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Phototactic responses in the gametes of the brown alga, *Ectocarpus siliculosus*

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Abstract. The action spectrum of phototaxis was determined and the photoreceptive mechanism was studied in *Ectocarpus* gametes (Ectocarpales, Phaeophyceae) using a computerized cell-tracking system. The fine structures of the stigma and the flagellar swelling were analyzed, and the reflective function of the stigma was demonstrated for the first time. Under monochromatic light stimulation, *Ectocarpus* gametes show mainly positive phototaxis between 370 nm and 520 nm. The action spectrum has a minor peak near 380 nm, and two major peaks at 430 nm and 450 nm or 460 nm and a shoulder at 470 nm adjoining a remarkable depression near 440 nm. Under unilateral stroboscopic illumination with more than four pulses per second, the gametes show clear phototaxis. However, the response is disturbed at lower frequencies. Addition of methyl cellulose, which increases the viscosity of the medium and slows down gamete rotation, decreases the threshold frequency. These results indicate that rotation of the gamete plays an essential role in the photoreceptive mechanism. Under equal intensities of bilateral illumination at an angle of 90°, most of the gametes swim on the resultant between the two light beams. This response is disturbed when the angle of the two light beams is as large as 120°. Observations by transmission electron microscopy show that the flagellar swelling fits precisely into a concave depression of the chloroplast at the central region of the stigma. Electron-dense material is present in that sector of the flagellar swelling which faces away from the stigma. Epifluorescence microscopy without a barrier filter and epipolarization microscopy reveal that stigmata reflect blue light. A hypothesis is formulated which discusses the possibility that the reflected light is focused onto the flagellar swelling.

Key words: Blue light response (cryptochrome) – *Ectocarpus* (phototaxis) – Phaeophyceae – Phototaxis (photoreception) – Stigma (blue-light reflection)

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Introduction

Although flagellated cells of many brown algae are known to show obvious phototaxis, not much is known about their photoreceptive mechanism. Recently, several papers concerning this subject have been published. Müller et al. (1987) have studied the photoaccumulation of *Ectocarpus* gametes and suggested that it is a blue-light response. Several authors have reported the presence of a flavin-like autofluorescent substance in the posterior smooth flagellum of phototactic flagellated cells of brown algae; the anterior flagellum or the stigma showed no autofluorescence (Müller et al. 1987; Kawai 1988). Such autofluorescence has also been observed in the smooth flagellum of some related chlorophyll-c-containing algae (Müller et al. 1987; Coleman 1988; Kawai 1988; Kawai and Inouye 1989). Kawai et al. (1990) studied the action spectrum of phototaxis in zoospores of *Pseudochorda* sp. (Laminariales, Phaeophyceae) and confirmed that it is a blue-light-mediated phototactic response. Here we present a further study on the action spectrum obtained with different methods and some experiments to provide more information on the phototactic mechanism using the gametes of the brown alga, *Ectocarpus siliculosus*. We also report the reflective nature of the stigma and discuss its possible function.

Material and methods

Suspensions of swimming gametes. Male gametophytes of a clonal isolate of *Ectocarpus siliculosus* (Dillw.) Lyngbye (Ectocarpales, Phaeophyceae) originating from Texas (PA-27a, male, Müller 1979) were cultured at 20° C, in PES medium (enrichment for seawater; Starr 1978) and irradiated with white light from fluorescent lamps (Osram, München, FRG; 13.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$). To release synchronously the gametes, mature gametophytes were placed in a cold (approx. 3° C) dark place for one to three nights, then transferred to 20° C and irradiated with white light from one side. Released gametes, which accumulated in the unilateral light, were collected by pipette and kept in a cold dark place (0–5° C) until subjected to the experiments.

