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High Light Exposure Leads to a Sign Change of Gravitaxis in the Flagellate *Euglena gracilis**

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Summary. The unicellular flagellate *Euglena gracilis* orients itself in the water column by means of pronounced phototaxis and gravitaxis. The antagonism of phototaxis and gravitaxis brings the cells in a position in the water column providing them with optimal light conditions for their photosynthetic apparatus (about 30 Wm^{-2}). Long exposure to solar or artificial radiation induces a loss of the negative gravitactic orientation in *Euglena gracilis* or very often a pronounced, persistent (> 4 h) sign change in gravitaxis. The effects on gravitaxis are exclusively due to UV and intensive blue light as experiments with different light qualities revealed. This phenomenon is not caused by the phototaxis photoreceptor or chloroplast processes, because also the colorless and blind (no photoreceptor) *Euglena gracilis* 1f mutant and *Astasia longa* reverse the sign of gravitaxis upon strong radiation. The sign change is oxygen-dependent, because gravitaxis is not affected in oxygen free medium (in wild type *Euglena gracilis*, as well as in the 1f mutant and *Astasia longa*). This indicates the involvement of oxygen radicals as a trigger of gravitaxis sign reversal. As the destruction of the photoreceptor molecules by light leads to a loss of phototaxis, the switch from negative to positive gravitaxis might be an adaptation mechanism of the cells to escape from deleterious radiation even after loss of the ability to perceive the light direction.

Key words: *Astasia longa*, *Euglena gracilis*, gravitaxis, oxygen, phototaxis, sign change, solar radiation, UV.

INTRODUCTION

The unicellular flagellate *Euglena gracilis* uses external stimuli to control its position in the water column. Negative gravitaxis is a very important mechanism, which enables the cells to orient themselves towards the water surface. In addition, the cells show positive phototaxis at low irradiances (1-10 Wm^{-2}) and increasingly pronounced negative phototaxis at higher irradiances. The interaction of phototaxis and gravitaxis allows the cells to find an optimal position in the water column for growth and reproduction (about 30 Wm^{-2}) (Häder and Griebenow 1988). Phototaxis as well as gravitaxis are most likely active physiological mechanisms. The photoreceptor of *Euglena gracilis* is the paraxonemal body, which is attached to the trailing flagellum. The photoreceptor was found to have a paracrystalline structure and to consist of chromoproteins containing pterins and flavins as chromophoric groups (Sineshchekov *et al.* 1994, Brodhun and Häder

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* Dedicated to Dr. Francesco Lenci on the occasion of his 60th birthday

1995). While flavins are identified as the primary photoreceptors of phototaxis, the pterins seem to act as bulk antenna pigments funneling the adsorbed energy to the flavins. The knowledge on the signal transduction chain from the photoreceptor to the flagellum is still limited. Recently, it was found that the flavoproteins have an adenylate cyclase activity (Iseki *et al.* 2002). The current status of research in phototaxis in *Euglena gracilis* is thoroughly described in a recent review article (Lebert 2001).

The mechanism of gravitaxis in *Euglena gracilis* is not fully elucidated. Formerly, gravitaxis was thought to be a physical phenomenon based on a buoyancy effect due to an unequal mass distribution within the cell body (Brinkmann 1968). But the results of many experiments and movement analyses make a physiological mechanism of gravitaxis very likely (Machemer and Bräucker 1996; Lebert and Häder 1996, 1999a; Häder 1997; Häder *et al.* 1997; Lebert *et al*. 1997, 1999; Porst 1998; Tahedl *et al.* 1998; Kamphius 1999; Richter *et al*. 2001a, b).

According to a recent working model of gravitaxis in *Euglena gracilis* the sedimenting cell body (denser than the surrounding medium) exerts a force on the lower membrane and most likely calcium channels. The resulting change of calcium conductance leads to a change of the membrane potential which in turn triggers reorientational movements of the trailing flagellum. Phototaxis as well as gravitaxis were found to be sensitive to UV radiation (Häder and Liu 1990a, Brodhun and Häder 1995). Gravitaxis was shown to be abolished after about 2 h of exposure to solar radiation (Häder and Liu 1990a). Reanalysis of the experimental data indicate that some of the cells in the irradiated culture show an active positive gravitaxis. Subsequent experiments showed that *Euglena gracilis* often shows a sign change in gravitaxis after exposure to solar radiation. The aim of the current paper is to elucidate these effects in detail.

MATERIALS AND METHODS

Organisms and growth conditions

The flagellate *Euglena gracilis* Z was obtained from the algal culture collection of the University of Göttingen (Schlösser 1994). The cells were grown in a mineral medium as described earlier (Starr 1964, Checcucci *et al.* 1976) in stationary cultures in 100 ml Erlenmeyer flasks at about 20°C under continuous light of about 18 Wm^{-2} from mixed cool white and warm tone fluorescent lamps. The colorless mutant *Euglena gracilis* 1f and *Astasia longa* (obtained from the same source as *Euglena gracilis* Z) were grown in complex medium (Starr 1964) in 100 ml Erlenmeyer flasks at about 20°C in the dark.

