

## The mechanics of gravitaxis in *Paramecium*

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### SUMMARY

**An analysis of swimming patterns in the ciliate *Paramecium* shows that the ability to swim preferentially upwards (negative gravitaxis) is primarily the result of upwardly curving trajectories. The trajectory characteristics are consistent with those produced by mechanical orientation. Cell profile measurements from microscope images suggest that the characteristic front–rear body asymmetry accounts for the observed orientation rates. Gravitaxis may result from interactions between the propelling cilia and the sedimentary flow around the cell, and it seems unlikely that an internal physiological gravity receptor exists in *Paramecium*.**

**Key words:** gravitaxis, gravikinesis, biomechanics, hydrodynamics, cilia.

### INTRODUCTION

All free-swimming microorganisms live and move around under the influence of Earth's gravity. Although most are denser than their surrounding medium, some, such as the ciliate *Paramecium*, are readily able to swim upwards and accumulate at the top of the water column. This ability, termed negative gravitaxis, is clearly of biological advantage to motile organisms that can then range, unhindered by the sedimenting effects of gravity, over all parts of their aqueous environment.

The mechanism underlying gravitaxis has been in dispute ever since the phenomenon was first reported in *Paramecium* (Jennings, 1906). Two purely physical mechanisms have been proposed in the past: the buoy effect, produced by density variations within the cell (Verworn, 1889; Wager, 1911; Dembowski, 1931), and shape orientation (sometimes called hydrodynamic drag orientation) produced during sedimentation by front–rear body asymmetry (Roberts, 1970). These two mechanisms can in principle be distinguished by immersing cells in a medium of the same density as the cell itself; shape orientation should then not occur (because there is no sedimentation) whereas orientation caused by the buoy mechanism should remain unchanged. Such experiments on non-motile specimens of *Paramecium* led Mogami et al. (Mogami et al., 2001) to conclude that the principal mechanism is shape orientation, although back-heaviness might play a minor role. Fukui and Asai (Fukui and Asai, 1985) showed that organisms tend to orientate upwards as they swim, and, together with Machemer and Bräucker (Machemer and Bräucker, 1996) and Nagel and Machemer (Nagel and Machemer, 2000), concluded that gravitaxis in *Paramecium* is mediated, at least in part, by physical processes.

It has been widely assumed that passive mechanisms cannot account for all the characteristics of gravitaxis (see review by Häder et al., 2005), leading to suggestions that cells can detect gravity directly. Gebauer et al. (Gebauer et al., 1999) reported a small shift in cell membrane potential when impaled specimens of *P. caudatum* were rotated in a vertical plane through 180 deg, potentially indicating sensitivity to the direction of gravity. The phenomenon of gravikinesis, whereby cells swim upwards at a faster rate than

downwards once sedimentation has been allowed for, is well established in this organism (Machemer et al., 1991; Nagel and Machemer, 2000), and Machemer and Bräucker (Machemer and Bräucker, 1992) have suggested that stretch-sensitive ion channels in the *Paramecium* cell membrane may actively respond to the vertical hydrostatic pressure difference across the organism. One difficulty with this specialised statocyst hypothesis is that the available pressure differences are extremely small, and some unknown amplifying mechanism would seem to be necessary (Takeda et al., 2006). Measurements of gravitaxis using whole population studies have led to the conclusion that the activation and relaxation times of gravitaxis and gravikinesis in *Paramecium* are different, suggesting that two separate gravity response systems may be involved (Bräucker et al., 1998; Krause et al., 2006).

It has been proposed that shape orientation is primarily responsible for gravitaxis in the flagellate *Chlamydomonas* (Roberts, 2006) and the aim of this study was to investigate whether this may also be the case in *Paramecium*. This mechanism should cause cell trajectories to curve upwards during swimming with a rate of orientation proportional to the sine of the angle between the long axis of the organism and the upward vertical, with the maximum rate of turning occurring when the cell is horizontal. The rate of turning should also be independent of swimming velocity. The objective was to determine whether cell trajectories have these characteristics, and to consider whether the familiar asymmetric slipper-shaped profile of *Paramecium* can account for any observed curvature.