Measurement of phototaxis and action spectrum. The orientation of swimming gametes under unilateral illumination was studied at various wavelengths (350–650 nm) at $4 \cdot 10^{-3}$ to $3.6 \cdot 10^{3}$ μmol·$m^{-2} \cdot s^{-1}$. Movement vectors were determined using a computerized cell-tracking system (Häder and Lebert 1985). A slide projector with a halogen lamp (24 V, 250 W) was used as light source and the light was focused on the cuvette by a biconvex lens (f = 200 mm, diameter 76 mm). The fluence rate was modified by inserting neutral-density filters. Interference filters (Schott and Gen. Mainz, FRG) were used for monochromatic light. The illuminance was measured with a luxmeter (Mavolux, Gossen, Erlangen, FRG), and the energy measurements were made with a thermopile (CA 1; Kipp and Zonen, Delft, The Netherlands) connected to a microvoltmeter (Model 155; Keithly). A cuvette made from microscope slides and cover slips ($68 \cdot 18 \cdot 0.17$ mm^3) was used for unilateral-light experiments. The cuvette containing gametes was placed on the object stage of a Zeiss inverted microscope (ICM 405; Zeiss, Oberkochem, FRG). To restrict the observation light to the infrared, a cutoff filter (RG 705) was used. The image of the swimming gametes was viewed by an infrared-sensitive charge coupled device (CCD) camera (LDH 0600; Philips, Hamburg, FRG) and analyzed by a computer program (Häder and Lebert 1985). Most of the measurements were made within 6 h after gamete release. Methyl cellulose (approx. 0.1%) was added to the medium to slow down the gamete movement slightly.

Stroboscopic- and bilateral-illumination experiments. For experiments involving stroboscopic pulse illumination, a Strobex Model 100 (Chadwick-Helmuth, Cal, USA) was used. For bilateral-illumination experiments, two slide projectors were used to provide two light beams with the following angles; ±45° (45° and 315°), ±60° (60° and 300°) and ±90° (opposite lights, 90° and 270°). The experiments were done under 1000 lx (±45°), 1600 lx (60°, 90°), 3000 lx (±90°), 6000 lx (±60°), 6200 lx (±45°) and 8000 lx (±45°) for each unilateral illumination. An experiment using bilateral lights of unequal intensities (1 klx and 2.3 klx) was done at 45°. For bilateral-illumination experiments, triangular or quadratic cuvettes with 5 cm side length and 0.17 mm thickness were used to avoid refraction.

Observations by transmission electron microscopy (TEM) epifluorescence and epipolarization microscopy. For TEM observations, swimming gametes were fixed simultaneously in a mixture of 2% glutaraldehyde and 1% OsO_4 in sea water, pre-embedded in 2% agar, dehydrated in an acetone series and embedded in Spurr's epoxy resin (Spurr 1969). They were sectioned with a diamond knife, and stained with uranyl acetate and lead citrate. In order to observe the reflected light from the stigma, a Zeiss epifluorescence microscope was used in modified blue-excitation mode (450–490 nm exciter filter, FT510 dichroic mirror, no barrier filter). A Zeiss Axioplan epipolarization microscope was also used for the selective observation of the polarized reflected light.

Results

General features of phototaxis. Under unilateral illumination at effective wavelengths and sufficient fluence rates, the gametes of *Ectocarpus* essentially showed positive phototaxis, swimming in a very straight course toward the direction of the light source. However, some gametes showed negative phototactic movement as well. There was no clear relation between intensity or wavelength of the stimulation light and a positive or negative phototactic tendency. The cells were often observed to turn to the opposite direction even under unilateral illumination, switching between positive and negative.

Action spectrum. Action spectra were based on the quantum flux required for threshold and 50% responses of phototaxis (50% of the cells swim phototactically, e.g., the angle of the track to the light is 0° ± 30° or 180° ± 30° in Fig. 1). Within the wavelengths examined, *Ectocarpus* gametes showed phototaxis between 370 nm and 520 nm (Fig. 1, Fig. 2b). In the action spectra, there is a minor peak near 380 nm and two major peaks at 430 nm and 450 nm (50% efficiency) or 460 nm (threshold) with a shoulder at 470 nm (50% efficiency). A remarkable depression exists near 440 nm (Fig. 1). No phototactic responses were observed in the infrared range (Fig. 2a).

Stroboscopic-illumination experiments. Under unilateral stroboscopic illumination with more than four pulses per second, *Ectocarpus* gametes showed clear phototactic responses (Fig. 3a, b) as in continuous illumination. However, at very low frequencies, the response was obviously disturbed (Fig. 3c). In experiments with methyl cellulose, where the rotation of the gametes is slowed down, the threshold was around two pulses per second. In experiments without methyl cellulose, the frequency threshold was between four and five pulses per second.

