

## GRAVITY-INDUCED CHANGES IN PROPULSION OF *PARAMECIUM CAUDATUM*: A POSSIBLE ROLE OF GRAVIRECEPTION IN PROTOZOAN BEHAVIOUR

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### Summary

The swimming behaviour of *Paramecium* was analyzed under natural and experimental hypergravity conditions. *Paramecium* that swam upwards (in the opposite direction to the gravitational force) along a straight path (straight swimmers) swam more slowly than those swimming downwards. This dependence of the swimming velocity on its direction relative to gravity can be partly interpreted as the consequence of sinking due to gravity if the propulsive force does not vary. The effect was different for *Paramecium* swimming along a circular path (curved swimmers). The difference in velocity between those swimming upwards and those swimming downwards was substantially smaller than would have been expected from sinking effects with invariant propulsion even after correcting for maximal hydrodynamic wall effects, indicating that *Paramecium* compensate for sinking caused by gravity by controlling their propulsion. The propulsive velocity evaluated by vector calculus increased both as *Paramecium* swam more sharply upwards and as the experimental gravitational force was increased. The dependence of propulsion on the swimming direction and on gravity was reduced in a high-density medium of nearly neutral buoyancy, suggesting that the site of gravireception is unlikely to be in the interior of the cell. The differences between straight and curved swimmers are discussed in terms of rapid adaptation of gravireceptors in the cell membrane, desensitization of mechanosensory channels and hyperactivation of ciliary activity in straight swimmers.

### Introduction

It has been suggested that ciliates are capable of modulating their swimming behaviour by controlling ciliary activity in response to sensory input (Machemer, 1988; Naitoh, 1984; Poff *et al.* 1984). This sensory input may be chemical, tactile, thermal or optical. A possible role of sensory input from gravitational acceleration

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(gravireception) has also been postulated for loxodid Protozoa, which possesses vacuoles containing concretions of a barium compound (Finlay *et al.* 1983; Rieder *et al.* 1982) that bear a morphological resemblance to hydrozoan statoliths (Bedini *et al.* 1973; Horridge, 1969; Markl, 1974) and come into close contact with the vacuolar membrane when the cell is rotated in the gravitational field (Fenchel and Finlay, 1986). Capillary tube inversion experiments with a horizontal microscope have demonstrated that individual cells of *Loxodes striatus* swimming upwards under anoxic conditions react to rapid upside-down inversion by turning themselves back to their original direction of swimming; cells under aerobic conditions prefer downward swimming and show similar turning responses to changes in the direction of the cell body relative to gravity (Fenchel and Finlay, 1984).

Evidence for gravireception and candidates for gravireceptive organelles in non-loxodid Protozoa, e.g. *Paramecium*, have also been looked for (Bean, 1984; Machemer and de Peyer, 1977; Machemer *et al.* 1991; Markl, 1974; Mogami *et al.* 1988a). Our present analysis of the swimming velocity of *Paramecium caudatum* in experimental gravitational fields reveals gravity-induced changes in propulsion, and suggests that ciliary activity is modified by sensory input due to gravity. In view of the results from similar experiments but in a high-density medium, we propose a new model for gravireception in *Paramecium*: the anterior and posterior membranes react by depolarizing and hyperpolarizing, respectively, in response to stretching as a result of a local pressure difference caused by the density difference between the interior and the exterior of the cell when it is placed in a gravitational field. This shift in the membrane potential may modulate ciliary activity, as it does in voltage-clamped cells (Machemer, 1988). This causes an increase in speed, resulting from hyperpolarization, in cells swimming upwards and a decrease in speed, resulting from depolarization, in those swimming downwards in accordance with the present results and those of recent experiments with galvanotactically aligned *Paramecium* (Machemer *et al.* 1991). A possible role for gravity-induced modulation of the membrane potential and for its coupling to the ciliary beat polarity (Machemer, 1988) and to the pitch angle of helical trajectories (Machemer, 1989, 1990; Machemer and Sugino, 1989; Crenshaw, 1990) in negative geotactic orientation is also discussed. Parts of this work have been presented elsewhere (Baba *et al.* 1989a,b; Izumi-Kurotani *et al.* 1989; Ooya *et al.* 1988, 1989a,b,c).

## Materials and methods

### *Culture and preparation*

All experiments were carried out with *Paramecium caudatum* Ehrenberg (syngen 3, mating type V, a gift from Dr M. Takahashi of Tsukuba University), grown at 24°C in a hay infusion in Dryl's medium (2 mmol l<sup>-1</sup> sodium citrate, 1.2 mmol l<sup>-1</sup> Na<sub>2</sub>HPO<sub>4</sub>, 1.0 mmol l<sup>-1</sup> NaH<sub>2</sub>PO<sub>4</sub>, 1.5 mmol l<sup>-1</sup> CaCl<sub>2</sub>, pH 7.1; Dryl, 1959). Cells grown to the stationary phase (10–14 days after incubation) were

collected through a nylon mesh (pore size 42  $\mu\text{m}$ ), washed in fresh Dryl's medium and adapted to room temperature (23–24.5°C) before use (normally at least 1 h). In some experiments, cells were adapted in high-density Dryl's medium containing Percoll prepared by diluting a 23 % (w/w) colloidal solution of Percoll (Sigma) to 25 % v/v (specific gravity 1.033, relative viscosity 1.12 at 24.5°C).

*Paramecium* in these media were transferred into a chamber made of a slide and/or cover slip and silicone rubber spacer (inner dimensions 13 mm  $\times$  13 mm  $\times$  1 mm) and kept free of air bubbles with no particular sealant. They remained healthy for several hours under these conditions.

