GRAVIRESPONSES IN *PARAMECIUM CAUDATUM* AND *DIDINIUM NASUTUM* EXAMINED UNDER VARIED HYPERGRAVITY CONDITIONS

RICHARD BRÄUCKER, SIGRUN MACHEMER-RÖHNISCH
AND HANS MACHEMER*

*Arbeitsgruppe Zelluläre Erregungsphysiologie, Fakultät für Biologie, Ruhr-Universität,
D-44780 Bochum, Germany*

Summary

The swimming behaviours of two species of ciliates characterized by different mechanosensory and ciliary motor properties were investigated under hypergravity up to $5.4\,g$. The experiments were designed to examine large numbers of cells using video recording, digital data processing and statistics for the documentation of the rates and orientations of swimming. The gravikinetic responses (change in active swimming rates) were calculated from (1) the velocities of vertical swimming in the gravity field, (2) sedimentation of Ni^{2+}-immobilized cells and (3) the intrinsic rate of propulsion, independent of gravity. Propulsion was determined from the intersection of regression lines of the gravity-dependent upward and downward swimming velocities. The rates of swimming and sedimentation, and consequently the gravikineses, were linear functions of gravitational acceleration. Comparisons of cell populations from different cultures suggest that there is an age-dependent change in gravikinesis. In starved *Paramecium caudatum* (7-day cultures), the kinetic responses antagonizing sedimentation (negative gravikinesis) increased with acceleration. In *Didinium nasutum*, negative gravikinesis was documented at $1\,g$ in downward-swimming specimens only, which agrees with the mechanosensory organization of this cell. Hypergravity induced the gravikinesis of *Didinium* to change sign. In both species, and at all accelerations tested, a neutral gravitaxis was documented. Such behaviour incorporates distinct acceleration-dependent orientational and velocity responses, keeping populations of cells stationary in the gravity field (taxis coefficients close to zero).

Introduction

All ciliates so far investigated respond to the natural gravity vector. The most obvious response, swimming away from the centre of gravity ('negative gravitaxis'), has long been known and described in detail (see Machemer and Bräucker, 1992, for references).

*To whom reprint requests should be addressed.

Responses to gravity at the cellular level have appeared in a new light since Naitoh and Eckert (1969) discovered the electrophysiological basis of mechanosensitivity in *Paramecium* and since the presence of an intricate gradient-type distribution of depolarizing and hyperpolarizing mechanoreceptor channels was established in the membranes of *Stylonychia* and *Paramecium* (De Peyer and Machemer, 1978; Ogura and Machemer, 1980). The topographical pattern of mechanosensitivity in a ciliate determines the sign and amplitude of the receptor potential following local stimulation. If the gravity vector can deform mechanically sensitive structures on the membrane, the resulting potential may be predicted to depend on cell orientation in the gravity field. Moreover, this hypothetical 'gravireceptor potential' carries valuable information on the position of 'up' and 'down' in the environment (Machemer *et al.* 1991). Extensive investigations of the control of ciliary activity in ciliates have established a close coupling between ciliary beating and membrane potential: in *Paramecium*, a hyperpolarization augments the rate of beating, whereas a small depolarization slows down ciliary frequency (see Machemer, 1986, 1988*b*). The mechanosensitive organization and electromotor coupling properties formed the basis of a previous hypothesis predicting that upward-swimming cells increase, and downward-swimming cells decrease, active locomotion in response to the gravity vector (Machemer, 1989; Baba *et al.* 1991). Kineses of this kind have been isolated repeatedly in *Paramecium*, *Didinium* and *Loxodes* (see Machemer and Bräucker, 1992, for references). We have termed this type of response 'negative gravikinesis' because it tends to offset, by analogy with 'negative gravitaxis', the sedimenting effect of the gravitational pull. A gravikinetic response of a cell results from (1) the mechanosensory organization of the cell and (2) its orientation in space. Like classical kinesis, gravikinesis has no direct cell-orientating capacity.

Using hypergravity is a promising approach to the investigation of gravikinetic responses. Enhancing gravitational acceleration may establish more pronounced graviresponses depending on type, number, location and activation state of receptor channels. In a previous investigation of vertically gliding *Loxodes striatus*, it was shown that constant velocities of vertical gliding at 1*g* (Bräucker *et al.* 1992) gave way to diverging velocities under hypergravity: the rates of upward gliding were unchanged, but those of downward gliding rose with the gravity vector (Machemer-Röhnisch *et al.* 1993). The latter investigation also established that both graviorientation and gravikinesis in *Loxodes* prevent net upward or downward shifts of a cell population ('neutral gravitaxis').

The present study uses two species of ciliates, *Paramecium caudatum* and its predator *Didinium nasutum*, for a comparative study of the effect of hypergravity on the graviresponses. *Didinium* differs from *Paramecium* in two fundamental ways: (1) it can generate depolarizing, but not hyperpolarizing, mechanoreceptor responses (Hara and Asai, 1980; Hara *et al.* 1985); (2) modulation of its ciliary activity is limited to activation by depolarizing stimuli: induced hyperpolarization from the resting potential does not change ciliary frequency (Mogami *et al.* 1990; Pernberg, 1993). We have evaluated the effects of integration of gravikinesis and graviorientation on the position of cell populations of *Paramecium* and *Didinium* using the taxis coefficient for quantification. The results confirm the persistence of 'neutral gravitaxis' even under hypergravity.

Materials and methods

Cells and experimental solutions

Paramecium caudatum, line G3, were reared in Cerophyl solution (Cerophyl Laboratories, Inc., Kansas City, USA), 0.2 % (w/v) cerophyl powder in double-distilled water that had been autoclaved, buffered at pH 7.0 by modified Sörensen buffer (1.38 mmol l^{-1} Na$_2$HPO$_4$ + 0.62 mmol l^{-1} NaH$_2$PO$_4$) and bacterized with *Aerobacter aerogenes*. The *Paramecium* were cultured at 22 °C in a 14 h:10 h light:dark regimen and harvested in the early (3 days, 'well-fed cells') or late (7 days, 'starved cells') stationary phase. The cells were collected by negative gravitactic accumulation in the experimental solution (in mmol l^{-1}: CaCl$_2$, 1; KCl, 1; MgSO$_4$, 0.1; Mops, 1.5; buffered at pH 7.0). Transfer to the experimental chamber (volume 0.86 ml) was performed by a syringe (1 mm terminal inner diameter), avoiding mechanical agitation.

Didinium nasutum, wild type, was grown in Sörensen-buffered Pringsheim solution [in mmol l^{-1}: Ca(NO$_3$)$_2$, 0.85; KCl, 0.35; MgSO$_4$, 0.08; Na$_2$HPO$_4$, 0.41; NaH$_2$PO$_4$, 0.18; pH 7.2] at 19 °C and fed every 24 h with cells of *Paramecium caudatum*. Prior to an experiment, *Didinium* cells were starved for 12–16 h. Gravitactic accumulation and transfer were carried out in the experimental solution (see *Paramecium*).

Experimental chamber

The acrylic chamber enclosed an experimental fluid space of 25 mm× 25 mm×1.6 mm, equivalent to 1 ml. The upper (=centripetal) and lower (=centrifugal) borders of this space were lined by agar blocks (1.5 % in experimental solution; 0.8 ml each) to increase the buffering volume of the medium.

Equilibration

After transfer into the chamber, the cells were equilibrated for 4 h in experimental solution. The O$_2$ level within the closed chamber was ≥93 % air saturation (polarographic sensor and amplifier, type 170, Ingold, Germany). In a large-scale reference chamber with the same surface-to-volume ratio, the O$_2$ concentration decreased by ≤5 % over 4 h at the same cell density [cell/water: 1/16 000 (v/v)].

