay infusion in Dryl’s solution (2 mM sodium citrate, 1.2 mM Na₂HPO₄, 1.0 mM NaH₂PO₄, 1.5 mM CaCl₂, pH 7.2). Cells grown to the early stationary phase (14–20 d after incubation) were collected and adapted in the experimental solution (KCM; 1.0 mM KCl, 1.0 mM CaCl₂, 1.0 mM MOPS, pH 7.2). After the adaptation, cells gravitactically accumulating beneath the water surface were collected and immobilized in the KCM containing 5 mM NiCl₂. Hyper-density KCM (P-KCM) was prepared by substituting a colloidal solution of Percoll (Sigma) for water up to 60% (v/v) in KCM. At 24 °C, the specific gravity and relative viscosity of KCM were 1.00 and 1.02, respectively; those of P-KCM were 1.06 and 1.57. Specific gravity of the experimental solutions was determined by weighing the known volume, and viscosity was measured by means of an Ostwald viscometer.

Larvae of the sea urchin *Hemicentrotus pulcherrimus* were grown at 17 °C (Degawa et al., 1986). Larvae at the mid- to late gastrula stage (ca. 24 and 48 h after insemination, respectively) were collected by hand centrifuge and washed once with artificial seawater (ASW; 450 mM NaCl, 10 mM KCl, 10 mM CaCl₂, 25 mM MgCl₂, 28 mM MgSO₄, 10 mM Tris-HCl, pH 8.0). For immobilization, larvae were immersed in ASW containing 2 mM KCM. Hyper-density ASW (P-ASW) was prepared by substituting Percoll for water up to 22% (v/v) in ASW. At 25 °C, the specific gravity and relative viscosity of ASW were 1.01 and 1.07, respectively; those of P-ASW were 1.04 and 1.14.

**Recordings and analyses of gravity-dependent orientation**

Ni²⁺-immobilized *Paramecium* cells and KCN-immobilized sea urchin larvae were transferred, with experimental solutions to be tested, into a chamber made of a slide and coverslip and silicone rubber spacer (inner dimension 12 × 24 × 1 mm for *Paramecium* and 16 × 16 × 1 mm for sea urchin larvae) and kept air bubble-free without any particular sealant. The chamber was set on a horizontal microscope equipped with a rotating stage. After trapping immobilized specimens at the bottom or the top of the chamber (depending on the density of the medium), the chamber was rotated upside down, and the orientation motion during vertical movement due to gravity was recorded with a video camera (XC-77, Sony, Tokyo) and a videotape recorder. To avoid the hydrodynamic interactions between nearby moving objects, we chose organisms moving down (or up) far from neighbors (>1 mm, about 5 body lengths, apart). For measuring the orientation angle, we selected recordings in which the orientation motion was observed in a single focal plane.

The orientation angle as a function of time (θ, t) was measured directly on the video monitor. The rotational velocity as a function of orientation angle (dθ/dt, θ) was obtained as an average velocity ((θᵢ₊₁ − θᵢ)/Δt) at the angle of geometrical average ((θᵢ + θᵢ₊₁)/2) between every successive datum of inclination angle versus time. β in Equation 8 was obtained by nonlinear least-squares regression of the velocity data (dθ/dt, θ) to the equation

\[
\frac{d\theta}{dt} = \beta \sin (\theta + \alpha),
\]

where α is a factor to adjust the angle between the morphologically defined fore-axial axis and the mechanically defined axis.

**Results**

The drag-gravity model is the major mechanism of *Paramecium*

When *Paramecium* was immobilized by Ni²⁺, it maintained an anterior-thinner cell shape. This shape was preserved in P-KCM as well as in KCM: cells showed no significant changes in axial length (162 ± 17 μm [n = 30] and 163 ± 16 μm [n = 21], P = 0.64, for cells in KCM and P-KCM, respectively) or in maximum width (47.2 ± 6.9 μm and 46.5 ± 4.7 μm, P = 0.69). Thus it is highly likely that rotational motion of the immobilized cell occurs with the same coefficient of resistance in both media.

Typical recordings of gravity-dependent orientation of immobilized paramecia in the hypo- and hyper-density media are shown in Figure 2a and b. In KCM (ρ < ρ₀), paramecia oriented upwards during sinking due to gravity, whereas in P-KCM (ρ > ρ₀) they oriented downwards during floating up. As shown in Figure 2c, plots of orientation rates (dθ/dt) against orientation angle (θ) fit well to the sinusoidal function of Equation 11. Values for β obtained by least-square regression were positive in the control hypo-density medium and negative in the hyper-density medium (Table 1). Negative values of β in the hyper-density medium indicate that the drag-gravity model is the major mechanism of mechanical gravitactic orientation in *Paramecium*.