#### *Recording and analysis of the swimming paths*

The swimming behaviour of *Paramecium* in the chambers was recorded either by multiple-exposure photography under dark-field stroboscopic illumination (Dagawa *et al.* 1986) or by video-microscopy with a charge-coupled device (CCD) camera (XC-77, Sony, Tokyo) and a video tape recorder (VTR, EDV-5000, Sony) under bright-field illumination with a light-emitting diode (MU02-2205, Stanley, Tokyo). The multiple-exposure photographs were analyzed as described previously (Degawa *et al.* 1986). The video tape recordings were analyzed by means of digital image analysis as described in detail elsewhere (Baba *et al.* 1991). Successive frames from the VTR at constant time intervals (usually 0.6 s) were fed into an image processor (Nexus 6510, Nexus, Tokyo) to give a multiple-exposure photo-like image, which was analyzed with laboratory-made software (VIDEOB Ver. 1.13) on an IBM 4381-R24 computer.

The swimming vector velocity,  $v$ , of cells was computed from the difference between successive Cartesian coordinates of the centre of the cell body divided by the time. The magnitude of the velocity,  $v$ , of individual cells or of a population specified by the average curvature of the swimming path was averaged and plotted against the angular direction,  $\theta$ , of swimming with respect to gravity. This presentation facilitated comparison of data with a theoretical curve for cells swimming with constant propulsive force in gravitational fields expressed by the equation:

$$v = \sqrt{(p_i^2 - s^2 \sin^2 \theta) + s \cos \theta}, \quad (1)$$

where  $p_i$  and  $s$  are the magnitudes of the invariant propulsive and sinking velocities, respectively (Roberts, 1970; Mogami *et al.* 1988b). To plot this curve,  $p_i$  was calculated from the data as follows:

$$p_i = (v_{\text{dn}} + v_{\text{up}})/2, \quad (2)$$

where  $v_{\text{dn}}$  and  $v_{\text{up}}$  are the magnitudes of the downward and upward velocities, respectively;  $s$  was evaluated from recordings of the rate of sinking of cells from the same culture immobilized in Dryl's solution containing 5 mmol l<sup>-1</sup> NiCl<sub>2</sub>. The actual propulsive vector velocity,  $\mathbf{p}$ , was also evaluated by vector calculus from  $v$  and  $s$  and analyzed as a function of its angular direction,  $\phi$ , with respect to gravity.

*Hypergravity experiments*

Hypergravity was achieved by using a swing-basket-type centrifuge, which had two baskets with inner dimensions of 400 mm×210 mm horizontal and 320 mm vertical at each edge of 2-m arms. One of the baskets carried *Paramecium* in the chamber described above and recording equipment, including the CCD camera with the VTR placed outside and connected through slip rings at the centre of the centrifuge. The wider wall of the chamber, to which the optical axis of the horizontal microscope was perpendicular, was vertical with respect to the floor of the basket and parallel to the arm. The resultant force of the earth's gravitational force and the centrifugal force, which will be referred to as 'experimental' gravity or simply gravity, was designed to be perpendicular to the floor of the baskets and was monitored with a load cell carried in the second basket. It was found that the experimental gravity was steady at scheduled set-points in the range 2–5 *g* and was always directed perpendicular to the floor, and hence downwards in the picture taken through the horizontal microscope. Recording for 1 *g* was performed inside the basket before and after operation of the centrifuge.

Sinking velocities under hypergravity conditions were also measured using nickel-immobilized cells. When the experimental gravity had reached a given value, the chamber was gently inverted, with the axis of rotation being kept on the optical axis of the horizontal microscope by means of a controlled d.c. motor. The sinking velocity was, however, routinely evaluated by extrapolation from the mean value measured under 1-*g* conditions, since that measured after the initial perturbation had settled was found to be exactly proportional to gravity at least up to 5 *g* (not shown).

**Results***Long-term adaptation*

*Paramecium* swam at 1–2 mm s<sup>-1</sup> frequently (about 80 % of observations) along a straight line (short-diameter helical path) immediately after they were transferred into an experimental chamber. After adaptation (usually 1 h), they swam along either a straight or a circular path (long-diameter helical path) with nearly equal frequency. The swimming velocity decreased to a steady level within this period of adaptation and remained steady, as reported by other authors (Machemer, 1989; Oka *et al.* 1986). It was this velocity that was measured.

Gravity was increased step by step with ample time for recording at each steady state and then decreased in a similar way. There was no systematic variation in the results between experiments in which gravitational force was increased and those in which it was decreased. Therefore, data from cells under experimental gravitational conditions were not thought to be affected by long-term hysteretic effects of gravistimulation (stimulation by gravity) and will be referred to only by the value of the gravitational force.

*The effect of gravitational force on the swimming velocity of individual cells*

Individual cells swimming along a circular path, usually viewed from the top or

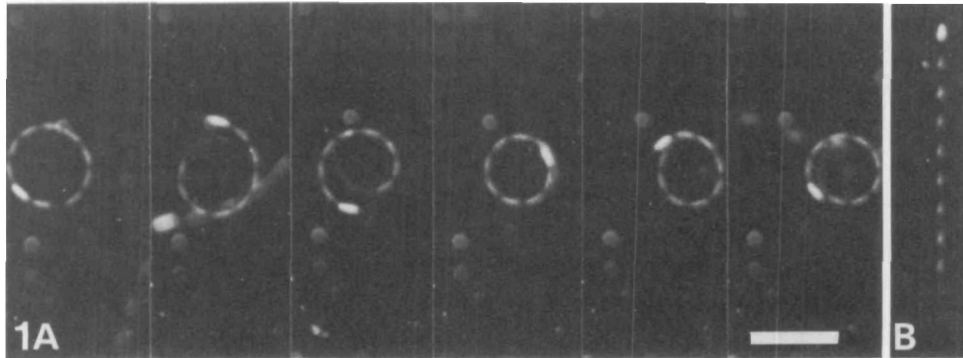


Fig. 1. Multiple-exposure photographs of swimming and falling *Paramecium* under natural gravity conditions at 24 °C. (A) Six successive frames (from left to right at about 7 s intervals) of a single *Paramecium* swimming along a circular path in a vertical plane (upwards is towards the top of the page) in Dryl's solution. (B) An immobilized *Paramecium* falling in Dryl's solution containing 5 mmol l<sup>-1</sup> NiCl<sub>2</sub>. Flash intervals: 0.5 s (A) and 4.0 s (B); the first flash is the brightest. Scale bar, 1 mm.

bottom of the helical axis, were found to maintain their average vertical position (Fig. 1A). Consistent downward drifts were not detected during the observation time. During the same period, immobilized cells fell a substantial distance (Fig. 1B). These circular swimmers occasionally even drifted upwards (not shown).