Centrifugation and recording

For the recording of cellular behaviour at gravity values above 1*g*, the chamber was mounted in a horizontal centrifuge (NIZEMI, Dornier GmbH, Germany) designed to generate highly controlled accelerations of microorganisms (Briegleb and Hemmersbach, 1987; Kreuzberg *et al.* 1991). The centrifuge consisted of a belt-driven disc, to which the chamber was fixed in a radial plane so that the resultant acceleration vector was parallel to the plane of the chamber (Fig. 1). The disk-shaped chamber was free to pivot about its central axis (perpendicular to the chamber surface) in the radial plane of the centrifuge. An eccentric mass connected to the chamber could swing out depending on the angular velocity of the centrifuge. The design of the centrifuge ensures that the major axis of the chamber is in line with the axis of the pendulum so that the resulting acceleration acts
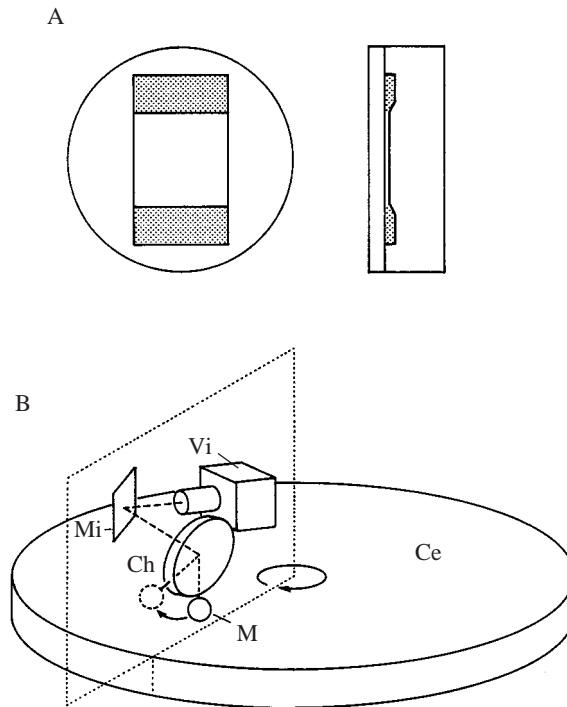
Fig. 1. Experimental arrangement. (A) Acrylic chamber in surface view (left) and cross section (right). Fluid volume, 1 ml; white rectangular field, 25 mm×25 mm×1.6 mm. Sides perpendicular to the gravity vector are lined by agar (1.5 % in experimental solution; stippled). (B) The instruments mounted on the horizontal platform of the centrifuge (Ce). The chamber (Ch) is in the radial plane of the centrifuge (dotted) and mounted so as to allow rotation about the central axis of the chamber. An eccentric mass (M) fixed to the chamber swings out centrifugally in parallel with the resultant gravitational vector. Swimming of cells is recorded by videocamera (Vi) using a mirror (Mi) for reflection of the light path. After Machemer-Röhnisch *et al.* (1993).

parallel to that axis (Machemer-Röhnisch *et al.* 1993). The angle of centrifugal swing (γ) was used to calculate the resulting factor (x) of terrestrial acceleration (g):

$$x = g\sqrt{1 + [\tan\gamma/(1 + l\sin\gamma/R)]^2}, \tag{1}$$

where l is the distance between the centres of mass of the chamber and the pendulum and R represents the distance between the centre of the chamber and the axis of the centrifuge. The accuracy of equation 1 for the determination of acceleration was checked against an equation using the angular velocity of the centrifuge and R.

The central recording area (8.5 mm×11.5 mm) was illuminated by a ring of 48 light-emitting diodes generating a dark-field illumination in the experimental plane of 800 lx. The emission wavelength (565 nm; half-width 28 nm) is near the minimum spectral sensitivity for photoresponses in *Paramecium caudatum* (Iwatsuki and Naitoh, 1982). Spectral sensitivity in the colourless *Didinium nasutum* is yet to be determined. The

macrolens of a high-resolution video camera (HR600 M, 25 Hz) was focused on the central plane of the fluid volume 0.8 mm away from the inner surfaces of the chamber. Images (8.5 mm×11.5 mm) of the field were projected into the video camera *via* a mirror. The video signals were transmitted to a recorder outside the centrifuge. The positions of cells in the vertical plane were defined with respect to the angle made by the trajectory of the cell with the vector of acceleration.

Cell immobilization and sedimentation

Paramecium cells from the experimental solution were exposed to 0.5 mmol l^{-1} NiCl$_2$ dissolved in experimental solution and were immediately transferred into the chamber. After allowing 20 min for immobilization, sedimenting cells were recorded. *Didinium* cells were treated with 1 mmol l^{-1} NiCl$_2$ in experimental solution and transferred to the chamber 45 min after nickel application. Sedimenting cells were recorded following 1 h of immobilization. For both species, recording time was limited to approximately 20 min. A criterion for completeness and quality of cell immobilization, apart from direct inspection of the sedimentation traces, was the shape of the velocity distribution: samples showing a steady and preferably Gaussian distribution were considered as acceptable (see inset to Fig. 6). Uneven distributions of velocities may be caused by Ni^{2+}-induced cell deformations (swelling) and/or residual ciliary activity.

Experimental protocol

A sample of 150–200 cells was transferred into the chamber. The temperature inside the chamber was between 22 and 23 °C. Swimming in *Paramecium* was recorded for periods of 2 min in two sequences of g values: 'type 1', 1→1.5→2.1→3.3→4.3→5.4→1; 'type 2', 1→5.4→4.3→3.3→2.1→1.5→1. Changes in gravitational acceleration between two predetermined g levels were set at 1 g min^{-1}. A 2 min recording of cell locomotion was evaluated to generate five separate representations of 4 s tracks using digital image analysis (Machemer *et al.* 1991). From each of these images, at least 10, and commonly about 30, tracks were evaluated. With *Paramecium*, 12 experiments were carried out for each value of hypergravity (1 g: 24 experiments). Assuming that each specimen entered the recording area and was measured 1.3 times, data points are based on at least 1200 individual cells. With a similar protocol, about 1000 individual *Didinium* cells were evaluated up to gravities of 2.1 g. Beyond 2.1 g, cell numbers declined because they showed rapid downward swimming activity and escaped from the recording area (see Figs 3, 9).

Data evaluation

For evaluations of tracks, we used 16 markers on each track separated by the same time interval. Velocity and orientation were determined for each trace. The averaging of velocities within an orientation class was carried out irrespective of the cell count. Non-parametric statistics (calculation of median values; confidence ranges; *U*-test) were applied because Gaussian distribution of the data could not be guaranteed (see Figs 3 and 9, for example).

Theory

Investigations of a gravikinetic response in motile microorganisms raise conceptual questions. Vertical downward (V_D) or upward (V_U) locomotion rates are the sums of the rates of propulsion (P) unrelated to gravity, sedimentation (S) and a gravikinetic increment or decrement in velocity (Δ), according to the equation (Machemer *et al.* 1991):

$$(V_D - V_U)/2 = S + \Delta, \tag{2}$$

which determines the value and sign of Δ. Variables in this equation are velocities, which are proportional to forces of active propulsion or gravitational pull according to the Stokes equation of flow at low Reynolds numbers (Wu, 1977). An observed downward swimming rate may exceed, correspond to, or fall below the upward swimming rate. Unless the rate of sedimentation (S) is determined, an observed response to gravity has only descriptive value. With the value of S known, the amount and sign of gravikinesis can be calculated. The term 'negative gravikinesis' indicates, by analogy with an orientational (=tactic) response, that upward or downward velocity is regulated so that the cell moves *away from the centre of gravity*. With a 'positive gravikinesis', the probability of locomotion *towards the centre of gravity* is raised.

Gravikinesis may differ in upward-swimming (Δ_U) and downward-swimming cells (Δ_D). Determination of the velocity of intrinsic propulsion (P) allows calculation of Δ_U and Δ_D because an observed velocity is the vector sum of propulsion, gravikinesis and sedimentation (Machemer *et al.* 1991). Hence, using the equations:

$$\Delta_U = P - S - V_U \tag{3}$$

and

$$\Delta_D = V_D - P - S, \tag{4}$$

the sign and size of the kinetic responses may be determined. The arithmetic mean of Δ_U and Δ_D gives the generalizing term Δ as represented in equation 2.

Graviorientation, integrated over time and with many individuals, is expressed by the orientation coefficient, r_o, which incorporates the stimulus direction and can vary between +1 (all cells strictly orientated upwards) and −1 (all cells strictly orientated downwards), with zero designating no orientation. The combined effects of swimming velocity and orientation are expressed by the taxis coefficient, r_t, which weighs individual swimming orientation by individual swimming rate. A negative taxis coefficient indicates a net upward shift of a cell population, and a positive taxis coefficient indicates a net downward shift. A taxis coefficient close to zero indicates that the centre of a population of cells probably maintains a stable vertical position (see Machemer and Bräucker, 1992).

