Phototactic Responses of *Chlamydomonas eugametos* Gametes Before and After Fusion

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Abstract. The phototactic behavior of *Chlamydomonas eugametos* gametes and vis-à-vis pairs was quantitated using a fully automated, computer-controlled microvideo image analysis system. Two different mt^- (mating type minus) and one mt^+ (mating type plus) strain, together with the two combinations of pairs were studied. One mt^- strain of dark-adapted gametes was non-phototactic while the others were positively phototactic at all effective intensities of white light. The mt^+ strain exhibited one of the strongest positive responses that has so far been reported in algae (r-values > 0.7). After sexual fusion, the mt^+ cell powers the swimming vis-à-vis pair. Its phototactic behavior reversed on fusion, with the pairs swimming away from all effective light intensities, irrespective of whether its partner was formerly phototactic or not. However, when adapted to the dark for an hour or more, vis-à-vis pairs swam positively to the light. The ecological consequence could be that pairs settle and develop into zygotes under intermediate light intensities or at light-dark interfaces.

Key words: *Chlamydomonas eugametos* — Phototaxis — Image analysis — Sexual fusion — Vis-à-vis pairs

Introduction

One of the favourite single-celled green algae for studying phototaxis is the biflagellate *Chlamydomonas* (for a recent review, see Nultsch and Häder 1988). Usually *C. reinhardtii* is used, but an alternative is *C. eugametos*. It is interesting because it offers the possibility of studying phototaxis in gametes before and

Abbreviations: $mt^{+\,-}$ — mating type plus or minus

after cell fusion, for the gametes only fuse partially to form cell tandems that remain motile for several hours after fusion.

At the start of sexual reproduction, when mt^+ (mating type plus) and mt^- (mating type minus) gametes are mixed, they adhere together via their flagella (van den Ende et al. 1988). They form clumps of agglutinating cells, in which pairs sort themselves out and fuse together by means of protoplasmic papillae that protrude from the anterior end of the cell bodies. After fusion, each cell-tandem loses its flagellar adhesiveness, the original mt^+ cell regains its normal beating (Lewin 1952) and the so-called vis-à-vis pair swims away from the gametes that are still agglutinated. Only several hours later the motile pairs settle to the substrate, retract their flagella and slowly undergo plasmogamy to form primary zygotes. Both the free-swimming gametes and the vis-à-vis pairs are phototactic, but as we shall illustrate, they respond very differently to light.

Light is an important factor in the sexual reproduction of *C. eugametos* and at least three different stages in the reproductive cycle are dependent upon light. Firstly, the sexual competence of the gametes of many strains is light-dependent in that they are non-agglutinable in the dark and thus unable to recognize and bind to a potential partner (Kooijman et al. 1986). Secondly, vis-à-vis pairs kept in the dark remain motile and fail to develop into primary zygotes (Lewin 1957). Thirdly, zygotes matured in the dark only undergo meiosis and germinate when returned to the light (Gowans 1960).

Phototaxis in gametes could also be important for their reproduction, for they have to be present in densities above 2×10^5 cells/ml for sexual contacts to result in stable unions (Tomson et al. 1986). Thus, any phenomenon such as phototaxis or gravitaxis (Kessler 1985) that helps to concentrate the cells in their aqueous environment can promote sexual reproduction. Even after cell fusion, phototaxis appears to be important for further development because the motile mt^+ partner in the pairs changes its photoresponse, and consequently the pairs swim away from the gametes and become concentrated in other locations where they aggregate and develop into zygotes (Musgrave et al. 1985). For example, the mt^+ gamete strain used in this study is positively phototactic, but upon fusion with the mt^- gamete it becomes negatively phototactic. Thus, within minutes of cell fusion, the response of the mt^+ gamete is completely reversed and this seems to happen irrespective of the phototactic behavior of the mt^- gamete with which it fuses.

Materials and Methods

Cell cultures

The mt^+ strain 17.17.2 and the mt^- strain 5.39.4 were created as described by Schuring et al. (1987).

The mt⁻ strain UTEX 793 was obtained from the Algal Collection of the University of Texas at Austin, USA. All strains were cultured on an agar-containing mineral medium under a light intensity of 4 klx provided by Philips TL65 W/33 fluorescent tubes, using a 12 h light/ 12 h dark regime (Mesland et al. 1976). Gamete suspensions were obtained by flooding 2- to 4-week-old cultures at room temperature with distilled water. Gametes used for phototaxis experiments were diluted in sterilised pond water (final concentration 2 – 5 × 10^5 cells/ml) and equilibrated for 30 min at 16° C, in the dark.

