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Phototaxis in the gliding flagellate, *Euglena mutabilis**

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Abstract. Due to the lack of an emergent flagellum the green flagellate *Euglena mutabilis* is restricted to gliding motility. During forward movement, the organisms orient positive phototactically in the presence of a suitable light stimulus. The cell contains both a stigma and a paraflagellar body which differ in shape and size from the organelles found in *E. gracilis*. The degree of orientation in white light follows an optimum curve with a maximum at about 100 lx. The spectral sensitivity shows a number of prominent peaks in the blue and green regions and extends well into the red region of the visible spectrum. Since the cell does not rotate during locomotion a periodic shading mechanism cannot account for phototactic orientation. Thus, phototaxis in the related species, *E. gracilis* and *E. mutabilis* differ in their photoreceptor molecules, their sensory transduction chains and their strategies of light direction detection.

Key words: Electron microscopy – *Euglena mutabilis* – Flagellate – Photomovement – Photoreceptor – Phototaxis – Single-cell analysis – Videomicroscopy

Photomovement in flagellates has been studied for well over a century (Treviranus 1817). One of the model systems which has been studied extensively is *Euglena* (Diehn 1979, Colombetti et al. 1982). The photoautotrophic species, *E. gracilis*, swims using its one emerging flagellum. It shows positive and negative phototaxis (Diehn 1973; Häder et al. 1981) as well as step-up and step-down photophobic responses (Diehn et al. 1975; Doughty et al. 1980).

The action spectra of these responses resemble each other and show maximal activity in the blue and near UV regions of the spectrum; there is no response to radiation > 530 nm. The similarity of the action spectra indicates that phototaxis and the phobic response share the same photoreceptor, but differences in the zero thresholds and in the sensitivities towards drugs suggest that the photoresponses differ in the subsequent steps of the sensory transduction chain. The action spectra have been interpreted to indicate the activity of flavins in the photoreception (Diehn 1972; Lenci and Colombetti 1978). The photoreceptor has been suggested to be localized in the paraflagellar body, a swelling at the basis of the emerging flagellum within the reservoir adjacent to the end of the non-emerging flagellum (Benedetti and Checcucci 1975; Melkonian et al. 1982). Whether or not the paraflagellar rod, a paracristalline structure within the flagellum next to the paraflagellar body, is involved in the sensory process cannot yet be decided (Hyams 1982). In addition to swimming by means of the trailing flagellum, *Euglena gracilis* is capable of gliding on a substratum using body contractions (Mikolajczyk 1975).

The related species, *Euglena mutabilis*, does not have an emerging flagellum and moves exclusively by euglenoid contractions. Due to the different mechanism of propulsion, this species must have a different sensory transduction chain than *Euglena gracilis*: the light stimulus is converted into a signal which controls the body contractions rather than the flagellar reorientation as a means to adjust the direction of swimming with the incident light rays. Whether or not differences in the sensory processes include differences in the photoreceptor pigments can be studied by comparing the action spectra for phototactic orientation in the two species.

Materials and methods

The gliding flagellate, *Euglena mutabilis*, was isolated from a small pond near Abisko (Sweden) and an axenic culture was kindly provided from Dr. Surek (Münster). The cells grew at 16° C in a light-dark cycle of 16 h white light (500 lx from fluorescent lamps) and 8 h dark. The cells were cultivated in 100 ml Erlenmeyer flasks in a rather acidic medium (pH 3.3) described by Greenblatt and Schiff (1959) and were used 3–4 weeks after inoculation.

