J Comp Physiol A (1993) 171:779–790

Journal of Comparative Physiology A Sensory, Neural, and Behavioral Physiology

Neutral gravitaxis of gliding *Loxodes* exposed to normal and raised gravity

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Accepted June 24, 1992

Summary. In the statocystoid-bearing, flat ciliate *Loxodes*, the peculiar steady locomotion on submersed substrates (called "gliding") was investigated between 1 *g* and 5.4 *g* under controlled environmental conditions in a centrifuge microscope. Videorecordings of the movements of large cell populations were processed with an automated analysis procedure. At 1 *g*, possible sedimentation was fully compensated, and vertical shifts of the population were neutralized because upward and downward orientations of the cells occurred at equal proportions ("neutral gravitaxis"). With rising gravity the resultant velocity of upward-gliding cells remained unchanged, whereas the velocity of downward-gliding cells increased continuously. Long-term exposure to hypergravity did not generate detectable signs of adaptation. The bipolar orientation of *Loxodes* persisted even under fivefold normal gravity, but the axis of orientation rotated from the gravity axis in the counterclockwise direction. The data suggest that both gravikinesis and graviorientation of gliding *Loxodes* are instrumental in perfect neutralization of sedimentation at terrestrial conditions.

Key words: Locomotion by cilia – Gravikinesis – Gravitaxis – Hypergravity – *Loxodes*

Introduction

Motor responses in ciliates to a variety of stimuli including gravity are beneficial for the discovery of food sources and for survival. *Paramecium* exhibits a negative gravitaxis during steady swimming. The analysis of gravitaxis is complicated because gravity tends to pull heavy bodies downwards. Methods have been developed to separate passive downward motion of cells (sedimentation) from active graviresponses. Exclusively physical mechanisms counteracting sedimentation were identified in *Paramecium*, such as a minor posterior shift of the centre of gravity reorienting forward swimming upward (Verworn 1889; Fukui and Asai 1985). This mechanism does not apply to starving *Paramecium* (Taneda et al. 1987). Gravitaxis in *Loxodes* (Fenchel and Finlay 1984) is most interesting because this cell bears statocyst-like organelles (Fenchel and Finlay 1986a). The slender, willow-leaf-shaped ciliate occurs preferentially in sediments where it moves close to solid surfaces. During this "gliding" locomotion *Loxodes* faces the substrate with its planar, densely ciliated right body side. Under increasingly low O_2 concentration, *Loxodes* leaves the sediment and migrates in the water column. Vertical migration was shown to be guided by O_2 tension and light intensity (Fenchel and Finlay 1986b; Finlay and Fenchel 1986a, b; Finlay et al. 1986).

In search of active responses of cells to gravity – apart from sedimentation and possible orientation by physical principles – the gravikinetic behaviour of protozoans was investigated. It is well established that in ciliates such as *Paramecium* and *Stylonychia*, the rate of ciliary beating during forward locomotion is a monotonous function of the membrane potential: negative shifts from the resting potential raise the ciliary frequency and swimming rate; depolarizations depress the rate of forward locomotion (Machemer 1986; Machemer and Sugino 1989). Our hypothesis, that gravity modulates the membrane potential analogous to deformation of mechanically sensitive membrane sites in ciliates (Machemer and Deitmer 1985), opens a new perspective to experimental analysis of gravitaxis (Machemer 1989). Previously, it was shown in *Paramecium* that after accounting for sedimentation the rate of upward swimming exceeded the rate of downward swimming. This suggests the existence of a physiologically mediated response to gravity (Machemer et al. 1991; Ooya et al. 1992).

Recently, we investigated the gravity-dependence of *Loxodes* gliding velocity (Bräucker et al. 1992). This steady type of locomotion in two dimensions is well suited for a quantitative study of gravikinesis and preferable to swimming in the fluid space which takes a more

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irregular course due to intervening ciliary reversals (Fenchel and Finlay 1984). It was shown that *Loxodes* maintains a constant velocity irrespective of whether it moves up or down, or in any direction in space. Because immobilized specimens of *Loxodes* sediment (Fenchel and Finlay 1984) it is possible that a gravikinesis cancels sedimentation. The present work investigates the gliding behaviour of *Loxodes* under artificially raised gravity anticipating more pronounced sedimentation and kinetic responses than under terrestrial gravity, and demonstrates that active compensation of the sedimentation rate can be driven to its physiological limit. Furthermore, a preferred bipolar vertical orientation and an increasing unidirectional angular offset from the vertical with rising gravity is reported for gliding *Loxodes*.

Materials and methods

Culture. Wild-type *Loxodes striatus* was supplied by B.J. Finlay, Institute for Freshwater Ecology, Ambleside, Cumbria, and reared in soil solution (Erdschreiber) with reduced O_2 by gassing with N_2 and CO_2 at pH 6.8. A new culture was kept for 48 h at 12 °C, and then transferred to 22 °C for further culturing in the dark. Cultures of *Euglena gracilis* (10 days old) were used for feeding. The *Loxodes* cultures were in their stationary phase and ready for the experiments 2 weeks after inoculation.

Experimental chamber. The chamber (Fig. 1A) was made of Plexiglass (60 mm diameter) enclosing a rectangular fluid space of $25 \times 25 \times 1.6$ mm, equivalent to 1 ml. The upper (= centripetal) and lower (= centrifugal) border of this space was lined by agar blocks (1.5% in experimental solution; 0.8 ml each) to increase the buffering volume of the medium.

Experimental solution and equilibration. Cells were concentrated by circular swinging of the culture dishes and transferred by pipette to an experimenting solution of 1 m*M* $CaCl_2 + 0.5$ m*M* $KCl + 0.1$ m*M* $MgSO_4 + 0.41$ m*M* $Na_2HPO_4 + 0.19$ m*M* NaH_2PO_4 at pH 7.0. This procedure was done 3 times. From the experimental solution, samples of cells were gently infused into the experimental chamber using a syringe. The cells equilibrated 4 h in the experimental solution. The O_2 level within the closed environment of the chamber was >93% air saturation (polarographic sensor and amplifier, Type 170, Ingold, FRG) and decreased by <5% within 4 h using the same cell density [cell/water: 1/16.000 (v/v)] in a large-scale reference chamber with same surface-to-volume ratio.

Centrifuge and recording. For the recording of cellular behaviour at gravity ≥ 1 *g*, the chamber was mounted in a horizontal centrifuge (NIZEMI, Dornier GmbH, FRG) designed to generate highly controlled accelerations of microscopic organisms (Briegleb and Hemmersbach 1987; Kreuzberg et al. 1991). The centrifuge consists of a belt-driven disc, to which the chamber was fixed in a radial plane (Fig. 1B) such that the resultant acceleration vector was parallel to the plane of the chamber. The disk-shaped chamber was free to rotate about its central axis (= normal to the disk) in the radial plane of the centrifuge. An eccentric mass connected to the chamber swang out like a pendulum dependent on angular velocity of the centrifuge. The construction of the centrifuge ensures that the major axis of the chamber (= vertical in Fig. 1A) is in line with the axis of the pendulum so that the resulting acceleration acts parallel to that axis. The angle of centrifugal swing (γ) was used to calculate the resulting acceleration):

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

where l is the distance between the centres of mass of the chamber and the pendulum, and R represents the distance of the centre of the chamber to the axis of the centrifuge. Correctness of Eq. 1 for determinations of acceleration was checked against an equation using the centrifuge angular velocity and R.

