Eur Biophys J (1991) 20: 101–107

European Biophysics Journal

Responses of the photosynthetic flagellate, *Euglena gracilis*, to hypergravity

D.-P. Häder [1], E. Reinecke [1], K. Vogel [1], and K. Kreuzberg [2]

[1] Institut für Botanik und Pharmazeutische Biologie der Friedrich-Alexander-Universität, Staudtstrasse 5, W-8520 Erlangen, Federal Republic of Germany
[2] DARA, Deutsche Agentur für Raumfahrtangelegenheiten, Plitersdorfer Strasse 93, W-5300 Bonn 2, Federal Republic of Germany

Received October 18, 1990/Accepted in revised form May 7, 1991

Abstract. Motility and orientation has been studied in the unicellular photosynthetic flagellate, *Euglena gracilis*, using real time image analysis capable of tracking up to 200 cells simultaneously in the slow rotating centrifuge microscope (NIZEMI) which allows one to observe the cells' swimming behavior during centrifugation accelerations between 1 g and 5 g. At 1 g the cells show a weak negative gravitaxis, which increases significantly at higher accelerations up to about 3 g. Though most cells were capable of swimming even against an acceleration of 4.5 g, the degree of gravitaxis decreased and some of the cells were passively moved downward by the acceleration force; this is true for most cells at 5 g. The velocity of cells swimming against 1 g is about 10% lower than that of cells swimming in other directions. The velocity decreases even more drastically in cells swimming against higher acceleration forces than those at 1 g. The degree of gravitactic orientation drastically decreases after short exposure to artificial UV radiation which indicates that gravitaxis may be due to an active physiological perception rather than a physical effect such as an asymmetry of the center of gravity within the cell.

Key words: *Euglena gracilis* – Gravitaxis – Image analysis – Microgravity – NIZEMI (slow rotating centrifuge microscope) – Velocity of movement

Introduction

The photosynthetic unicellular flagellate, *Euglena gracilis*, can orient its motion in response to a number of external chemical and physical stimuli (Nultsch and Häder 1981). While some motile microorganisms have been found to orient in the water column with the aid of chemical (MacNab 1985) and thermal (Poff 1985) gradients, the magnetic field of the earth (Esquivel and de Barros 1986) and even electrical currents (Mast 1911), *Euglena* mainly orients with respect to light (Colombetti et al. 1982; Lenci et al. 1983) and gravity (Häder 1987a). In addition to both step-up and step-down photophobic responses (Shimmen 1981; Doughty and Diehn 1983) and a weak photokinetic effect (Wolken and Shin 1958) the most important light responses in this organism are positive and negative phototaxis (Mast 1911; Häder et al. 1981).

The gravitactic response of *Euglena* had already been observed more than a century ago (Aderhold 1888; Verworn 1889). In addition to a few other species (see Bean (1988 for review) mainly ciliates (Fenchel and Finlay 1986; Taneda 1987) and flagellates have been studied (Creutz and Diehn 1976; Kessler 1986). Like many other microorganisms, older cultures of *Euglena* show an exclusive negative gravitaxis (Creutz and Diehn 1976) which takes the organisms to the surface of the water column and which operates antagonistically to the negative phototaxis by which the cells move downward in the water column. The negative gravitaxis is further supported by a less well oriented positive phototaxis at low illuminations. The antagonism of the orientation responses causes the cells to accumulate in a band of suitable light conditions for growth and survival (Häder and Griebenow 1988). This behavior is of important ecological consequence not only for photosynthetic microorganisms, since cellular chromoproteins are easily photobleached by the too bright light intensity at the surface of the water column (Nultsch and Agel 1986; Ekelund and Häder 1988). In addition, the UV-B component in solar radiation has been found to damage both photoorientation and motility in *Euglena* (Häder 1986; Häder and Häder 1989) and other photosynthetic and non-photosynthetic microorganisms (Häder 1984; Häder et al. 1986).

In contrast to higher plants, neither the gravireceptor organelle nor the sensory transduction chain for stimulus transmission have been identified. It is still an open question whether graviorientation in flagellates is brought about by a passive physical process such as an asymmetric mass distribution or whether an active physiological gravireceptor is responsible for the orientation (Brinkmann 1968; Kuroda et al. 1986). In the past experimentation has been hampered by the difficulty of measuring graviorientation in the ranges both above and below 1 g.

Offprint requests to: D.-P. Häder

Roberts (1970) assumes a hydrodynamic interaction between the medium and the specific cell form to be the reason for gravitactic orientation in the ciliate *Paramecium*, while a model by Winet and Jahn (1974) tries to explain gravitaxis by a non-equilibrium ratio between sedimentation and rotation during propulsion. The hypothesis that buoyancy is the only source for negative gravitaxis (Fukui and Asai 1989) has been invalidated by Taneda who also excluded an effect of the hydrostatic pressure (Taneda 1987). The aim of this paper is to quantify and characterize the orientation and velocity distribution during hypergravity conditions in the freshwater alga, *Euglena gracilis*.