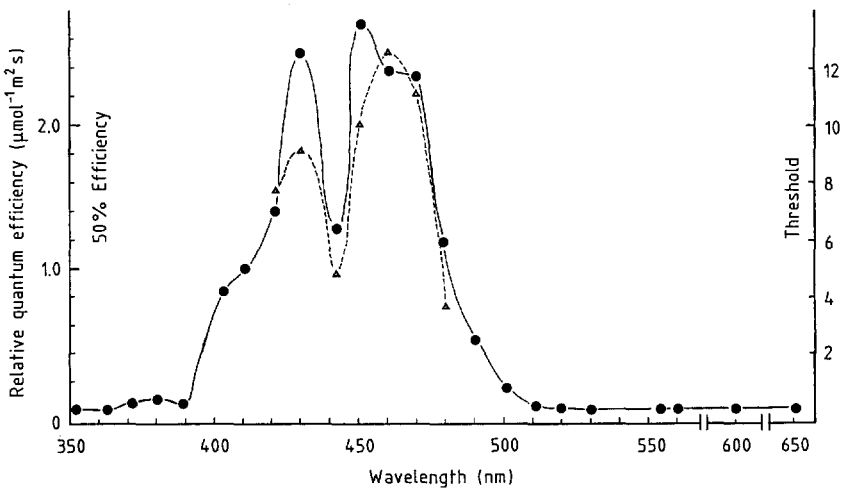


Fig. 1. Action spectra of phototaxis in *Ectocarpus* gametes based on the quantum flux density required for threshold or 50% efficiency of the response. △ Threshold; ● 50% efficiency

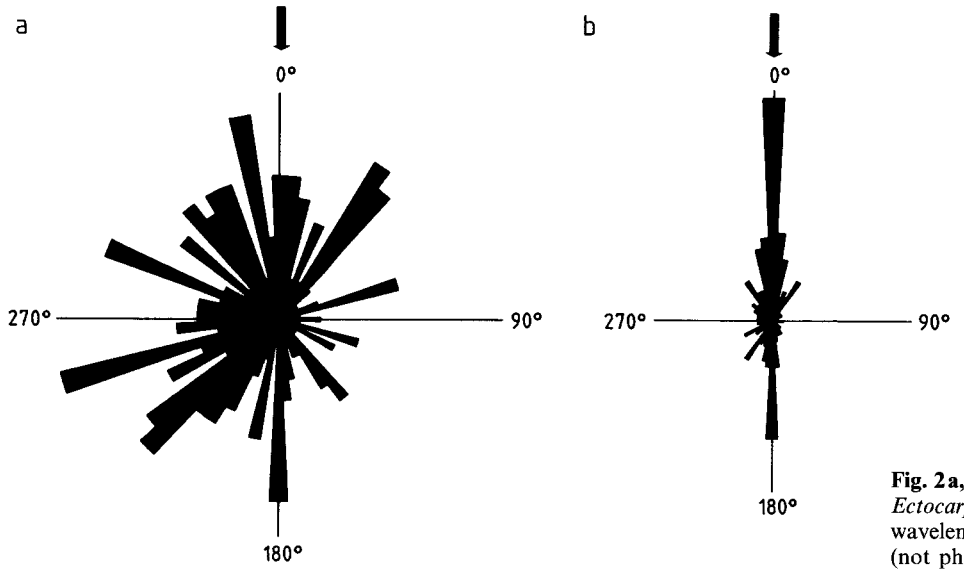


Fig. 2a, b. Histograms of phototaxis in *Ectocarpus* showing the effect of different wavelengths of the stimulation light; **a** 600 nm (not phototactic), **b** 411 nm (phototactic)