The central recording area of the chamber was illuminated by a ring of 48 LEDs generating a light intensity of 800 lx in the experimental plane in a darkfield configuration. The green emission wavelength (565 nm) is near the minimum of the absorbance and

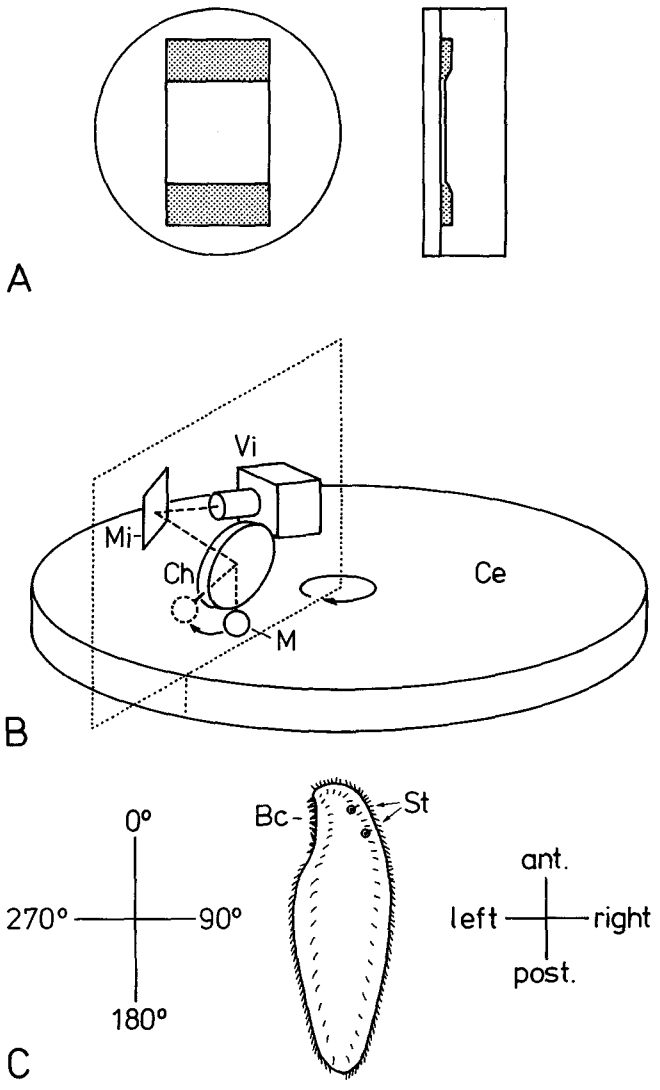

Fig. 1A–C. Experimental setup: **A** Plexiglass chamber in surface view and cross-section. The chamber encloses a fluid volume of 1 ml (rectangular field: 2.5 × 2.5 cm; depth 1.6 mm) with sides perpendicular to the direction of gravity lined by agar (1.5% in experimenting solution); **B** Principle of horizontal centrifuge platform (*Ce*). The chamber (*Ch*) is vertically mounted in the radial plane of the centrifuge (*dotted*) allowing rotation about the central axis of the chamber. An eccentric mass (*M*) fixed to the chamber swings outward in line with the resultant gravitational vector. Gliding motion of *Loxodes* along the proximal inner surface of the chamber is recorded by videocamera (*Vi*) using a mirror (*Mi*); **C** definitions of angles (*left*) and cell topography (*right*). *Loxodes* glides with the ciliated and flattened side facing the plexiglass surface. Angles are referred to the resultant gravity vector (0° = up or centripetal; 180° = down or centrifugal). Gliding of *Loxodes* occurs with the anterior end heading. Lefthand rotation in the direction of the buccal cavity (*Bc*), righthand rotation in the aboral direction (or toward the statocystoids, *St*). Polar histograms of *Loxodes* motion (Figs. 4, 10) use the view normal to cell and chamber surface

behavioural action spectra of *Loxodes* (Finlay and Fenchel 1986a). The macro-lens of a CCD high-resolution video camera (HR 600 M, Dornier, 25 Hz) was focused at the proximal inner surface of the chamber thereby selecting for gliding cells. Swimmers, which are identified by their helical pathway, and gliders on the distal surface were out of focus. Images (8.5 × 11.5 mm) of the field were projected into the video camera via a mirror. The video signals were transmitted to a video recorder outside the centrifuge. Displays of the recorded images showed the cells with their oral side to the left and their flattened ciliated surface for gliding away from the viewer (Fig. 1C). This view is a precondition for judgment of orientational responses regarding the cell coordinates. Positions of cells on the vertical surface are defined with respect to the angle the gliding cell makes with the "vertical" ("up" = 0°, angles proceeding clockwise).

Cell immobilization and sedimentation. Samples of cells in experimental solution were exposed for 10 min to 1 m*M* $NiCl_2$ in the same solution and were then transferred to the chamber; 20 min were allowed for complete immobilization. Sedimentation of cells was recorded with the camera focused on the 1.6 mm deep fluid body of the chamber.

Experimental protocol. Specimens (150–200) in experimental solution were transferred to the chamber with as little agitation as possible. The temperature inside the chamber was between 22 and 23 °C (room temperature controlled at 22 ± 0.5 °C). Gliding locomotion and sedimentation were recorded for periods of 2 min in two sequences of *g*-values: (1) "type 1" 1→2.1→3.3→4.3→5.4→1; (2) "type 2" 1→5.4→4.3→3.3→2.1→1. Changes in acceleration between two predetermined *g*-levels were set at 1 *g* min^{-1}. A 2-min record of cell locomotion was evaluated to generate five separate representations of 4-s tracks from the video tapes by computer analysis (Machemer et al. 1991). From each of these images at least 10, commonly about 30, tracks were used for evaluation. Twelve experiments were done for each value of gravity (1 *g*: 24 experiments). Assuming that each specimen entered measurement 1.3 times, data points are based on at least 1200 individual cells.

Data evaluation. For evaluations of tracks, three markers were used on each track separated by the same time interval. In digital image analysis these identified points served to test for steady gliding at little or no curvature, and to eliminate reversals or sharp turns of *Loxodes*. The evaluation criteria have been described in detail elsewhere (Bräucker et al. 1992). For separation of kinetic and orientational responses, velocity in 24 orientational sectors of 15° was determined separately, and averaging of sector velocities was done irrespective of the cell count within one sector. Non-parametric statistics (calculation of median values; 95% confidence range; *U*-test) were applied because Gaussian distribution of the data (see Fig. 7 for example) was not assured.

Theoretical considerations

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

$$(V_D - V_U/2 = S + \Delta \quad [\mu m \cdot s^{-1}], \qquad (2)$$

which determines the value and sign of Δ. Dimensions in this equation ($\mu m \cdot s^{-1}$) are linear velocities which are proportional to forces of active propulsion or gravitational pull according to the Stoke's equation of flow at low Reynolds numbers (Wu 1977). The downward swimming rate may exceed, correspond to, or fall below upward swimming rates (Fig. 2, observation 1). According to conventions, the first case may be called a positive gravitaxis, and the latter case a negative gravitaxis. Unless sedimentation rate is determined (Fig. 2; observation 2), the generalized term of gravitaxis has only descriptive value. The flow diagram in Fig. 2 shows that an observed net downward migration of cells is compatible with $\Delta = 0$, or $\Delta > 0$, or $\Delta < 0$, depending on the sedimentation rate. Conversely, a gravikinesis with negative sign ($\Delta < 0$), counteracting sedimentation, may be extracted from cells exhibiting positive ($V_D > V_U$) or negative gravitaxis ($V_D < V_U$), or no gravitaxis at all ($V_D = V_U$). The term "negative gravikinesis" indicates, in analogy to a genuine orientational (= tactic) response, that the kinesis acts to remove the cell from the vectorial stimulus source. For guidance of the analysis of the gliding behaviour in *Loxodes*, we follow the emphasized pathways (1) and (2) of Fig. 2.

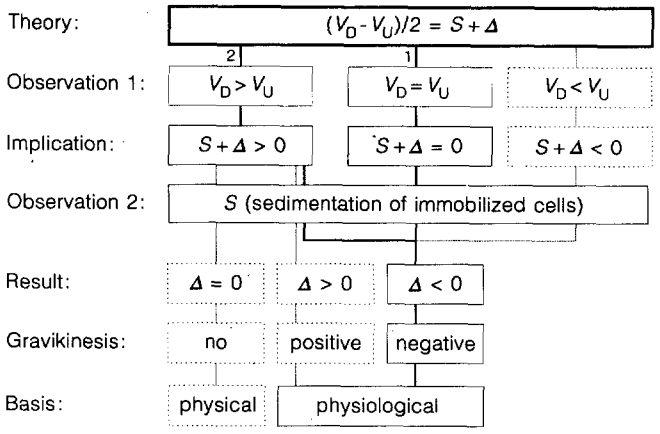

Fig. 2. Flow diagram to illustrate steps in isolation of gravikinesis in *Loxodes*. Theory predicts that vector addition of the downward (V_D) and upward (V_U) rates of locomotion, divided by 2, equals the sum of the sedimentation rate and a gravity-induced component of active propulsion (Δ = gravikinesis). The downward sequence of boxes starting with (1) summarizes data of gliding *Loxodes* under terrestrial gravity (1 *g*): here, the rates of downward and upward gliding are identical implying cancelling of sedimentation and gravikinesis. Determination of the cell sedimentation rate (S, positive sign) established gravikinesis (Δ, negative sign). The present paper, in applying hypergravity, shows (2) that downward gliding can exceed upward gliding, with the sum of S and Δ being positive. It is seen that after inclusion of sedimentations under hypergravity, values of Δ rise with the gravity vector but increasingly fail to compensate sedimentation (Table 3). *Dotted boxes* are theoretically possible events which have not been observed

Results

Behaviour of Loxodes *under hypergravity*

Effects on velocity. Table 1 lists the medians of gliding rates obtained under different *g*-values. Up to about threefold normal gravity the mean gliding rates were virtually unchanged. At 5.4 *g* the rates had risen by up to 12%. Differences between the median rates at 1 *g* and 5.4 *g* occurred in cells exposed to rising *g*-sequence (7%) and falling *g*-sequence (12%).