Exposure of cells to natural solar radiation

Samples used for one experiment were prepared from the same culture. The cells were transferred into custom-made cuvettes, designed for the Erlanger flagellate test (EFT, Häder *et al.* 1997). Each cuvette consists of a holder with four independent compartments. Each compartment is covered with a different filter: WG 280 (transmits all solar radiation), WG 320 (transmits PAR and UV-A), GG 400 (PAR only) and UG 11 (UV-A and UV-B only). All filters are from Schott and Gen., Mainz, Germany. Water was pumped through the stainless steel body of the cuvette holder in order to warrant a stable temperature of 20°C (thermostatically controlled water bath). Three of these cuvettes were used in parallel for one experiment. Samples were taken at defined time intervals from the cuvettes after gentle mixing with a suction pipette. The samples were transferred into slide cuvettes sealed with silicon (see below), and gravitaxis was measured in a dark room (see below). In addition, the recovery of the cells from illumination was determined in darkness. The irradiances during solar exposure were determined with an ELDONET instrument in the UV-B, UV-A and PAR ranges located on the roof of the Department of Biology at the University of Erlangen (Häder *et al.* 1999).

Exposure to artificial radiation

The light source was a Hönle lamp (Dr. Hönle, Martinsried, Germany), which produces a spectrum similar to the solar spectrum (Klisch *et al.* 2001). The irradiances were: PAR 321 Wm^{-2}, UV-A 67 Wm^{-2} and UV-B 1.9 Wm^{-2} at a distance of 65 cm. About 30 ml of cell suspension were transferred into small black plastic boxes, which were placed in a temperature-controlled water bath (20°C). To determine the effects of different light qualities, different samples were covered with different cut-off filters. The following cut-off filters were used for the experiments: filter foil 295 nm (transmits UV-B, UV-A and PAR, Digefra, Munich, Germany), 320 nm (Montagefolie, Nr. 10155099, Folex, Dreieich, Germany), 395 nm foil (PAR only, Digefra) and the glass cut-off filters (Schott and Gen., Mainz, Germany) UG 11 (UV-B and UV-A only), GG 280 (UV and PAR), GG 400 (PAR only), GG 440, OG 540 and RG 645. Dark controls were covered with aluminum foil. Samples were drawn at predefined time intervals after gentle mixing of the cell suspension. The cells were filled into a cuvette and subsequently analyzed with the image analysis software WinTrack 2000 (see below).

Oxygen was removed by addition of NaS_2O_3 (final concentration 3 µM). Experiments concerning oxygen were performed with the following samples: (1) cells + NaS_2O_3 + light, (2) cells + light (no NaS_2O_3), (3) cells + NaS_2O_3 (no light, covered with aluminum); (4) dark control (no NaS_2O_3 and no light).

Motion analysis

Samples of cells were transferred into a custom-made cuvette (0.1 mm depth and 20 mm diameter) made from stainless steel with glass windows (Daimler-Benz Aerospace, Bremen, Germany). In the case of the solar exposure experiments (see above) some drops of

Euglena cell suspension were sealed between two slides by means of silicon (Bayer Silone, high viscous, Bayer, Leverkusen, Germany). Motion analysis was performed with a recently developed cell tracking system (WinTrack 2000, Lebert and Häder 1999b). The system is based on a video A/D flash converter (Meteor, Matrox, Canada) connected to a PCI slot of an IBM compatible computer which digitizes the analog video images from a CCD camera mounted on a horizontally oriented microscope.

The digitized images are transferred to the computer memory. Objects are detected by brightness differences between cells and background. The movement vectors of all motile cells on the screen are determined by subsequent analysis of five consecutive video frames (movement vectors of the objects from frame 1 to frame 5). In addition to orientation and velocity of the cells, motility, area and cell form are determined as well as several statistical parameters. The *r*-value indicates the precision of (gravitactic) orientation and ranges from 0 (random orientation) to 1 (precise orientation):

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

where α is the deviation from the stimulus direction (here acceleration) and n the number of recorded cell tracks. The angle theta indicates the mean movement direction of a cell culture. The increment of the angle is clockwise (see one of the circular histograms in the figures).

Image analysis was performed in darkness to avoid any phototactic or photophobic effect on the orientation of the cells. To exclude the evaluation of immotile cells, which sediment in the vertical cuvette, the software accepted only cells with a speed faster than the sedimentation velocity (about 20 μms^{-1}). In all experiments the movement of the cells was visually monitored by the experimenters on screen in order to avoid any mistakes of data acquisition of the obtained cell tracks by the software.