Results

Paramecium caudatum

Distribution of swimming velocities at normal and raised acceleration

The velocities of cells from 3-day cultures were plotted using polar histograms for orientation (class width, 15°; Fig. 2). The rates of locomotion within these sectors are
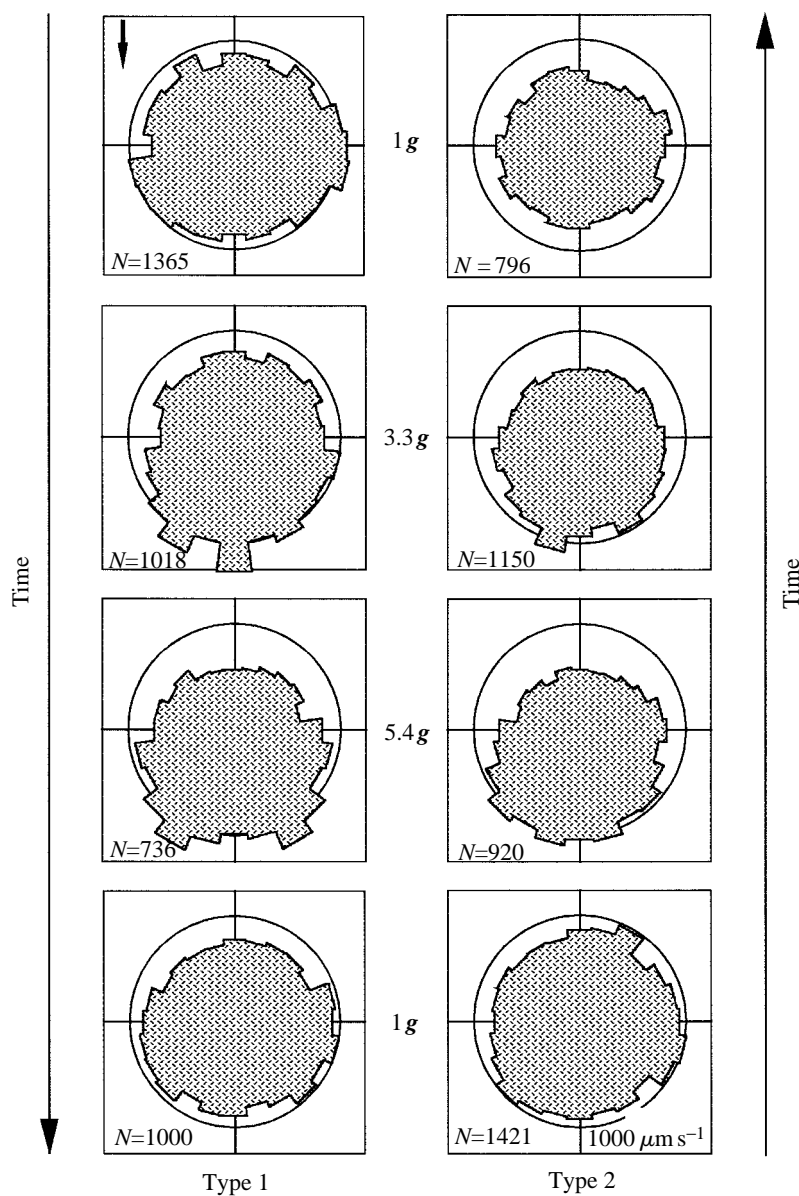
Fig. 2. Velocity of *Paramecium caudatum* (from 3-day cultures) in the vertically oriented chamber during two experimental series, applying gravity in rising (type 1) and falling sequences (type 2; time indicated by arrows). The vertical arrow represents the vector of acceleration for all polar velocity histograms (calibration, $1000\,\mu\mathrm{m\,s^{-1}}$). Two out of five hypergravity steps applied are represented in this figure. The $1\,g$ reference levels before and after application of hypergravity suggest a time-dependent decrease in absolute swimming rates in both the type 1 and the type 2 experiments. *N*, number of evaluated traces.

represented by medians. The velocity polarograms show a minor increase in downward rates over the upward rates at $1g$. This tendency is much enhanced with rising acceleration. At $5.4g$ the velocities tend to grow continuously with a more 'downward' orientation of the cell. This would be expected from vector addition of increasing sedimentation to cellular propulsion.

Gravity-dependent distributions of velocity are independent of previous stimulation. This applies to both experimental series employing rising (type 1) and falling (type 2) acceleration. A small time-dependent decrease in absolute velocity is apparent in both type 1 and type 2 experiments (Fig. 2). This decay may be due to the partial loss of rapid swimmers, which were able to leave the central recording area (Fig. 3, $1g$ before, $1g$ after).

Graviorientation of swimming

Distributions of orientation of *Paramecium* applying gravitational acceleration between $1g$ and $5.4g$ are shown in Fig. 4. The polar histograms suggest a small tendency for upward orientations ('negative gravitaxis') at normal gravity. With acceleration rising to $5.4g$, negative graviorientation is clearly evident. Correspondingly, the orientation coefficient (r_o), which integrates all swimming directions, rises from near zero to almost $+0.2$ (see Table 2).

Comparison of the polarograms of velocity and orientation (Figs 2, 4) suggests an inverse relationship between the g-dependent proportion of cells found in a given orientational sector and their speed: many 'slow' cells swim 'upwards' and a smaller proportion of 'fast' cells swim 'downwards'. This diverse behaviour of a cell population is integrated by the taxis coefficient (r_t; see Machemer and Bräucker, 1992). At a taxis coefficient close to zero (see Table 2), the probability of a gravity-dependent shift of the cell population is low. Thus, the data suggest that the orientational and kinetic behaviours in *Paramecium* tend to neutralize the effects of gravity on a cell population at all g values.

Velocities in well-fed and starved cultures

Medians of the vertical and horizontal velocities ($\pm15°$) plotted as a function of acceleration appear to change in a linear fashion (Fig. 5), especially if the values at $1g$ are not used in the regressions (Table 1). We have tentatively excluded the $1g$ data from the calculation of the g-dependent slopes of velocity because an unknown mechanical factor (possibly vibration) might have influenced the locomotor activity of *Paramecium* while the centrifuge was running (open symbols in Fig. 5). A comparison of the swimming rates in well-fed and starved cells (Fig. 5A,B) reveals differences in the slopes of the regression lines of velocities: the positive slope of downward swimming (V_D) increased, and the negative slope of upward swimming (V_U) decreased, in starved cells. No change occurred in the slopes of horizontal swimming rates.

Gravity-independent propulsion rate

The observed relationship between swimming velocity and acceleration suggests velocities below $1g$ can be estimated by extrapolation. The inferred swimming rate at $0g$ then equals the propulsion rate, P, of *Paramecium*, which is defined as being unaffected
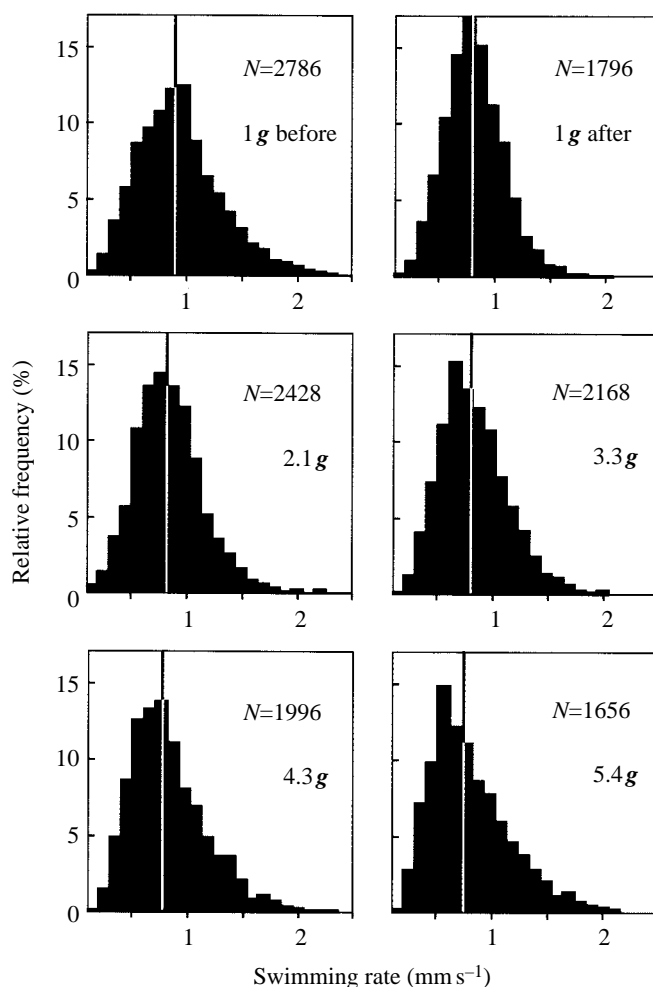
Fig. 3. Class distributions of swimming rates of *Paramecium caudatum* from 3-day cultures at different *g* values. The distributions did not follow the Gaussian algorithm and, therefore, require application of non-parametric statistics for calculation of medians (vertical bars). Note the difference in distributions and medians of velocities at normal gravity at the beginning (1 *g*, before) and at the end of the hypergravity experiment (1 *g*, after). The medians shown are not orientation-independent and cannot be used for calculations of gravikinesis. *N*, number of evaluated traces.

by gravity. Theory predicts that the curves of horizontal and vertical swimming rates must intersect at 0 *g*. Fig. 5 shows only an approximation to this prediction. In order to minimize errors in determinations of *P* from the regression lines, we calculated the velocity at the intersection of the V_D and V_U slopes near 0 *g* (arrows in Fig. 5). These inferred velocities during weightlessness agree with the observed horizontal swimming velocity of *Paramecium* at 1 *g* within close limits (Fig. 5A, well-fed cells, −1 %; Fig. 5B, starved cells, −5 %).