These cells enabled us to analyze the swimming vector velocity for a full range of values of  $\theta$ . As might be expected from the observations described above,  $v$  remained nearly constant, far from the variation predicted by equation 1 (Fig. 2). This constancy in  $v$  with respect to  $\theta$  suggests that gravity-induced changes in propulsive force exist and can compensate for the effect of sinking due to gravity.

*The effect of gravitational force on the swimming velocity of a population of Paramecium specified by path curvature*

Preliminary analyses of  $v$  vs  $\theta$  for a given population of cells revealed that  $v$  varies as an inverse sigmoid function of  $\theta$ , as expected from equation 1. The discrepancy between this result and the finding described above for single cells led us to the idea that gravity-induced changes in propulsion may depend on path curvature. Under either natural or experimental gravitational conditions, a plot of  $v$  vs  $\theta$  for a population specified by a path curvature of less than 0.1 mm<sup>-1</sup> (cells in this population will be referred to as straight swimmers) nearly matched that predicted by equation 1 (Fig. 3A), while  $v$  for cells with a curvature greater than 0.85 mm<sup>-1</sup> (curved swimmers with about one-third of the curvature of the cell shown in Fig. 1A) remained nearly constant when plotted against  $\theta$  (Fig. 3B). The critical curvatures were selected arbitrarily, but equal numbers of cells were included in each population. Sorting by curvature might select cells that tend to be close to the chamber wall and hence are more subject to a reduction in  $s$ , as a result

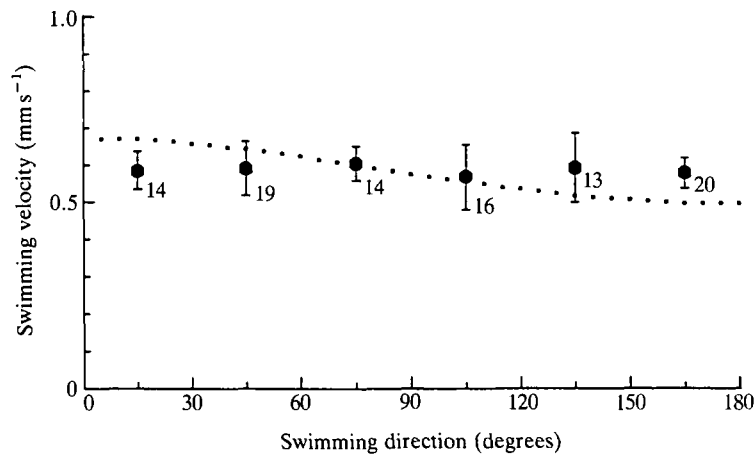


Fig. 2. Velocity of a *Paramecium* swimming along a circular path in a vertical plane. The magnitude of the velocity,  $v$ , is shown as a function of the angular direction of swimming,  $\theta$ , with respect to gravity. Values of  $v$  are averaged within each range of  $\theta$ , at  $30^\circ$  intervals from  $0^\circ$  (downwards) to  $180^\circ$  (upwards); bars indicate s.d. Values of  $N$  are given beside the points. The dotted line represents the prediction if there were no regulation of propulsive force (from equation 1) (data from Fig. 1).

of an increase in viscous resistance from hydrodynamic wall effects. However, a modified form of equation 1 with a 0.435-fold reduction in  $s$  did not match the data for curved swimmers. This reduction is the maximum possible assuming that all immobilized cells fell midway between opposite chamber walls and that all curved swimmers swam in contact with a wall (based on calculations using equations cited in Mogami *et al.* 1988*b*). In addition, preliminary analyses of the motion of cells far from chamber walls by means of three-dimensional video-microscopy demonstrated plots of  $v$  vs  $\theta$  similar to those in Fig. 3B (Izumi-Kurotani *et al.* 1989). It is therefore unlikely that the deviation from equation 1 of the measurements for curved swimmers is due to hydrodynamic wall effects.

The deviation of the data from equation 1 was measured by plotting  $(v_{\text{dn}} - v_{\text{up}})/2$  against  $s$ , with  $s$  being varied by changing the gravitational force (Fig. 4). If the propulsive force were invariant, irrespective of the swimming direction and gravity,  $(v_{\text{dn}} - v_{\text{up}})/2$  should be equal to  $s$ , as indicated by the dashed line in Fig. 4. The downward shift of the data points from the dashed line,  $\Delta$  of Machemer *et al.* (1991), is assumed to be a measure of the gravity-induced active control of propulsion. It should be noted that this shift for curved swimmers under natural gravity conditions is about  $750 \mu\text{m s}^{-1}$  (from data shown in Fig. 4), ten times as large as that reported for cells whose orientation has been fixed galvanotactically (Machemer *et al.* 1991) (see Discussion).

In a high-density Percoll medium, which nearly neutralizes the buoyancy of cells, horizontal swimming velocities ( $\theta = 90 \pm 30^\circ$ ) of curved and straight swimmers under natural gravitational conditions were  $0.461 \pm 0.081 \text{ mm s}^{-1}$  and