Materials and methods

Organisms and experiment preparation

The unicellular photosynthetic freshwater flagellate, *Euglena gracilis*, strain Z, was inoculated into 40 ml of a medium described in the literature (Checcucci et al. 1976) contained in 100 ml Erlenmeyer flasks and grown for about 6 weeks under continuous light of about 600 lx ($=2.5\ W\,m^{-2}$ from mixed cool white and warm tone fluorescent lamps) at about 23 °C. All experiments were carried out with the cells in their original growth medium. Cell suspensions were removed from the cultures and transferred into a circular chamber (12 mm in diameter and 0.2 mm in thickness, made from two glass plates pressed together by a metal frame, which was mounted onto the object table of the microscope within the NIZEMI, see below).

Construction of the NIZEMI

The slow rotating centrifuge microscope (NIZEMI) was developed and constructed by Dornier (Friedrichshafen, FRG) on behalf of the Deutsche Versuchsanstalt für Luft- und Raumfahrt (DLR, Köln, FRG) and the Federal Minister for Research and Technology (BMFT). The NIZEMI consists of a Zeiss (Oberkochen, FRG) Axioplan light microscope accomodated horizontally on a circular rotating steel table driven by an electric motor (Fig. 1). The rotating table is balanced during operation and the excess heat produced from the microscope lamp is guided by a central pipe to an opening in the top. The specimen on the microscope table can be moved by remote control via a joystick which drives the object stage by servo motors. In addition focussing is performed using another remotely controlled servo motor. The acceleration depends on the rotation rate of the instrument and is read from a nomogram listing the dependence. Since the gravitational force and the acceleration are perpendicular to each other, the resultant vector of the two is calculated for each acceleration (these values are listed for the histograms). The optical axis of the monitoring beam is perpendicular to the resultant acceleration vector.

The image of the organisms was recorded on-line by a CCD camera (Aqua TV HR 600, Aqua TV, Kempten, FRG) and sent to the control unit with the video screen by a set of brushes which also carried the servo motor voltages. In addition, an independent macro unit allowed the analysis of larger objects under increased acceleration forces; however, this system was not used for this purpose. The organisms were observed using the microscope lamp as light source in combination with a red cut-off filter (RG 665, Schott & Gen., Mainz, FRG). The instrument provides a vectorial addition of the gravity and acceleration forces.

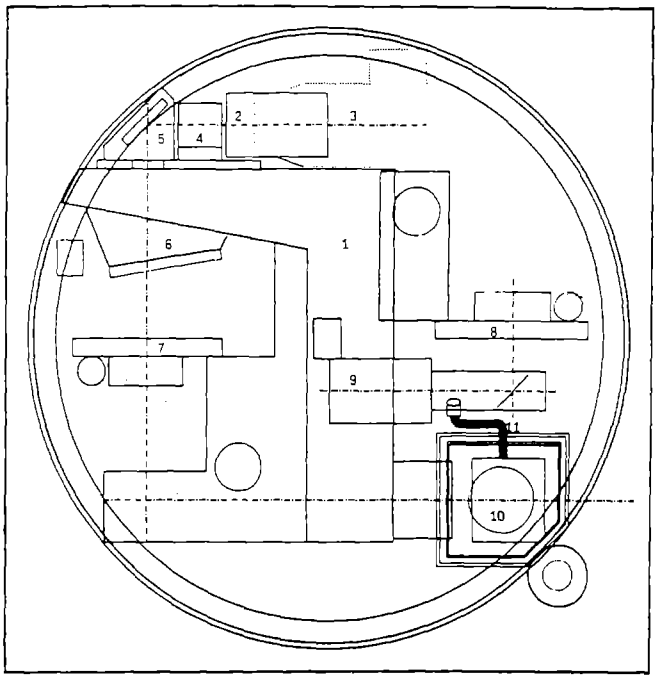


Fig. 1. Principal schematic diagram of the NIZEMI showing the main components of the micro and macro observation unit. 1 = microscope, 2 = micro CCD camera, 3 = photo camera (optional), 4 = beam splitter, 5 = mirror, 6 = objective holder, 7 = micro stage, 8 = macro stage, 9 = macro CCD camera, 10 = lamp, 11 = light tube

Automatic analysis of organism tracks

The video sequence was either recorded on a VHS video recorder (Mitsubishi HS 3600 E) or analyzed on-line during the experiment. The video signal was digitized at a spatial resolution of 512×512 pixels with 256 possible gray levels each (Matrox digitizer card PIP-1024, Quebec, Canada). The card plugged into an IBM AT compatible microcomputer (Tatung 7000, Taipei, Taiwan) and allowed real time digitization by A/D conversion of a full video frame every 40 ms. The digitized image was manipulated by on board look-up tables (LUTs, (Häder 1988)) and stored in a dedicated video memory. The computer had access to the image in memory and could both read and write pixels at random and display the image on an analog monitor in pseudocolor graphics using a second set of LUTs.