*Sea urchin larvae change the mechanical mechanism of gravitactic orientation during development*

When sea urchin larvae were treated with KCN, their cilia ceased beating and stood nearly perpendicular to the larval surface. The outer morphology of the larvae was observed to be well preserved in P-ASW as well as in ASW: for gastrulae, axial length was 151 ± 7.6 μm (n = 16) and 145 ± 6.1 μm (n = 13), P = 0.19, in ASW and P-ASW,
respectively, and the maximum width was 135 ± 3.7 μm and 132 ± 5.7 μm, \( P = 0.06 \); for plutei, axial length was 235 ± 19 μm (\( n = 26 \)) and 240 ± 13 μm (\( n = 18 \)), \( P = 0.29 \), in ASW and P-ASW, respectively, and the maximum width was 175 ± 13 μm and 175 ± 12 μm, \( P = 0.98 \). This may justify the common basis for drag coefficients in rotation in the different density media, as in Paramecium.

The gravity-dependent orientation of immobilized larvae is shown in Figure 3a to d, which demonstrates the clear difference between gastrula and pluteus. In ASW (\( \rho < \rho_s \)), both gastrula and pluteus oriented upwards while sinking; in hyper-density P-ASW, however, gastrula oriented downwards but pluteus upwards while floating up. As shown in Figure 3e and f, the orientation rate appears to be a sinusoidal function of the orientation angle; although data from larvae fitted less closely to Equation 11 than did those from Paramecium, this was probably due to the uncertainty in measuring the orientation angle of the larvae. We sometimes observed that larvae rotated slowly around the fore-aft axis during sedimentation. This slow axial rotation made it difficult to determine the fore-aft axis of the larvae.

As shown in Table 1, values of \( \beta \) obtained from gastrula larvae were positive in the control medium and negative in the hyper-density medium. Thus, in gastrulae as in Paramecium, the drag-gravity model is the major mechanism of passive gravitactic orientation. However, pluteus larvae have positive values of \( \beta \) both in the control and in the hyper-density medium (Table 1). The relatively weak dependency of \( \beta \) of plutei on the density of the external medium indicates that the gravity-buoyancy model is the major mechanism of passive gravitactic orientation in these larvae. These results indicate that sea urchin larvae change the mechanical mechanism of gravitactic orientation during development.

**Discussion**

Estimation of the contribution of the mechanical models in the gravitactic orientation

The Reynolds number of rotational motion (\( Re_r \)) of the microorganisms is defined as

\[
Re_r = \frac{l^2 \omega \rho}{\eta},
\]

where \( l \) is a characteristic body length and \( \omega \) is the angular velocity of rotation (Happel and Brenner, 1973). From the maximum velocity of rotation (ca. 0.2 rad s\(^{-1}\), Table 1), \( Re_r \) of Paramecium or sea urchin larvae is calculated to be about 2 × 10\(^{-3}\), which is sufficiently smaller than unity. This means that the linear assumption of Equation 7 (see the Theory section) is valid to formulate the rotational motion of these microorganisms.

The orientation torque generated as a result of the combination of the torque originating from different mechanical sources causes the passive orientation of the immobilized organisms. It is difficult to formulate the combination, because we know little about the density distribution within an organism and its geometrical asymmetry. The simplest assumption for the combination of the rotational torque is that \( G, B, \) and \( H \) are located on the geometrical fore-aft axis of the organisms. This gives a sinusoidal function as a linear summation of the sinusoidal equations, each of which is deduced from the gravity-buoyancy and drag-gravity model, respectively. As a result, the orientation rate is given as
Orientation rate ($\beta$), in rad $\cdot$ s$^{-1}$, measured in different density media

| Organism       | Normal medium | Percoll-containing medium |
|----------------|---------------|---------------------------|
|                | Mean ± SD     | Range                     | Mean ± SD     | Range                     |
| Paramecium     | 0.090 ± 0.033 | 0.043 – 0.183             | –0.104 ± 0.058 | –0.257 – –0.041 |
| Sea urchin larvae |             |                           | 0.140 ± 0.032  | 0.107 – 0.197       |
| Gastrula       | 0.140 ± 0.032 | 0.107 – 0.197             | –0.120 ± 0.020 | –0.150 – –0.090 |
| Pluteus        | 0.157 ± 0.031 | 0.105 – 0.190             | 0.110 ± 0.013  | 0.097 – 0.137  |

\[
\frac{d\theta}{dt} = (\beta_v + \beta_R) \sin \theta. \tag{13}
\]