To obtain vis-à-vis pairs, the mt⁺ strain 17.17.2 was mixed with either of the mt⁻ strains in 14 cm plastic Petri dishes. The dishes were then placed in dim light. The pairs that were formed during the following 3 – 4 h swam to the darker side of the dish, while the non-fused, free-swimming gametes swam towards the light. The pairs then slowly aggregated on the plastic in large clusters in the darker half of the dish (Musgrave et al. 1985). Pairs were removed from these patches with a Pasteur pipette, collected into a beaker, diluted in pond water and maintained in the laboratory under fluorescent room lighting.

Determination of phototaxis

The orientation of swimming cells was determined by using a fully automated, computer-controlled micro-video image analysis system (Häder and Lebert 1985). Dark-adapted cells at 16° C were unilaterally illuminated in a flat glass cuvette (75 mm × 18 mm × 0.17 mm, inner dimensions) on the stage of a light microscope (Zeiss Universal, Oberkochen, FRG). The cells were observed using an infrared dark field technique (built-in microscope light source with an infrared passing cut-off filter RG 715, Schott & Gen., Mainz, FRG) in order to enhance the optical contrast and to avoid any influence of the monitoring beam on the cells. Care was taken to avoid trapping any air within the cuvette that could lead to an aerotactic response.

The video image was digitized in real time with a spatial resolution of 512 × 512 pixels at 256 possible grey levels and stored in a dedicated video memory on the digitizer to which a microcomputer had access. The software was developed in Assembly language in order to allow high speed analysis and was designed to select a cell at random and follow it for a predetermined period in order to analyze its movement vector as a deviation angle from the direction of the light source. The data were stored in a disk file for a later statistical analysis (Mardia 1972; Batschelet 1981; Häder and Lipson 1986).

The phototactic light was produced from a 250 W halogen projector (Prado, Leitz, Wetzlar, FRG) in combination with a heat absorbing filter (KG 2, Schott & Gen.). The illuminance level was selected using neutral density absorbing type filters (Schott & Gen.) and measured using a luxmeter (Mavolux digital, Gossen, Erlangen, FRG) previously calibrated against a photomultiplier (PM217 F) connected to a power supply (IL 760) and a radiometer (IL 1500, all from International Light).

Results

Figure 1 illustrates the general appearance (*A*) and phototactic behaviour (*B*) of the *C. eugametos* cells. When a suspension of cells was placed near a window, some gamete strains were attracted to the light (Fig. 1*B*, *left*) while others were nonresponsive (Fig. 1*B*, *right*). In contrast, vis-à-vis pairs always seemed to be repelled by the light (Fig. 1*B*, *middle*) and this has been how we previously

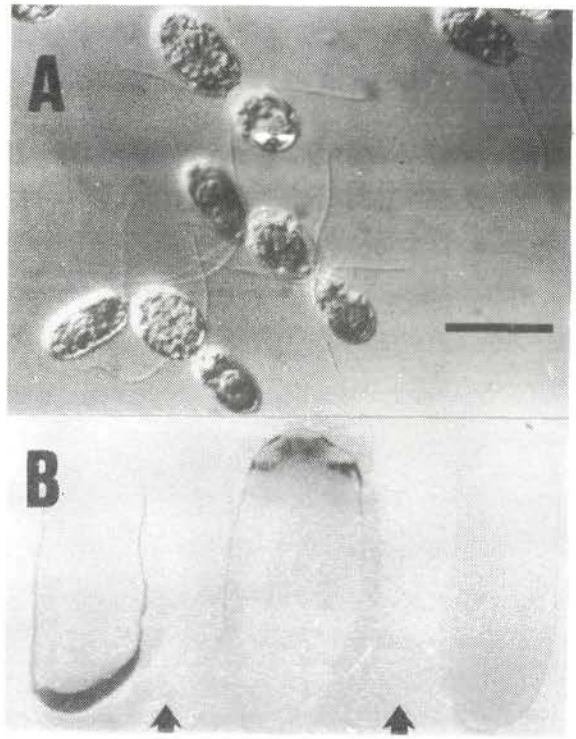

Fig. 1. *Chlamydomonas eugametos* gametes together with vis-à-vis pairs (*A*). Bar = 10 μm. When a suspension of strain 17.17.2 (mt$^+$) gametes was placed on a microscope slide exposed to daylight, they concentrated at the edge of the drop nearest to the light source (*B, left*). In contrast, gametes of the strain 5.39.4 (mt$^-$) were non-responsive (*B, right*), while pairs formed from these gametes became concentrated away from the light (*B, middle*). Arrows represent the direction of light.

separated vis-à-vis pairs from free swimming gametes (Musgrave et al. 1985).