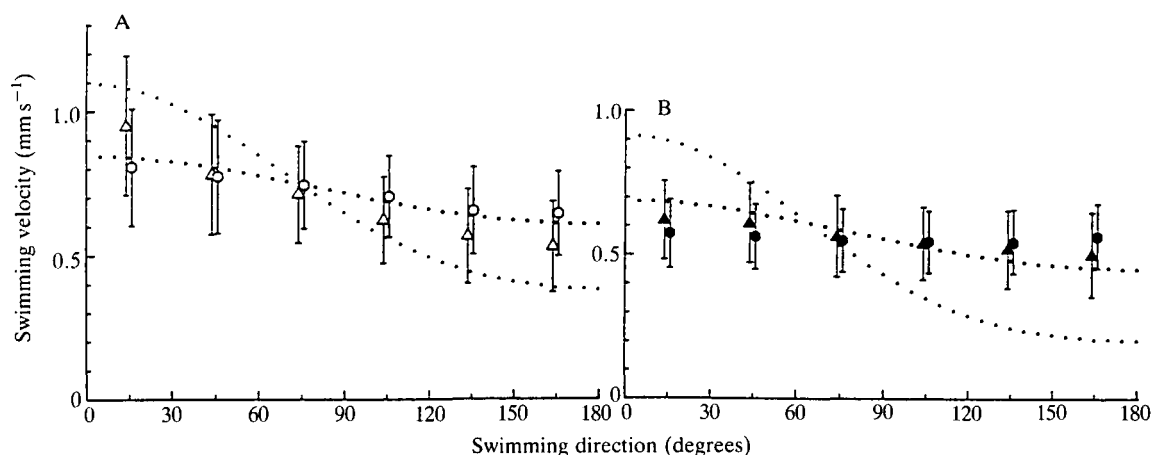


Fig. 3. Swimming velocity of *Paramecium* under natural and experimental gravitational conditions at 24°C. Curves are for populations of cells specified by path curvature; (A) curvature  $< 0.10 \text{ mm}^{-1}$  and (B) curvature  $> 0.85 \text{ mm}^{-1}$ ; circles under natural and triangles under  $3g$  conditions. The magnitude of the swimming velocity,  $v$ , is shown as a function of the angular direction,  $\theta$ , with respect to gravity. Values of  $v$  from about 2000 cells per graph for a given population with (usually) seven measurements per cell are pooled and averaged within each range of  $\theta$ , at  $30^\circ$  intervals from  $0^\circ$  (downwards) to  $180^\circ$  (upwards); bars indicate s.d. The dotted lines represent predicted curves if there were no regulation of propulsive force (from equation 1); values of  $p_i$  in equation 1 are 0.725 (A, 1g), 0.741 (A, 3g), 0.569 (B, 1g) and 0.560  $\text{mm s}^{-1}$  (B, 3g), and  $s$  at 1g is  $0.119 \pm 0.032 \text{ mm s}^{-1}$  (mean  $\pm$  s.d.,  $N=475$ ).

$0.701 \pm 0.135 \text{ mm s}^{-1}$ , respectively (mean  $\pm$  s.d.,  $N=13$  replicates of experiments similar to that shown in Fig. 3). These velocities were not significantly different from those measured in a normal-density medium,  $0.506 \pm 0.042 \text{ mm s}^{-1}$  and  $0.619 \pm 0.098 \text{ mm s}^{-1}$  for curved and straight swimmers, respectively ( $N=18$ ,  $P>0.05$ ). Analyses similar to those shown in Fig. 3 were made with cells in the Percoll medium under natural and experimental gravitational conditions. However, we found that it was more informative to analyze propulsive velocities, as described below.

#### *Gravity-induced changes in calculated propulsive velocity*

The preceding analyses revealed that the propulsive velocity  $\mathbf{p}$  tends to be either increased or decreased as cells swim upwards or downwards, respectively, probably by gravistimulation depending on the cell orientation. The magnitude of the propulsive velocity,  $p$ , increases with increasing values of  $\phi$  (more-upward swimming) and decreases with decreasing values of  $\phi$  (more-downward swimming) (Fig. 5). This tendency becomes more prominent as gravitational force increases and is always larger for curved swimmers (compare Fig. 5A and 5B), as might be expected. Regression lines for data from cells under natural and experimental gravitational conditions cross at a value of about  $120^\circ$ . This indicates

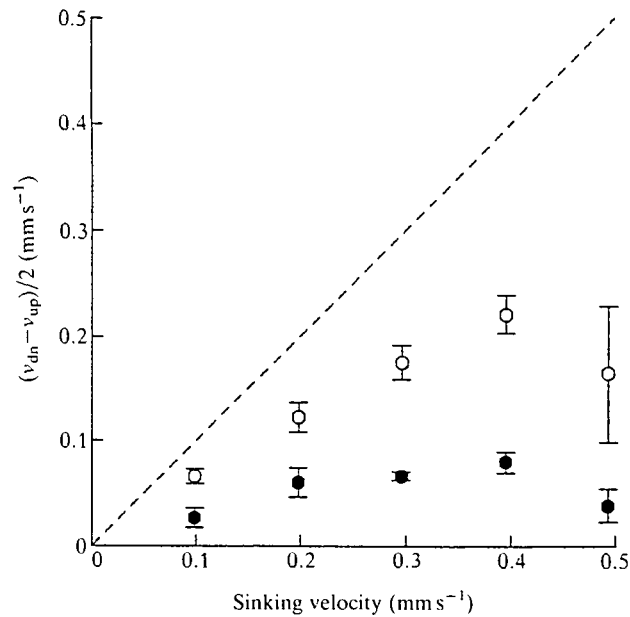


Fig. 4. Compensation for sinking effects due to gravity by *Paramecium* at 24°C. Half the difference between downward and upward swimming velocities,  $(v_{dn} - v_{up})/2$ , is plotted against sinking velocity,  $s$ , varied by experimentally manipulating the gravitational force (up to 5 g). Values are mean  $\pm$  s.e. from nine replicates from experiments similar to that shown in Fig. 3. Open symbols, cells with a path curvature of less than  $0.10 \text{ mm}^{-1}$ ; filled symbols, cells with a path curvature greater than  $0.85 \text{ mm}^{-1}$ . The dashed line represents the theoretical line for no compensation.

that the degree of suppression of propulsion induced by gravistimulation of downward-orienting cells is larger than that of potentiation of propulsion of upward-orienting cells. It should be noted that this feature of the gravisensitivity of cells is consistent with the findings of Machemer *et al.* (1991), i.e.  $\Delta_a > \Delta_p$  using their terminology. The slopes of the regression lines for  $p$  vs  $\phi$ , which is an overall measure of orientation-induced changes in propulsion and will be referred to as  $\Delta p / \Delta \phi$ , increased linearly with gravitational force (Fig. 6). This indicates that the orientation-induced changes in propulsive force are genuine responses to gravistimulation.