The movement of the cells was studied with a videomicroscope system. An infrared-sensitive video camera (National WV 1350E) was mounted on top of an inverted microscope (Zeiss ICM 405). The monitoring beam was produced from a quartz-halogen lamp and a cut-off filter which passed wavelengths > 780 nm. The video signal was recorded on a $^1/_2$ inch time-lapse videorecorder (National NV 8030E) which allowed time compression by a factor of up to 80. The actinic beam was produced with a quartz halogen slide projector (Prado, Leitz) and hit the sample cuvette at an angle of 15° above surface. White light intensities were measured with a luxmeter (Lange, Berlin). Monochromatic radiation was produced with interference filters (Schott & Gen.) and measured using a thermopile (Kipp and Zonen, CAI) connected to a microvoltmeter (Keithley, type 155). The movement of the cells was analyzed on a video monitor (Sony PVM 201 CE) by tracing their shape and position at constant time intervals on a transparent acetate overlay.

Phototactic orientation was determined from the drawings by computer-aided data acquisition: The positions of the cells

*Dedicated to Prof. Dr. H. A. von Stosch on the occasion of his 75th birthday

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at the beginning and end of a chosen time interval were taken using a bit pad (Summagraphics Bit Pad One), input into a Z80 microcomputer (SD Systems) and stored on 8 inch flexible disks.

The light-dependent movement was analyzed by directional statistics (Batschelet 1965) using computer programs recently described (Häder 1981). For each data point, the angle of deviation θ from the light direction of >1000 independent traces (n) was calculated and the mean angle $\bar{\theta}$ determined using:

$$\bar{\theta} = \frac{\arccos \frac{\Sigma \cos \theta}{n}}{\bar{r}}. \quad (1)$$

The degree of phototactic orientation $\bar{r}$ was quantified by an algorithm which is based on the standard deviation of the means in a linear distribution wrapped around a circle.

$$\bar{r} = \sqrt{\left(\frac{\Sigma \cos \theta}{n}\right)^2 + \left(\frac{\Sigma \sin \theta}{n}\right)^2}. \quad (2)$$

The value of $\bar{r}$ is a non-linear measure in the interval [0,1] for the directedness of the traces with respect to the light source where a value near 1 indicates a high degree of orientation and a value near 0 a nearly random distribution.

Light microscopy was carried out with a Zeiss Standard 16 microscope fitted with phase contrast optics and photographic unit MC 35. For electron microscopy, cells were fixed in 2.5% glutaraldehyde (made up in culture medium, pH 6.5) for 30 min at room temperature. Postfixation was with 0.5% OsO_4 (in culture medium) at 4°C for 30 min. Further processing of the samples was standard (Melkonian 1975). Sections were viewed with a Siemens Elmiskop 102.

Results

Movement of *Euglena mutabilis* does not consist of a simple continuous forward gliding but is rather complex: often the cells adhere to the substratum with their tail and circle their body around this point of fixation (Fig. 1). Simultaneously, the cellular shape can change remarkably. Interspersed between phases of adhesion there are intervals of linear forward movement during which the cell can orientate its movement with respect to the light direction (Fig. 2).

In lateral white light, the organisms show exclusively positive photoaxis over the range of 1 to 5,000 lx (which equals 4.22×10^{-3} to 21.09 Wm^{-2} for the light source used). Even in higher and lower illuminances negative phototaxis was never observed. The degree of phototactic orientation depends on the illuminance and follows an optimum curve (Fig. 3). Optimal orientation with a rather high degree of $\bar{r} = 0.71$ was induced by 100 lx.

The fluence rate-response curves for monochromatic radiation show a log/linear dependency over at least two orders of magnitude (Fig. 4). In all cases of monochromatic irradiation, the $\bar{r}$ value was smaller than in white light independent of the fluence rate. While the phototactic orientation at 100 lx white light had a value of about 0.71, an energy fluence rate of 0.42 Wm^{-2} (which corresponds to the white light illuminance of 100 lx) at 417 nm caused an orientation with an $\bar{r}$ value of 0.37. Even the saturation values of the fluence rate-response curves for monochromatic irradiation were not higher than 0.55.