The analysis program has been developed in the computer language C which handles the input and output routines as well as the mathematical analysis of the data (Häder and Vogel 1990), while all time consuming proce-

dures such as manipulation of image pixels, outline detection and calculation of centroid and area were written in 80286 ASSEMBLY Language.

Four snapshots are taken from the video sequence at 80 ms intervals and the first image is scanned sequentially line by line until an object is found which differs from the background by a predefined threshold. The outline of the organism is analyzed using the chain code algorithm (Freeman 1961) which has the advantage of being a robust technique and that each cell position can be described uniquely by its edge vectors. In addition, it is very fast and allows one to reduce drastically the amount of information to process. Several algorithms have been developed for calculating the circumferences, areas and centroids of the detected objects (Freeman 1980). After one organism has been analyzed, the search is continued until the whole frame has been covered. The centroids and areas of all organisms are stored in an array.

Then the positions of all organisms are determined in the next frame (80 ms later) in a similar fashion as described above using the stored centroids as starting points. Organisms which are not found within close vicinity (three organism diameters) of their position in the last frame are considered lost as are all the organisms colliding with the image boundaries. The positions and areas of all successfully found organisms are stored in a second array and the process is repeated for the third and fourth digitized image, overwriting the second array. The direction of movement is no longer defined when two organisms meet; therefore an unexpected increase in the area of an organism is taken as a signal to discard this organism. Upper and lower limits for the area allow the system to distinguish cells from debris or noise in the image. The system analyzes up to 500 organisms per minute under optimized conditions. The movement vectors can be determined for all the organisms from the data stored in the two arrays and the values are stored in terms of the deviation angles from the stimulation direction, the combined vector of gravity and acceleration (defined as 0°). The speed of movement of all the cells can be calculated, since the individual distances moved are known as well as the time elapsed between the first and final frame, determined from the built-in hardware clock of the computer.

Circular histograms of the direction are calculated off-line using a resolution of 64 sectors of 5.6° each by means of programs written in Turbo Pascal (Borland) (Häder 1985). In each experiment the data are presented in such a way that the resultant vector of gravity and acceleration point to 180°. Rayleigh tests are performed to determine the directedness of the moving organisms (Mardia 1972; Batschelet 1981). The Rayleigh test provides an r-value between 0 (random distribution) and 1 (perfect orientation of all organisms in one direction):

$$r = \frac{\sqrt{(\sum \sin \alpha)^2 + (\sum \cos \alpha)^2}}{n}$$

where α is the individual angle of movement of each track segment. The direction of movement is determined using a Fast Fourier Analysis described previously (Häder and Lipson 1986).

The mean velocity of all organisms moving in each sector is plotted in the corresponding sector in circular diagrams. The average velocity of all organisms moving in the direction of $0° \pm 30°$ is calculated and compared to the average velocity of all organisms moving in the opposite direction. In order to confirm statistically significant differences a Chi-square test was performed.

Inhibition of graviorientation by ultraviolet radiation

Ultraviolet radiation was produced by a transilluminator (Bachhofer, Reutlingen, FRG). The wavelength range is limited to 280–380 nm with a fluence rate of 16.4 mW m^{-2} at 312 nm as controlled by UV transmitting neutral density filters. The fluence rate was measured with an Optronics (model 742) spectroradiometer under computer control.

Results

In a preliminary experiment the gravitactic orientation of *Euglena gracilis* was studied (Fig. 2a). The cells show a moderate gravitactic orientation with an r-value (Rayleigh test (Batschelet 1981)) of about 0.52. Cells swimming against the gravity vector have a lower velocity than cells swimming in the opposite direction (Fig. 2b). The significance of the difference was confirmed using the Chi-square test on a 95% confidence level.

Under the acceleration force in the NIZEMI the cells were capable of orienting even at higher g values. The precision of graviorientation even increased up to 2 g (Fig. 3) after which it declined again. At 4.5 g most cells were still capable of moving against the acceleration vector though the degree of orientation was rather low (r-value of 0.17) (Fig. 4), while at 5 g most cells moved in the direction of the resultant vector of acceleration and gravity (Fig. 5).