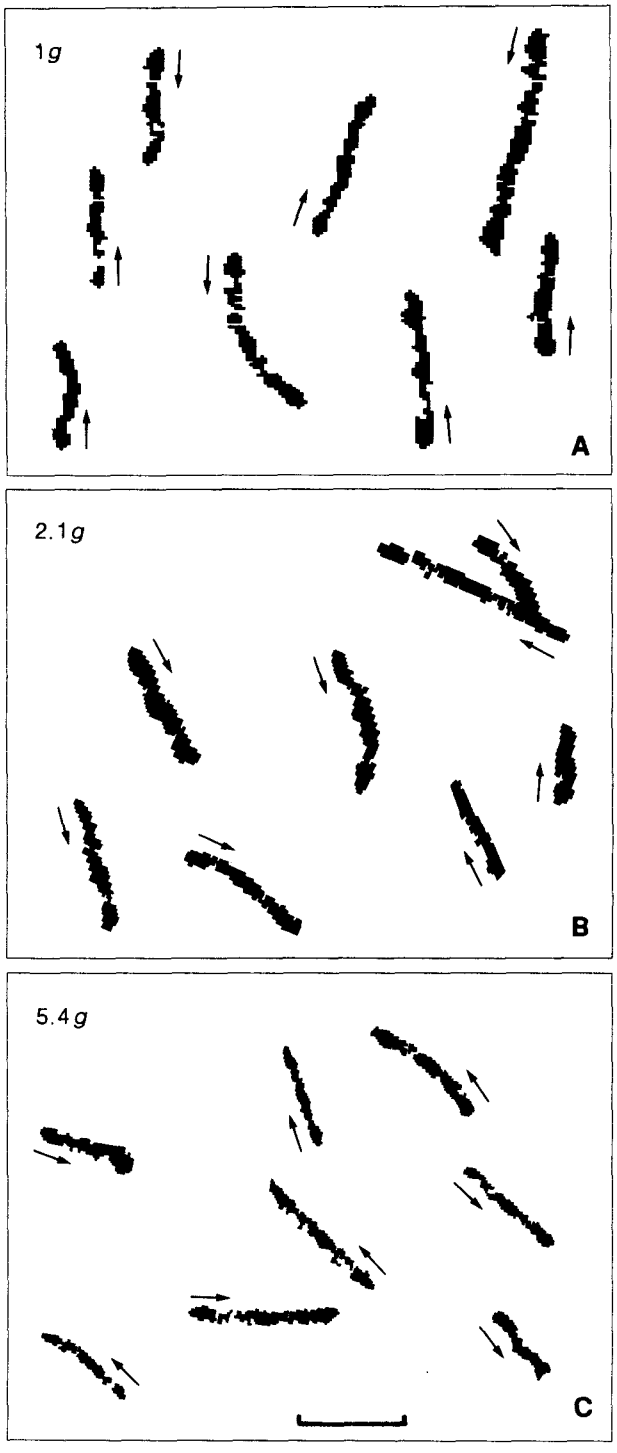

Fig. 3A–C. Examples of gliding *Loxodes* at normal (**A**) and raised levels of gravity (**B, C**). The 4-s tracks were generated from superimposed video images. *Arrow* near start of track indicates gliding direction. The resultant *g*-force is downward (also in following figures). The traces reveal a *g*-dependent counterclockwise reorientation of the cells. Scale bar: 500 µm

Velocities compensated for orientational bias. The data of Table 1 are medians over all individual velocities in any direction; they include an orientational bias because some gliding directions were preferred (Figs. 3, 11). Plotting the median gliding rates, as evaluated within orientational sectors of 15°, gives a more informative representation of velocities (Fig. 4). It is seen that beyond 2 *g* centrifugal rates increasingly exceeded centripetal rates; this effect was pronounced in the sequence of rising *g* (Fig. 4A) but less apparent with falling *g* (Fig. 4B). Surprisingly, centripetal rates were unchanged between 1 and 5.4 *g*.

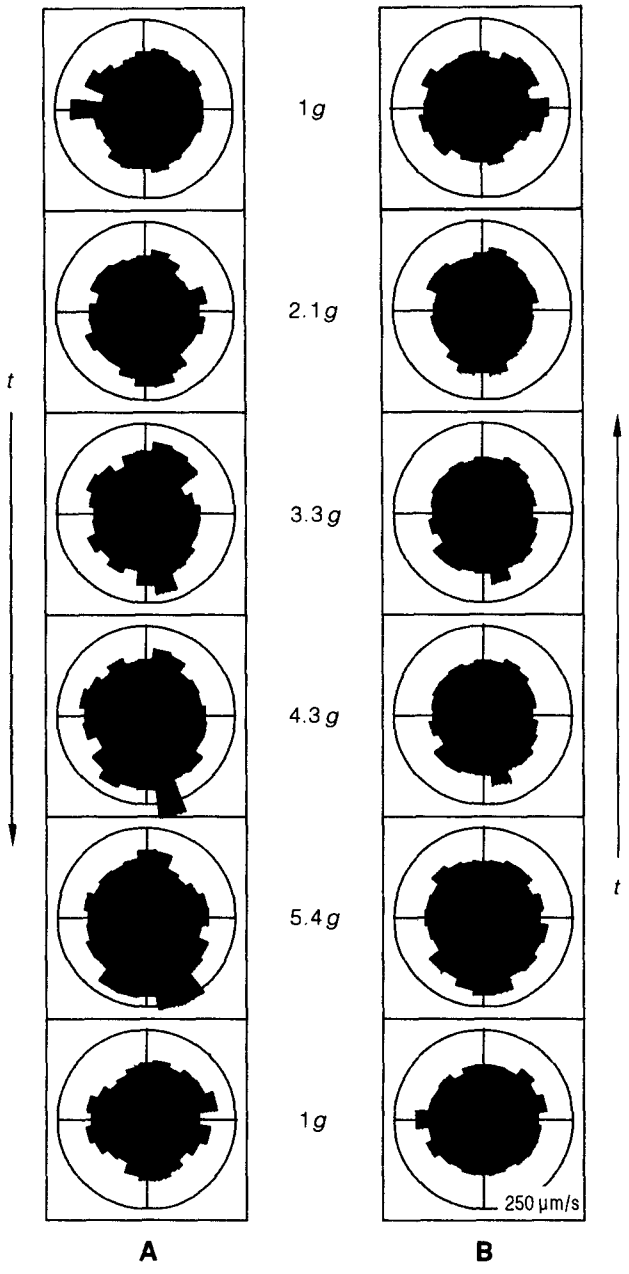

Fig. 4A, B. Gravity-dependent median gliding velocities in 24 orientational sectors of the vertical plane: **A** data from experiments (type 1) employing a sequence (*t*) of rising gravity and conclusion with 1 *g*; **B** data from experiments (type 2) starting with 1 *g*, switching to 5.4 *g*, and continuing with decreasing gravity. It is seen that in both types of experiments upward (centripetal) rates were unmodified, whereas downward (centrifugal) rates increased with the *g*-vector

In averaging the median gliding rates of 15°-sectors, preferences in orientation are neutralized (Fig. 5A).