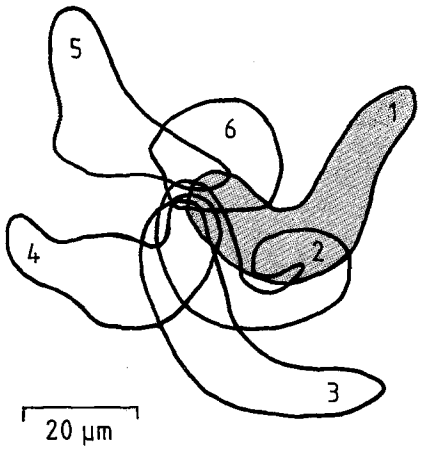


Fig. 1. Movement of *Euglena mutabilis* in medium on a glass surface. The cell is attached with its rears pole, circles clockwise around the point of fixation and undergoes metabolic changes of the cell shape. Phases drawn every 60 s, initial position marked by hatching. The scale corresponds to 20 µm

Fig. 2. Subsequent phases of movement at 60 s intervals showing periods of circling and forward movement in lateral light (arrow) of 100 lx. The scale indicates 50 µm

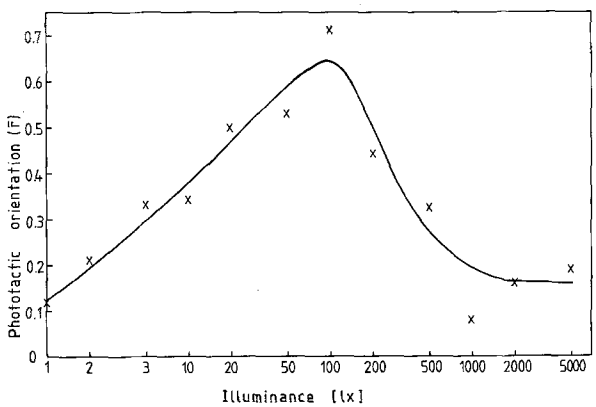


Fig. 3. Dependence of the degree of phototactic orientation ($\bar{r}$) on the illuminance (in lx) of the laterally impinging continuous white light

In order to determine the spectral sensitivity of the positive phototaxis in *Euglena mutabilis*, fluence rate-response curves were measured for each wavelength. Since a photon fluence rate of 46.6 mmol m^{-2} s^{-1} (which has an energy fluence rate of 1 Wm^{-2} at 555 nm) caused responses

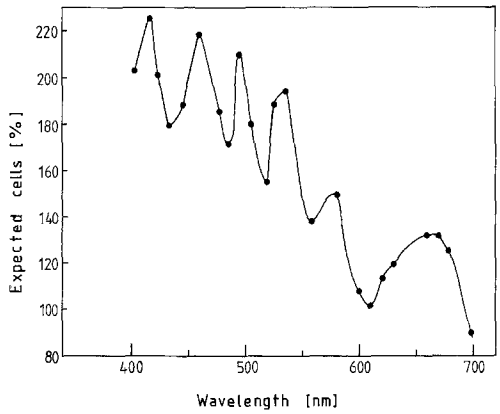


Fig. 4. Fluence rate-response curves for phototactic orientation ($\bar{r}$, ordinate) of *E. mutabilis* to monochromatic light at three wavelengths. Abscissa, energy fluence rate

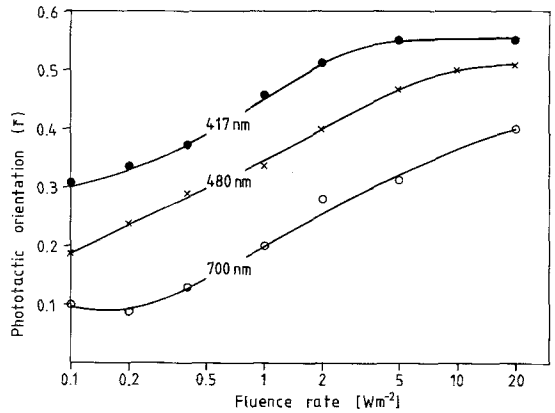


Fig. 5. Spectral sensitivity of the phototactic orientation. Abscissa wavelength (in nm), ordinate fraction of cells moving toward the light source (equal quantum flux density based on 1 Wm^{-2} at 555 nm) within the sector $\pm 30°$ as percentage of the fraction expected in this sector in a randomly oriented population