Table 1. *Vertical swimming velocities (V$_U$, V$_D$), intrinsic propulsion rates (P), sedimentation rates (S) and calculated gravikinetic responses (Δ$_U$, Δ$_D$) in* Paramecium *and* Didinium *as a function of gravitational acceleration*

	V$_U$		V$_D$		P	S		Δ$_U$	Δ$_D$	Δ
g	Int (μm s^{-1})	Med (μm s^{-1})	Int (μm s^{-1})	Med (μm s^{-1})	Int (μm s^{-1})	Int (μm s^{-1})	Med (μm s^{-1})	Int (μm s^{-1})	Int (μm s^{-1})	Int (μm s^{-1})
Paramecium caudatum 3-day cultures										
1.0		769 (+31 −34)		826 (+31 −36)		98	89 (+2 −1)			−60
1.5	804	791 (+36 −48)	908	903 (+64 −80)		123	130 (+3 −3)	−52	−90	−71
2.1	768	780 (+27 −46)	924	916 (+86 −89)	875	154	165 (+5 −4)	−47	−105	−76
3.3	694	702 (+40 −31)	957	973 (+90 −124)		215	217 (+5 −6)	−34	−133	−84
4.3	633	632 (+35 −37)	985	998 (+60 −72)		266	269 (+8 −7)	−24	−156	−90
5.4	566	561 (+33 −38)	1016	1001 (+118 −57)		321	314 (+6 −7)	−12	−180	−96
Paramecium caudatum 7-day cultures										
1.0		708 (+32 −30)		767 (+37 −38)						−60
1.5	718	701 (+33 −48)	846	797 (+45 −77)				−63	−55	−59
2.1	700	690 (+39 −23)	866	883 (+47 −40)	778			−76	−66	−71
3.3	665	703 (+25 −28)	904	939 (+68 −64)				−102	−89	−96
4.3	636	652 (+27 −37)	937	995 (+122 −113)				−124	−107	−116
5.4	605	577 (+21 −31)	972	912 (+235 −94)				−148	−127	−138
Didinium nasutum										
1.0	1519	1524 (+58 −62)	1621	1636 (+49 −76)		74	90 (+2 −2)	−1	−45	−23
1.5	1475	1466 (+53 −36)	1638	1639 (+59 −79)		94	79 (+5 −5)	+23	−48	−13
2.1	1422	1420 (+49 −59)	1658	1654 (+94 −138)	1592	117	121 (+6 −7)	+53	−51	+1
3.3	1315	1323 (+51 −33)	1699	1610 (+201 −155)		165	161 (+5 −3)	+112	−58	+27
4.3	1226	1230 (+35 −56)	1734	1851 (+104 −264)		205	189 (+9 −24)	+161	−63	+49
5.4	1129	1123 (+70 −44)	1771	1731 (+147 −169)		249	264 (+5 −6)	+214	−70	+72

The data from *Paramecium* are separated for well-fed (3-day cultures) and starved cells (7-day cultures).

Experimental data are represented as **g**-dependent interpolations (Int) from linear regressions of the medians (Med) in addition to the medians (with 95 % confidence range; in parentheses).

Propulsion (P) was determined from the intersection of the regression lines for V$_U$ and V$_D$, as shown in Fig. 5.

Values of gravikinesis are calculated from P and the interpolated data of V and S.
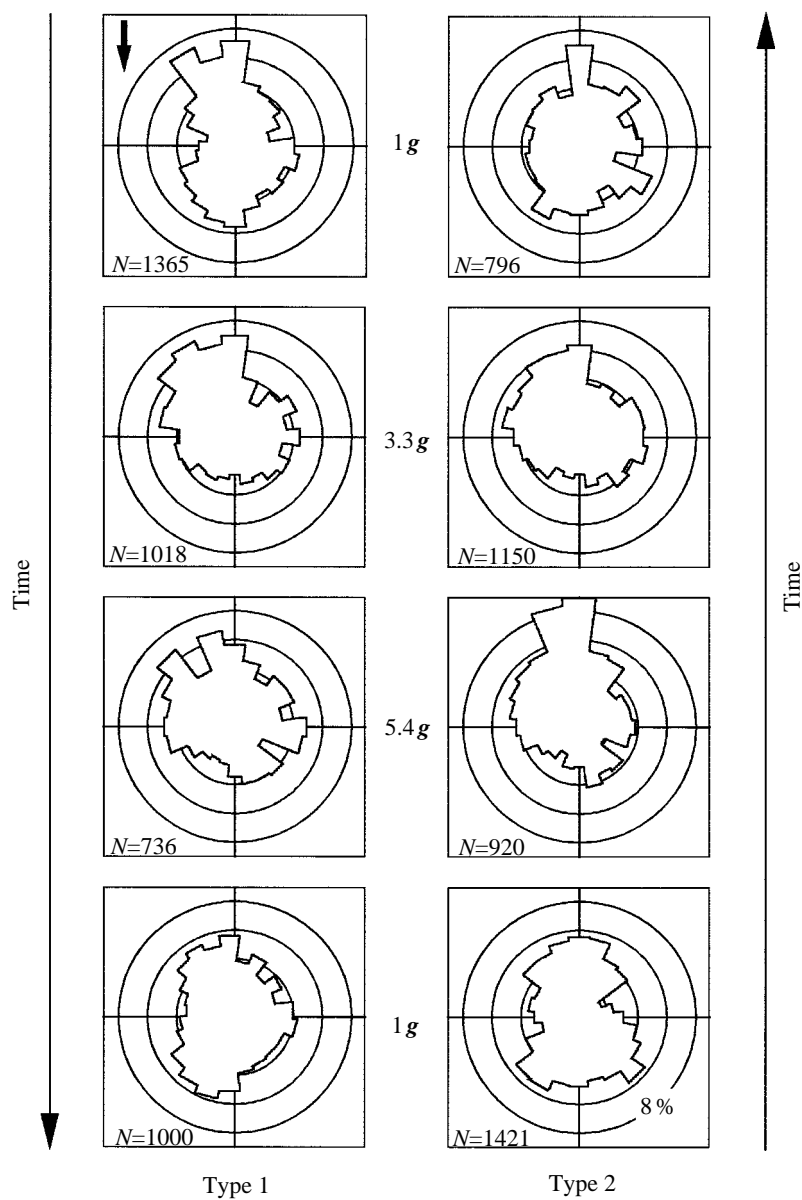
Numbers of measurements (N): see Table 2.