Fig. 6 also shows that  $\Delta p / \Delta \phi$  decreases significantly for curved swimmers when the cells are immersed in a high-density medium and that  $\Delta p / \Delta \phi$  for straight swimmers is relatively insensitive to the medium density. It should be noted that  $\Delta p / \Delta \phi$  for curved swimmers drops when buoyancy is neutralized to the level for straight swimmers.

### Discussion

The present analysis of the swimming velocity of *Paramecium* under natural and



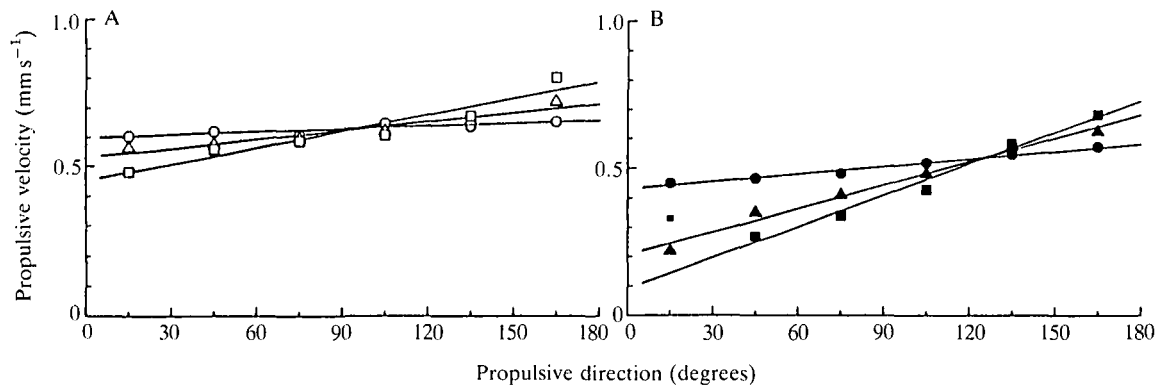


Fig. 5. Propulsive velocity of *Paramecium* under natural and experimental gravitational conditions at 24°C. The magnitude of propulsive velocity,  $p$ , is plotted against its angular direction,  $\phi$ , with respect to gravity, for cells with a path curvature of less than  $0.10 \text{ mm}^{-1}$  (A) and for those with a path curvature of more than  $0.85 \text{ mm}^{-1}$  (B). Mean values of  $p$  from nine replicates from experiments similar to that shown in Fig. 3 are represented by different symbols according to gravity conditions (circles: 1 g; triangles: 3 g; squares: 5 g) and regression lines are drawn; the linear correlation coefficients are 0.87 (A, 1 g), 0.95 (A, 3 g and 5 g) and 0.99 (B, 1 g, 3 g and 5 g). s.e. bars are omitted for clarity. The point indicated by the small square in B was ignored for the calculation of the regression line, because of its large deviation from the line. See text for the calculation of propulsive velocity.

experimental gravitational conditions revealed direction-dependent changes in propulsion that are responsible for the adjustment of the speeds of upward- and downward-swimming cells. One possible explanation is that the cells can sense their speed relative to the water from the changes in drag experienced by cilia (Mogami *et al.* 1988b). It has been demonstrated that a natural or experimental tether changes flow around swimming planktonic organisms (Emler, 1990). It therefore seems plausible that changes in the forces on beating cilia could be the signal that the animal is sinking or rising. Using this signal, *Paramecium* could increase its propulsion when swimming upwards and decrease it when swimming downwards, thus keeping its speed constant. Regulation of this sort by negative feedback would be readily achieved when the loads are small. However, the compensation for sinking was more complete at 5 g than at 3–4 g (see Fig. 4).

An alternative explanation is that the cells can sense their orientation relative to gravity by the changes in stress due to gravity experienced by the body. If the site of gravireception is in the interior of the cell, e.g. in Müller vesicles in *Loxodes* (Fenchel and Finlay, 1984, 1986), in the nucleo-nucleolar sensor (Moroz, 1984), in the concretion vacuole (Anderson and Dumont, 1966) or other statolith/statocyst-like structures, the magnitude of gravistimulation will be determined by the mass of the statolith-like structure, the density of the cellular fluid surrounding it and by gravity, and hence cannot be influenced directly by the external medium. If the site of gravireception is exposed to the exterior of the cell, the magnitude of

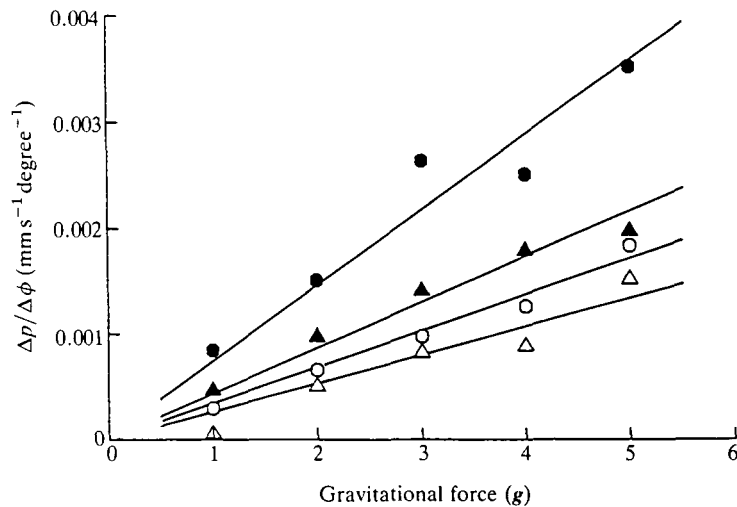


Fig. 6. The dependence of orientation-induced changes in propulsion in *Paramecium* on gravitational force at 24°C. The regression lines of  $\Delta p/\Delta\phi$  are plotted against gravity for cells with a path curvature of less than  $0.10\text{ mm}^{-1}$  (open symbols) and for those with a path curvature of more than  $0.85\text{ mm}^{-1}$  (filled symbols). Individual values of  $\Delta p/\Delta\phi$  are determined from replicates of experiments similar to that shown in Fig. 3 and are represented by different symbols according to medium conditions (circles, average of nine replicates of experiments in Dryl's solution; triangles, average of seven replicates in Dryl's solution containing Percoll at 25% v/v); the linear correlation coefficients are 0.99.