RESULTS

Effects of artificial radiation on gravitaxis

Green *Euglena gracilis* Z cells generally showed a pronounced sign change (negative to positive) of gravitaxis within 70-195 min of artificial solar radiation (Figs 1, 2). Positive gravitaxis persists for more than 150 min under dim light conditions but after 12 h the cells showed negative gravitaxis again (data not shown). In the colorless *Astasia longa* and *Euglena gracilis* 1f mutant the kinetics of gravitactic sign change was even faster. Most of the samples showed a pronounced positive gravitaxis after 30-45 min of radiation. But not all cells showed a fast reversal of gravitaxis. In some experiments (*Euglena gracilis* Z, mutant 1f, *Astasia longa*) a pronounced sign change in gravitaxis was obtained only after about 240 min of radiation or even longer (data not shown).

Determination of the effectiveness of light quality on gravitaxis

The light regime was the same as in the previous experiment. The samples were covered with different filters as described above. The calculated dose for each filter is shown in Table 1. After about 90 min most of the cells exposed to UV only (UG 11 filter) showed a pronounced positive gravitaxis. After 195 min also the 280 nm samples reversed their gravitactic orientation, while in the 400 and 440 nm samples a loss of orientation of the cell movement was visible (Fig. 3). During recovery in dim light the precision of positive gravitaxis in the samples, which performed a gravitactic sign change decreased within 4 h. After 12 h all samples showed negative gravitaxis again (data not shown).

Determination of the role of oxygen on the gravitactic sign change

The experiments were solely performed with colorless cells (*Astasia longa* and *Euglena gracilis* 1f), to avoid any influence of photosynthetically produced oxygen. In oxygen-free samples (addition of 3 μM Na_2SO_3) a sign change was never detected. The cells showed a pronounced negative gravitaxis during the course of the experiment, while the untreated light controls changed from negative to positive gravitaxis very fast. The gravitaxis of the dark controls (with and without Na_2SO_3) was not affected. Fig. 4 shows a representative result of an experiment with *Euglena gracilis* 1f. The swimming velocity of the cells in oxygen free medium was decreased. The responses of *Astasia longa* and *Euglena gracilis* 1f were very similar. At least four independent measurements were performed for each experiment.

Effects of solar radiation on gravitaxis

The cells which were exposed to solar radiation showed different responses to the radiation depending on the filter (Fig. 5). The perceived dose during the course of the experiment is shown in Table 2. The sign change in the samples exposed to solar radiation in the presented experiment was not as pronounced as in the experiments with artificial radiation. The tendency of positive gravitaxis was seen from the circular histograms (not shown). Although the percentage of upward swimming cells considerably decreased in all samples, a pronounced sign change in gravitaxis was only visible in samples, which were exposed to UV-A and PAR (320 nm cut-off filters). The samples covered with the 395 nm cut-off filters did not show a pronounced sign