Fig. 4. Orientation of *Paramecium caudatum* in the vertical plane during an experimental series, applying hypergravity in rising (type 1) and falling (type 2) sequences. The orientation polarograms (calibration, percentage of total) illustrate a weak negative gravitaxis at 1*g*, which was enhanced during hypergravity. The sequence of applied accelerations had no significant effect on the gravitactic orientation. *N*, number of evaluated traces. For further details, see legend to Fig. 2.
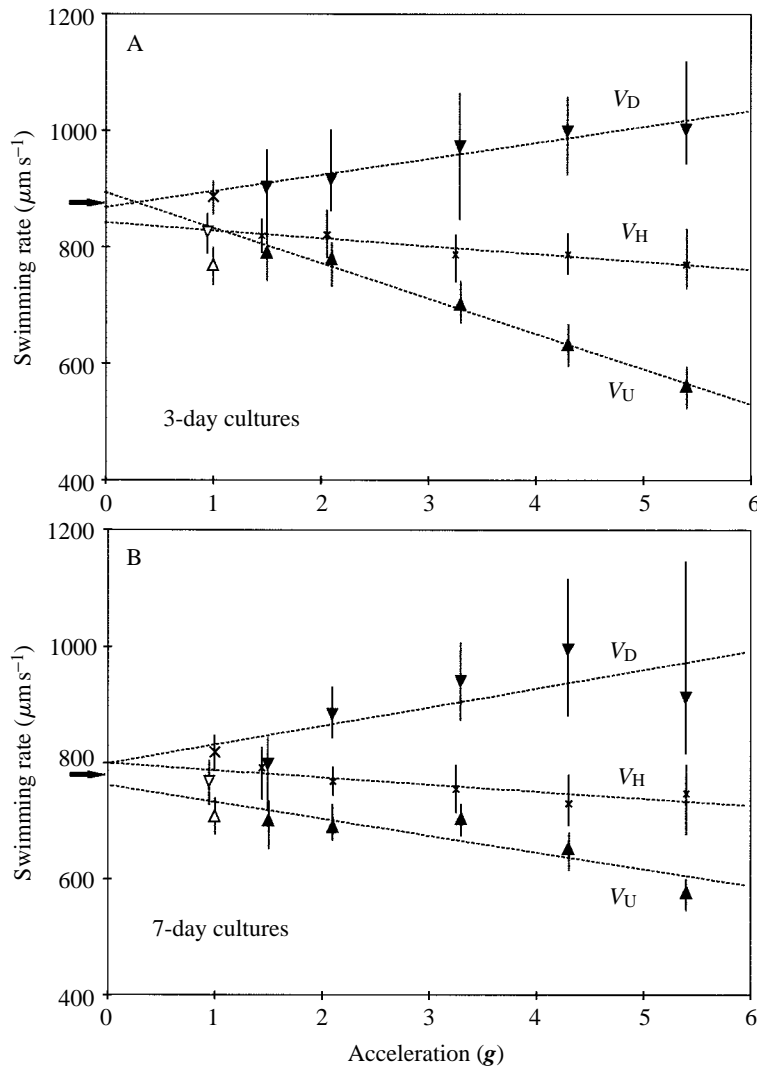
Fig. 5. (A,B) *Paramecium* swimming velocities as a function of acceleration in well-fed (A) and starved cells (B). The graphs give medians of the velocities in downward (V_D, ▼), upward (V_U, ▲) and horizontally swimming specimens (V_H, ×) with 95 % confidence ranges (see Table 2 for number of evaluated traces). Data from the type 1 and type 2 experiments (Fig. 2) are combined. Dashed lines are linear regressions of the velocity medians at hypergravity intersecting near $0g$. The $1g$ reference values from the unrotated centrifuge (open symbols) lie off the hypergravity regression lines. The intersection of the regressions of downward velocity (V_D) and upward velocity (V_U; see arrow) is used for determination of the propulsion rate (P). (A) Swimming velocities of cells 3 days after inoculation. Arrow, approximate value of P ($875 \, \mu m \, s^{-1}$). Correlation coefficients: V_D, $r=+0.96$; V_H, $r=-0.96$; V_U, $r=-0.99$. (B) Swimming velocities of cells 7 days after inoculation. Arrow, approximate value of P (778 $\mu m \, s^{-1}$). Correlation coefficients: V_D, $r=+0.70$; V_h, $r=-0.84$; V_U, $r=-0.87$. Note that (1) hypergravity changed horizontal swimming rates very little; (2) starvation tended to increase the g-dependent slope of downward swimmers and to reduce the slope of the upward swimmers.
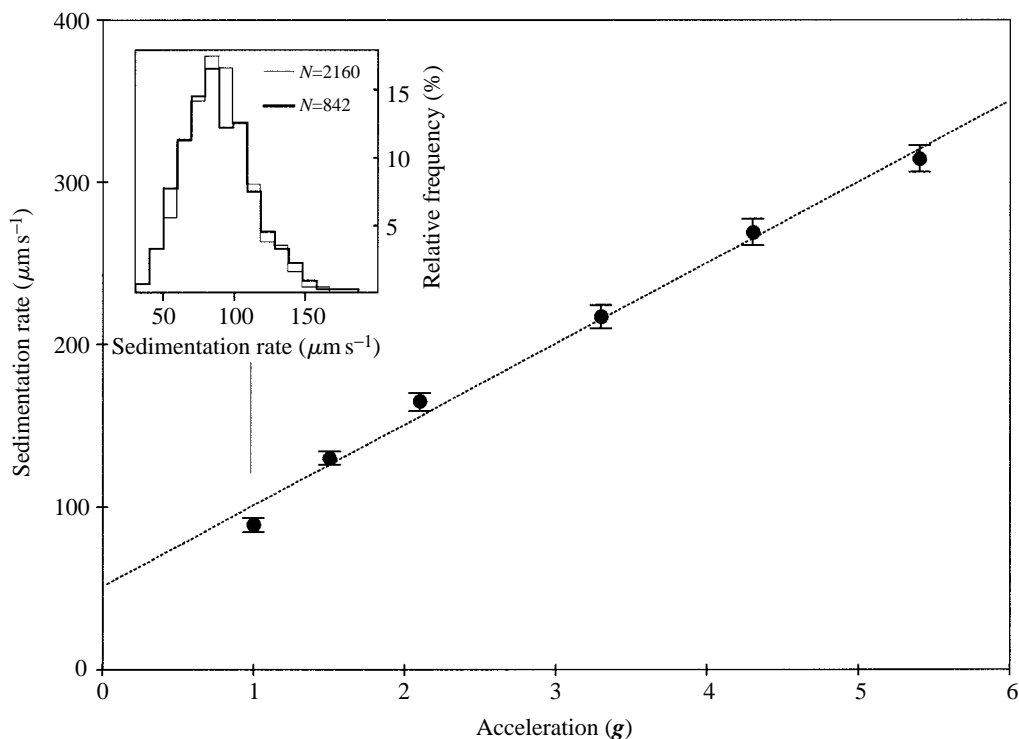
Fig. 6. Median sedimentation rates and confidence ranges (bars) of Ni^{2+}-immobilized, well-fed *Paramecium* (3-day culture) at g values rising from 1 to 5.4 g. A linear function is well approximated (r=+0.995), but the regression line does not intersect with the origin. The data include the option that sedimentation follows a non-linear function intersecting with the origin. The inset gives the velocity distribution of immobilized cells from two samples at 1 g (median in both samples was 89 μm s^{-1}).

An interesting observation is that hypergravity was virtually ineffective in modulating horizontal velocity: at 5 g, the horizontal swimming velocity was reduced by 6.5 % (in well-fed cells; 6.2 % in starved cells). This strengthens the conclusion from previous work (Machemer *et al.* 1991, 1993) that the bipolar gravisensory input to horizontally swimming *Paramecium* is neutralized at 1 g. Assuming that the slopes of V_H in Fig. 5A,B are real, the swimming velocity, P, during weightlessness will, nevertheless, exceed horizontal velocity at 1 g conditions by 1.5 %.

Sedimentation rates

Swimming velocities of cells are not directly amenable to determinations of gravikinesis because they include the sedimentation rate. Fig. 6 shows that immobilized cells from 3-day cultures settled at a median rate of 90 μm s^{-1}. We were unable to determine sedimentation in starved, 7-day cells because they tended to disintegrate during the nickel treatment. Individual sedimentation rates were quite different owing to varying cell size and cytoplasmic inclusions (inset Fig. 6). The distribution histogram of

sedimentation rates may even include contributions from rudimentary ciliary activity of the gullet region of the immobilized *Paramecium*. With rising acceleration, the sedimentation rate rose in a linear fashion (correlation coefficient 0.995). The extrapolated regression line, however, does not intersect with the origin, suggesting the possibility that the sedimentation curve is non-linear below 1g.

Experimental limitations make it impossible to investigate the association of swimming velocity with sedimentation at the single-cell level. Therefore, determinations of median rates of swimming and sedimentation are important for the calculation of gravikinesis. Median sedimentation rates at various levels of hypergravity and the establishment of a gravity–sedimentation relationship limit experimental errors. We use interpolations of the sedimentation rate from the regression line for measurement of gravikinesis under hypergravity. The 1g data are again excluded from this procedure because they were not obtained while the centrifuge was running.

Gravikinesis

Hypergravity between 1.5 and 5.4g increased the negative gravikinetic response of *Paramecium*, that is its ability to counteract sedimentation (Fig. 7, see generalized value of Δ). Gravikinesis rose markedly with hypergravity in starved cells (Fig. 7B). This positive correlation was less pronounced in well-fed cells (Fig. 7A). The basis of this difference in responsiveness to hypergravity is, primarily, the gravikinetic response of the upward swimmers (Fig. 7, Δ_U). Δ_U decreased with rising acceleration in well-fed cells (Fig. 7A), whereas in starved cells, it rose with hypergravity running nearly parallel to the gravikinetic response of the downward swimmers (Δ_D; Fig. 7B).

<div align="center">Didinium nasutum</div>

Swimming velocities

The experiments in the vertical chamber at 1g show that downward swimming rates are slightly greater than upward velocities (Fig. 8A). The decrease in upward swimming rates and the increase in downward swimming rates grew with rising acceleration. At 5.4g, the downward velocity was 150 % of the upward velocity. In contrast to *Paramecium caudatum*, a plot of the distributions of velocities revealed an over-representation of comparatively higher velocities up to accelerations of 3.3g (Fig. 9).