In order to quantitate phototactic responses, the direction in which individual cells swam was recorded, compiled and automatically analyzed (Häder and Lebert 1985) with respect to the position of a unilateral light source. The data are represented in circular histograms as shown in Fig. 2. Since each graph is the summation of the orientation of several hundreds of cells, it represents the behaviour of the cell population in question. The histograms of cells swimming in the dark showed a random distribution, irrespective of whether the cells had fused or not. Thus, in the dark, cells did not exhibit a preference for a particular direction (Fig. 2, *left*). In contrast, the histograms of cells subjected to unilateral light were strongly biased towards or away from the direction of the light

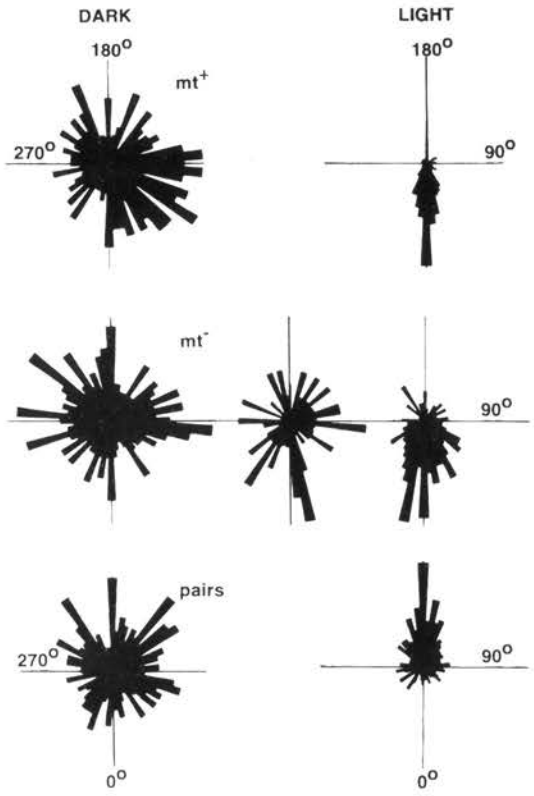

Fig. 2. Circular histograms of *C. eugametos* gametes and vis-à-vis pairs showing the orientation of swimming cells in the dark or when exposed to a unilateral light source of 50 klx at 0°. When illuminated, the mt^+ strain 17.17.2 responded within seconds and swam towards the light source. Under similar conditions, the mt strain UTEX 793 did not respond during the first 60 s of illumination (middle histogram) but responded positively thereafter. Vis-à-vis pairs (17.17.2 × UTEX 793) were maintained under fluorescent room lights, but when introduced into the experimental system and subjected to unilateral illumination, they responded quickly, swimming away from the light. The tracks of more than 300 cells were binned in 64 sectors for each treatment.

(Fig. 2, *right*, 0° represents the direction of the light). The mt^+ strain was strongly attracted to the light source, while the vis-à-vis pairs, which were propelled by this same mt^+ cell, were clearly repelled by the light. The response of the mt^- strain (UTEX 793) was more complicated. When transferred from the dark to the microscope stage in the light, the cells continued to swim at random for the first minute of illumination (Fig. 2, *middle*) and only thereafter

tively to a unilateral light source, as already described, but after 30 min in the dark, the same population became non-responsive, while a longer period resulted in more of the pairs swimming positively to the light. Thus, while cell fusion immediately reversed the nature of the photoresponse of the mt^+ gamete, it was not permanent. Pairs first swam away from the light but once in darkness, they slowly reversed and became positively phototactic.