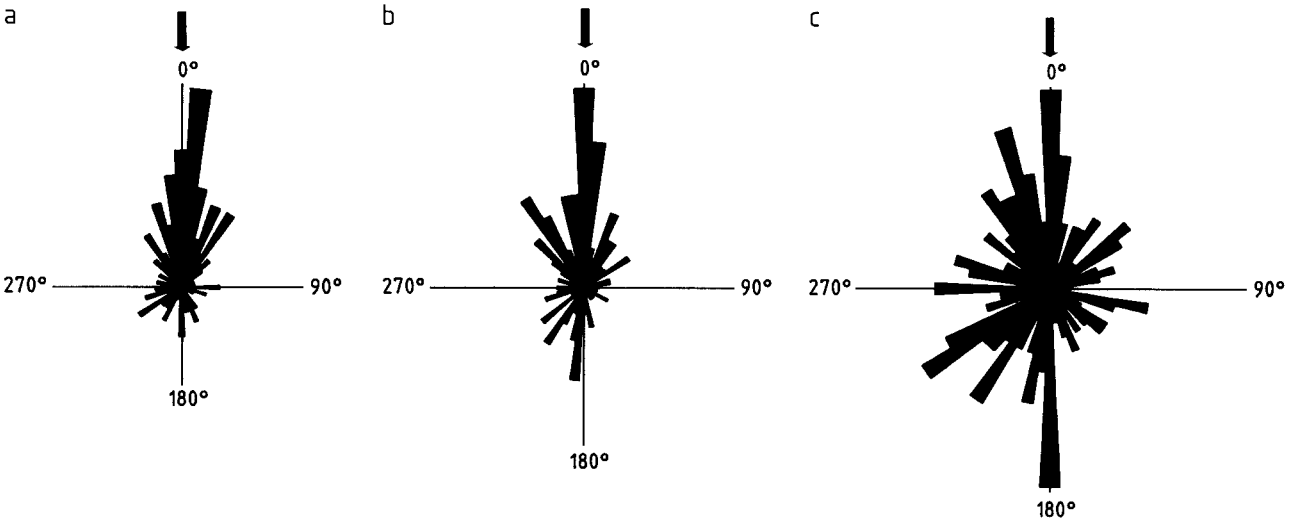


Fig. 3a–c. Histograms of phototaxis in *Ectocarpus* under stroboscopic illumination; **a** approx. 200 pulses·s^{-1} (phototactic), **b** 4 pulses·s^{-1} (phototactic), **c** 2 pulses·s^{-1} (disturbed)

Bilateral-illumination experiments. Under bilateral illumination of equal intensity from ±45°, most of the gametes swam on the resultant between the two light beams (Fig. 4c, 0° or 180°). A 20% increase in the intensity of the 315° light beam caused some gametes to swim towards or away from the direction of the light beam of higher intensity, while the others swam on the resultant between the two light beams.

Under equal intensities of bilateral illumination from ±60°, many of the gametes swam in the middle between the light beams (0° and 180°) as in ±45°, however, some swam in the directions of either light beam, or in random directions. Under opposite illumination from ±90°, some gametes swam in the directions of the light beams (90° and 270°), however, many swam in random directions (data not shown).

Observations by TEM, epifluorescence and epipolarization microscopy. The gametes of *Ectocarpus* have a single chloroplast with a stigma (Figs. 5, 6). The stigma is composed of a single layer of large osmiophilic globules of almost equal diameters inside the chloroplast membrane. The central region of the stigma shows a pronounced depression, into which the swelling at the basal part of the posterior flagellum fits. Electron-dense material is distributed at the sector of the flagellar swelling away from the stigma, adjacent to the axonema.

In epifluorescence microscopy without a barrier filter, and epipolarization microscopy, the stigma strongly reflects the blue excitation light (Figs.7–10). The stigma shows no autofluorescence under blue excitation light. The reflection occurred in swimming cells as well as in settled cells. Even young germlings still retained