### MATERIALS AND METHODS

#### Swimming track analysis

Cultures of wild-type *Paramecium caudatum* Ehr. were grown in boiled hay infusion. Organisms from 1-week-old cultures were transferred directly (using a glass dropper) into a small observation chamber formed from two microscope slides separated by a rubber spacer 1.1 mm thick, moistened with silicon fluid, held together by two spring clips. A 14 mm square cut-out in the rubber formed the chamber itself which, held in either horizontal or vertical planes, was illuminated by white light LEDs and observed with a low-power microscope ( $\times 10$  objective) and attached video camera. The

apparatus could be rotated as a whole about horizontal or vertical axes, thus enabling the direction of gravity with respect to the viewing chamber to be varied at will. Cell movements were recorded by video camera, and long-exposure photographs of the video screen during playback were used to record trajectories of individual organisms.

Track curvature produced by purely physical mechanisms is described by the equation:

$$\frac{d\theta}{dt} = -\beta \sin(\theta), \quad (1)$$

(Roberts, 1970) that gives the rate of change of orientation ( $\theta$ ) of the organism to the upward vertical as a function of time  $t$ .  $\beta$  is the maximum rate of orientation at the instant the cell is horizontal (at  $\theta=90^\circ$ ). Trajectories were assessed by fitting them to computer-generated tracks that assumed organisms swim at a constant swimming speed  $U$  in direction  $\theta$  with constant downward sedimentation velocity  $S$ , while reorientating to the vertical according to Eqn 1. Initial values of  $\theta$  were determined by aligning the actual and computed tracks at the start. Estimates of  $\beta$  and  $U$  were then deduced for each track by iteration until the best-fit curve was obtained. All computations and image processing were carried out using Mathcad 14 (www.mathcad.com).

#### Cell shape measurements

Cells were photographed swimming in culture medium between the coverslip and the microscope slide. Images were downloaded into Mathcad to determine boundary coordinates. These were then fitted to the three-parameter equation:

$$r(\varphi) = \frac{ab}{\sqrt{a^2 \sin^2(\varphi) + b^2 \cos^2(\varphi)}} + c \times \cos(\varphi). \quad (2)$$

The first term on the right hand side generates a cigar-shaped prolate spheroid with semi-major and semi-minor axes  $a$  and  $b$ , respectively, and the second term adds a degree of front-aft asymmetry determined by the length  $c$ ;  $r(\varphi)$  represents the distance from the centre of the coordinate system to the cell surface at angle  $\varphi$ . The direction  $\varphi=0$  is taken to point to the back end of the object, so a cell with a slightly wider posterior half possesses a positive value of  $c$ .

A least-squares procedure was used to obtain the best fit between the cell profile and that generated by Eqn 2. The origin of the coordinate system was initially placed arbitrarily somewhere within the cell perimeter, a profile calculated for initial estimates for  $a$ ,  $b$  and  $c$  values and then compared with the actual cell outline. The sum of the squared differences in  $r$  for the two profiles was then minimised, using a non-linear optimising algorithm, by varying the values of  $a$ ,  $b$  and  $c$  together with the position of the coordinate origin to find the best-fit curve. This then gave a best estimate for the asymmetry parameter  $c$ .

Experiments on sedimenting scale models of uniform density and with profiles described by Eqn 2 have shown that the magnitude of any shape orientation depends at low Reynolds numbers only on the asymmetry parameter  $c$  (Roberts and Deacon, 2002). The angular orientation rates of such models, as they sediment downwards between vertical walls a few body lengths apart, were found to be described by Eqn 1, the value of  $\beta$  being given by:

$$\beta = (0.056 \pm 0.004) \frac{\rho - \rho_0}{\eta} g c, \quad (3)$$

where  $\rho$  is the density of the body,  $g$  is the acceleration due to gravity and  $\rho_0$  and  $\eta$  are the density and viscosity, respectively, of the

surrounding fluid medium. Taking typical values appropriate for *Paramecium* [ $g=9.8 \text{ m s}^{-2}$ ,  $(\rho-\rho_0)=40 \text{ kg m}^{-3}$  (Machemer and Braucker, 1992) and  $\eta=10^{-3} \text{ Pas}$  for a dilute aqueous medium] Eqn 3 becomes:

$$\beta = (22,000 \pm 1600) c, \quad (4)$$

with  $\beta$  in  $\text{radians s}^{-1}$  and  $c$  in metres, or:

$$\beta = (1.26 \pm 0.09) c, \quad (5)$$

where  $\beta$  is in  $\text{deg s}^{-1}$  and  $c$  is in microns. The procedure thus offers a practical method of estimating the degree of shape orientation of such cells from their microscopic profiles, subject to the proviso that the shape described by Eqn 2 is an acceptable representation of the cell profile itself. Unfortunately it was not possible for technical reasons to measure simultaneously and with sufficient accuracy both the shapes and rates of orientation of individual organisms.