which fell into the linear part of the fluence rate-response curves for each wavelength, this irradiance was selected as reference. Figure 5 shows the fraction of cells moving towards the light source within the sector of $\pm 30°$ as a percentage of the expected value in this sector (taken as 100%) during unstimulated movement in random directions. The spectral sensitivity shows major peaks in the blue and green regions but extends as well into the red region with a maximum around 665 nm.

Since the individual traces represent constant time intervals and the beginning and end coordinates had been stored in computer files the net speed could be determined. Although no action spectrum has been calculated the data indicate a correlation between the net speed and the phototactic orientation: the fastest movement was observed at wavelengths which cause a high degree of orientation. Whether an increase in the net speed is due to a photokinetic effect or due to a straighter path could not be determined from the traces.

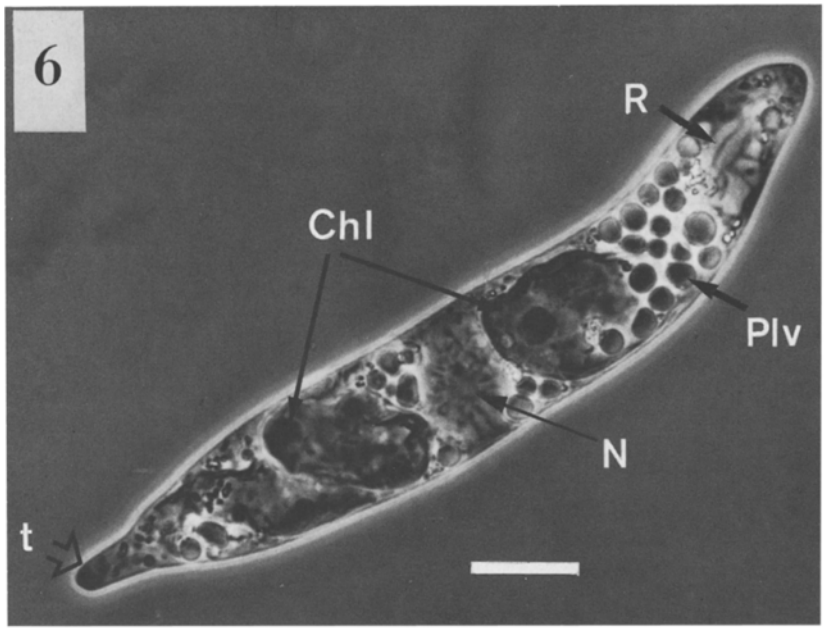
Since *Euglena mutabilis* has no emergent flagellum it is restricted to gliding movements. It attaches itself to the substrate by means of its posterior end ('tail') which secretes localized mucus to facilitate movement (Fig. 6, t). Despite the fact that flagella play no role in movement of *E. mutabilis* it has a well developed paraflagellar body (PFB) and stigma (Figs. 7, 8). The PFB of *E. mutabilis* has enormous dimensions ($1 \times 0.8 \times 0.9$ nm). It has a characteristic shape (a nose-like projection, Fig. 8) and is attached to the axoneme of the principal flagellum via the paraflagellar rod as in *E. gracilis* (Fig. 7). In one localized area (not illustrated), the membrane of the paraflagellar body is in contact with the reservoir membrane. The stigma of *E. mutabilis* consists of a few large lipid globules enclosed by separate membranes (Fig. 7). The space between the stigma globules and the reservoir membrane is occupied by a greater number of cytoplasmic microtubules. Both the principal and the minor flagellum do not project beyond the flagellar canal and therefore do not emerge beyond the cell proper.