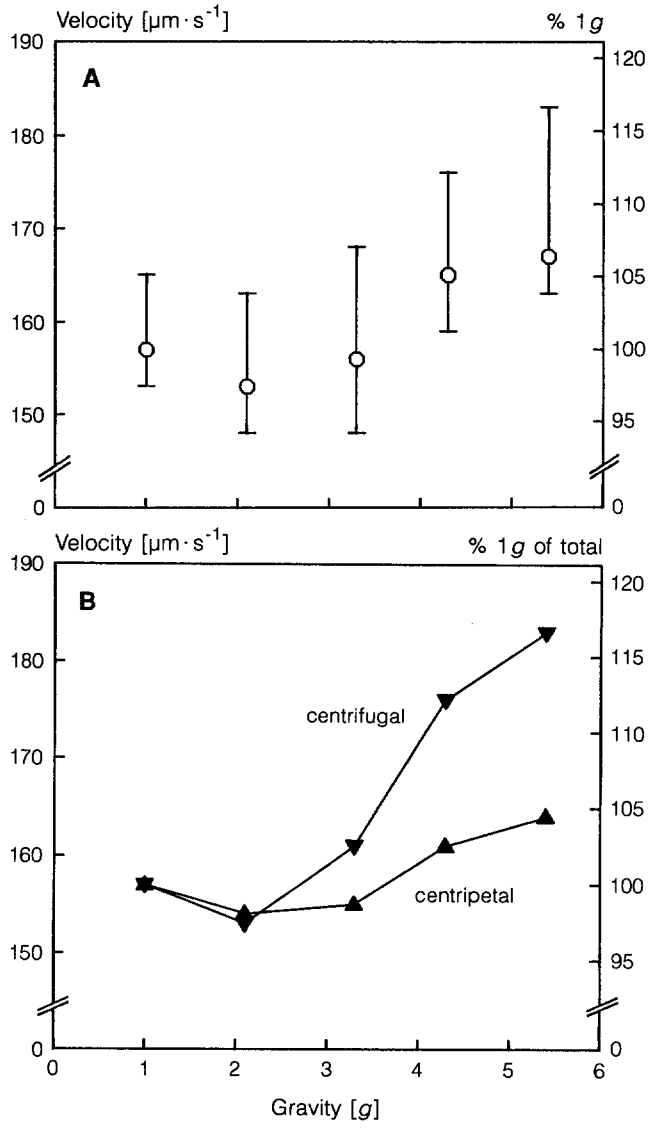

Fig. 5A, B. Gliding velocities after removal of the orientational bias (Fig. 10): **A** medians of all directions. *Error bars* (95% confidence range) based on 24 orientational sectors; **B** separation into median centrifugal and centripetal orientations shows that centripetal rates were largely unchanged throughout the range of gravity applied. Note interruption of *y*-axes

Table 1. Omnidirectional gliding velocities of *Loxodes* as modulated by different values of gravity

Medians of gliding velocity [μm · s⁻¹]			
g	total (*n*)	*g*-rising (*n*)	*g*-falling (*n*)
1	156^{+1}_{-2} (4554)	160^{+2}_{-3} (1480)	153^{+2}_{-1} (3074)
2.1	156^{+2}_{-2} (2003)	164^{+6}_{-5} (588)	154^{+2}_{-3} (1415)
3.3	159^{+3}_{-2} (1804)	164^{+6}_{-5} (493)	156^{+4}_{-3} (1311)
4.3	171^{+3}_{-3} (1705)	175^{+6}_{-7} (322)	170^{+4}_{-4} (1383)
5.4	173^{+3}_{-4} (1655)	173^{+6}_{-5} (322)	172^{+3}_{-4} (1333)

Values are medians ± 95% confidence; *n* = number of cell counts. Experiments with *g*-rising and *g*-falling sequence are separately listed. The velocities include an orientational bias (see Fig. 10). Each individual cell was evaluated about 1.3 times (see Methods)

Table 2. Rates of sedimentation (μm · s⁻¹) of suspended cells (*S*; *n* = 175–370), gliding downward (or centrifugal, V_D) and upward (or centripetal, V_U) listed as functions of gravity (*g*)

g	*S*	V_D	V_U	*Δ*
1	48	159	153	−45
2.1	84	186	148	−65
3.3	108	196	155	−87
4.3	112	204	152	−86
5.4	135	221	158	−104

The gravikinetic component (*Δ*, μm · s⁻¹) results form V_D, V_U and *S* according to Eq. 2. Median velocities are from orientational sectors ±15° from the vertical axis

Separation of the data into centripetal (270°→0°→90°) and centrifugal (90°→180°→270°) locomotion shows that centripetal velocities were little modulated throughout the range of hypergravity applied; however, centrifugal gliding rose continually beyond 2 *g* (+17%, Fig. 5B). Figure 5 is a quantitative abstract of Fig. 4 and suggests, more reliably than Table 1, a minor tendency for *g*-dependent increase in velocity.

Sedimentation. The sedimentation rate of viable but immobilized, floating cells increased from 48 μm · s⁻¹ at 1 *g* to 135 μm · s⁻¹ at 5.4 *g* (Table 2). Contrary to expectation, sedimentation rates did not grow in proportion to gravity. Wall effects (Wu 1977) may have reduced sedimentation rates during hypergravity.

Gravity-dependent vertical gains of cell total. Medians of the vertical components from all velocity sectors suggest little or no increase between 1 *g* and 5.4 *g* (Fig. 6A). Hypergravity did not alter the vertical rates of movement during *centripetal* gliding. During *centrifugal* motion, however, vertical displacement medians rose from 107 μm · s⁻¹ to 130 μm · s⁻¹ between 1 *g* and 5.4 *g* (Fig. 6B). Centrifugal motion was below the total medians at ≤2 *g* and positive to total medians at ≥3 *g*.

Cell loss under hypergravity. Data of gliding movement under hypergravity suffer from an increasing loss of available cells. It is possible that "slow" cells preferentially drift from the recording window in the centrifugal direction and are not counted in the records; hence, a positive selection of those specimens which successfully cope with additional load from gravity might shift the slopes of the velocity plots upward. Figure 6B represents the relative loss in cell counts *(dashed line)* which steadily rose to 35%. Splitting these data into separate experiments (type 1: rising *g*-sequence, 30% cell loss between 2.1 and 5.4 *g;* type 2: falling *g*-sequence; 6% cell loss between 2.1 and 5.4 *g*) shows, however, that the velocity characteristics of centrifugal and centripetal displacements were not produced by an increasing selection for particular cells (data not shown). We have also inspected the velocity distributions at different *g*-values to test for possible hypergravity- and time-dependent shifts of these distributions in the type-1 and type-2 experiments. Sustained hypergravity depressed the median of the 1-*g* rate

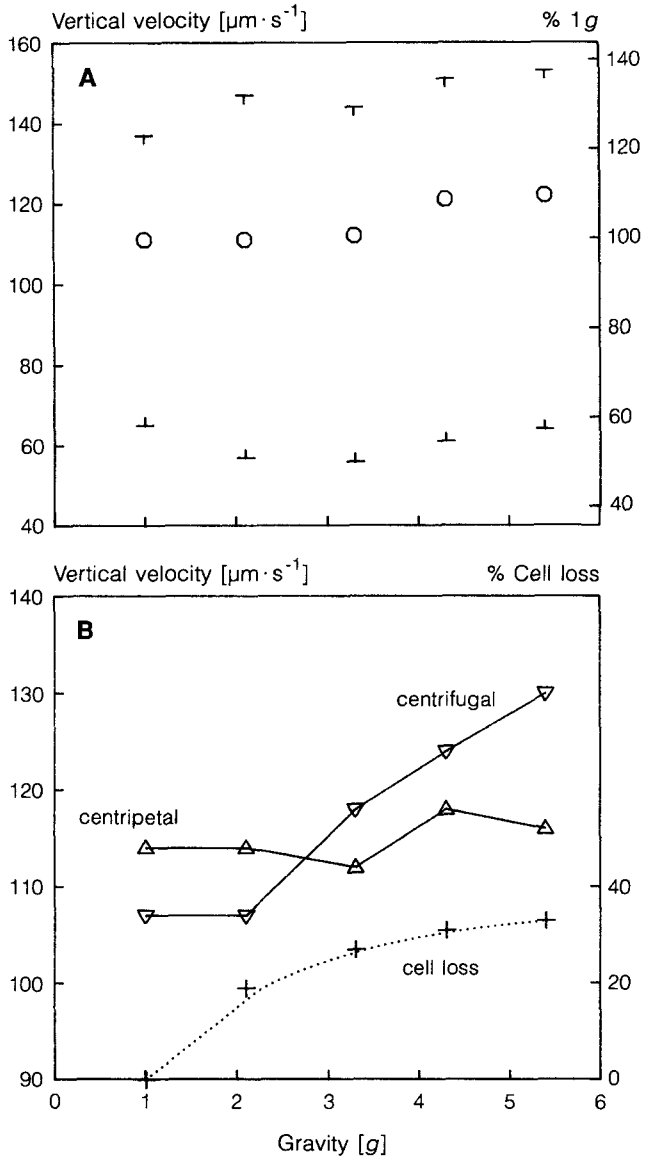

Fig. 6A, B. Vertical velocities at various levels of *g* after removal of orientational bias: **A** all cells; large 95% confidence range due to restriction a 24 sectors; **B** vertical velocities of upward (centripetally) gliding cells were presumably unaffected by hypergravity, whereas the vertical rates of downward (centrifugally) gliding cells rose beyond 2 *g*. *Dotted curve* gives percentage of cell loss from recording area following exposure to hypergravity (see text)

