Negative photomovements of desmid *Micrasterias rotata* as response to strong light

Negativní fotopohyby u *Micrasterias rotata* (Desmidiales) jako odpověď na silné světlo

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Abstract

We describe two types of photomovement of *Micrasterias rotata* cells in the presented study. When using relatively high irradiance (145 μmol.m^{-2}.s^{-1}), about 30 % of tested cells reacted by negative phototaxis (gliding) or by rising to avoid strong incident light. No marked responses were recorded under lower irradiance (15 μmol.m^{-2}.s^{-1}) and in the darkness. Alternation of higher and lower irradiances resulted in a rising – laying down cycle. Moreover, light responses of this cycle markedly delayed when repeating irradiances alternation. Thus, for both tested photomovements we have observed primarily negative response of *Micrasterias rotata* cells, suggesting an adaptation to low light intensities habitats of this species.

Introduction

The unicellular green alga *Micrasterias rotata* (GREV.) RALFS (Desmidiales, Zygnematophyceae) seems to be a suitable desmid material for photomovement testing because of its size, shape and easy determination. Various experiments were carried out using genus *Micrasterias* as an experimental organism for the movement research (HÄDER & WENDEROTH 1977, LÜTZ-MEINDL & BROSCH-SALOMON 2000, NOSSAG & KASPRIK 1984, NOSSAG & KASPRIK 1993, WENDEROTH & HÄDER 1979). Those published papers dealt with photomovement of desmid cells or with intracellular movements (e.g. nuclear and chloroplast movements during cell division, vesicle and organelle transport, cytoplasm streaming).

There are 3 types of photomovement described by desmids: phototaxis, photokinesis and photophobic reactions. Phototaxis is an oriented movement in relation to the direction of light, as revealed by light traps experiments with

various desmid species in genera *Cosmarium*, *Euastrum* and *Micrasterias*. Cells move towards the applied white light and their response is light dependent. Photokinetic experiments showed increased velocity of movement with increased light intensity. Photophobic reactions are described as an active reorientation of cells at light-dark borders (HÄDER & WENDEROTH 1977) and generally defined as a quick reversal of movement induced by a sudden light intensity change (VAN DEN HOEK et al. 1995). When various wavelengths of light were tested in *Cosmarium* cells, there was a significant phototactic and photophobic response to the blue (443 nm) and red (545 nm) light with no substantial differences between them. There was only a weak response to the green light (668 nm). This phenomenon indicates that some chlorophyll pigments could serve as photoreceptors (WENDEROTH & HÄDER 1979). Concentric rings of cells were seen around light traps during the experiment, which indicated some circadian rhythm of the cell movement (HÄDER & WENDEROTH 1977, WENDEROTH & HÄDER 1979). Slime extrusion from one of the apical poles of the desmid cells is responsible for these movements generally described as gliding.

Gliding movement of *Micrasterias* cells is caused by slime secretion, which alternates from one or the other of two groups of pores at the left and right side of the polar lobe. It swells and pushes the cell forward resulting in the wavelike path of the cell (WENDEROTH 1983). Positioning (rising reaction) is another type of desmid cells movement. Orientation of flat *Micrasterias* cells in line with the direction of the strong blue light (390-450 nm) occurred in individual cells and it is time- and light intensity-dependent. Photoreceptors responsible for this reaction could possibly be flavonoid pigments because there is no remarkable response for the red light at 670 nm (NOSSAG & KASPRIK 1984). Slime production was not observed during positioning events (NOSSAG & KASPRIK 1993). Although various experiments have been carried out with movements of *Micrasterias* cells, intracellular mechanisms of both types of photomovement have not been studied in detail yet. It is known that both actin filaments and myosin are involved as kinetic proteins in cytokinetic chloroplast movements (OERTEL et al. 2003) or postmitotic nuclear migration (OERTEL et al. 2003, MEINDL et al. 1994). The presence of the actomyosin motor system in *Micrasterias* cells indicates its possible role in both main types of photomovement.

In the present study we tested photomovement reactions (rising and gliding) of *Micrasterias rotata* cells to two various intensities of light. We presume different reactions of cells with respect to light intensities and generally negative response to strong light considering the light intensity conditions in the natural habitat.

Material and methods

The experimental alga *Micrasterias rotata* (GREV.) RALFS was isolated from a peat pool in the Úpolínová louka natural reservation in the Slavkovský les region (western Bohemia). Algae were maintained in a sterilized natural medium, which was obtained directly from the locality. For the movement experiments a simply micro-chamber was constructed. It consisted of two microscope slides knit by epoxy resin to leave the gap with diameter of 0,2 mm. The suspension of *M. rotata* cells has been pretreated in the dark for 2 days to increase the sensitivity (HÄDER & WENDEROTH 1977) and loaded to the micro-chamber with a micropipette. The chamber was filled with the medium described above and sealed by the paraffin film. The cells were observed in OLYMPUS BX 60 microscope at constant temperature of 23.5 ± 0.5 °C. The microscope light source was used for the illumination (light direction perpendicular to the micro-chamber) and two irradiance levels were adjusted, 145 or 15 $\mu mol.m^{-2}.s^{-1}$. The irradiance was measured using Luxmetr PU 550 equipped with an appropriate light sensor. All photographs were taken with the digital camera OLYMPUS CAMEDIA C-5050 ZOOM.