## RESULTS

### Swimming track analysis

Fig. 1 shows organism tracks in the observation chamber within minutes of their introduction. Cells swim around extremely rapidly (at up to  $1.7 \text{ mm s}^{-1}$ ) while exhibiting wild side-to-side oscillations, probably stimulated by the shear fluid flows experienced during the transfer operation. Upward curvature of smoothly swimming cells appears very gentle because of their relatively high speed, and that of oscillating cells is impossible to estimate. After a few hours, however, swimming speeds drop (to  $\sim 0.5 \text{ mm s}^{-1}$ ) and organisms move around quite smoothly. Fig. 2 shows such cells, initially travelling horizontally, exhibiting a dramatic and clear-cut systematic upward curvature during the 13 s sequence. This and other similar sequences were obtained by first rotating the chamber through  $180^\circ$  in the vertical plane. Organisms previously near the top (as a result of negative gravitaxis) are then at the bottom,

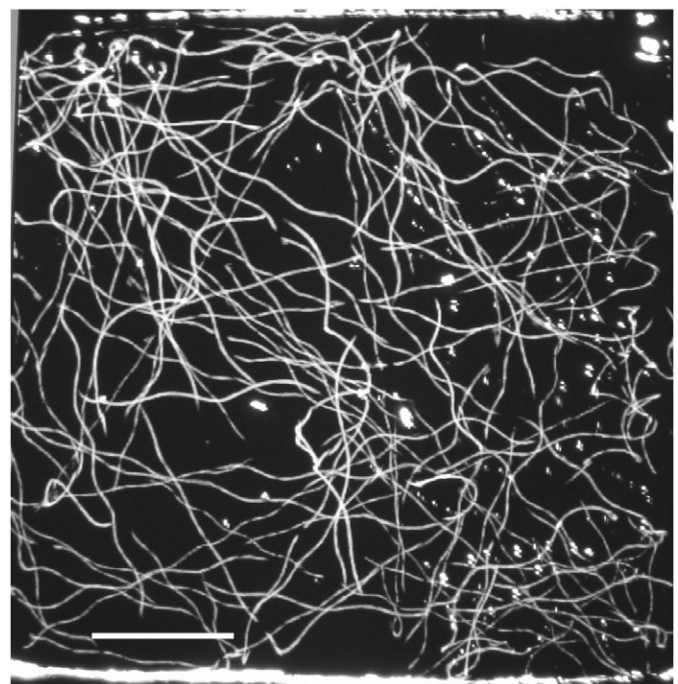


Fig. 1. Time exposure photograph of organism tracks in the vertical observation chamber 5 min after their introduction. Gravity is directed down the page. Exposure time: 5 s; scale bar: 3 mm.

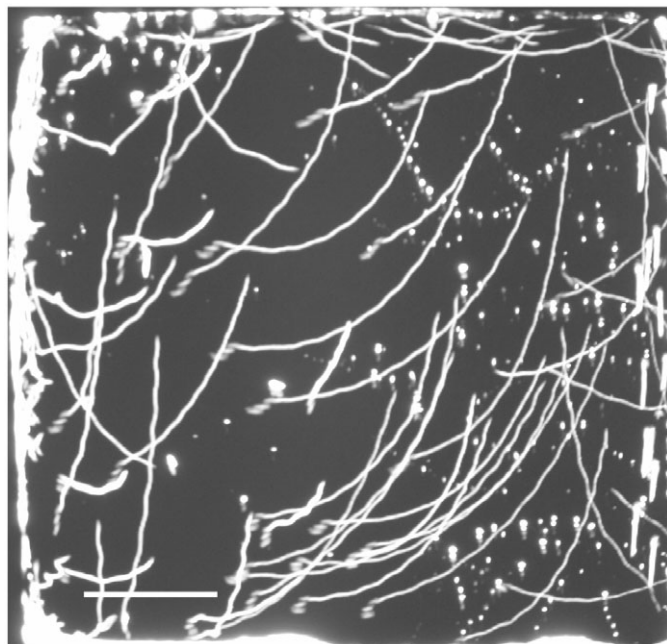


Fig. 2. Time exposure photograph of organism tracks in the vertical observation chamber a few hours after their introduction. Gravity is directed down the page. At the beginning of this sequence most cells were moving from left to right. Exposure time: 13 s; scale bar: 3 mm.

and quickly start swimming upwards. About 30 s later, when most cells are about halfway up the chamber, the chamber is rotated (within about 2 s) a further 90 deg, still in the vertical plane. Cells now find themselves swimming horizontally, and immediately respond to the new direction of the gravity vector. The tracks shown in Fig. 2 commenced within 1 s of the final chamber rotation.