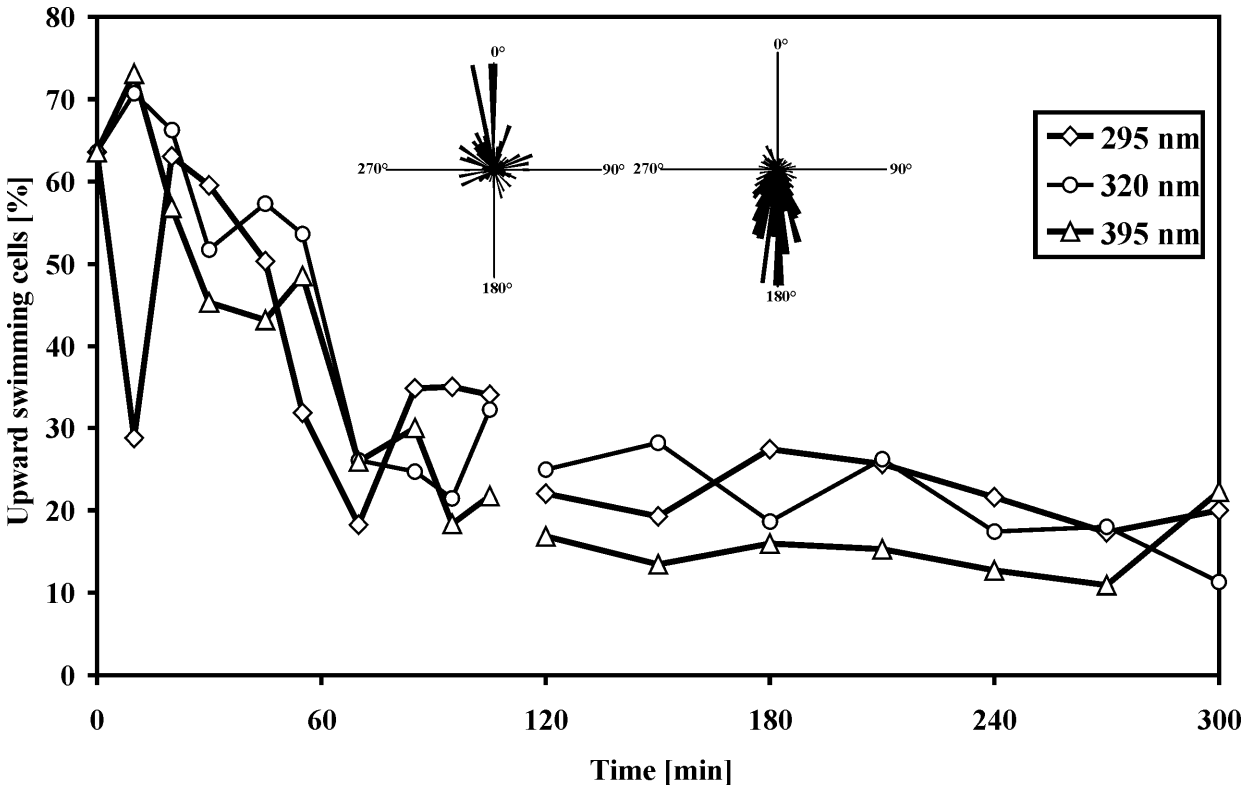

Fig. 1. Effect of artificial simulated solar radiation on gravitactic orientation of *Euglena gracilis*. The diagram shows the percentage of upward swimming cells (120° cone around the vertical) after radiation. The samples were covered with different cut-off filters (295 nm, 320 nm and 395 nm). The decrease in upward swimming cells in combination with the corresponding circular histograms reveal the sign change to positive gravitaxis. Theta indicates the mean movement direction of the cell culture and is indicated by the lines in the histograms

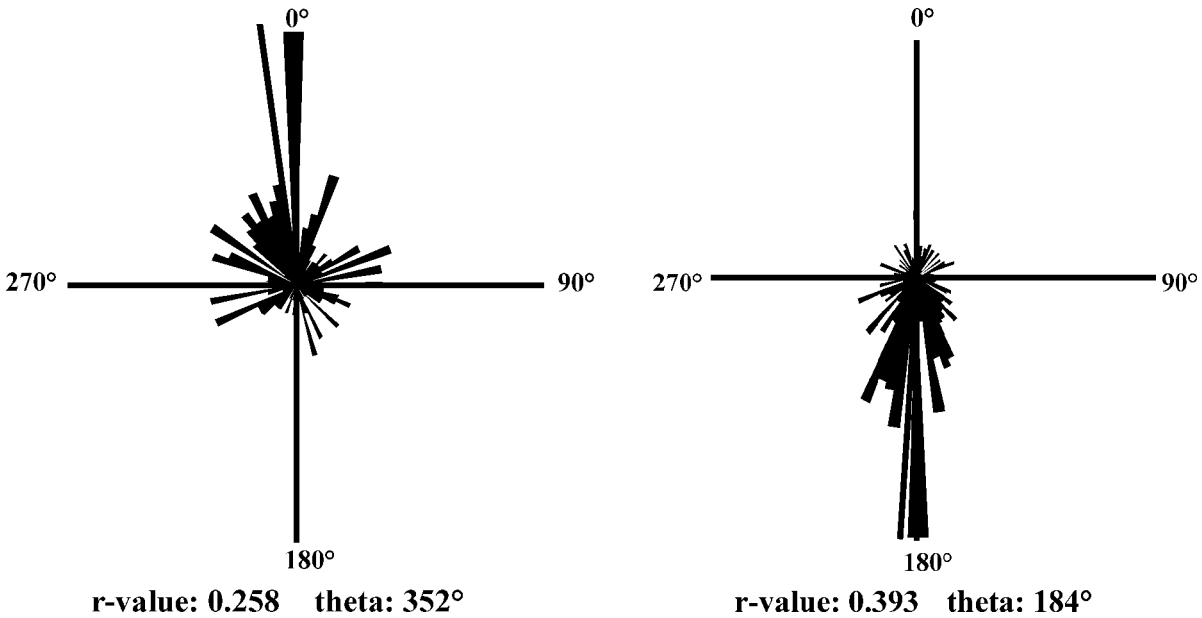

Fig. 2. Typical example of light-induced sign change of *Euglena gracilis* gravitaxis after irradiation shown as circular histograms. Here the effect of 90 min UV-A and PAR irradiation is shown. The length of each single sector indicates the amount of cells swimming in this direction (details see text). The r-value indicates the precision of orientation; theta indicates the mean movement direction of the population (for details see text), and is indicated by the lines in the histograms