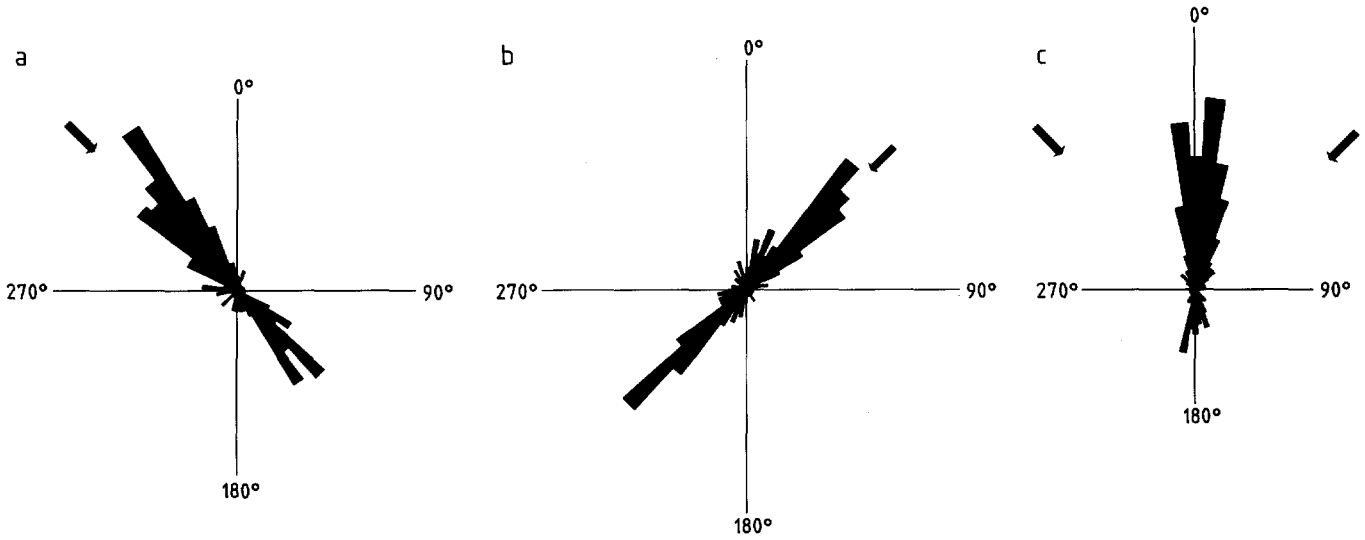
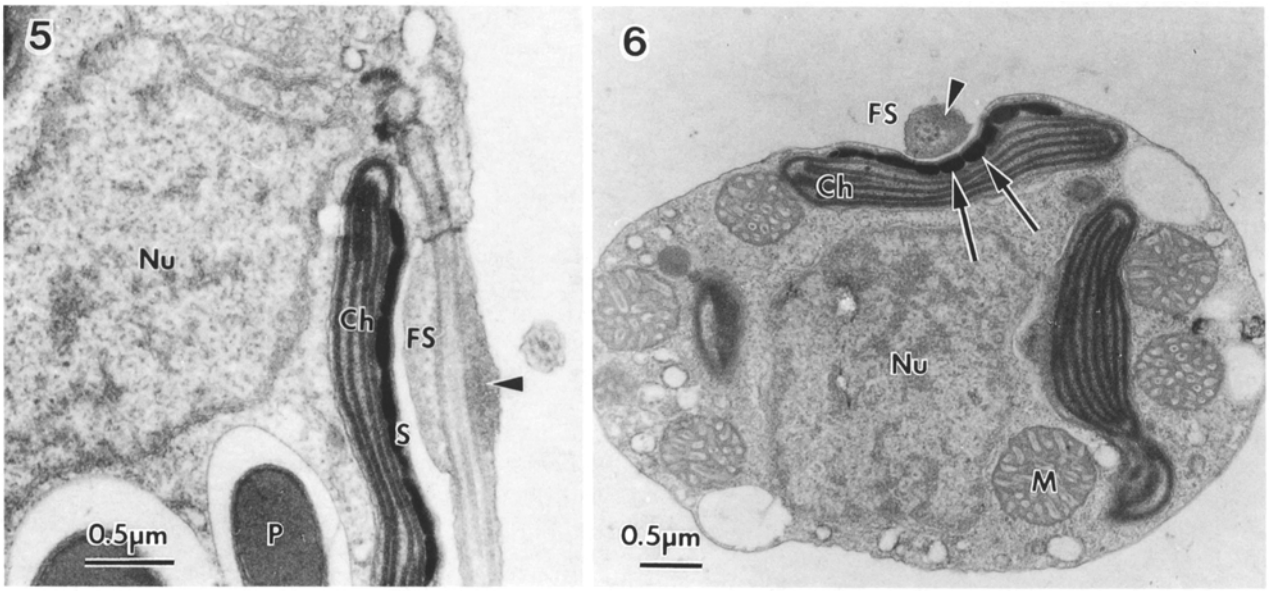


Fig. 4a–c. Histograms of phototaxis in *Ectocarpus* with one or two equal light sources (8 klx); **a** unilateral from −45° (315°), **b** unilateral from +45°, **c** bilateral from ±45° (45°, 315°). Gametes swim in the direction of the light beam in unilateral light, but swim on the resultant of the two light beams in ±45° bilateral lights