stimulation will be determined by a balance between the internal and external fluid densities and gravity. When the external density approaches the internal density, the magnitude of stimulation, and hence the orientation-induced changes in propulsion, will decrease to zero. Therefore, the observation that  $\Delta p/\Delta\phi$  for curved swimmers decreased substantially when they were immersed in a nearly neutrally buoyant medium indicates that most of the sites of gravireception are exposed to the exterior of the cell. The plasma membrane itself is a primary candidate for this 'exposed' gravireceptor; its possible roles in gravireception have been discussed for cytoplasmic streaming in *Nitellopsis obtusa* (Wayne *et al.* 1990) and for geotactic reorientation in *Paramecium* (Machemer and de Peyer, 1977). We will discuss only this type of gravireception, although the residual  $\Delta p/\Delta\phi$  after neutralization of buoyancy for curved swimmers and the insensitivity of  $\Delta p/\Delta\phi$  to gravitational force in straight swimmers suggest that some internal gravireceptors may exist.

We propose a new model for the control of ciliary activity by the cell membrane through sensory input due to gravity, which will result in gravikinesis (regulation of propulsion by gravity) and gravitaxis (regulation of trajectory direction with respect to gravity).

*Upward swimming*

*Up1.* When the cell swims upwards or obliquely upwards, the posterior cell membrane is stimulated by gravity, because the membrane tension is increased as a result of the pressure difference between the exterior and the interior of the cell.

*Up2.* In response to this stimulation, mechanosensitive  $K^+$  channels, whose sensitivity is maximal at the posterior end of the cell (Ogura and Machemer, 1980), increase their open probability and, hence  $K^+$  efflux increases, resulting in hyperpolarization (Naitoh, 1984; Naitoh and Eckert, 1969).

*Up3.* Hyperpolarization induces changes in ciliary activity; an increase in beating frequency and a more posterior beating direction together increase propulsion (Machemer, 1976) (gravikinesis).

*Up4.* Hyperpolarization-induced changes in ciliary activity also cause changes in the rate of cell rotation (rolling, yawing and possibly pitching), resulting in a reduction of the pitch angle of the helical trajectory (Machemer, 1989, 1990; Machemer and Sugino, 1989), which will reorientate the axis of the path helix closer to the direction of the instantaneous swimming velocity vector (Crenshaw, 1989, 1990).

*Downward swimming*

*Dn1.* When the cell swims downwards or obliquely downwards, the anterior cell membrane is stimulated by gravity, as a result of increased membrane tension.

*Dn2.* In response to this stimulation, mechanosensitive  $Ca^{2+}$  channels, whose sensitivity is maximal at the anterior end of the cell (Ogura and Machemer, 1980), increase their open probability and, hence,  $Ca^{2+}$  influx increases, resulting in depolarization (Naitoh, 1984; Naitoh and Eckert, 1969).

*Dn3.* Depolarization induces changes in ciliary activity; a decrease in beating frequency and a more sideways beating direction together reduce propulsion (Machemer, 1976) (gravikinesis).

*Dn4.* Depolarization-induced changes in ciliary activity also cause changes in the rate of cell rotation, resulting in an increase in the pitch angle of the helical trajectory (Machemer, 1989, 1990; Machemer and Sugino, 1989), which will reorientate the axis of the path helix farther from the direction of the instantaneous swimming velocity vector (Crenshaw, 1989, 1990).

*Helical swimming*

*Hx1.* Cells swimming along a helical path will experience continuous changes in the direction of the cell body relative to gravity. When the direction of the cell body is upwards, gravity-induced hyperpolarization will orientate the axis of the path helix closer to the instantaneous swimming direction, i.e. upwards (*Up4*), and when it is downwards, gravity-induced depolarization will orientate the axis of the path helix farther from the instantaneous swimming direction, i.e. downwards (*Dn4*).

*Hx2*. This will shift the helical axis of the cell trajectory farther upwards (gravitaxis).

Assumptions *Up1* and *Dn1* require extremely sensitive mechanoreceptors (stretch receptors?) in the cell membrane of *Paramecium*. The magnitude of stimulation by gravity can be estimated to be 0.08 Pa at 1 g, the pressure difference calculated from the average density of *Paramecium* measured with Percoll,  $1.033 \text{ g ml}^{-1}$  [a similar value of  $1.04 \text{ g ml}^{-1}$  has been reported by Taneda (1987) using deuterium oxide] and the cell length ( $230 \mu\text{m}$ ). Sackin (1989) has demonstrated for *Necturus* proximal tubule that a negative pressure of 588.6 Pa ( $6 \text{ cmH}_2\text{O}$ ) is sufficient to increase the open probability of stretch-activated  $\text{K}^+$  channels by a factor of 4. Therefore, it is possible that *Paramecium* mechanoreceptor channels have a sensitivity four orders of magnitude higher than that of *Necturus* proximal tubule. It should be noted, however, that evaluation of the proximal tubule's sensitivity is based on measurements of an ionic current induced by tonic suction using the patch-clamp technique. It is intriguing in this context that the bullfrog sacculus is sensitive to vibratory accelerations four orders of magnitude smaller than the static acceleration due to gravity (Assad *et al.* 1989). This type of adaptation, if it exists, in gravireceptors may explain the much lower sensitivity to gravistimulation of *Paramecium* fixed in orientation either by galvanotaxis (Machemer *et al.* 1991) or swimming straight compared with those changing orientation by curved swimming. The trajectory and higher speed of straight swimmers may indicate a slightly more negative membrane potential compared with that of curved swimmers (Machemer, 1989, 1990; Machemer and Sugino, 1989). This shift in membrane potential might reduce gravisensitivity in straight swimmers by desensitizing either the anterior or the posterior mechanoreceptors, since hyperpolarization is known to decrease the  $\text{Ca}^{2+}$ -dependent depolarizing conductance (Machemer and Deitmer, 1985) and the  $\text{K}^+$  driving force (Machemer *et al.* 1991; Ogura and Machemer, 1980). The more hyperpolarized state in straight swimmers may also reduce gravisensitivity by shifting the working range to a saturated zone, where a given shift in the membrane potential induces a smaller change in ciliary activity (de Peyer and Machemer, 1983; Nakaoka and Machemer, 1990). Thus, adaptation, desensitization or saturation of gravireceptors in straight swimmers may underlie the different extent of gravity-induced control of propulsion found in the present study.