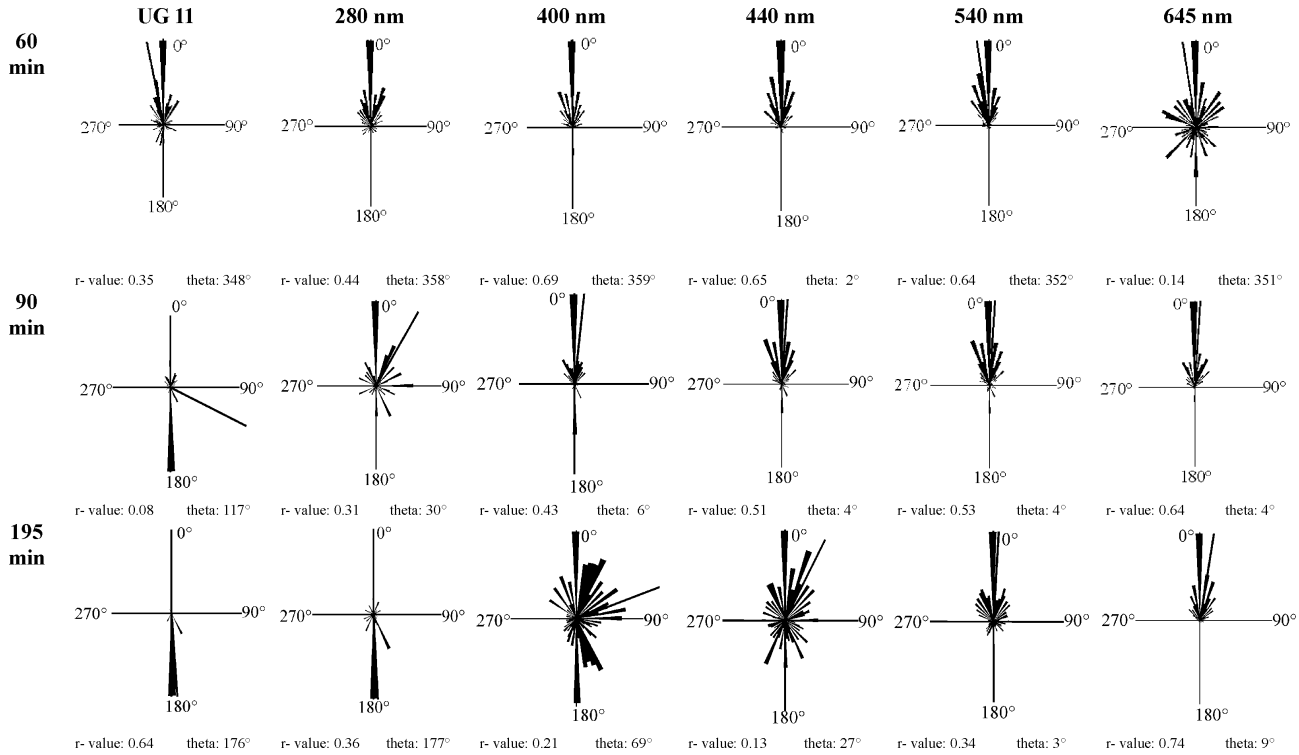

Fig. 3. Effect of light quality on graviorientation in *Euglena gracilis*. A pronounced sign change in gravitaxis (negative to positive) occurred in the samples which were exposed to UV only or to UV plus PAR. The movement behavior is represented as circular histograms. The length of each individual sector indicates the percentage of cells swimming in this direction. The r-value indicates the precision of orientation; (for details see text), theta indicates the mean movement direction of the cell culture and is indicated by the lines in the histograms

Table 1. Calculated radiation doses [J m^{-2}] of artificial radiation under the various filters used in the experiment presented in Fig. 3

Time	UG11	WG 280	WG 400	WG 440	OG 540	RG 645
45 min	74774	879956	670216	511007	319151	77197
90 min	149547	1759913	1340431	1022015	638302	154395
195 min	324019	3813144	2904268	2214366	1382988	334522

Table 2. Calculated radiation doses [J m^{-2}] of solar radiation under the various filters used in the experiments presented in Figs 5 and 6

Time	30 min	120 min	240 min	360 min
Dose PAR [J m^{-2}]	759534	1575479	3206058	4483337
Dose UV-A [J m^{-2}]	173651	362318	735939	1014727
Dose UV-B [J m^{-2}]	5630	12007	24560	32508