Careful analysis of the video record, of which Fig. 2 is part, showed that not a single cell in this sequence ( $N=50$ ) reversed or reoriented suddenly when the direction of gravity was changed. This strongly suggests that ciliary reversals are not primarily involved in the orientational process.

The characteristics of 42 tracks were analysed in detail. Each track was compared with a computed track obtained by choosing swimming parameters ( $U$ ,  $S$ ,  $\beta$  and initial value of  $\theta$ ) that gave the closest fit. In each case examined, the fit was found to be extremely good, suggesting that curvature is entirely consistent with Eqn 1. Fig. 3 shows three examples of computed tracks fitted to cell trajectories.

The sedimentation rates  $S$  of individual cells cannot be deduced directly from the tracks themselves, so various values were initially assumed. The best-fit value of  $U$  depends on the value assumed for  $S$ , because the resultant upward swimming velocity when vertical is  $(U-S)$ . By contrast, the value of  $\beta$  does not depend significantly on  $S$ , but does depend on the accuracy with which the simulated track can be aligned with the actual track; the uncertainty in  $\beta$  for individual tracks is estimated to be about  $\pm 0.5 \text{ deg s}^{-1}$ .

A histogram of the measured orientation rates is shown in Fig. 4. The average rate of reorientation was found to be about  $7 \text{ deg s}^{-1}$ , with individual values varying greatly from one organism to another. No evidence could be found for any systematic variation of  $\beta$  with swimming velocity.

These results suggest that upward swimming in *Paramecium* is caused by a mechanism involving curvature of the swimming

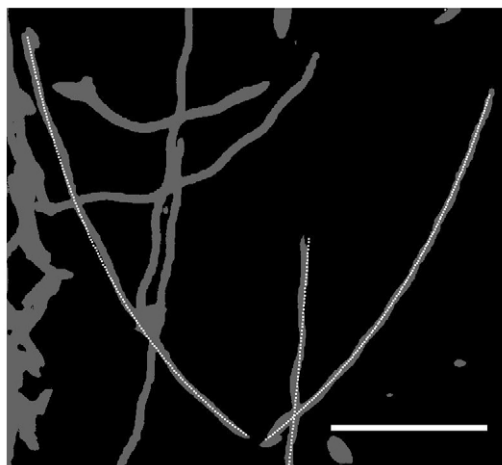


Fig. 3. Illustrative best-fit curves superimposed on three tracks from Fig. 2. Scale bar: 1.5 mm.

trajectories as described by Eqn 1, and that the observed responses do not involve ciliary reversals with upwardly directed reorientations when the direction of gravity is changed.

#### Measurement of cell shape

Specimens of *Paramecium* taken from continuous culture were found to vary widely in both overall size and shape, with cells invariably being significantly wider towards the rear. Fig. 5 shows three photographs of cells with their superimposed best-fit profiles, and the full results of the analysis are summarised in Table 1.

Fig. 6 shows the distribution of values of  $\beta$  as calculated using Eqn 5, together with the measured  $c$  values. The average value of  $\beta$  for axially symmetric bodies is predicted to be about  $9 \text{ deg s}^{-1}$ . However, this is likely to be an upper value; cells are slightly flattened, so that on average the orientation will be somewhat less than that predicted for axially symmetrical bodies; unfortunately there seems at present no way of obtaining a more accurate prediction of orientation rates for actual body shapes. Nevertheless there seems to be a compelling case for arguing that shape orientation is probably sufficiently great to account for trajectory curvature in *Paramecium*.

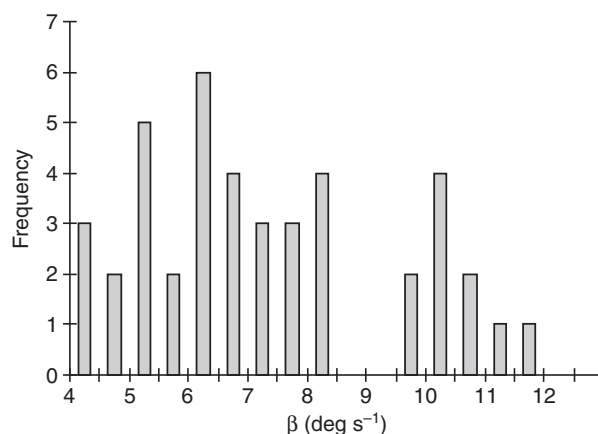


Fig. 4. Histogram illustrating the range of measured values of  $\beta$ , the maximum rate of orientation at the instant the cell is horizontal. The mean ( $\pm$ s.d.) value of  $\beta$  is  $6.9 \pm 2.3 \text{ deg s}^{-1}$  ( $N=42$ ).