Gravity-induced changes in the membrane potential have recently been measured directly with a glass microelectrode inserted into elongating cortical cells of mung bean roots, although direct gravistimulation of these cells has not been verified (Ishikawa and Evans, 1990). Gravity-induced hyperpolarization and depolarization in *Paramecium* postulated in *Up2* and *Dn2* and by Machemer *et al.* (1991) can only be the result of direct gravistimulation of the cell but they remain to be measured directly.

Hyperpolarization- and depolarization-induced changes in ciliary activity have been demonstrated (*Up3* and *Dn3*). However, the changes in the helical variables postulated in *Up4* and *Dn4* are based on observations of trajectories of cells

immersed in hyperpolarizing or depolarizing media only under natural gravity conditions (Machemer, 1989, 1990; Machemer and Sugino, 1989). Measurements of these variables under microgravity (0g) and neutral-buoyancy conditions by means of three-dimensional video-microscopy (Baba *et al.* 1991) remain to be carried out.

In conclusion, the present study with *Paramecium* demonstrates a possible role of gravireception in inducing changes in ciliary activity that result in gravikinesis (Up3 and Dn3) and gravitaxis (Hx2). This fascinating adaptive behaviour was discussed more than a century ago (Verworn, 1889), but its mechanisms are still disputed (Fukui and Asai, 1985; Roberts, 1970; Taneda, 1987; Taneda *et al.* 1987; Winet and Jahn, 1974).

### References

- ANDERSON, E. AND DUMONT, J. N. (1966). A comparative study of the concrement vacuole of certain endocommensal ciliates – a so-called mechanoreceptor. *J. Ultrastr. Res.* **15**, 414–450.
- ASSAD, J. A., HACOHEM, N. AND COREY, D. P. (1989). Voltage dependence of adaptation and active bundle movement in bullfrog saccular hair cells. *Proc. natn. Acad. Sci. U.S.A.* **86**, 2918–2922.
- BABA, S. A., INOMATA, S., OOYA, M., MOGAMI, Y. AND IZUMI-KUROTANI, A. (1991). Three-dimensional recording and measurement of swimming paths of micro-organisms with two synchronized monochrome cameras. *Rev. sci. Instrum.* **62**, 540–541.
- BABA, S. A., OOYA, M., MOGAMI, Y., IZUMI-KUROTANI, A., YAMASHITA, M. AND OKUNO, M. (1989a). Control of swimming speed in microorganisms in the gravity field. *Joint Meeting ELGRA Jap. Soc. biol. Sci. Space*, Toulouse, March 29–31 (in press).
- BABA, S. A., OOYA, M., MOGAMI, Y., OKUNO, M., IZUMI-KUROTANI, A. AND YAMASHITA, M. (1989b). Gravireception in *Paramecium* with a note on digital image analysis of videorecords of swimming *Paramecium*. *Biol. Sci. Space* **3**, 285 (Abstr.).
- BEAN, B. (1984). Microbial geotaxis. In *Membrane and Sensory Transduction* (ed. G. Colombetti and F. Lenci), pp. 163–198. New York, London: Plenum Press.
- BEDINI, C., LANFRANCHI, A. AND NOBILI, R. (1973). The ultrastructure of the Müller body in *Remanella* (Ciliata Holothrica Loxodidae). *Monitore Zool. Ital.* **7**, 87–95.
- CRENSHAW, H. C. (1989). The kinematics of the helical motion of microorganisms capable of motion with four degrees of freedom. *Biophys. J.* **56**, 1029–1035.
- CRENSHAW, H. C. (1990). Helical orientation – A novel mechanism for the orientation of microorganisms. *Lecture Notes Biomath.* **89**, 361–386.
- DEGAWA, M., MOGAMI, Y. AND BABA, S. A. (1986). Developmental changes in Ca<sup>2+</sup> sensitivity of sea-urchin embryo cilia. *Comp. Biochem. Physiol.* **85A**, 83–90.
- DE PEYER, J. E. AND MACHEMER, H. (1983). Threshold activation and dynamic response range of cilia following low rates of membrane polarization under voltage-clamp. *J. comp. Physiol. A* **150**, 223–232.
- DRYL, S. (1959). Antigenic transformation in *Paramecium aurelia* after homologous antisera treatment during autogamy and conjugation. *J. Protozool.* **6**, 25 (Suppl.).
- EMLET, R. B. (1990). Flow fields around ciliated larvae: effects of natural and artificial tethers. *Mar. Ecol. Prog. Ser.* **63**, 211–225.
- FENCHEL, T. AND FINLAY, B. J. (1984). Geotaxis in the ciliated protozoan *Loxodes*. *J. exp. Biol.* **110**, 17–33.
- FENCHEL, T. AND FINLAY, B. J. (1986). The structure and function of Müller vesicles in loxodid ciliates. *J. Protozool.* **33**, 69–76.
- FINLAY, B. J., HETHERINGTON, N. B. AND DAVISON, W. (1983). Active biological participation in lacustrine barium chemistry. *Geochim. cosmochim. Acta* **47**, 1325–1329.
- FUKUI, K. AND ASAI, H. (1985). Negative geotactic behaviour of *Paramecium caudatum* is