Even under 1 g conditions, cells swimming against the gravity vector move slower than those moving in any other direction. The mean velocities of cells swimming against the acceleration vector (0°) and deviating no move than ±30° from this direction are compared with those of cells swimming with the acceleration vector (180°) deviating no move than ±30° are listed in Table 1. This effect is even more pronounced at higher accelerations up to 4.5 g (Fig. 3b, 4b). Cells which still actively swim against the acceleration vector at 5 g are even slower (Fig. 5b).

Table 1. Mean velocities [μm s^{-1}] of cells swimming against the acceleration vector (0°) deviating no more than ±30° and with the acceleration vector (180°) deviating no move than ±30° at various accelerations [g]

Acceleration	0°±30°	180°±30°
0	50	49
1	42	56
2	40	60
4.5	32	63
5	28	64

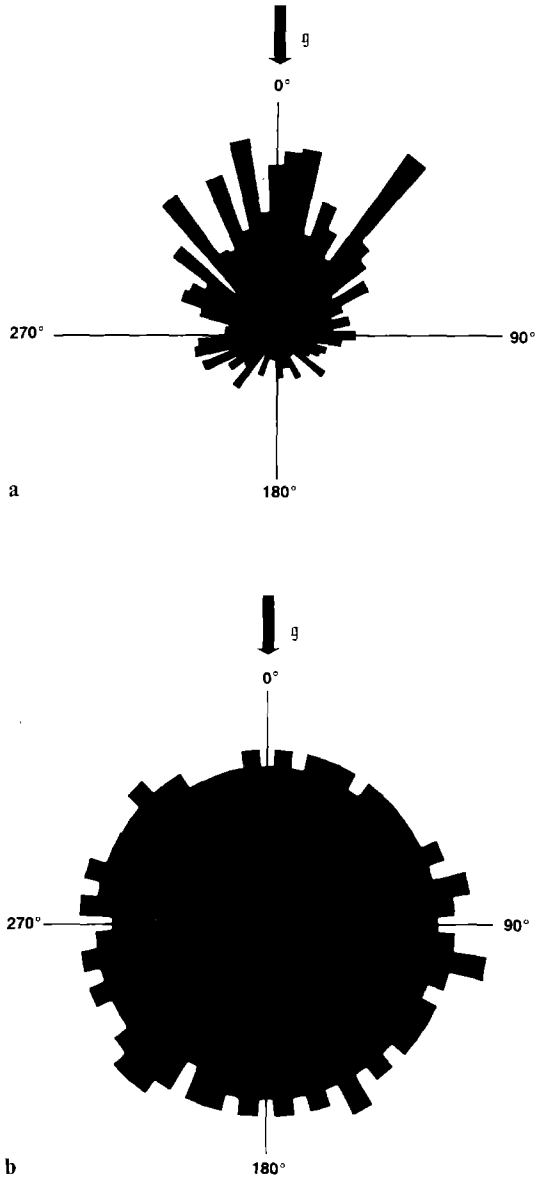


Fig. 2. Circular histogram of the movement vectors (**a**) and velocity diagram (**b**) of a population of *Euglena gracilis* under 1-g conditions. 1 000 tracks have been analyzed