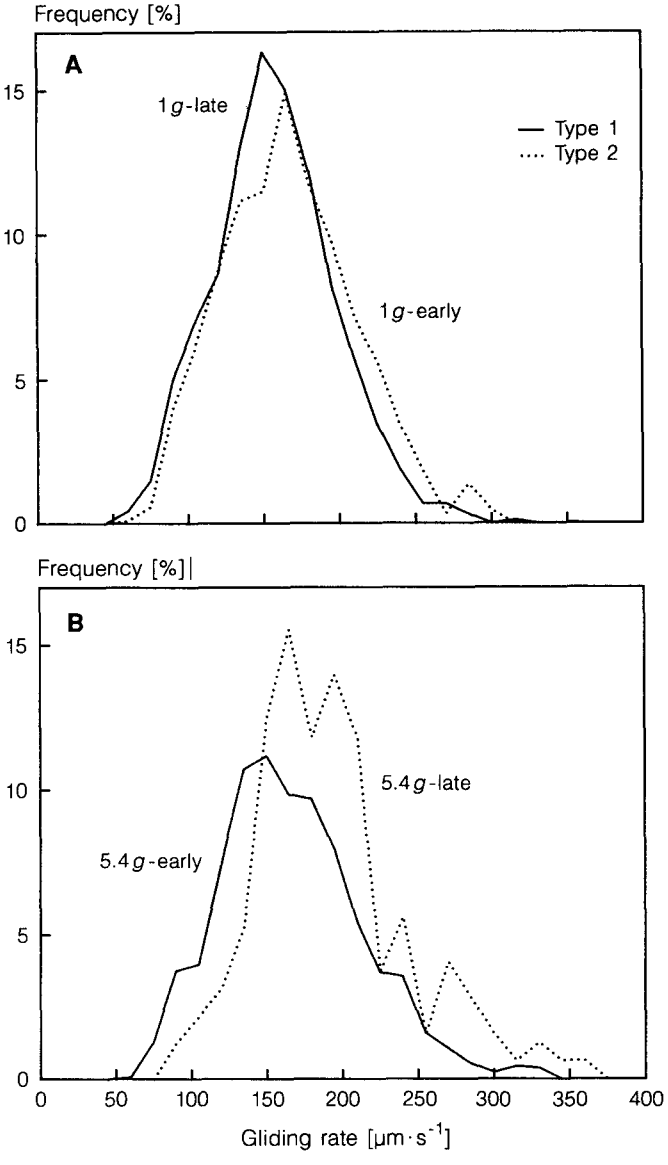

Fig. 7A, B. Test of velocity-class distributions of *Loxodes* as being potentially affected by gravity and time (no orientational bias; see Fig. 4); **A** 1-*g* velocities in type-2 experiments (1 *g*-early) and type-1 experiments (1 *g*-late; reduction in rate); **B** 5.4-*g* velocities in type-1 experiments (5.4 *g*-early) and type-2 experiments (5.4 *g*-late; increase in rate). Although minor differences exist between the median velocities of the type-1 population ($n=1480$) and type-2 population ($n=3074$), a tendency for gravity- and time-dependent adaptation of the kinetic response cannot be derived from the data

by 5%, and increased the median of the 5.4-*g* rate by 4% with corresponding shifts in distributions (Fig. 7). This suggests that hypergravity and elapsed time did not select for "fast" cells. Figure 7 shows that small differences in frequency peaks relate to two different populations of cells (experiment type 1:1 *g* early→5.4 g late; type 2:5.4 *g* early→1 *g* late).

No adaptation to hypergravity

The *g*-dependent increases in gliding rate and graviki nesis might be affected by previous experience of hypergravity. It is possible that cells which have been exposed to hypergravity for some time change their behavioural response to gravity. Comparison of type-1 and type-2 experiments (Fig. 7) suggests that adaptation to hypergravity is unlikely. We have tested, in addition, the stability of velocities of a population exposed to 4.3 *g* for up to 52 min. A *g*- and time-dependent relaxation of a negative gravikinetic response predicts a decrease in centripetal gliding rates, and an increase in centrifugal rates. The median total gliding velocities, corrected for orientational bias, rose from 143 to 150 μm · s^{-1} after 2 min and gradually declined to 144 μm · s^{-1} after a further 50 min. During the same period, the centripetal vertical velocity

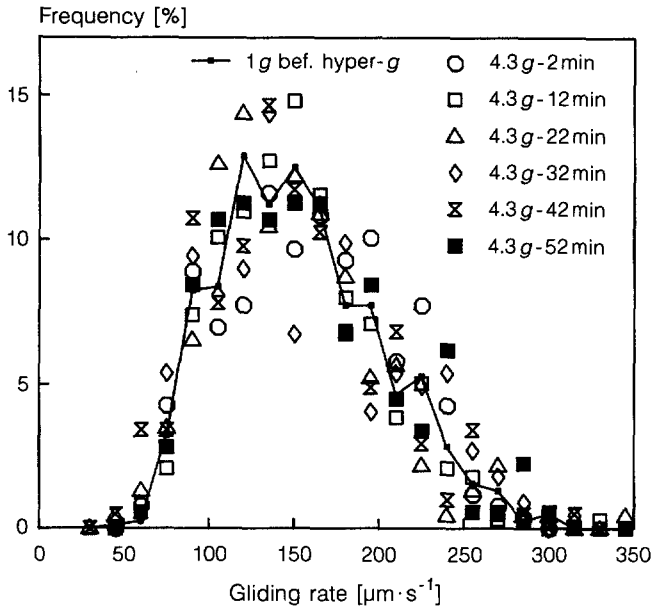

Fig. 8. Effects of sustained exposure to hypergravity on gliding rate ($n=776$; orientational bias removed). Centering of data points about the velocity distribution at 1 *g* suggests that the characteristics of gliding locomotion were not affected by long-term exposure to 4.3 *g*

decreased from 96 to 84 μm · s^{-1}, and the centrifugal vertical rate also decreased from 106 to 99 μm · s^{-1}. A minor general decline of rates over extended periods of hypergravity does not suggest sensory adaptation. This view is supported by velocity-class distributions in the course of 52 min of exposure, which all centre at the velocity prior to hypergravity (Fig. 8).

Isolation of the gravikinetic response

Theory predicts that the gravikinetic velocity component (Δ) of an actively moving cell can be derived from the rates of vertical downward and upward swimming and sedimentation (Eq. 2). It is not known whether cilia in gliding *Loxodes* adhere to the solid substrate; considering the typical beating rate of cilia in ciliates (15–40 Hz), adhesion by currents of water from beating cilia is more likely than physical contact. In gliding cells, sedimentation has not so far been determined experimentally. Therefore, as a first approximation for determination of Δ, the settling rate of suspended, immobilized specimens is used (see also Discussion).

Vertical velocities are directly measured in the vertically oriented cells ($0\pm15°$, $180\pm15°$, Table 2). The data suggest, in agreement with previous observations (Bräucker et al. 1992), that at 1 *g* gliding velocity of *Loxodes* (V_U, V_D) can compensate effects of sedimentation. This conclusion applies irrespective of the correctness of the empirical value of the sedimentation rate. Table 2 shows that Δ approximated the sedimentation rate at 1 *g*, and fell behind the sedimentation rate (by $<23\%$) with rising hypergravity.

Table 3. Two independent approximations of active propulsion rate of *Loxodes*, as unaffected by gravity (P_{1g}: median of omnidirectional cell velocities at 1 *g*; P_{hor}: median of horizontal gliding rates ($\pm7.5°$) at all values of gravity applied

g	P_{1g} (*n*)	P_{hor} (*n*)	*P*	Δ (Eq. 2)	Δ_D (Eq. 3)	Δ_U (Eq. 4)
1	157^{+8}_{-4} (24)	↑	↑	−45	−45	−45
2.1		|	|	−65	−54	−76
3.3		155^{+3}_{-6}	156	−87	−68	−107
4.3		(620)	|	−86	−64	−108
5.4		↓	↓	−104	−70	−137

The generalized term of *P* is the mean of P_{1g} and P_{hor}. Using values of V_D, V_U, and *S*, the gravikinesis during downward gliding (Δ_D) and during upward gliding (Δ_U) are calculated (Eq. 2, 3, 4). It is seen that Δ_D and Δ_U correspond to Δ at 1 *g*. Beyond normal gravity, Δ_D and Δ_U diverge. The dimension of *P* and Δ is μm · s^{-1}