Graviorientation

Polarograms plotting the orientations of *Didinium* at different accelerations (Fig. 8B) are the inverse of the distributions of velocities (Fig. 8A). A small preference for upward orientation at 1g (r_o=0.031) was continuously transformed to obvious negative gravitactic orientation above 3.3g ($r_o \geqslant 0.15$; Table 2). The taxis coefficients (r_t) show that the orientational response effectively counterbalanced the effects of the enhanced downward velocities: during hypergravity, r_t shifted from near zero at 1g to small positive values, maximally +0.08. These observations on the gravitactic behaviour in *Didinium* correspond to those in *Paramecium* (Table 2).
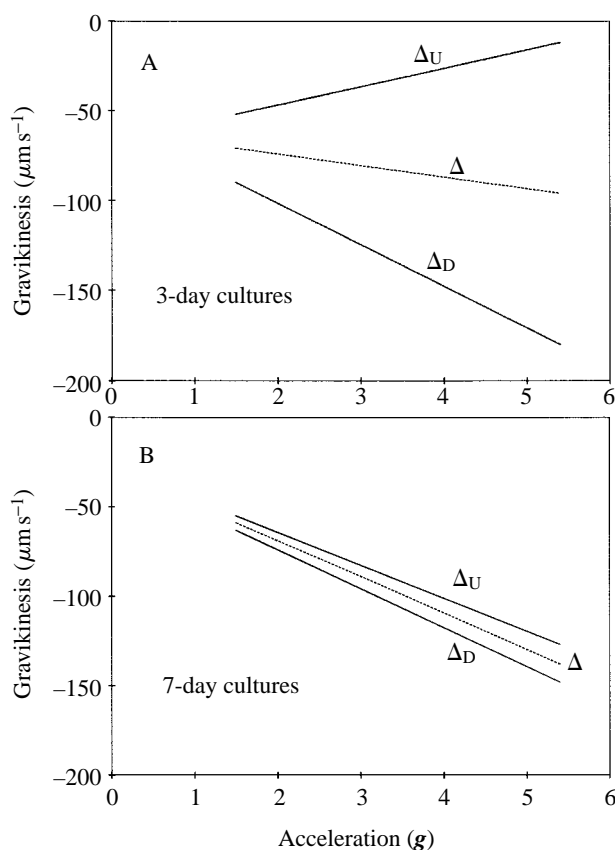
Fig. 7. Hypergravity-dependent change in gravikinesis (Δ) of 3-day cultures (A) and 7-day cultures (B). Gravikinesis was determined from gravity-free propulsion (*P*, see Fig. 5), and the regression lines of upward and downward swimming rates (V_U, V_D), and of the sedimentation rates (*S*; equation 2). Δ_U, kinesis of upward-swimming cells; Δ_D, kinesis of downward-swimming cells (equations 3, 4); Δ, a generalized term (arithmetic mean of Δ_U and Δ_D). Note that negative gravikinesis tended to rise with increasing hypergravity with the exception of that for upward swimmers (Δ_U) in 3-day cultures.

Gravity-independent propulsion rate

Plotting swimming rates as a function of acceleration gives linear relationships with different correlation coefficients (upward swimmers, $r=-1.00$; downward swimmers, $r=0.65$; horizontal swimmers, $r=-0.88$) and varying ranges of confidence (Fig. 10). The reason is that, as hypergravity increased, an increasing number of cells swam upwards, whereas horizontal and downward swimmers were registered less frequently (Fig. 8; Table 1). However, the regression lines intersect with the ordinate ($0\,g$) at very similar velocities, suggesting that the gravity-free propulsion rate can be determined reliably. Intersection of the 'downward' with the 'upward' regression is at $1592\,\mu\mathrm{m\,s}^{-1}$ (the definition of *P*). The intersection of the 'horizontal' regression line with $0\,g$ is at $1583\,\mu\mathrm{m\,s}^{-1}$. In contrast to *Paramecium*, the horizontal swimming rate is strongly

Table 2. *Coefficients of orientation (r_o), kinesis (r_k) and gravitaxis (r_t) in* Paramecium *and* Didinium *calculated for different accelerations and separated for well-fed (3-day cultures) and starved* Paramecium *(7-day cultures)*

g	r_o	r_k	r_t	N_U	N_D	N_H	N_S
Paramecium caudatum 3-day cultures							
1.0	−0.003	−0.069	−0.040	486	448	562	842
1.5	0.074	−0.085	0.039	283	192	472	773
2.1	0.117	−0.111	0.056	281	140	409	457
3.3	0.141	−0.094	0.057	269	132	356	512
4.3	0.132	−0.098	0.012	281	144	303	513
5.4	0.170	−0.107	0.022	238	124	271	455
Paramecium caudatum 7-day cultures							
1.0	0.045	−0.077	0.012	558	421	502	
1.5	0.063	−0.105	0.028	240	191	382	
2.1	0.088	−0.089	0.037	250	158	398	
3.3	0.139	−0.127	0.067	205	104	300	
4.3	0.131	−0.126	0.030	175	77	229	
5.4	0.188	−0.189	0.059	169	70	212	
Didinium nasutum							
1.0	0.031	−0.021	0.005	132	110	169	1229
1.5	0.049	+0.005	0.023	128	118	193	98
2.1	0.080	−0.003	0.039	143	91	185	96
3.3	0.126	−0.011	0.053	141	67	169	433
4.3	0.178	+0.077	0.081	102	42	85	59
5.4	0.151	+0.025	0.025	75	34	62	639

N, number of evaluated tracks in upward (N_U), downward (N_D) and horizontally swimming (N_H) cells and during sedimentation (N_S), as used in the experiments.

See Materials and methods for an approximation of the number of individual cells.

dependent on acceleration. The value of P cannot be determined from V_H because the distribution of gravireceptor conductances in *Didinium* is still unknown (see Discussion).

Sedimentation rates

Median sedimentation rates of Ni^{2+}-immobilized *Didinium* are a linear function of acceleration (Fig. 11; correlation coefficient: $r=+0.98$). The good correlation enables the use of interpolated sedimentation rates between 1 and 5.4g to compensate for experimental limitations and errors (Table 1). As in the experiments with *Paramecium*, the regression line does not intersect with the origin (zero sedimentation at 0g) and thereby restricts our conclusions to the range of accelerations used in the experiments.

Gravikinesis at normal gravity

Using interpolations of the vertical velocities (V_U, V_D) and sedimentation (S), a generalized gravikinesis (Δ) of $-23\ \mu m\ s^{-1}$ results for normal gravity (Fig. 12, Table 1). Gravikinesis was virtually absent in upward swimmers ($\Delta_U=-1\ \mu m\ s^{-1}$), whereas it was
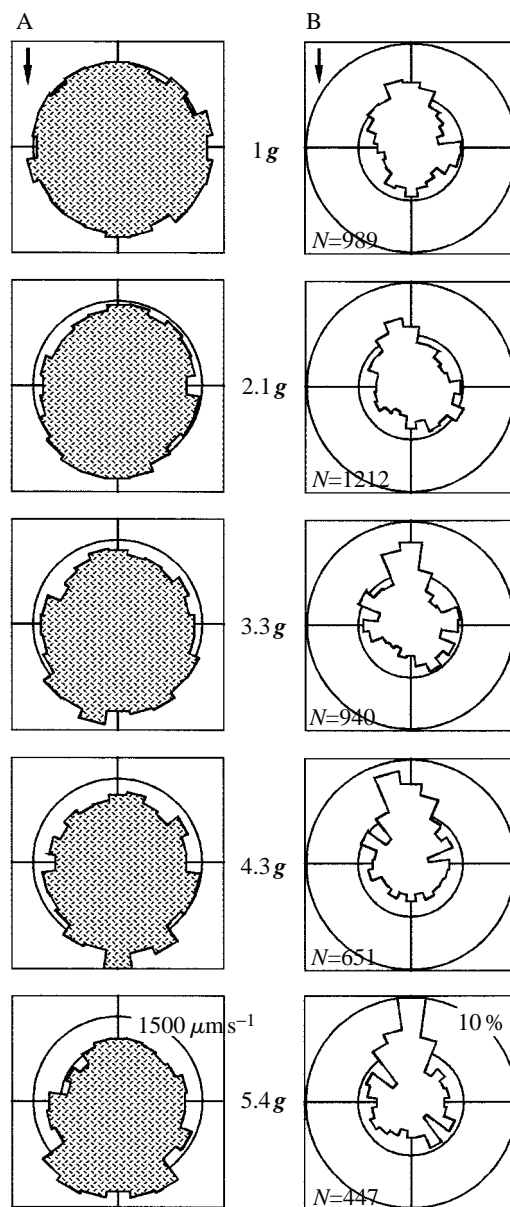
Fig. 8. Swimming velocity (A) and orientation of *Didinium* in the vertical plane (B) at accelerations rising from 1 to 5.4 *g* (the arrow marks the direction of the gravity vector). (A) The prevalence of the downward over the upward swimming rates grew between 1 and 5.4 *g* (calibration, 1500 μm s^{-1}). (B) Upward orientation was weak at 1 *g* and rose to a maximum with increasing gravity (calibration, percentage of total). See Table 2 for coefficients.