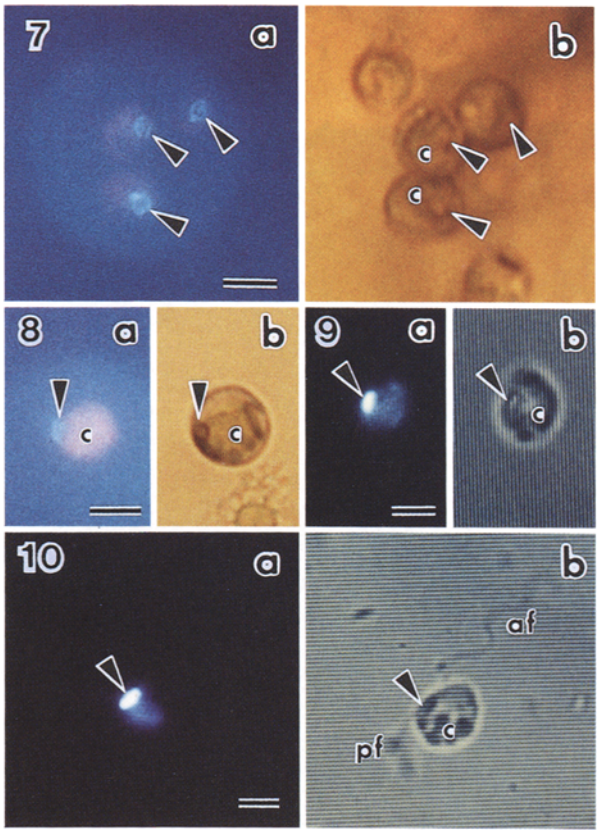
Figs. 5, 6. Transmission electronmicrographs of *Ectocarpus* gametes in longitudinal (**Fig. 5**) and cross (**Fig. 6**) sections through the flagellar swelling and stigma. *Ch*, chloroplast; *FS*, flagellar swelling; *M*, mitochondrion; *Nu*, nucleus; *P*, pyrenoid; *S*, Stigma; *arrow*, large osmiophilic globules composing the stigma; *arrowhead*, electron-dense material in the flagellar swelling

the stigma and its reflective property. In swimming cells, the reflected light from the stigma appeared as periodic flickering during the rotation of the cell. There was a circular darker region in the central part of the stigma (Fig. 7a).

Discussion

The action spectrum of phototaxis in *Ectocarpus* gametes generally agrees with that of *Pseudochorda* sp. zoospores (Kawai et al. 1990), with most effective wavelengths between 400 and 480 nm and a depression at 440 nm. However, in *Pseudochorda* shorter wavelengths, between 340 mm and 400 nm, were more effective. Müller et al. (1987) used the same strain as in the present work for a study on photoaccumulation. They found the effective wavelengths to be between 380 and 540 nm (maximum 430–450 nm). This agrees with our action spectrum, although the obvious depression near 440 nm was not observed. However, since energies were not measured in their experiments, the results can not be compared to detail.

The facts that lower-frequency stroboscopic light is not effective in eliciting phototaxis, and that the threshold frequency decreases when the rotation is slowed



Figs. 7a, b, 8a, b. Reflection of blue excitation light visualized in epifluorescence microscopy by the stigma (**a**) and the same cells in normal transmission illumination (**b**). Note the circular darker area in the reflected light of the stigma. Scale bar = 5 μm. *c*, chloroplast and its red autofluorescence; *arrowhead*, stigma

Figs. 9a, b, 10a, b. Light reflected from the stigma under epipolarization microscopy (**a**) and the same cell in transmission light illumination (**b**), photographed from the video screen. Scale bar = 5 μm *af*, anterior flagellum; *c*, chloroplast; *pf*, posterior flagellum; *arrowhead*, stigma