Discussion

C. eugametos gametes are fast-swimming cells suitable for studying phototaxis. The mt^+ strain used here (17.17.2) exhibited the strongest positive response that we have ever witnessed, with *r*-values above 0.7. After dark-adaptation, none of the three gamete strains showed a negative response even at high fluence rates. In this respect, *C. eugametos* resembles some marine dinoflagellates (Ekelund and Häder 1988) but differs from vegetative cells of its closer relative *C. reinhardtii* which swim towards low intensity light but away from high intensity light (Feinleib and Curry 1971; Stavis and Hirschberg 1973). If *C. eugametos* gametes swim towards high light intensities and accumulate in the surface water layers, there could be deleterious effects due to photobleaching and UV-damage but, on the other hand, it could concentrate the cells above 2×10^5/ml and thus promote sexual reproduction (Tomson et al. 1986). In this way, phototactic strains have a reproductive advantage over non-responsive strains. Many *C. eugametos* gametes are non-agglutinable in the dark because their flagellar agglutinin is inactive (Kooijman et al. 1986). This means that at the start of each new light period, sexual activity is promoted by gametes becoming agglutinable and phototactically concentrated at the same time. It is also promoted indirectly by the light regime, which imposes a diurnal rhythm on the cells. Gametes reach a peak of sexual competence within the first few hours of the light period (Demets et al. 1987), their competence then declines, only to recover in the dark period. Of course, if the cells are light-sensitive, the recovery of sexual competence is only expressed when they are illuminated. It seems that sexual reproduction is strongly geared to start at daybreak. We do not know why, but the differentiation of vis-à-vis pairs into zygotes is also light-dependent (Lewin 1957), and it may be important to reach a critical stage of zygote development within the first day.

The main purpose of this paper is to bring attention to the change in phototactic behaviour in mt^+ gametes of *C. eugametos* on cell fusion. As far as we are aware, similar phototactic reversals have only been reported for gametes of *Ulva* and other green algae (Haupt 1959). The behaviour of *C. reinhardtii* gametes and dikaryons has been described (Hirschberg and Hutchinson 1980a);

in this species however, gametes fuse immediately to form a quadriflagellate cell and the phototactic behaviour of the latter is intermediate between that of its parents. *C. eugametos* pairs are therefore unusual and worth of more attention, especially since they can so easily be produced in various combinations and, due to their unusual phototactic response and their tendency to accumulate in dense patches, they are easily purified in large numbers. They are intriguing because they would seem to possess the photo-detection and transduction systems of both gametes, yet they can only be expressed via the movement of the mt^+ flagella. The strains used were those popular for studying cell recognition but many others are available (Schuring et al. 1987) even though their phototactic behavior has not yet been determined.

The response of the mt^+ strain was dramatically reversed on fusion with either of its partners, becoming negatively phototactic. To our suprise, when these pairs were kept in the light, they swam away from all effective light sources. This suggested that pairs seek very low light intensities, which was unexpected because they need light to develop into zygotes (Lewin 1957). This paradox is explained by the pairs adapting to the dark and becoming positively phototactic. Before developing into zygotes, pairs settle to the substrate. In our experience, pairs do not settle in the lightest part of the culture. Therefore we assume that the positive photoresponse seen on dark-adaptation can again be modified in the light. Since pairs swim more slowly with age, especially in the light, this behavior will result in their settling down in light of intermediate intensities, or at light/dark interfaces.

In *C. reinhardtii* phototactic reversals as shown here are also known, for example, a positive response to intermediate light intensities is reveresed if the cells are exposed to higher intensities (Feinleib and Curry 1971) or if they are treated in the presence of chlorpromazine (Hirschberg and Hutchinson 1980b). This treatment is known to affect the intracellular Ca^{2+} concentration in *C. reinhardtii* (Hutchinson and Hirschberg 1985) and indeed, several studies have made it clear that the free calcium level not only dictates the nature of flagellar movement (Hyams and Borisy 1978; Nultsch 1979; Bessen et al. 1980) but also the differential behavior of each cell's flagella dependent on whether they lie cis or trans in relation to the eyespot (Kamiya and Witman 1980; Morel-Laurens 1987). Thus, the change in behavior in the mt^+ gamete on fusion could reflect a change in the intracellular calcium environment. Another explanation comes from the presence of two photoreceptors in vis-à-vis pairs. If the mt^- light receptor with its transduction system is still operative, it will not lie "in phase" with the mt^+ flagella and this could cause the reversed orientation. However, this possibility does not account for the change in phototaxis when the mt^+ gamete was fused with a non-phototactic mt^- strain.

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