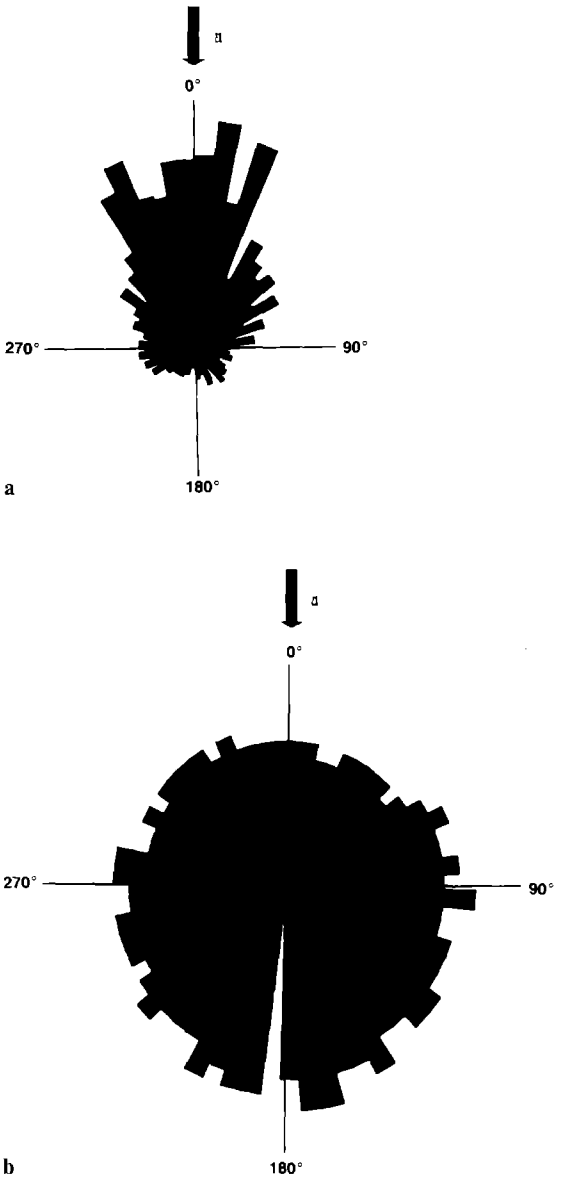


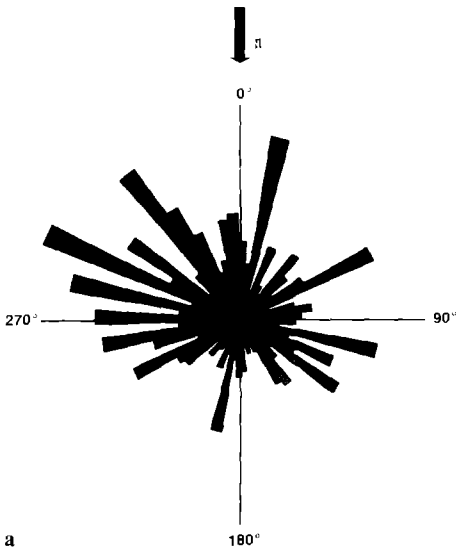
Fig. 3. Circular histogram of the movement vectors (**a**) and velocity diagram (**b**) of a population of *Euglena gracilis* at an acceleration of 2 g in the NIZEMI. 1 000 tracks have been analyzed. Since gravitactic orientation is fairly high and fewer cells swim in the lower half of the diagram, there are no organisms in the sector next (clockwise) to the 180° sector

Figure 6 plots the mean velocities of the cells moving against the acceleration vector ($0° \pm 30°$) in dependence of the acceleration. For comparison, an additional value has been inserted for microgravity conditions (ca. 10^{-5} g) which has been measured during a previous TEXUS flight providing weightlessness for up to 6 min (Häder et al. 1990).

When cells were exposed to ultraviolet radiation for predefined periods of time, the degree of orientation decreased significantly even after short exposure times and the cells moved almost randomly after an exposure of about 90 min (Fig. 7). The data points represent the average of three experiments at each experimental situation.

Discussion

The pronounced decrease in the gravitactic orientation observed after exposure to artificial ultraviolet radiation within even short exposure times indicates that gravity may be controlled by an active process depending on a distinct gravireceptor, though the organelle responsible for this function has not been identified in *Euglena gracilis*. A passive orientation of the cell, e.g., due to an asymmetric center of gravity in the cell (Brinkmann 1968), seems unlikely since the mass distribution within the cell is assumed not to be changed so drastically by a short UV exposure. Microscopic observation revealed no visible



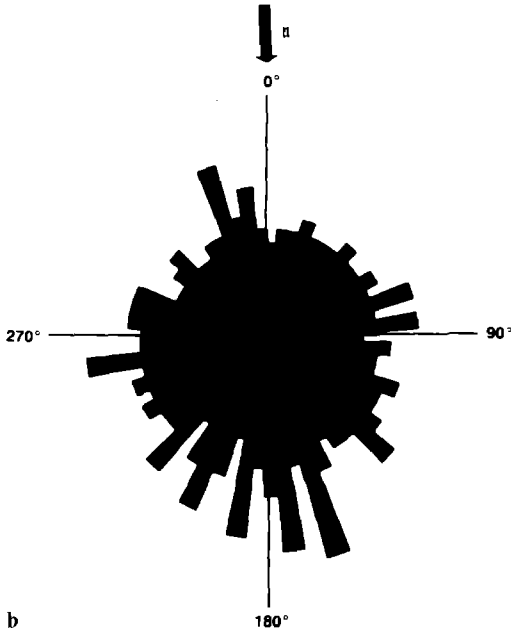
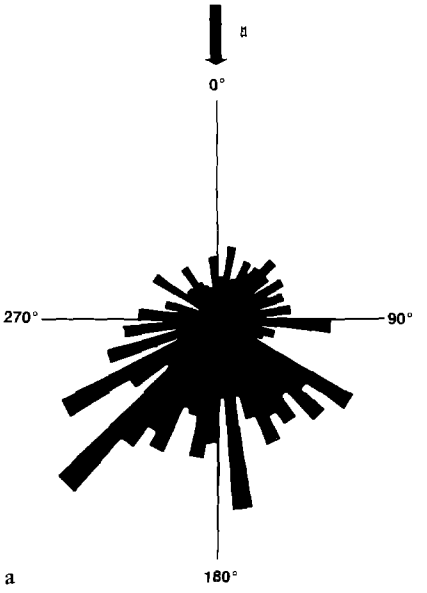