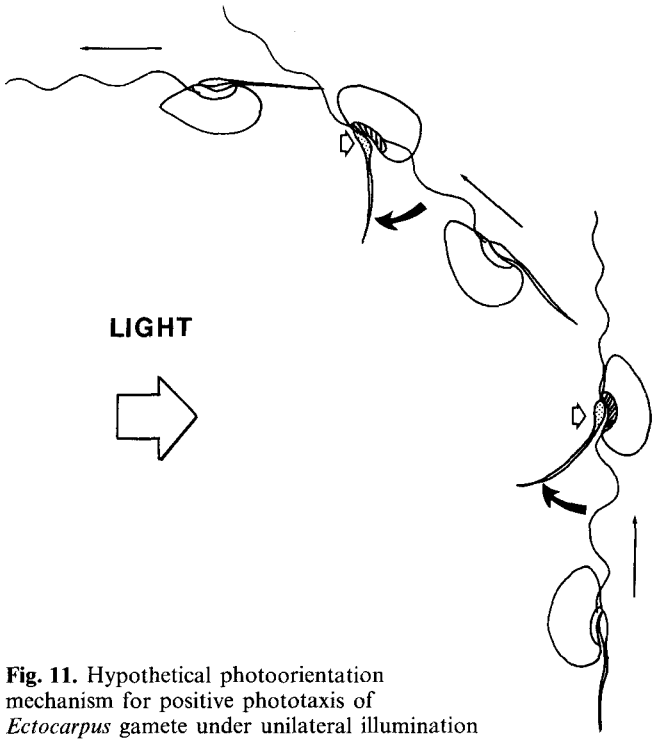


Fig. 11. Hypothetical photoorientation mechanism for positive phototaxis of *Ectocarpus* gamete under unilateral illumination

down by adding methyl cellulose, indicate that the rotation of the gamete is essential for the receptive mechanism. This fits with the requirements of the "periodic shading mechanism" (Buder 1919; Diehn 1973). In *Euglena*, the paraflagellar body located on the locomotive flagellum is suggested to contain flavins and to act as a photoreceptor. The stigma was thought to act as a shading device which can cast a periodic shadow onto the paraflagellar body during the rotation of the cell. Periodic shading of the photoreceptor could be recognized by the cell as a signal and results in changes of the flagellar beat pattern to correct the swimming direction. However, recently Häder et al. (1986) and Häder (1987) showed evidence which argues against the periodic-shading mechanism in *Euglena* and proposed that the cells orient using a dichroic orientation of the photoreceptor pigments.

The spatial arrangement of the structures in *Ectocarpus* has superficial similarities with *Euglena*, but in *Euglena* the stigma is located in the cytoplasm facing the reservoir, and is not associated with a chloroplast. Furthermore, the stigma of *Ectocarpus* is reflective, while in *Euglena* it is not (Walne and Arnold 1967). Judging from the morphological situation the concave face of the stigma in *Ectocarpus* could focus the reflected light onto the flagellar swelling. The circular darker area in the reflected light from the stigma may be caused by the fact that less light is reflected to the observer from the concave area compared with that from the outer flat area, due to the focusing effect. Chloroplasts in brown algae, which are associated with a flagellar swelling, can have a shading function. In contrast, the stigma of the *Ectocarpus* gamete is considered to act as a reflector to focus the light to the side of the flagellar swelling facing the stigma, instead of being a shading device. A similar reflective function is reported in *Chlamydomonas* and *Volvox*, although their flagella are not associated with the stigma and have no flagellar swelling (Foster and Smyth 1980).

The asymmetrically localized electron-dense material in the flagellar swelling of *Ectocarpus* may play an important role in light perception. Such electron-dense materials are found widely in Phaeophyceae, as well as in Chrysophyceae, Xanthophyceae, Eustigmatophyceae and Euglenophyceae, and are suggested to be involved in photoreception (Dodge 1875; Moestrup 1982). On the other hand, there is evidence that the posterior flagellum of phototactic flagellate cells of brown algae contains a flavin-like substance in its entire length (Müller et al. 1987; Kawai 1988), which could be involved in photoreception. The electron-dense material may possibly represent a specialized form (e.g. protein-bound complexes) of the flavin-like substance.

In the chemotactic responses, *Ectocarpus* gametes are known to change their swimming direction by deflec-

tions of the posterior flagellum (Geller and Müller 1981). If the turning reaction in photoaxis is based on the same mechanism as in chemotaxis, photoorientation of *Ectocarpus* gametes may be explained as follows: When the swimming path of the cell is parallel to the axis of the light, the flagellar swelling is not illuminated by the reflected light from the stigma. In this situation, the posterior flagellum keeps its normal passive position and the cell swims straight forward. When the swimming path is sufficiently inclined to the light direction, the flagellar swelling is illuminated periodically by the focused, reflected light from the stigma. This stimulation causes the rapid deflection of the posterior flagellum, which results in a change of the swimming direction. If the turning occurs without delay after the pulse illumination by the reflected light, the cell will turn toward the light direction (Fig. 11). The results of bilateral-illumination experiments agree with this hypothesis since, if the gametes change their swimming directions repeatedly to both of the stimulation lights ($\pm 45°$, $\pm 60°$), the swimming track will be on the resultant between the two light beams.

This hypothesis does not explain the observations that some cells changed their swimming directions between positive and negative phototaxis under unilateral illumination. Further information on this topic may be obtained with a species which shows exclusively negative phototaxis.

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