For determinations of the gravikinetic velocity components (Δ_D, Δ_U) during vertical downward (V_D) and vertical upward locomotions (V_U) under gravity, the following equations are applied:

$$V_D = P+S-\Delta_D \text{ or: } \Delta_D = P-V_D+S, \ (\mu m \cdot s^{-1}) \quad (3)$$

and

$$V_U = P-S-\Delta_U \text{ or: } \Delta_U = V_U-P+S, \ (\mu m \cdot s^{-1}) \quad (4)$$

where *P* is the rate of active propulsion of the cell as unaffected by gravity. Downward velocities have a positive sign and upward velocities a negative sign. Table 3 lists two independent assessments of *P:* one using the median of all individual gliding rates at 1 *g* ($P_{1g}=157$ μm · s^{-1}) according to Bräucker et al. (1992), and the other employing medians of the horizontal velocities ($P_{hor}=155$ μm · s^{-1}) which were not modified by gravity [Machemer et al. (1992); see also Fig. 4]. In fixing *P* at the common mean of P_{1g} and P_{hor} (156 μm · s^{-1}), Table 3 shows that Δ_D and Δ_U were identical at 1 *g* (−45 μm · s^{-1}) approximating sedimentation ($S=48$ μm · s^{-1}). With rising gravity, Δ_D and Δ_U grew at different rates: Δ_D saturated between 65 and 70 μm · s^{-1}, whereas Δ_U steadily rose attaining similar rates as seen in sedimentation (5.4 *g:* $S=135$ μm · s^{-1}; $\Delta_U=137$ μm · s^{-1}). The growing divergence in the gravikinetic subcomponents indicates different capacities in *Loxodes* for active compensation of sedimentation during downward and upward locomotion: Δ_D and Δ_U compensate *S* at 1 *g*, Δ_U compensates *S* perfectly up to 5.4 *g*. The scalar values of Δ_D and Δ_U then determine the generalized term of Δ according to Machemer et al. (1991):

$$\Delta = (\Delta_D+\Delta_U)/2 \quad (\mu m \cdot s^{-1}) \quad (5)$$

Figure 9 summarizes the vertical gliding behaviour of *Loxodes* showing the contributions of rates of propulsion (*P*), gravikinesis (Δ_U, Δ_D) and sedimentation (*S*) to resulting vertical velocity (V_U, V_D) in four apposed bars. The full histogram illustrates how *Loxodes* was able to maintain the centripetal gliding rate throughout the applied range of hypergravity, and how centrifugal velocity gradually rose to a maximum of 140% of the 1-*g* reference (Table 2). The graph also shows that a possible

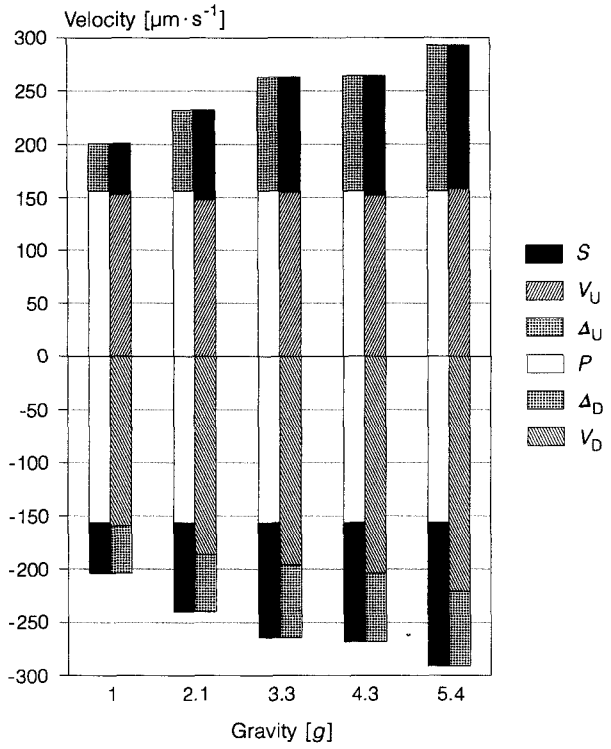

Fig. 9. An overview of hypergravity-induced vertical gliding rates (V_U, up; V_D, down; *hatched histograms*) together with contributions of *g*-free active propulsion (*P*), suspended sedimentation (*S*), and gravikinetic component (Δ_U, Δ_D) to this motion (Eqs. 2, 3, 4). Median values of gravikinesis were determined within $\pm 15°$ of the vertical axis. Values of gravikinesis may be somewhat smaller, if sedimentation during gliding is less than sedimentation of suspended cells

error in experimental determination of the sedimentation rate affects only the length of the terminal bars. The asymmetry of vertical locomotion rates rejects the view that sedimentation and gravikinesis are absent in gliding *Loxodes* (see Discussion).

Orientation with respect to the gravity vector

Cells of *Loxodes* which glide on a vertical plane prefer upward or downward orientation and avoid horizontal locomotion. This orientational behaviour is established within 1 min and remains independent of time and previous gravitational stimulation (Fig. 10). In order to detect a possible spatial bias or a chemical gradient, which might emanate from the agar (Fig. 1A), the orientation of *Loxodes* was tested after rotating the vertically fixed chamber by 90° so that the agar boundaries were at right angles to the gravity vector. Again, the cells preferentially oriented themselves parallel to the gravity vector (Fig. 10C).

With rising gravitational acceleration the behaviour of *Loxodes* changed in a peculiar way: the cells reoriented in the counterclockwise direction (or toward the oral side; see Fig. 1C) and away from the major axis of the chamber and direction of gravity (Figs. 1A, B, 3), respectively. At the same time, the orientation was less pro-

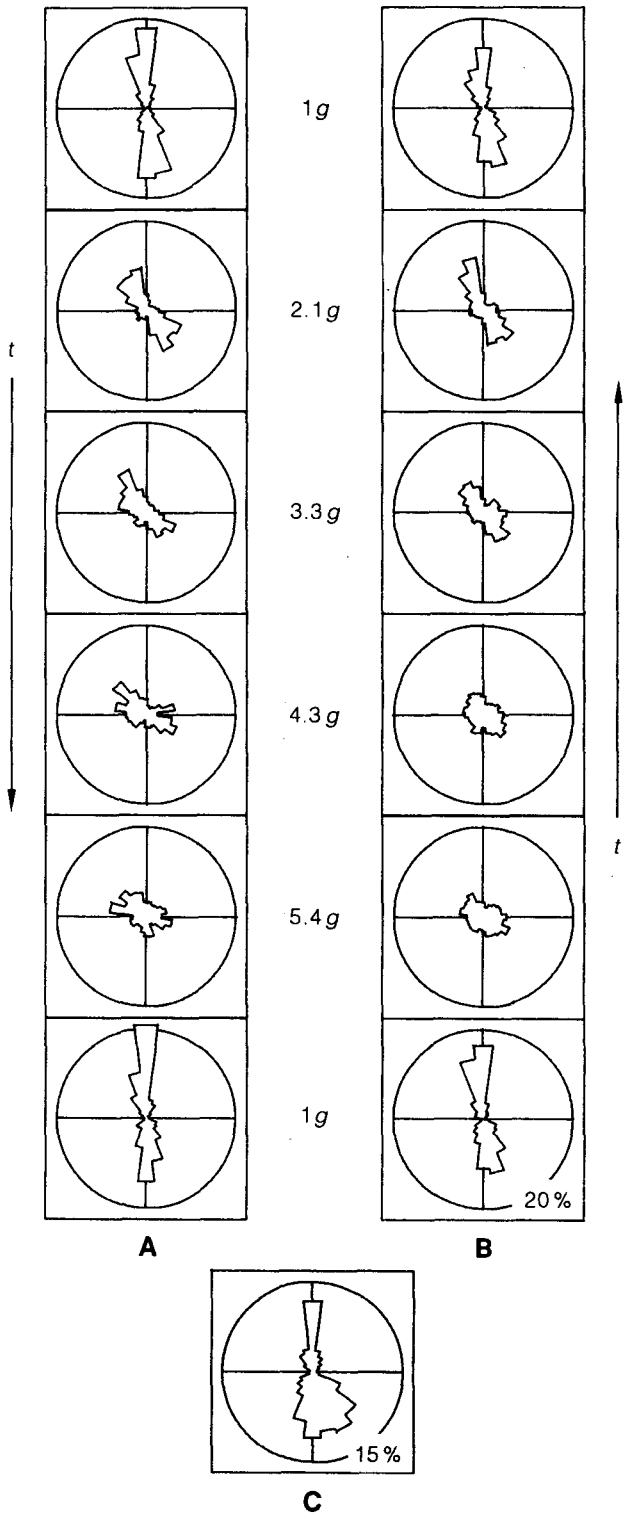

Fig. 10A–C. Orientational distributions of gliding *Loxodes* at different *g*-vectors (in percent of total; resultant gravity force down): **A, B** experimental sequences (*t*) according to Fig. 4. With rising hypergravity the original vertical orientation of *Loxodes* increasingly turned in the counterclockwise direction. At the same time, the accuracy of orientation decreased. The 1-*g* records before and after applied hypergravity suggest reproducibility of the *g*-induced shifts. No difference exists in the results using a rising or a falling *g*-sequence; **C** a test for local bias of orientation: the vertically held chamber (Fig. 1A) was rotated by 90° but the cells continued to show vertical up or down orientations