This simple linear assumption seems to be supported by the fact that $\alpha$ in Equation 11 was calculated on average as nearly zero (0.00 ± 0.26 rad $n = 37$) for *Paramecium*, 0.03 ± 0.18 ($n = 15$) for gastrula, and 0.06 ± 0.21 ($n = 16$) for pluteus). Therefore, it is likely that the morphologically defined fore-aft axis almost coincides with the mechanically defined axis. According to the assumption above, $\beta_N$ obtained in the different density media are given by

\[
\beta_N = \frac{V \rho_p g L_G}{R \eta_N} + \frac{V (\rho_r - \rho_N) g L_H}{R \eta_N}, \tag{14}
\]

\[
\beta_R = \frac{V \rho_p g L_G}{R \eta_R} + \frac{V (\rho_r - \rho_R) g L_H}{R \eta_R}, \tag{15}
\]

where $\beta_N$ is the maximum orientation velocity measured in the normal density ($\rho_N$) medium (KCM or ASW) of the viscosity of $\eta_N$, and $\beta_R$ is that measured in the hyper-density ($\rho_R$) medium (P-KCM or P-ASW) of the viscosity of $\eta_R$. Equations 14 and 15 give $L_H$, the distance from B to H, as

\[
L_H = \frac{\eta_N \beta_N - \eta_R \beta_R}{\rho_r - \rho_N} \frac{R}{V g}, \tag{16}
\]

and, thus, $\beta_R$ and $\beta_V$ are given by:

\[
\beta_R = \frac{\rho_r - \rho_N}{\rho_r - \rho_N} \left( \frac{\beta_N - \beta_R}{\eta_R} \right) \tag{17}
\]

\[
\beta_V = \beta_N - \beta_R. \tag{18}
\]

For *Paramecium*, $\rho_N = 1.00$, $\rho_R = 1.06$ and $\rho_l = 1.03$ g $\cdot$ cm$^{-3}$ (Ooya et al., 1992), and $\eta_d/\eta_N = 1.53$. For sea urchin larvae, $\rho_N = 1.01$, $\rho_R = 1.04$, and $\rho_l = 1.03$ and 1.03 g $\cdot$ cm$^{-3}$, for gastrula and pluteus, respectively (values were obtained by sedimentation equilibrium experiments; data not shown), and $\eta_d/\eta_N = 1.07$. Using these values and $\beta_N$ and $\beta_R$ in Table 1, Equations 17 and 18 can be used to obtain values for the contribution of the two mechanisms to negative gravitaxis in normal-density medium. The upward orientation of *Paramecium* in KCM, corresponding to $\beta_N = 0.09$ rad $\cdot$ s$^{-1}$, is the result of an upward drag-gravity component ($\beta_R = 0.12$ rad $\cdot$ s$^{-1}$) combined with a smaller downward gravity-buoyancy component ($\beta_V = –0.03$ rad $\cdot$ s$^{-1}$). The situation is similar for sea urchin gastrulae. The upward orientation with $\beta_N = 0.14$ rad $\cdot$ s$^{-1}$ results from an upward drag-gravity component ($\beta_R = 0.18$ rad $\cdot$ s$^{-1}$) combined with a small downward gravity-buoyancy component ($\beta_V = –0.04$ rad $\cdot$ s$^{-1}$). However, the upward orientation of pluteus larvae with $\beta_N = 0.16$ rad $\cdot$ s$^{-1}$ reflects a very different situation. The gravity-buoyancy component has reversed direction from downward to upward, and has increased to $\beta_V = 0.13$ rad $\cdot$ s$^{-1}$. The upward drag-gravity component has diminished greatly, to $\beta_R = 0.03$ rad $\cdot$ s$^{-1}$, so that it now makes only a small contribution to the upward orientation.

The mechanical property of *Paramecium*

There have been several investigations on the mechanical basis of the passive upward orientation of *Paramecium*. Most of them favored the gravity-buoyancy model as a major mechanism of gravitactic orientation. Fukui and Asai (1980) reported that Triton-treated immobilized cells oriented mostly upwards at the sedimentation equilibrium in sucrose density gradient. This upward orientation was evident in well-fed cells but not in starved cells. The upward-orienting posture was found under centrifugal force in Ni$^{2+}$-immobilized cells in the isodensity medium (Taneda et al., 1987) and also in the cells swimming at isopycnic level in the density gradient with Ficoll or Percoll (Kuroda and Kamiya, 1989). It was also reported that upward orientation was induced by centrifugal force effectively in the cells at the early culture phase but not in those at the late phase, which showed little or no gravitaxis. These results appear to conform with the conclusion that the upward orientation of *Paramecium* is strongly biased by the torque resulting from the higher density of the posterior part of the organism; the increased density is mainly due to the accumulation of food vacuoles (Fukui and Asai, 1985).