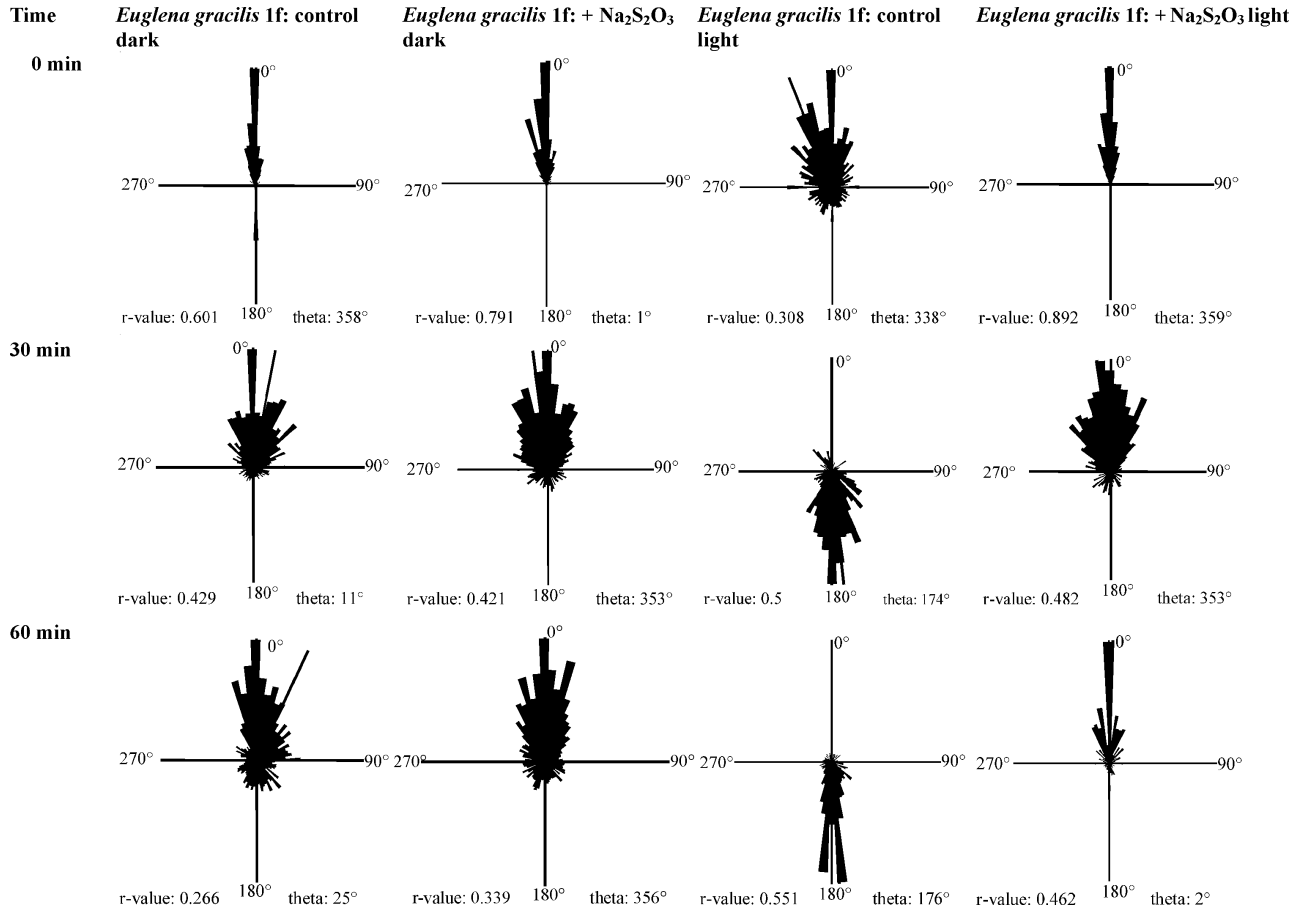

Fig. 4. Influence of oxygen on gravitactic sign change in *Euglena gracilis*. In the absence of oxygen (addition of 3 µM Na_2SO_3) no gravitactic sign change was detected (fourth column), while the control cells (oxygen + light) showed a pronounced reversal of gravitaxis. The dark controls (first and second columns) were not considerably affected. The r-value indicates the precision of orientation; (for details see text), theta indicates the mean movement direction of the cell culture and is indicated by the lines in the histograms

change. The data indicate a loss of gravitactic orientation in the course of radiation. But there is a tendency of positive gravitaxis after longer solar exposure in some of the samples (360 min). The samples which were exposed to UV-B, UV-A and PAR (280 nm cut-off filter) showed positive gravitaxis after 120 min, which disappeared in the subsequent measurements. Cells which perceived UV only (UG 11 filter) lost their orientation after long exposure (240 min) but did not show any indication of a sign change in gravitaxis. In contrast to the other samples, in which the cells were found to accumulate at the bottom of the cuvettes the cells of the UG11 samples were equally distributed in their medium. After 12 h in the darkness all samples had completely recovered (data not shown). The time course of sign change is also culture dependent, because in other experiments a much faster sign change of the cells was detected (data not shown).