substantial in downward swimmers ($\Delta_D = -45$ μm s^{-1}). These data suggest that *Didinium* does not actively respond to gravity while swimming upwards at 1 *g*.
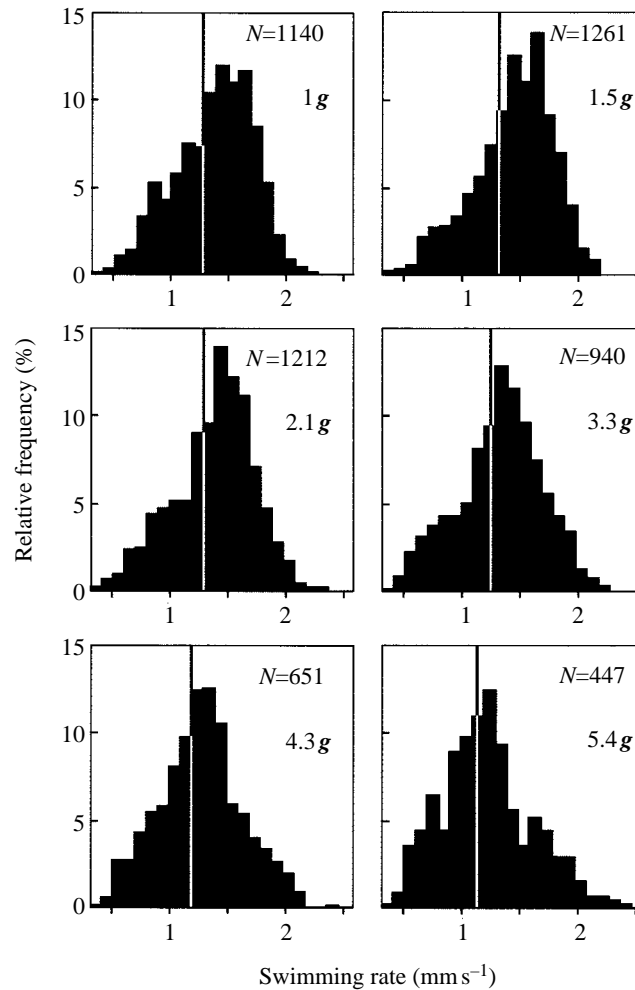
Fig. 9. Class distribution of swimming rates of *Didinium* at different g values. Higher velocities are over-represented at most g values (non-Gaussian distributions). Compare with distributions in *Paramecium* (Fig. 3).

Hypergravity induces bipolar gravikinesis

With acceleration exceeding $1g$, the upward swimmers showed an increasing positive value of kinesis ($5.4g$: $\Delta_U = +214\,\mu m\,s^{-1}$), i.e. the upward swimming rate was depressed by both sedimentation and an active reduction in forward propulsion (Fig. 12). In the downward-swimming cells, negative gravikinesis gradually increased in response to rising acceleration ($5.4g$: $\Delta_D = -70\,\mu m\,s^{-1}$). Consequently, the generalized term Δ changed from a negative value at $1g$ to a positive value at $5.4g$ ($+72\,\mu m\,s^{-1}$). Note that this bipolarity of the gravikinetic response was associated with artificial accelerations.
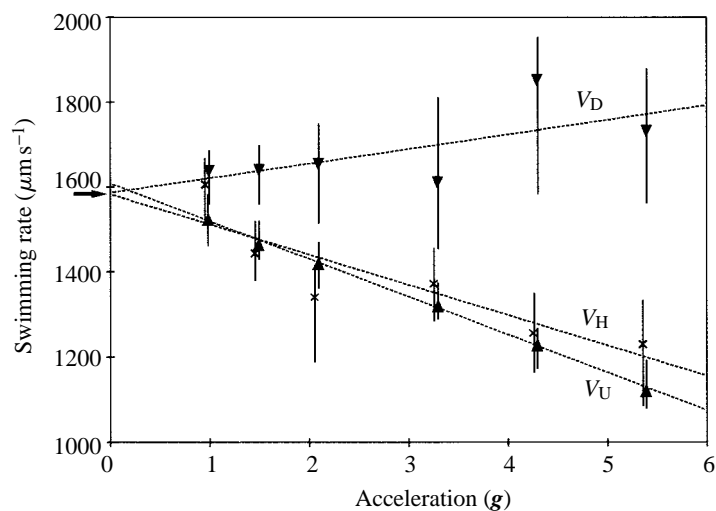
Fig. 10. Horizontal and vertical swimming velocities of *Didinium* as a function of acceleration. The graph shows medians of the velocities (95 % confidence ranges) of downward (V_D, ▼), upward (V_U, ▲) and horizontally swimming cells (V_H, ×). The intersection of the linear regressions of V_D and V_U (near 0g, arrow) define the gravity-free propulsion rate (P=1592 μm s^{-1}). Correlation coefficients: V_D, r=+0.65; V_H, r=−0.88; V_U, r=−1.00. Other definitions correspond to those given in the legend to Fig. 5. Note that, in *Didinium*, horizontal swimming rates (V_H) were inseparable from the upward swimming rates (V_U).
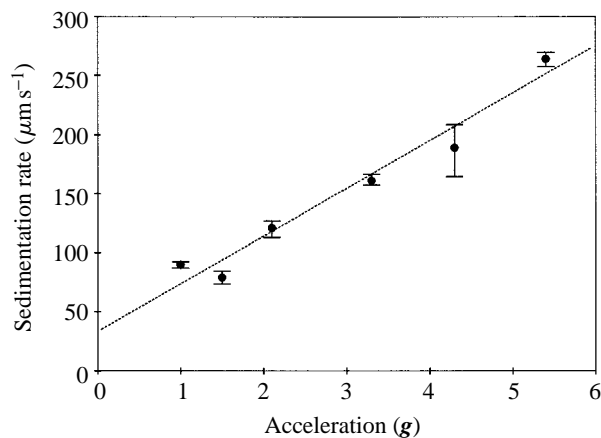

Fig. 11. Median sedimentation rates of Ni^{2+}-immobilized *Didinium* at g values rising from 1 to 5.4 g. Fluctuations in medians and confidence ranges (bars) are due to the varying size of populations (see Table 2). As with *Paramecium* (Fig. 6), the regression line (r=+0.98) does not pass through the origin.
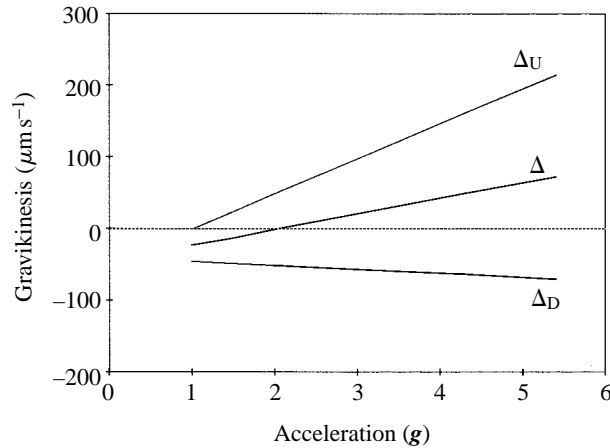
Fig. 12. Graphical representation of gravikinesis (Δ) in *Didinium* as modulated by hypergravity. Gravikinesis was determined from the regression lines of upward and downward swimming rates (V_U, V_D, Fig. 10), and sedimentation rates (S, Fig. 11), and from the value of gravity-free propulsion rate (P, Fig. 10, Table 1). Δ_U, gravikinesis of upward-swimming cells (equation 3); Δ_D, gravikinesis of downward-swimming cells (equation 4); Δ, a generalized term (arithmetic mean of Δ_U and Δ_D); equation 2. Note change of Δ from negative values (i.e. sedimentation antagonized) at normal gravity to positive values (i.e. synergistic with sedimentation) at hypergravity.

Discussion

Isolation of the physiological response to gravity from behaviour in ciliates requires standardized experimental conditions and large cell numbers because swimming velocity and direction, the rate of intrinsic propulsion and sedimentation cannot all be determined in the same cell. At terrestrial gravity level, the kinetic response in *Paramecium* and *Didinium* is a small fraction of locomotory velocity (Table 1). Since gravikinesis is calculated from three observed variables (equations 2–4), possible experimental errors in their determination are summed. An additional problem is the unknown individual variability in a population of cells. In spite of these complications, the present study confirms the existence and magnitude of gravikinesis in *Paramecium* and *Didinium* at natural gravity (Table 2 in Machemer and Bräucker, 1992). Moreover, by using hypergravity up to 5*g*, we have been able to study the modulation of both gravikinesis and gravitaxis as a function of acceleration.