Fig. 4. Circular histogram of the movement vectors (**a**) and velocity diagram (**b**) of a population of *Euglena gracilis* at an acceleration of 4.5 g in the NIZEMI. 1000 tracks have been analyzed



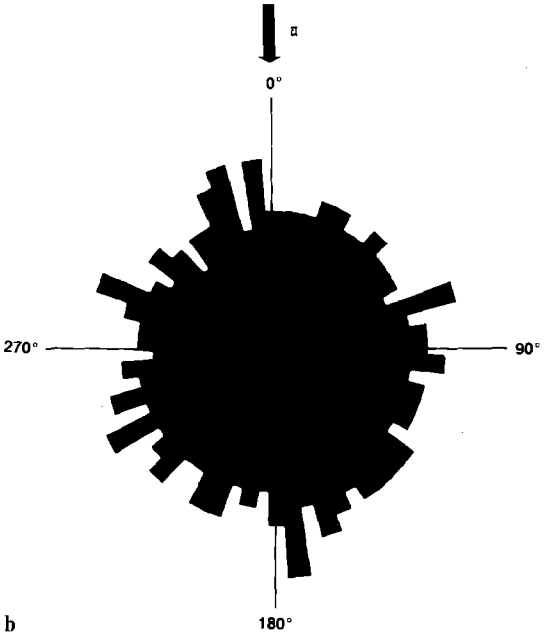


Fig. 5. Circular histogram of the movement vectors (**a**) and velocity diagram (**b**) of a population of *Euglena gracilis* at an acceleration of 5 g in the NIZEMI. 1000 tracks have been analyzed

changes in the cell proportions. In addition, the experimental situation excludes thermal or gaseous gradients in the cuvette, which theoretically could also induce a vectorial movement. However, additional experiments have indicated that *Euglena* does not respond to thermal gradients up to 2 °C per cm.

The fact that the degree of orientation increases with the acceleration force could be explained by both an active physiological and a passive physical mechanism. This question is also discussed in ciliates, where the degree of orientation depends on the oxygen concentration in the medium (Fenchel and Finlay 1986).

It is interesting to note that the cells are capable of swimming against acceleration forces up to almost 5 g and orienting in the acceleration fields even though they have never been subjected to such conditions during evolution. The observed dependence of the velocity of the cells on acceleration can be employed to estimate the force required for locomotion. During forward propulsion the cells encounter a frictional component which is equal at all acceleration forces. In contrast, the cells have to supply additional energy to move against the acceleration vector which depends on the acceleration force in each experiment. The specific density of the cells has been

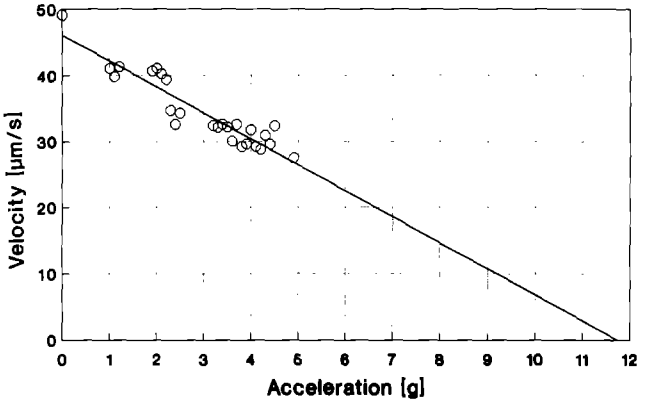


Fig. 6. Mean velocities (ordinate in $\mu m\ s^{-1}$) of *Euglena gracilis* moving against the acceleration vector ($0° \pm 30°$) in dependence of the acceleration