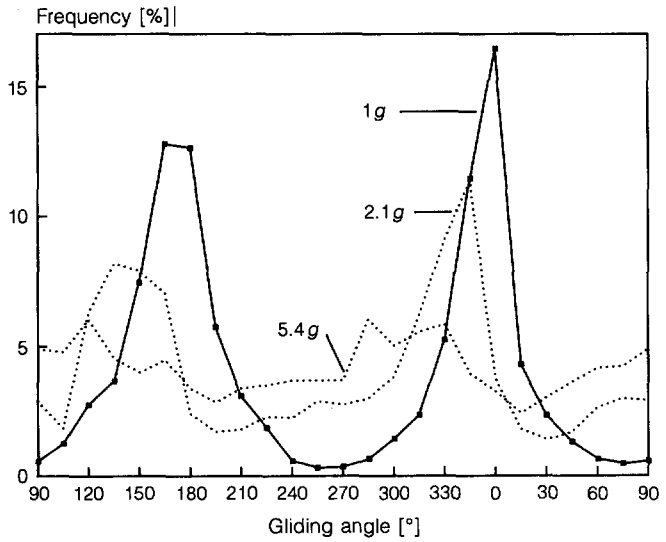

Fig. 11. Distributions of gliding angles in response to gravity. The bipolar orientations ("up", "down") at 1 *g* are characterized by 180° separation with a slightly higher accuracy in the "up" direction (=0°). With rising gravity, this pattern increasingly shifted toward smaller angles (counterclockwise direction), and the preciseness of orientation decreased (compare Fig. 10)

nounced (Fig. 10). However, at 5.4 *g* most cells were counted in the upper left and lower right quadrant of the orientational polarogram. Plotting the frequencies of orientational distribution underscores the similarity of "downward" and "upward" distribution patterns (Fig. 11).

Integration of kinesis and orientation

The gravity-induced active modulation of the gliding rate may be expressed by a *kinesis coefficient* (r_k) which is the ratio of Δ over propulsion rate (P):

$$r_k = \Delta/P \quad (6)$$

Table 4 lists the kinesis coefficients for *vertical gliding* under different *g*-values. Between 1 *g* and 5.4 *g* the kinesis coefficient more than doubled (from 0.29 to 0.67). Differentiating between downward (centrifugal) and upward (centripetal) gliding rates: the increase was 3-fold for locomotion antiparallel to gravity, and only 1.5-fold parallel to the gravity vector.

The *coefficient of orientation* with respect to gravity (r_o) is expressed as follows (Machemer et al. 1991):

$$r_o = R \cos \Phi \quad (7)$$

where R and Φ designate the polar coordinates of the mean of all summed individual orientational vectors with unity value (Batschelet 1981). With all cells strictly oriented up (0°), $r_o = +1$; if all cells are oriented down (180°), $r_o = -1$. Randomly or horizontally oriented cells have a coefficient $r_o = 0$. In gliding *Loxodes* r_o equals 0.047 at 1 *g*, corresponding to absence of orientation with respect to gravity. This lack of net orientation is due to

Table 4. Coefficients of kinesis, orientation, and taxis in gliding *Loxodes* as functions of gravity between 1 *g* and 5.4 *g*

g		*n*	Coefficient of		
			Kinesis r_k	Orientation r_o	Taxis r_t
1	total	4554	0.29	+0.047	−0.046
	down	2402	0.29	−0.884	
	up	2152	0.29	+0.904	
2.1	total	2003	0.42	+0.041	+0.006
	down	985	0.35	−0.697	
	up	1018	0.49	+0.732	
3.3	total	1804	0.56	+0.037	−0.014
	down	875	0.44	−0.651	
	up	929	0.69	+0.669	
4.3	total	1705	0.55	+0.007	−0.055
	down	855	0.41	−0.636	
	up	850	0.69	+0.629	
5.4	total	1655	0.67	+0.014	−0.015
	down	812	0.45	−0.621	
	up	843	0.88	+0.625	

The vertical *kinesis coefficient* ($r_k = \Delta/P$, Eq. 6) is large and continuously rises with gravity, in particular during upward locomotion. The *orientation coefficient* (r_o, Eq.7) of all cells is near zero corresponding to absence of net orientation of the population, although downward and upward moving cells were highly oriented. The *taxis coefficient* (r_t, Eq. 8) is close to zero. Neutral gravitaxis results from this combination of coefficients (r_k, r_o and r_t). Note that determinations of r_t refer to orientation *and* velocity data, the latter including rates of sedimentation and kinesis

a near-perfect cancelling of downward ($r_o = -0.884$) and upward ($r_o = +0.904$) orientation rather than random orientation over all directions (Table 4).

With raised gravity the orientation coefficient was even smaller than seen at 1 *g*, suggesting that net orientation is absent in *Loxodes*. Separate inspection of the centrifugally (down) and centripetally (up) gliding cells shows, however, that these orientations were increasingly less precise with rising *g* (decrease of r_o from near 0.9 to near 0.6; Table 4).

Integration of kinesis and orientation leads to determination of the *taxis coefficient* (r_t) (Machemer et al. 1991). The taxis coefficient combines the orientational response (cosine of mean orientation angle) with the velocity response of gliding *Loxodes* according to the equation:

$$r_t = -(R \cdot n \cdot \cos \Phi)/\Sigma v_i \quad (8)$$

where the vertical mean of all orientational responses ($R \cos \Phi$) is weighted by the mean gliding rate ($\Sigma v_i/n$). In the case of a perfect *negative gravitaxis* (all cells moving upward), the value of r_t equals −1; with perfect *positive gravitaxis* (all cells moving downward), $r_t = +1$. Note that the sign of r_t corresponds to the direction of taxis and is inverse to the sign of r_o. Table 4 shows that the taxis coefficient ($< |0.055|$) roughly corresponds to the orientation coefficient ($< |0.047|$). This finding suggests that no gravitaxis occurs in a population of gliding *Loxodes* even at hypergravity of up to 5 *g*.

Discussion

Gravikinesis and sedimentation

The experiments under artificially raised gravity confirm a conclusion from a previous analysis of gliding rates at 1 *g*: at normal gravity gliding *Loxodes* is able to cancel sedimentation generating a kinetic counter-response, a "negative gravikinesis" (Bräucker et al. 1992). However, active compensation of sedimentation by speed regulation increasingly fails with rising gravity. The determination of sedimentation of gliding cells is central to quantitative treatment of gravitaxis in *Loxodes*. We have measured settling rates of suspended immobilized *Loxodes* because gliding motion cannot be mimicked by immobilized cells. According to the hydrodynamics of swimming at low Reynolds numbers, sedimentation may be reduced near walls (Wu 1977; Roberts 1981). In future experiments under short-term microgravity during free fall in a drop tower, it may be possible to determine the sedimentation rate of gliding *Loxodes* from vertical shifts in horizontally moving cells during transition from 1 *g* to microgravity. It should be noted that values of the sedimentation rate measured in suspended immobilized cells may be too large and thus lead to overestimates of Δ.

Hypergravity-induced divergence of vertical kinetic responses

The orientation coefficient (Table 4) suggests that the gravikinetic velocity component of Δ can amount to near 30% of active propulsion at 1 *g*, and that this value applies to downward as well as to upward vertical gliding motion ($\Delta = \Delta_D = \Delta_U$; Eqs. 3, 4, 5). In this respect *Loxodes* differs from *Paramecium* whose downward gravikinetic velocity prevailed over the upward gravikinetic response (Machemer et al. 1991; Ooya et al. 1992). The omnidirectional balance of gliding in *Loxodes* was disturbed by raising the gravity stimulus: gliding-down gravikinesis (Δ_D) was presumably already saturated at 1 *g*, whereas gliding-upward gravikinesis (Δ_U) continuously rose, showing no signs of saturation even at 5.4 *g*. The basis of the diverging centrifugal and centripetal overall gliding velocities (Fig. 5B) and vertical rates (Fig. 6B) is the discrepancy between Δ_D and Δ_U. Sedimentation affects upward and downward motion equally.

It might be argued that only those cells which "take off" from the substrate surface during elevated gravity can sediment. Several arguments reject this explanation: (1) for evaluations, we identified gliders and eliminated swimmers (see Methods); (2) methods of non-parametric statistics suppress extreme and rare events; (3) cell detachment was less frequently observed with rising gravity (Fig. 6B), which would tend to decrease rather than enhance the discrepancy in upward and downward gliders (Fig. 9); (4) unilateral orientational offsets from the gravity vector are reproducible and independent of time (Fig. 10). The asymmetry in stimulus-response relationship is reminiscent of molluscan, insect and vertebrate mechanoreceptors (Furakawa et al. 1972; Hudspeth and Corey 1977; Rüsch and Thurm 1990; Thurm et al. 1983; Williamson 1990) and might be a further cue of an electrophysiological basis of gravitransduction (Machemer et al. 1991).