DISCUSSION

The results of the experiments show that gravitaxis is influenced by light. UV and blue light lead to a loss of gravitactic orientation and very often also to a pronounced sign change in gravitaxis (from negative to

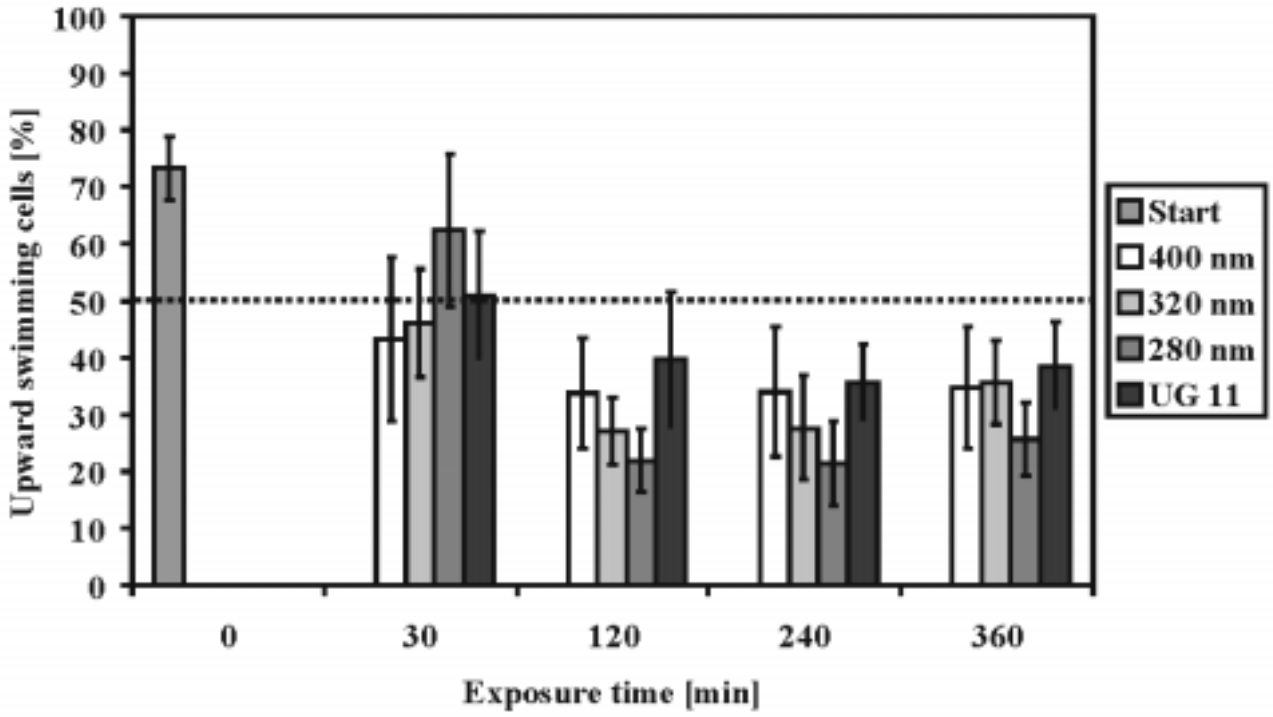

Fig. 5. Effect of solar radiation on the graviorientation of *Euglena gracilis*. The diagram shows the percentage of upward swimming cells (120° cone around the vertical). The samples were covered with different filters (cut-off filters WG 280, WG 320 and WG 400 nm; UG 11 exclusively transmits UV-B and UV-A). Although the percentage of upward swimming cells declined in all samples, only the 320-nm samples switched from negative to a pronounced positive gravitaxis (details see text)

positive), which persists for several hours in darkness or dim light, respectively. The time scale of this phenomenon is very variable, depending on the culture used for the experiments. In some experiments the cells totally reversed gravitaxis within 30 min, other samples needed longer irradiation times to perform a gravitactic sign change (up to 4 h). For this reason it was not possible to determine a dose response curve of gravitactic sign change. A role of the photoreceptor for phototaxis, the paraxonemal body, can be excluded, because also colorless mutants as well as *Astasia longa*, both of which lack a paraxonemal body showed a pronounced sign change. Also photoinduced reactions inside the chloroplasts can be ruled out, because these cells are chloroplast-free. But it is very obvious, that the phenomenon is based on light-induced reactions with oxygen. In the absence of oxygen (performed by addition of NaS_2O_3) no sign change was detected even after long exposure to light. Most likely oxygen radicals or reactive oxygen species trigger the gravitactic sign change. The oxygen receptor in the ciliate *Loxodes* is likely the cytochrome-c-oxidase (see below). The underlying mechanism in *Euglena* is currently intensively investigated by means of metabolic inhibitors.