Gravikinesis in vertically swimming cells

The bipolar organization of mechanosensitivity in *Paramecium* ensures that the sign of gravikinesis remains negative in upward and downward swimmers even under hypergravity (see Fig. 13, left-hand column). Under normal gravity, the unipolarity of the mechanoreceptor response in *Didinium* leaves upward swimmers without kinesis; downward swimmers are slowed down by negative gravikinesis (Fig. 13, right-hand column). We believe that this system, which is less effective in antagonizing
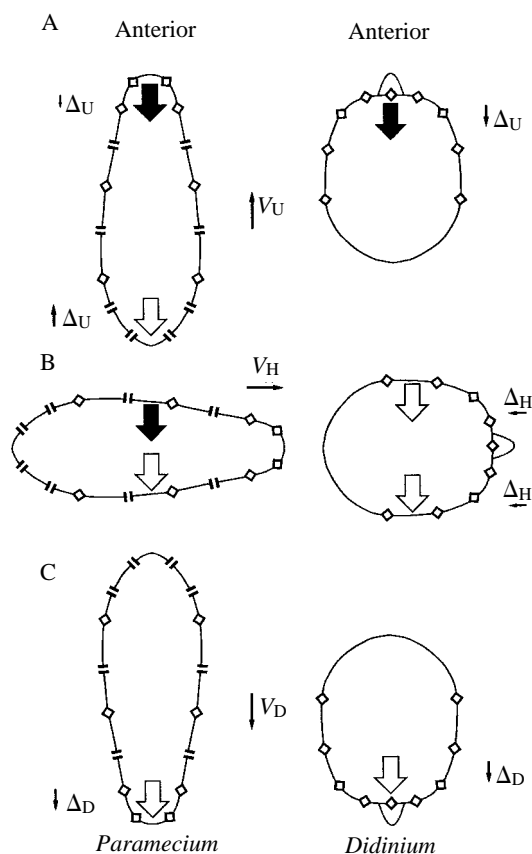
Fig. 13. Hypothetical scheme explaining the observed gravikinetic behaviour of *Paramecium* and *Didinium*. Three orientations are shown: cells during upward swimming (A), horizontal swimming (B) and downward swimming (C). Symbols incorporated in the cell membrane represent a gradient-type distribution of mechanically sensitive Ca^{2+} ($\diamond$) and K^+ ($=$) channels, according to electrophysiological evidence. Large vertical arrows show suggested sites of effective deformation of the membrane by gravity (filled arrows, presumed additional deformation under hypergravity). Long thin arrows show vectors of upward (V_U), horizontal (V_H) and downward (V_D) swimming rates. Short thin arrows show gravity-induced kinetic vectors in upward swimming (Δ_U), horizontal swimming (Δ_H) and downward swimming (Δ_D). Active swimming is enhanced or reduced by the gravikinetic response according to cell orientation, gravisensory channel distribution and acceleration.

sedimentation, is disturbed under hypergravity under which the upward swimmers generate a positive gravikinesis, enhancing the effect of passive sedimentation. Hypergravity was not experienced in the evolution of ciliates so that this behaviour has no impact on survival in common environments. Moreover, it may be seen from Fig. 8 and from the taxis coefficients in Table 2 that, in *Didinium*, negative graviorientation appears successfully to offset the less favourable gravikinesis. A downward drift of a cell population is therefore less probable even under hypergravity.

Gravikinesis in horizontally swimming cells

We have proposed that bipolar gravistimulation in horizontally swimming *Paramecium* cancels gravikinesis at 1g (Machemer *et al.* 1991). In confirmation of this view, the swimming rate of *Paramecium* under weightless conditions equalled the swimming rate in a horizontal direction at 1g (Machemer *et al.* 1992). Our present experiments test this hypothesis under hypergravity (Fig. 5). The horizontal velocity decreased by only 6 % between 1 and 5.4g in both well-fed and starved cells, confirming our hypothesis. It can be concluded from electrophysiological experiments in *Paramecium* that the ratio of summed mechanically induced conductances, g_{Ca}/g_K, corresponds to the conductance ratio of the unstimulated cell (Ogura and Machemer, 1980; Machemer, 1988*a*). This provides further support for the observed gravitational zero balance of horizontally orientated *Paramecium* (Machemer, 1994).

Inspection of the data from horizontally swimming *Didinium* (Fig. 10) immediately reveals that conclusions valid for *Paramecium* may not apply to other types of cells. In *Didinium*, the horizontal swimming rate decreased with rising gravitational acceleration, suggesting the existence of a depolarizing input in horizontal swimmers inhibiting ciliary frequency. Depolarizing mechanoreceptor potentials in *Didinium* were established, whereas hyperpolarizing mechanoreceptor potentials were not detected (Hara *et al.* 1985). In addition, hyperpolarizing voltage steps did not modify the ciliary frequency of unstimulated *Didinium* (Mogami *et al.* 1990). Under weightless conditions, *Didinium* swam faster than horizontally oriented specimens did under 1g (Machemer *et al.* 1992). Fig. 13 (right-hand column) accounts for these data by assuming that a gradient of hyperpolarizing mechanoreceptors is missing in *Didinium*. The scheme explains why $V_H < P$, why, under rising gravity, horizontally swimming *Didinium* cells slowed down and why the upward swimming rate was strongly decreased (Fig. 10).

Gravikinetic sensitivity presumably changes with cell age

Cells taken from our cultures at 3 days and 7 days show different kinetic behaviour under hypergravity (Fig. 7). Cells from these cultures differ in individual age. Interestingly, the data show the same value for generalized gravikinesis, ($\Delta = -60 \mu m s^{-1}$) for well-fed and starved *Paramecium* at normal gravity (Table 1). Under hypergravity, the slopes of Δ are primarily determined by the inversion of the slope of Δ_U (Fig. 7A). This cannot be explained by assuming saturation of the response of the hyperpolarizing gravireceptor, but a gravireceptor at the anterior cell end must be activated under hypergravity. The simultaneous activation of antagonizing (i.e. depolarizing and hyperpolarizing) mechanoreceptors is a fundamental property of *Paramecium* (Ogura and Machemer, 1980) and is likely to apply to gravireception (Machemer *et al.* 1991). We interpret the slope of Δ_U in well-fed cells (Fig. 7A) by assuming an additional depolarizing deformation of the anterior membrane of upwardly oriented *Paramecium* (filled arrow in Fig. 13A). The mechanically induced conductance ratio, $\Delta g_{Ca}/\Delta g_K$, decreases as a consequence of such dual gravireceptor activation. Hence, the resulting (hyperpolarizing) receptor potential is depressed, the frequency response of the cilia declines and the upward swimming rate is reduced. Surprisingly, our

data do not suggest a similar conclusion for starved cells, where gravikinesis was uniform in upward- and downward-swimming cells (Fig. 7B). This cannot be explained by assuming an age-dependent shift in the proportion of mechanically sensitive channels because the horizontal swimming rates were unchanged (Fig. 5). Possibly, these are age-dependent changes in the mechanical properties of the cell cortex, which may affect the gravireceptor conductances in different ways. Microtubules and microfilaments of the cortex of *Paramecium* (Allen, 1988) may change in ageing cells, and these elements of the cytoskeleton may play a role in mechanotransduction (Wang *et al.* 1993).

Combined orientational and kinetic responses neutralize sedimentation

The taxis coefficients integrate the active and passive components of velocity and orientational responses of cells. They are therefore a measure of the displacement of a cell population in the gravitational field (Machemer and Bräucker, 1992). The taxis coefficients of the present data are close to zero under natural gravity conditions in *Paramecium* and *Didinium*, and they vary little from zero (Table 2) under hypergravity. We conclude that the swimming ciliates so far investigated are able to offset sedimentation effects. Sedimentation of cells in the absence of any counterbalancing mechanism irreversibly moves them to the bottom of fluid environments. In addition, sedimentation of cells interferes with the identification of stimulus gradients in water (e.g. chemical, photic or thermic), which may be important for survival. It has been shown that *Loxodes* maintains the same gliding velocity irrespective of its orientation in space (Bräucker *et al.* 1992). The compensation of sedimentation effects by a negative gravikinesis response in *Loxodes* broke down under hypergravity. Nevertheless, the overall taxis was neutral up to $5\,g$ (Machemer-Röhnisch *et al.* 1993).

Our conclusion that gravitational pull is neutralized by orientational and velocity responses in *Paramecium* and *Didinium* applies only to fully equilibrated cells in their physiological steady state. Previously stimulated cells (for instance, by mechanical disturbances, transfer to different solutions, shifts in temperature) may show a transient, pronounced negative gravitaxis, which is not neutralized by velocity responses. As is well known to experimenters, this spectacular phenomenon is commonly limited to some minutes or tens of minutes. Neither experimentally induced gravitaxis nor swimming rate under steady-state conditions is fully representative of the behaviour of a ciliate in its natural environment.

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