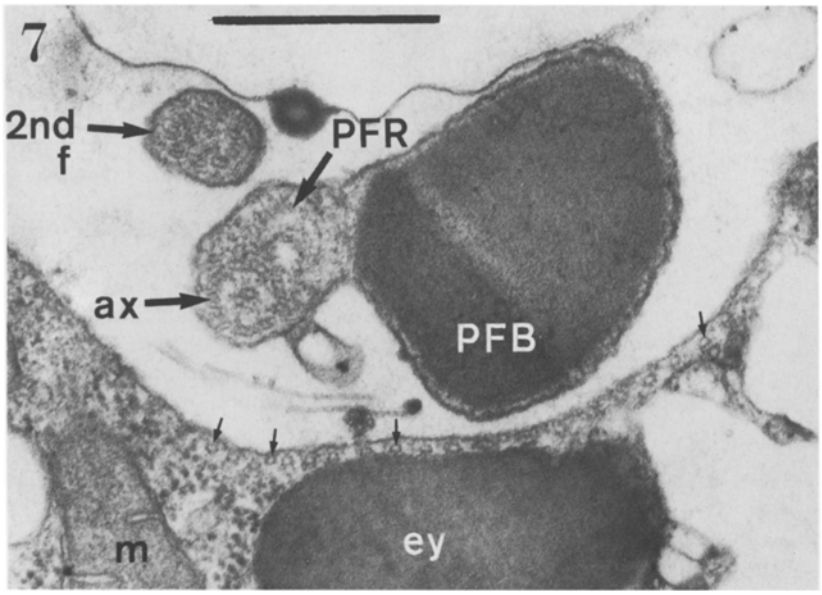
Discussion

Although *Euglena mutabilis* lacks an emergent flagellum, it contains both a paraflagellar body (PFB) and stigma, prerequisites which are regarded as essential for phototactic orientation in *Euglena gracilis* (Vavra 1962; Colombetti et al. 1982). The PFB of *E. mutabilis* appears especially large compared to that of *E. gracilis* (Kivic and Vesk 1972; Kronestedt and Walles 1975) and has an unusual hitherto unreported shape. Most authors agree that the photoreceptor is located in the PFB, while the stigma acts as a shading device. No phototactic orientation is found in wavelengths > 530 nm which is one of the reasons to assume a flavoprotein as the photoreceptor pigment.

Therefore, it is surprising that the phototactic action spectrum for *E. mutabilis* extends well into the red region. While the activity in the blue range could be due to an absorption by a flavin photoreceptor, the maxima at 530 nm and the broad band around 660 nm cannot be explained by assuming a flavoprotein as the sole photoreceptor. Checcucci et al. (1974) have found a weak accumulation of *E. gracilis* in red light fields. This effect was due to an aerotactic attraction of organisms outside the light field by the photosynthetically produced oxygen from the organisms within the light field. Such an explanation does not hold for the phototactic orientation of *E. mutabilis*, since all organisms were in the same light conditions so that no oxygen gradient could be produced. If the photosynthetic pigments are involved in the photoperception, their action seems to be mediated by a direct photoresponse as in the case of desmids or blue-green algae (Wenderoth and Häder 1979; Nultsch 1961). Flavins can be assumed to operate as additional photoreceptors to account for the strong activity in the blue region. The fact that white light induces a higher degree of phototactic orientation than any monochromatic irradiation – regardless of its fluence rate – indicates an interaction of more than one photoreceptor.