In addition, it can be stated that only UV and short-wavelength visible light (around 400 nm) can induce a gravitactic sign change. *Euglena* cells are very sensitive to solar UV radiation, and impacts on the photosynthetic apparatus and the photoreceptor pigments of *Euglena gracilis* have been demonstrated (Häder and Brodhun 1991, Brodhun and Häder 1995). Tirlapur *et al.* (1992) revealed an enormous increase in the intracellular calcium concentration, as well as a pronounced decrease in the calmodulin concentration and effects on the nucleus in *Euglena gracilis* upon UV-B radiation. So it is advantageous for the cells to switch to positive gravitaxis after exposure to high solar radiation. An impact on the motility and gravitactic orientation in *Euglena gracilis* was already detected in earlier studies. Gerber *et al.* (1996) measured a polychromatic action spectrum of inhibition in motility of *Euglena*. The experiments revealed a strong effect of UV-B and UV-A, but also the blue light range was effective. This is in good agreement with the results obtained in the present study. The effect

of artificial and solar radiation on gravitaxis has been demonstrated by Häder and Liu (1990a). The strong impact on gravitaxis was interpreted as a possible effect on an active gravireceptor or flagellar protein. The active sign change of gravitaxis after long UV exposure was already mentioned by the authors. It is possible that the reversal of the cells was not completed, because of the limited duration of the experiments (120 - 200 min). The circular histograms as well as the kinetic diagrams in this paper indicate a beginning gravitactic reversal. It is obvious that the positional regulation of *Euglena gracilis* due to the proposed interplay between phototaxis and negative gravitaxis (see introduction) is not the only control mechanism. In the case that the photoreceptors are destroyed by UV the cells are not longer able to regulate their position by means of gravi- and photoreceptors. If the cells were blinded by UV, as demonstrated by Häder and Liu (1990a) with a resulting loss in phototaxis, the negative gravitaxis would guide them to the water surface where they would encounter deleterious radiation. The described phenomenon might be an adaptation mechanism in the case that the cells cannot avoid a high radiation regime (e.g. shallow habitat, as simulated in the described experiments). Positive gravitaxis in *Euglena gracilis* can be found under certain circumstances. In fresh cultures *Euglena* cells very often show positive gravitaxis during the first days of cultivation. Also by manipulation of the properties of the medium gravitaxis can be switched to positive (Richter 2000). Increased salinity (10 g/l NaCl) often leads to positive gravitaxis. Even after transfer of the cells to the standard medium the positive gravitaxis persists for many days (unpublished observations). After recovery from the columella stage (permanent cysts), which can be induced by experimental increase of the osmolarity of the medium (Höfler and Höfler 1952), the cells show a pronounced positive gravitaxis (unpublished results). These observations make it very likely that *Euglena* is able, in analogy to phototaxis, to switch actively between negative and positive gravitaxis. At least under laboratory conditions negative gravitaxis is the favored of the two. Also other gravitactic microorganisms are described to show a sign change in gravitaxis upon radiation. *Prorocentrum micans*, a marine dinoflagellate, normally shows a pronounced negative gravitaxis, which switches to a positive one upon short exposure to solar or artificial UV radiation (Eggersdorfer and Häder 1991, Sebastian *et al.* 1994). Also the dinoflagellate *Gymnodinium* (Y-100), which has been shown to be sensitive to solar UV-radiation reversed actively from negative gravitaxis to positive gravitaxis (Tirlapur *et al.* 1993). The dinoflagellate *Peridinium gatunense,* in contrast, seems to sediment upon excessive exposure to solar radiation in order to avoid cellular light-induced damages (Häder and Liu 1990b). The negative gravitaxis of *Dunaliella bardawil* is not impaired by light; in this case avoidance is only due to negative phototaxis (Jimenez *et al.* 1996). The ciliate *Paramecium* was shown to change between positive gravitaxis upon illumination and negative gravitaxis in the darkness (Fox 1925). Also temperature was described to influence gravitaxis in *Paramecium*. A strong oxygen dependency of gravitaxis was reported in *Paramecium* and *Loxodes*. It was found that low oxygen concentrations of the medium induce a pronounced negative gravitaxis in *Paramecium*, while higher oxygen pressure (>1.2 mg/l) lead to a deterioration of gravitaxis (Hemmersbach-Krause *et al.* 1991). *Loxodes,* a unicellular ciliate normally shows a pronounced positive gravitaxis in the presence of oxygen, while under conditions of anoxia the cells were described to show negative gravitaxis (Fenchel and Finlay 1986). As *Loxodes* only shows a low activity of catalase and superoxide dismutase the cell are very sensitive to high oxygen pressure (Finlay *et al.* 1986). In *Loxodes* probably cytochrome-c-oxidase is the oxygen receptor, because incubation with KCN led to a loss of gravitactic orientation (Finlay *et al.* 1986). In *Anabaena variabilis* singlet oxygen generated in high light was found to be responsible for phototactic reaction by means of an unknown signal processor (Nultsch and Schuchart 1985).

The results obtained in the present study also strongly indicate, that gravitaxis is an active physiological mechanism and not a passive physical effect based, e.g., on buoyancy (Brinkmann 1968, Kessler 1992). Some experiments indicate that the mechanism of gravitactic orientation itself is probably sensitive to excessive UV-B radiation, because also positive gravitaxis disappeared after long exposure to UV radiation. This is another proof for active graviperception in *Euglena gracilis*.

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