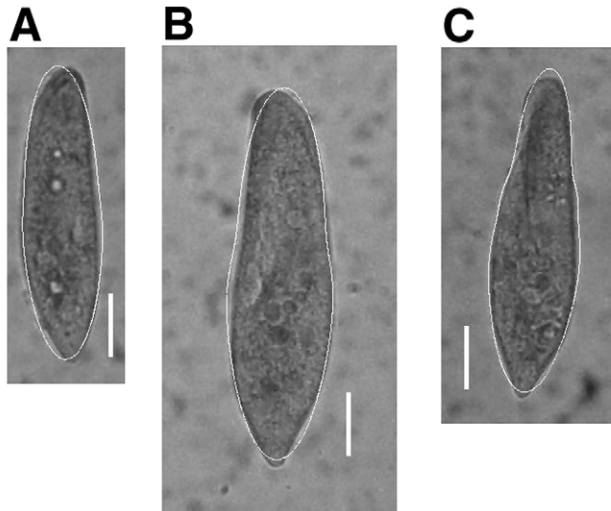


Fig. 5. Three examples of organisms with the best-fit shapes superimposed. (A) Length 233  $\mu\text{m}$ ,  $c=2.9 \mu\text{m}$ , predicted orientation rate of  $3.7 \pm 0.2 \text{ deg s}^{-1}$ . (B) Length 295  $\mu\text{m}$ ,  $c=7.0 \mu\text{m}$ , predicted orientation rate  $8.8 \pm 0.7 \text{ deg s}^{-1}$ . (C) Length 255  $\mu\text{m}$ ,  $c=12.4 \mu\text{m}$ , predicted orientation rate  $15.3 \pm 1.1 \text{ deg s}^{-1}$ . Scale bars: 50  $\mu\text{m}$ .

### DISCUSSION

The present results show that the primary response of *Paramecium* to gravity is upward curvature of the swimming trajectories, with horizontal orientation rates for individual cells lying within the range  $4\text{--}12 \text{ deg s}^{-1}$ . This is in general agreement with the results of others (Fukui and Asai, 1985; Taneda and Miyata, 1995; Mogami et al., 2001). Good agreement with Eqn 1 suggests that a purely physical mechanism is responsible, and it is difficult to envisage any physiological mechanism that could enable the cell to control its cilia to induce such track curvature. Furthermore, the size and spread of the front–rear asymmetries observed in populations of *Paramecium* appear sufficient to account for the observed orientation rate distribution.

Two other potential mechanical effects of sedimentation need to be considered in this context. Beating cilia produce fluid flow in the ciliary layer that varies between zero at the cell wall and a maximum value of about twice the swimming velocity at a distance of one extended cilium length (Jahn and Votta, 1972). For a ciliary layer  $10 \mu\text{m}$  thick and a swimming velocity of  $500 \mu\text{m s}^{-1}$  the corresponding velocity gradient within the layer is therefore  $\sim 100 \text{ s}^{-1}$ . Gravity also imposes a fluid velocity gradient in the same region; for a vertically oriented organism with a sedimentation velocity under normal gravity of  $\sim 100 \mu\text{m s}^{-1}$ , this is estimated, using standard hydrodynamic equations (Keller and Wu, 1977) to be  $\sim 2 \text{ s}^{-1}$  in the cell's equatorial plane. This flow is always upwards relative to the organism whereas the ciliary-induced flows are upward for down-swimming cells and downward for up-swimming cells. Cilia therefore experience a 4% change in hydrodynamic drag on