It should be noted, however, that the results of the sedimentation equilibrium experiments were ascribed only to the function of the gravity-buoyancy model and not to the contribution of the drag-gravity model, since $F_H = 0$ with buoyancy artificially balanced with gravity. Furthermore, it
seems likely that the gravity-buoyancy component of the orientation torque might be enhanced in these experiments. Since the center of gravity would shift in relation to the content and the distribution of organelles such as food vacuoles, it is probable that in the sedimentation equilibrium experiments, the intracellular distribution of the organelle was reorganized by gravity during long-lasting sedimentation of Triton-permeabilized cells through the sucrose density gradient (Fukui and Asai, 1980), or by a large centrifugal acceleration (100$ \times $ g, Taneda et al., 1987; 300–400 $ \times $ g, Kuroda and Kamiya, 1989). This may result in accumulation of organelles in the rear part of the cell, and may cause upward orientation, even if the cells originally have a slightly top-heavy organelle distribution that gives a negative $ \beta_V $ as estimated above. These facts suggest that the results of previous experiments are still equivocal for the contribution of the drag-gravity model in the gravitactic orientation of Paramecium.

The evidence presented in the Results, on the contrary, indicate that the drag-gravity model makes a major contribution to generating a torque for the gravitactic orientation. Although the possibility of a minimal contribution cannot be ruled out, it is clear that the gravity-buoyancy model cannot solely explain the alteration of the sign of the rotational torque in the hyper-density medium. In addition, paramecia were observed in P-KCM to swim mostly downwards (data not shown). Swimming cells changed the net direction of their helical swimming trajectory gradually downwards and accumulated at the bottom of the chamber against the strong floating bias. Positive gravitaxis of Paramecium in the hyper-density medium can be explained by the drag-gravity model, not by the gravity-buoyancy model.

Developmental changes in the mechanical property in sea urchin larvae

In the present paper we demonstrated a change in the mechanical basis for gravitactic orientation during the development of sea urchin larvae: from the drag-gravity model in gastrulae to the gravity-buoyancy model in plutei. Gastrulae have a thicker posterior part, similar to that of Paramecium, which is required for the drag-gravity model to function. Plutei, on the other hand, have a thicker anterior part. Therefore they may orient the rear end upwards if the rotational torque is generated according to the drag-gravity model. This was not the case for plutei. Regardless of the remarkable fore-aft asymmetry in morphology, plutei obeyed the gravity-buoyancy model. Gravitactic orientation by different mechanisms was also revealed in the gravitactic swimming behavior of the larvae in P-ASW. In spite of the strong floating bias, gastrulae swam preferentially downwards (positive gravitaxis) and accumulated at the bottom of the chamber, whereas plutei swam upwards (negative gravitaxis) and accumulated at the top of the chamber (data not shown).

Mogami et al. (1988) found that sea urchin larvae change their gravitactic behavior during development. Larvae at the blastula stage to the early gastrula stage swim preferentially
upwards. This may be explained by a major upward drag-gravity component of orientation torque. The negative gravitactic behavior becomes less remarkable in prism larvae: they tend to swim in random directions independent of the gravity vector. This transient disappearance of gravitaxis may correspond to the alteration of the orientation mechanism revealed in the present paper. At the pluteus stage, larvae again show negative gravitaxis as they acquire the orientation mechanism with a major upward gravity-buoyancy component. A strong separation between the centers of gravity and buoyancy may develop in association with the growth of skeletal structures. Rudiments of spicules growing, they may change the density distribution to shift the center of gravity toward the rear of the cell. If plutei hereafter maintained the rear-end-heavy mass distribution, they could maintain negative gravitactic behavior irrespective of pronounced morphological changes during the late larval stages.

Although the functional role of the drag-gravity model has been accepted in theory, it was not experimentally demonstrated in the orientation movement of organisms. In the present paper we present the first evidence that external forces may play a role in the orientation of larval organisms. The spicule is made of magnesian calcite with a density about three times higher than the average density (Okazaki and Inoué, 1976). As spicules grow, they may change the density distribution to shift the center of gravity toward the rear of the cell. If plutei hereafter maintained the rear-end-heavy mass distribution, they could maintain negative gravitactic behavior irrespective of pronounced morphological changes during the late larval stages.

In conclusion, the present study on the mechanical properties of gravitactic orientation in the gravity field demonstrates a relation between the morphology of microorganisms and their gravitactic behavior. This relationship might be instructive in researching cases of microbial gravitaxis whose mechanism is still disputed.

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