During locomotion, the cells do not rotate along their longitudinal axis unlike *E. gracilis*; therefore, a periodic shading effect can be excluded as the basis for phototactic orientation. Orientation with respect to the light direction could be achieved during the rotation of the cell around the attached rear end. Since the chloroplast extends well into the front region, it could be hypothesized that it could function – perhaps in addition to the stigma – as a shading device. During the rotation, the cell could scan the horizon for a maximum in light intensity. When the front end points toward the light source, the PFB receives the highest light





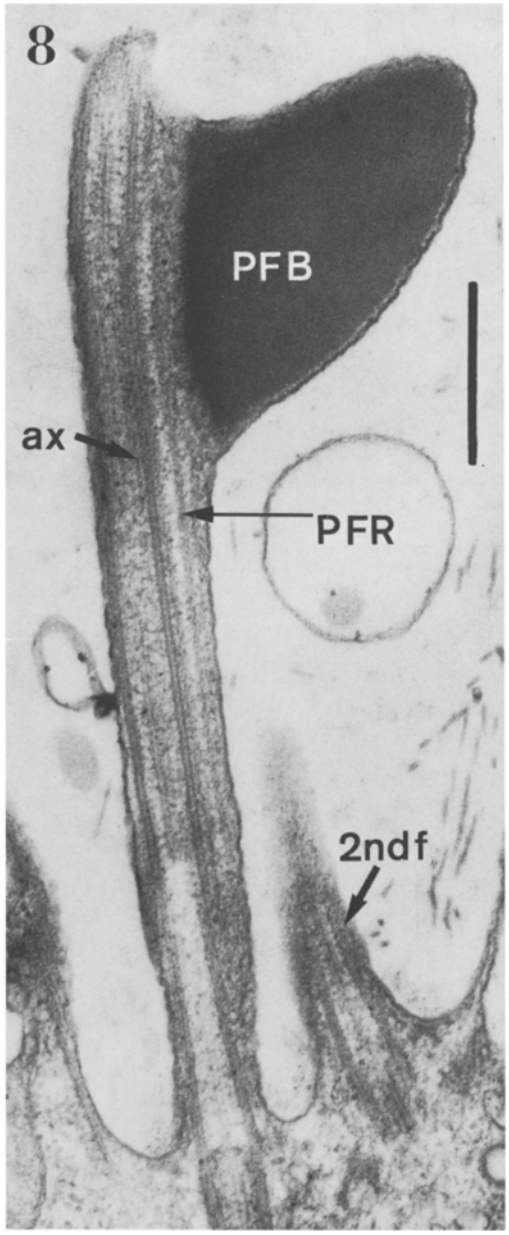


Fig. 6. Living cell of *Euglena mutabilis* seen in phase contrast, × 1,370. *R* Reservoir at the anterior end of the cell with one of the two flagella visible, *Plv* phospholipid vacuoles, *Chl* two chloroplasts with one pyrenoid each, *N* nucleus with condensed chromosomes, *t* 'tail'-region of the cell (point of attachment to the substrate). The bar represents 10 μm

Fig. 7. Cross section through the two flagella in the region of the paraflagellar body (*PFB*). *PFR* paraflagellar rod, *ax* axoneme of the principal flagellum, *2ndf* second, minor flagellum, *m* mitochondrial profile, *ey* one large stigma globule, small arrows indicate cytoplasmic microtubles lining the reservoir membrane between the stigma and the principal flagellum, × 60,000; the bar represent 500 nm

Fig 8. Longitudinal section through most of the principal flagellum of *E. mutabilis* inside the reservoir region. *PFB* paraflagellar body, *ax* axoneme, *PFR* paraflagellar rod, *2ndf* second, minor flagellum. × 48,000, the bar represents 500 nm

intensity (not shaded by either stigma nor chloroplasts) and this signal could serve as a trigger to induce forward movement.

In conclusion, the flagellates *E. gracilis* and *E. mutabilis*, although belonging to the same genus, differ in major aspects of their phototactic orientation: they use a different set of photoreceptor molecules, they orient with different mechanisms of propulsion and at least parts of their sensory transduction must be different. In additon, while *E. gracilis* shows both positive and negative phototaxis, negative phototaxis has not been observed in *E. mutabilis*.

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