Absence of adaptation to hypergravity

Several steps of the experimental protocol, i.e. separation of *g*-rising and *g*-falling experiments, long-term exposure to hypergravity, and tests for undesired selection of cells by hypergravity, were analyzed for possible signs of sensory adaptation but gave negative results. The lack of adaptation to gravity would be a straightforward assumption for 1-*g* conditions but cannot be expected a priori for hypergravity. The data (Fig. 8) imply that the gravisensory system of *Loxodes* tolerates sustained hypergravity of up to 4.3 *g* for at least 50 min without modification of the stimulus-response relationship. We have noted a small decline of the motor response of *Loxodes* (near 10%) after >50-min exposures to 4.3 *g*, but were unable to identify the basis of this reduction in activity. It is possible that cell metabolism (starvation?) contributes to this observation. Depletion in O_2 supply can be excluded (see Methods).

A physiological basis of vertical orientation in Loxodes?

In the vertical chamber at 1 *g* a population of cells falls into two classes of equal size: one moving up and the other moving down (Fig. 10). Possible factors emanating from the vertically aligned agar blocks (Fig. 1A) did not generate this bipolar property because a 90° rotation of the chamber left the vertical orientation unchanged (Fig. 10C). In addition, pieces of agar placed near a population of *Loxodes* neither attracted nor repelled cells. Furthermore, light is unlikely to induce the observed vertical orientation because an even illumination was provided by a ring of green LEDs. The data shown in Fig. 10 suggest that gravity was the orienting stimulus.

In *Paramecium*, upward orientation is thought to be due, at least in part, to the buoyancy principle (Verworn 1889; Fukui and Asai 1985). A similar explanation is difficult to apply to gliding *Loxodes* because buoyancy permits only one stable orientation of cells. For lack of an exclusively physical principle of bipolar orientation of *Loxodes*, we infer that detection of the gravity axis is mediated physiologically, allowing bistable vertical orientations and avoidance of horizontal positions. Equal proportions of upwardly and downwardly oriented cells can result from repetitive reorientations of downward and upward gliders, but our evaluations do not permit judgment on individual reorientations. An alternative explanation assuming two permanent groups of "down cells" and "up cells" is not applicable because we never saw these presumed groups separating.

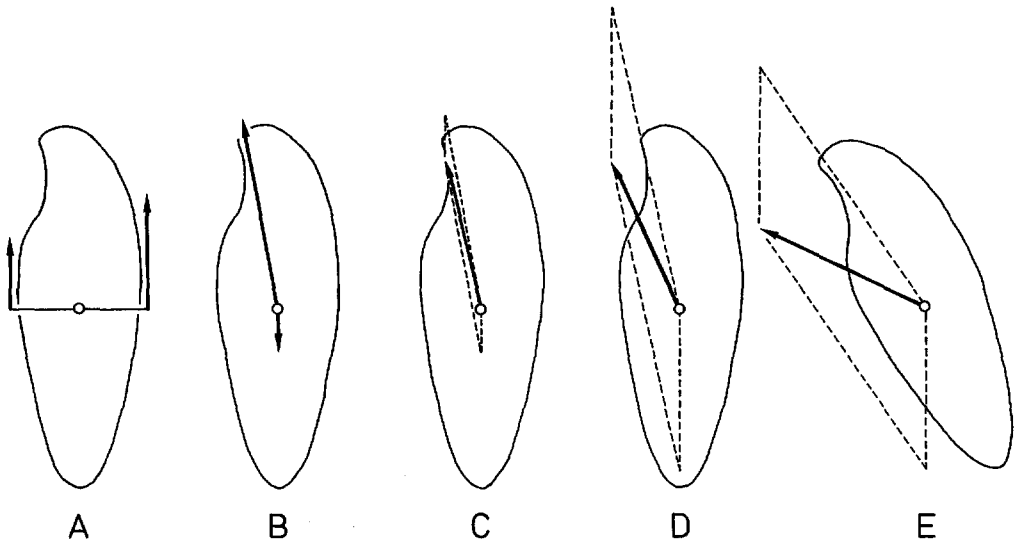

Fig. 12A–E. A model of hypergravity-induced counterclockwise angular offsets in graviorientation of *Loxodes:* **A** view down on gliding cell illustrating a minor imbalance of vectors of propulsion by cilia of the bilaterally asymmetric cell; **B** ciliary activity determines the lefthand curvature of the gliding path. Gravity at 1 *g* (down) causes sedimentation and generates the signal for continuous reorientation toward vertical posture; **C** resultant force of vertically gliding cell at 1 *g;* **D** resultant force of centripetally gliding cell at hypergravity, and gravikinetic increase in propulsion; **E** reorientation of the resultant propulsion vector tends to rotate the cell head-down. Hypergravity (with mechanical load on oral side of cell) generates compensating ciliary action so that further counterclockwise turn of the cell is cancelled. Note that stability of orientation is explained assuming feedback regulation of oral and aboral ciliary activity

Components of cellular reorientation by hypergravity

The striking *g*-dependent unidirectional turn of *Loxodes* (Fig. 10) is associated with the two-dimensional gliding mode, excluding lateral randomization of orientations due to helical swimming. Figure 1C suggests that forward gliding motion results from the balance of two different force vectors: a larger propulsion arising from the cilia of the right (= aboral) body side, and a smaller propulsion from the left (= oral) side of the cell (Fig. 12A); consequently, most gliding tracks curve toward the left (Fig. 3). Figure 12B schematizes the presumed locomotive partial forces in vertically oriented *Loxodes,* and Fig. 12C shows the resultant vector at 1 *g*. Raising the gravitational vector increases the counterclockwise turning of the cell (Fig. 12D). This mechanism tends to reorient the cell (Fig. 12D, E). Eventually, all cells would end up in head-down position with these assumptions. Because this is not observed, we postulate an orientation-sensitive gravitransduction-mediating feedback regulation of ciliary activity analogous a galvanotactic swimming of *Paramecium* (Machemer 1988). There is no explanation why, at various accelerations, inverse orientations of cells can occur at equal frequencies (Fig. 10).

Precision in kinetic and orientational responses

Loxodes can increase or reduce active propulsion in response to gravity to neutralize sedimentation. In addition to speed regulation, graviorientation of gliding *Loxodes* does not generate a common gravitaxis: the cumulative orientations parallel to gravity neutralize individual behaviour at one time so that a population of cells maintains its position in space. This conclusion agrees, in part, with observations by Fenchel and Finlay (1984) who found accumulations of *Loxodes* at low O_2 tension. Our data indicate that neutral gravitaxis may not be restricted to a particular O_2 tension. The present data support a tentative conclusion from a previous investigation in *Loxodes* (Bräucker et al. 1992) suggesting that active neutralization of sedimentation is a favourable basis for behavioural responses of *Loxodes* to vertical stimulus gradients such as of O_2 and light (Fenchel and Finlay 1984, 1986b; Finlay et al. 1986).

Which mechanism?

The orienting principle remains so far unknown. A tentative explanation would be that a gravity sensor receives and transmits an increasingly erroneous signal at hyper-*g* following overload of the local gravireceptor(s). Regarding the observed orientational error of the cell (Fig. 10), it is tempting to speculate that assessment of angular offsets plays a role in graviorientation (Lyon 1905). Graded depolarizing and hyperpolarizing shifts in membrane potential are linked to topographically specified mechanostimulation in *Stylonychia* and *Paramecium* (De Peyer and Machemer 1978; Ogura and Machemer 1980). By analogy, a gradient-type distribution of gravisensory channels corresponding to mechanosensitivity in other ciliates might be a basis of graviorientation in *Loxodes*. Alternatively, graded deformation of a sensory organelle (microtubules connecting "statolith" to ciliary base?) of the *Loxodes* "Müller vesicle" (Fenchel and Finlay 1986a;

Finlay and Fenchel 1986b) may mediate gravisensory transduction. The present analysis of gliding of *Loxodes* under hypergravity suggests action of a highly organized gravity sensor, the physiology of which still remains to be established.

Acknowledgments. This work was supported by the Deutsche Agentur für Raumfahrtangelegenheiten (DARA), grant 50 QV 8857 (to HM). We thank Prof. W. Haupt and Dr. P.F.M. Teunis for stimulating theoretical discussions and reading of drafts of the manuscript, and G. Krumbach, U. Schilken and P. Ullrich for careful assistance.

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