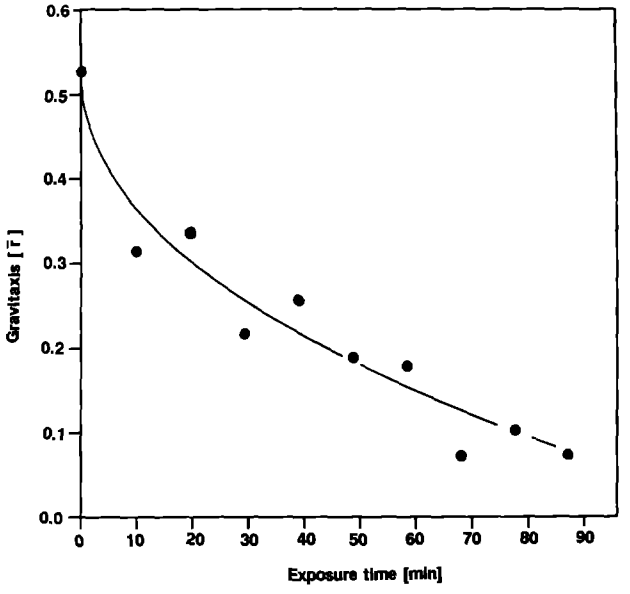


Fig. 7. Effects of ultraviolet radiation from a transilluminator (270–380 nm) on the degree of gravitactic orientation (*r*-value, [38, 39]) at 1 g in *Euglena gracilis* in dependence of the exposure time

determined to be 1.04 g/cm^3 and the force necessary to overcome the acceleration can be calculated from the excess weight above buoyancy. This can be determined by multiplying the density difference (0.04 g/cm^3) times the cell volume, measured to be about 2 450 µm^3 times the applied acceleration: at 1 g the force required to swim against the gravity vector amounts to about 9.7×10^{-13} N. The linear regression line through the data points can be extrapolated to a velocity of zero, which indicates the point where the sedimentation force compensates the force for upward swimming. The sedimentation force F for spherical cells can be calculated from

$$F = \frac{4}{3} \pi a^3 \Delta\varrho\, g$$

where a is the diameter of the cell and μ the viscosity. The sedimentation velocity is calculated from

$$v_{vel} = \frac{2}{9} \frac{a^2 \Delta\varrho\, g}{\mu}.$$

Acknowledgement. This work was supported by financial support from the Bundesminister für Forschung und Technologie. The authors gratefully acknowledge the skilfull technical assistance of B. Brodhun, O. Joop, H. Koch, W. Lork, R. Treichl, J. Schäfer, U. Schleier, S. Seeler and H. Vieten. The authors thank Prof. Dr. J. Kessler for his help in the physical calculations.

References

Aderhold R (1888) Beiträge zur Kenntnis richtender Kräfte bei der Bewegung niederer Organismen. Jenaische Z Med Naturwiss 22:311–342

Batschelet E (1981) Circular statistics in biology. Academic Press, London

Bean B (1984) Microbial geotaxis. In: Colombetti G, Lenci F (eds) Membranes and sensory transduction. Plenum Press, New York London, pp 163–198

Brinkmann K (1968) Keine Geotaxis bei *Euglena*. Z Pflanzenphysiol 59:12–16

Checcucci A, Colombetti G, Ferrara R, Lenci F (1976) Action spectra for photoaccumulation of green and colorless *Euglena*: evidence for identification of receptor pigments. Photochem Photobiol 23:51–54

Colombetti G, Häder D-P, Lenci F, Quaglia M (1982) Phototaxis in *Euglena gracilis*: effect of sodium azide and triphenylmethyl phosphonium ion on the photosensory transduction chain. Curr Microbiol 7:281–284

Creutz C, Diehn B (1976) Motor responses to polarized light and gravity sensing in *Euglena gracilis*. J Protozool 23:552–556

Doughty MJ, Diehn B (1983) Photosensory transduction in the flagellated alga, *Euglena gracilis*. IV. Long term effects of ions and pH on the expression of step-down photobehavior. Arch Microbiol 134:204–207

Ekelund N, Häder D-P (1988) Photomovement and photobleaching in two *Gyrodinium* species. Plant Cell Physiol 29:1109–1114

Esquivel DMS, de Barros HGPL (1986) Motion of magnetotactic microorganisms. J Exp Biol 121:153–163

Fenchel T, Finlay BJ (1986) Photobehavior of the ciliated protozoon *Loxodes*, taxic, transient, and kinetic responses in the presence and absence of oxygen. J Protozool 33:139–145

Freeman H (1961) On the encoding of arbitrary geometric configurations. IRE Trans EC-10:260–268

Freeman H (1980) Analysis and manipulation of lineal map data. Map data processing. Academic Press, New York, pp 151–168

Fukui K, Asai H (1985) Negative geotactic behavior of *Paramecium caudatum* is completely described by the mechanism of buoyancy-oriented upward swimming. Biophys J 47:479–482

Häder D-P (1984) Effects of UV-B on motility and photoorientation in the cyanobacterium, *Phormidium uncinatum*. Arch Microbiol 140:34–39

Häder D-P (1985) Photomovement in *Cyanophora paradoxa*. Arch Microbiol 143:100–104

Häder D-P (1986) Effects of solar and artificial UV irradiation on motility and phototaxis in the flagellate, *Euglena gracilis*. Photochem Photobiol 44:651–656