- completely described by the mechanism of buoyancy-oriented upward swimming. *Biophys. J.* **47**, 479–482.
- HORRIDGE, G. A. (1969). Statocysts of medusae and evolution of stereocilia. *Tissue & Cell* **1**, 341–353.
- ISHIKAWA, H. AND EVANS, M. L. (1990). Gravity-induced changes in intracellular potentials in elongating cortical cells of mung bean roots. *Plant Cell Physiol.* **31**, 457–462.
- IZUMI-KUROTANI, A., OOYA, M., MOGAMI, Y., YAMASHITA, M., OKUNO, M. AND BABA, S. A. (1989). Swimming behaviors of *Paramecium* under various gravitational environments. *ISAS Houkoku* **24**, 33–47 (in Japanese with abstract in English).
- MACHEMER, H. (1976). Interactions of membrane potential and cations in regulation of ciliary activity in *Paramecium*. *J. exp. Biol.* **65**, 427–448.
- MACHEMER, H. (1988). Electrophysiology. In *Paramecium* (ed. H.-D. Görtz), pp. 185–215. Berlin: Springer-Verlag.
- 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 DEITMER, J. W. (1985). Mechanoreception in ciliates. *Progr. Sensory Physiol.* **5**, 81–118.
- MACHEMER, H. AND DE PEYER, J. (1977). Swimming sensory cells: electrical membrane parameters, receptor properties and motor control in ciliated Protozoa. *Verh. dt. Zool. Ges.* **1977**, 86–110.
- MACHEMER, H., MACHEMER-RÖHNISCH, S., BRÄUCKER, 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, H. AND SUGINO, K. (1989). Electrophysiological control of ciliary beating: A basis of motile behaviour in ciliated Protozoa. *Comp. Biochem. Physiol.* **94A**, 365–374.
- MARKL, H. (1974). The perception of gravity and of angular acceleration in invertebrates. In *Handbook of Sensory Physiology*, vol. VI/1 (ed. H. H. Kornhuber), pp. 17–74. Berlin: Springer-Verlag.
- 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.
- MOROZ, P. E. (1984). The cell in the field of gravity and the centrifugal field. *J. theor. Biol.* **107**, 303–320.
- NAITOH, Y. (1984). Mechanosensory transduction in Protozoa. In *Membrane and Sensory Transduction* (ed. G. Colombetti and F. Lenci), pp. 113–135. New York, London: Plenum Press.
- NAITOH, Y. AND ECKERT, R. (1969). Ionic mechanisms controlling behavioral responses in *Paramecium* to mechanical stimulation. *Science* **164**, 963–965.
- NAKAOKA, Y. AND MACHEMER, H. (1990). Effects of cyclic nucleotides and intracellular Ca on voltage-activated ciliary beating in *Paramecium*. *J. comp. Physiol. A* **166**, 401–406.
- OGURA, A. AND MACHEMER, H. (1980). Distribution of mechanoreceptor channels in the *Paramecium* surface membrane. *J. comp. Physiol. A* **135**, 233–242.
- OKA, T., NAKAOKA, Y. AND OOSAWA, F. (1986). Changes in membrane potential during adaptation to external potassium ions in *Paramecium caudatum*. *J. exp. Biol.* **126**, 111–117.
- OYOA, M., BABA, S. A., MOGAMI, Y., IZUMI-KUROTANI, A., YAMASHITA, M. AND OKUNO, M. (1989a). Swimming behavior of *Paramecium* in the gravity field: Analysis using gravity as an experimental variable. *6th Space Util. Symp.* **6**, 91–95 (in Japanese), a18 (Abstr. in English).
- OYOA, M., BABA, S. A., MOGAMI, Y., IZUMI-KUROTANI, A., YAMASHITA, M. AND OKUNO, M. (1989b). Analysis of swimming behavior of *Paramecium* under hypergravity conditions. *Biol. Sci. Space* **3**, 320–321 (in Japanese).
- OYOA, M., MOGAMI, Y., BABA, S. A., OKUNO, M., IZUMI-KUROTANI, A. AND YAMASHITA, M.

- (1989c). Swimming speed regulation in *Paramecium* keeping the body from sinking. *Proc. VIII Int. Congr. Protozool.* **77** (Abstr.).
- Ooya, M., Mogami, Y., Okuno, M., Yamashita, M. and Baba, S. A. (1988). Swimming behavior of *Paramecium* at microgravity and hypergravity. *Biol. Sci. Space* **2** (Suppl.), 46–47 (in Japanese), 107 (Abstract in English).
- Poff, K. L., Fontana, D. R. and Whitaker, B. D. (1984). Temperature sensing in microorganisms. In *Membrane and Sensory Transduction* (ed. G. Colombetti and F. Lenci), pp. 137–162. New York, London: Plenum Press.
- Rieder, N., Ott, H. A., Pfundstein, P. and Schoch, R. (1982). X-ray microanalysis of the mineral contents of some Protozoa. *J. Protozool.* **29**, 15–18.
- Roberts, A. M. (1970). Geotaxis in motile micro-organisms. *J. exp. Biol.* **53**, 687–699.
- Sackin, H. (1989). A stretch-activated K<sup>+</sup> channel sensitive to cell volume. *Proc. natn. Acad. Sci. U.S.A.* **86**, 1731–1735.
- Taneda, K. (1987). Geotactic behavior in *Paramecium caudatum* I. Geotaxis assay of individual specimen. *Zool. Sci.* **4**, 781–788.
- Taneda, K., Miyata, S. and Shiota, A. (1987). Geotactic behavior in *Paramecium caudatum*. II. Geotactic assay in a population of the specimens. *Zool. Sci.* **4**, 789–795.
- Verworn, M. (1989). *Psychophysiologische Protistenstudien*. Jena: Gustav Fischer.
- Wayne, R., Staves, M. P. and Leopold, A. C. (1990). Gravity-dependent polarity of cytoplasmic streaming in *Nitellopsis*. *Protoplasma* **155**, 43–57.
- Winet, H. and Jahn, T. L. (1974). Geotaxis in Protozoa. I. A propulsion gravity model for *Tetrahymena* (Ciliata). *J. theor. Biol.* **46**, 449–465.