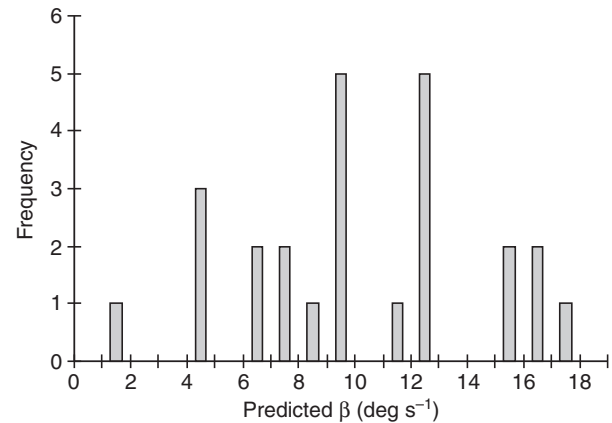


Fig. 6. Histogram showing the distribution of predicted values of  $\beta$ , the maximum rate of orientation at the instant the cell is horizontal, as deduced from the shape analysis ( $N=25$ ).

switching from up- to down-swimming to which, being mechanically sensitive, they may respond. This could be an explanation for gravikinesis if a reduction in hydrodynamic drag slightly depolarizes the cell membrane with a corresponding decrease in the strength of the ciliary beat. Rather than gravity-induced changes in the membrane modifying the ciliary beat (as supposed by the specialised statocyst hypothesis) cilia may simply be sensing and responding to orientation-dependent changes in fluid flow around them. Thus down-swimming cells (with a slightly depolarized membrane) would be more likely to reverse than up-swimming cells, as has been observed (Nagel and Machemer, 2000). This hypothesis could be investigated directly by measuring the effects of fluid flows of up to  $100 \mu\text{m s}^{-1}$  on the membrane potentials of impaled organisms using the procedure described by Gebauer et al. (Gebauer et al., 1999).

A second possibility is that the sedimentary flow affects the effectiveness of the propulsive system itself. The average force developed by each cilium depends on its velocity relative to the fluid in the ciliary layer; in a down-swimming cell the ciliary power stroke is upwards, and in the augmented upward fluid flow the relative velocity between itself and fluid, and so the propulsive force produced, is reduced. At the same time the drag on the cilium during the recovery phase is increased, further reducing the average propulsive force. If the sedimentary flow is increased (by increasing the  $g$ -force) the point must come – even if the ciliary beat pattern remains completely unchanged – where propulsive force is reduced to zero. The situation may be compared with that of an oarsman in a rowing boat being pulled along by a rope affixed to the bow, who can exert no additional forward propulsive force with his oar if the water is rushing back past him faster than the backward swing velocity of his blade. This is a possible explanation for the observation that an eightfold increase in the sedimentary flow reduces the propulsive force in down-swimming *P. caudatum* to zero while similarly augmenting up-swimming velocities (Guevorkian and Valles, 2006).

Both these mechanisms could potentially contribute to gravikinesis in *Paramecium*. However, it may be remarked that gravikinesis does not seem to confer much benefit on the organism. Net upward migration in a randomly moving population of organisms produced by gravikinesis alone can only be achieved if  $\Delta/S > 2$ , where  $\Delta$  is the magnitude of the gravikinetic increment in swimming velocity under normal gravity and  $S$  is the average

Table 1. Summary of shape data analysis results

	$a$ ( $\mu\text{m}$ )	$b$ ( $\mu\text{m}$ )	$c$ ( $\mu\text{m}$ )
Mean	123	29	6.9
s.d.	10	5	3.8

The mean value of  $c$  leads to a predicted mean ( $\pm$ s.d.) orientation rate, produced by shape asymmetry, of  $9.2 \pm 4.3 \text{ deg s}^{-1}$ ;  $N=25$ .

sedimentation rate, whereas reported values of  $\Delta/S$  in *Paramecium* are only around 0.6 (Bräucker et al., 1994; Nagel and Machemer, 2000; Guevorkian and Valles, 2006). Increased reversal rates in down-swimming cells could also produce a net upward drift, but ciliary reversal frequencies are extremely low in adapted cultures (Nagel and Machemer, 2000) and are unlikely to have a significant effect. This suggests that gravikinesis may be an incidental property of ciliary propulsion rather than a concerted effort by the organism to achieve effective upwards swimming.

It is concluded that shape orientation is the principal mechanism whereby *Paramecium* exhibits upward swimming. Gravikinesis may be the result of the interaction of the ciliary propulsion system with the surrounding sedimentary flow, suggesting that the organism need not possess any internal gravity-sensing mechanism. The much greater effectiveness of curved upward swimming implies that gravikinesis is unlikely to make any significant contribution either to gravitaxis or to the life of the cell. By evolving and maintaining an appropriate body profile *Paramecium* has itself become an efficient whole-body gravity detector that requires neither complex physiological gravity sensitivity nor the expenditure of metabolic energy.

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