Häder D-P (1987a) Polarotaxis, gravitaxis and vertical phototaxis in the green flagellate, *Euglena gracilis*. Arch Microbiol 147: 179–183

Häder D-P (1987b) Effects of UV-B irradiation on photomovement in the desmid, *Cosmarium cucumis*. Photochem Photobiol 46:121–126

Häder D-P (1988) Computer-assisted image analysis in biological sciences. Proc Ind Acad Sci (Plant Sci) 98:227–249

Häder D-P, Griebenow K (1988) Orientation of the green flagellate, *Euglena gracilis*, in a vertical column of water. FEMS Microbiol Ecol 53:159–167

Häder D-P, Häder MA (1988) Inhibition of motility and phototaxis in the green flagellate, *Euglena gracilis*, by UV-B radiation. Arch Microbiol 150:20–25

Häder D-P, Häder MA (1989) Effects of solar UV-B irradiation on photomovement and motility in photosynthetic and colorless flagellates. Environ Exp Bot 29:273–282

Häder D-P, Lipson E (1986) Fourier analysis of angular distributions for motile microorganisms. Photochem Photobiol 44:657–663

Häder D-P, Vogel K (1990) Simultaneous tracking of flagellates in real time by image analysis. J Math Biol (accepted for publication)

Häder D-P, Colombetti G, Lenci F, Quaglia M (1981) Phototaxis in the flagellates, *Euglena gracilis* and *Ochromonas danica*. Arch Microbiol 130:78–82

Häder D-P, Watanabe M, Furuya M (1986) Inhibition of motility in the cyanobacterium, *Phormidium uncinatum*, by solar and monochromatic UV irradiation. Plant Cell Physiol 27:887–894

Häder D-P, Rhiel E, Wehrmeyer W (1987) Phototaxis in the marine flagellate *Cryptomonas maculata*. J Photochem Photobiol 1:115–122

Häder D-P, Vogel K, Schäfer J (1990) Responses of the photosynthetic flagellate, *Euglena gracilis*, to microgravity. Micrograv Sci Technol 3:110–116

Kessler JO (1986) The external dynamics of swimming microorganisms. In: Round FE, Chapman DJ (eds) Progress in physiological research. Biopress, Oxford, pp 258–307

Kuroda K, Kamiya NMJA, Yoshimoto Y, Hiramoto Y (1986) *Paramecium* behavior during video centrifuge-microscopy. Proc Jpn Acad Ser B 62:117–121

Lenci F, Colombetti G, Häder D-P (1983) Role of flavin quenchers and inhibitors in the sensory transduction of the negative phototaxis in the flagellate, *Euglena gracilis*. Current Microbiol 9:285–290

MacNab RM (1985) Biochemistry of sensory transduction in bacteria. In: Colombetti G, Lenci F, Song P-S (eds) Sensory perception and transduction in aneural organisms. Plenum Press, New York London, pp 31–46

Mardia KV (1972) Statistics of directional data. Academic Press, London

Mast SO (1911) Light and behavior of organisms. Wiley, New York/Chapman & Hall, London

Nultsch W, Agel G (1986) Fluence rate and wavelength dependence of photobleaching in the cyanobacterium *Anabaena variabilis*. Arch Microbiol 144:268–271

Nultsch W, Häder D-P (1988) Photomovement in motile microorganisms II. Photochem Photobiol 47:837–869

Poff KL (1985) Temperature sensing in microorganisms. In: Colombetti G, Lenci F, Song P-S (eds) Sensory perception and transduction in aneural organisms. Plenum Press, New York London, pp 299–307

Rhiel E, Häder D-P, Wehrmeyer W (1988) Diaphototaxis and gravitaxis in a freshwater *Cryptomonas*. Plant Cell Physiol 29:755–760

Roberts AM (1970) Geotaxis in motile micro-organisms. J Exp Biol 53:687–699

Shimmen T (1981) Quantitative studies on step-down photophobic response of *Euglena* in an individual cell. Protoplasma 106:37–48

Taneda K (1987) Geotactic behavior in *Paramecium caudatum*. I. Geotaxis assay of individual specimen. Zool Sci 4:781–788

Taneda K, Miyata S, Shiota A (1987) Geotactic behavior in *Paramecium caudatum*. II. Geotaxis assay in a population of the specimens. Zool Sci 4:789–795

Verworn M (1889) Die polare Erregung der Protisten durch den galvanischen Strom. Pflügers Arch Physiol 45:1–36

Winet H, Jahn TL (1974) Geotaxis in protozoa: I. A propulsion-gravity model for *Tetrahymena* (*Ciliata*) J Theor Biol 46:449–455

Wolken JJ, Shin E (1958) Photomotion in *Euglena gracilis*. Photokinesis. II. Phototaxis J Protozool 5:39–46