Results and Discussion

Two types of *Micrasterias rotata* cell movement were observed: positioning (rising movement) and gliding. The positioning movement was manifested as a turning of the flat cell through 45-90º to the incident light mostly around its longer axis, sporadically around the shorter one. Gliding movement was observed as a shift of the cell to certain direction in the wavelike path. Both types of these movements were caused by light with markedly higher intensity (145 $\mu mol.m^{-2}.s^{-1}$) than measured in the natural peat habitats of *Micrasterias rotata* (10-60 $\mu mol.m^{-2}.s^{-1}$).

When we used relatively higher irradiance (145 $\mu mol.m^{-2}.s^{-1}$), one third of cells responded by rising. This is in agreement with previous experiments (NOSSAG & KASPRIK 1984, NOSSAG & KASPRIK 1993) on *Micrasterias thomasiana* where the increase of risen cells ratio was observed with augmentation of the blue light (450 nm) intensity. Maximum of risen cells (>30 %) occurred under maximal (60 $\mu mol.m^{-2}.s^{-1}$) light intensity used. Thus, rising movement is described as the strong-light response (NOSSAG & KASPRIK 1984). Moreover, when various strains of *Micrasterias thomasiana* were tested under similar conditions, most of strains responded by rising with 30-40% efficiency. It is believed to be a reaction to photooxidation and the strain-specific response by *Micrasterias thomasiana* species was reported (NOSSAG & KASPRIK 1993). The rising movement was reversible; when we used lower irradiance (15 $\mu mol.m^{-2}.s^{-1}$) or darkness, cells laid down. The cycle of rising and laying down movement repeated with continued irradiance alternation (Fig. 1). Alternation of

cell orientation in response to light somewhat resembles photoperiodic (circadian) reactions of many algae and higher plants. It was found that *Chlamydomonas reinhardtii* altered its resistance to UV-light depending on light/dark cycles (SUZUKI & JOHNSON 2001). Moreover, we found that intervals between individual orientation changes prolonged markedly. Especially the "laying down" reaction under lower irradiance was much slower than rising (Fig. 2). Previously, the circadian flower opening cycle in *Kalanchoë blossfeldiana*, which was induced by light/dark alternation, was described (light-open, dark-close). Then the plants were kept at continuous darkness and opening cycle continued with lesser intensity (BÜNNING 1967).

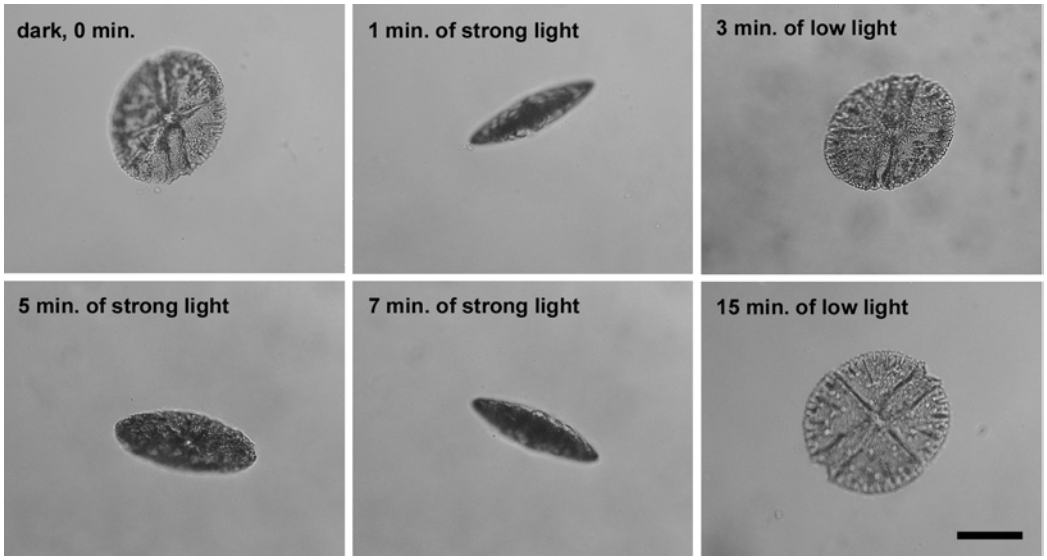

Fig. 1: Sequence of positioning movements of *Micrasterias rotata*. Cell orientation changes as a result of irradiance alternation (strong light = 145 μmol.m^{-2}.s^{-1}, low light = 15 μmol.m^{-2}.s^{-1}). The response time is markedly prolonged with repeating irradiance alternations. Bar represents 100 μm.

We observed a delay of rise/lay down movement as described above, nevertheless the cells transferred subsequently into darkness did not response (Fig. 3), *i.e.* no "time memory" was proven in *Micrasterias rotata*. Rising movement of *Micrasterias rotata* conspicuously resembles the reorientation movement of ribbon-shaped chloroplast in cylindrical cells of the genus *Mougeotia* (HAUPT 1959, WAGNER et al. 1984). In *Mougeotia* cells, the reorientation was induced by a pulse of red light (683 nm; 8,5 μmol.m^{-2}.s^{-1}) for 1 minute and allowed to proceed in darkness; the chloroplasts were reoriented within 5 minutes (WAGNER et al. 1984).

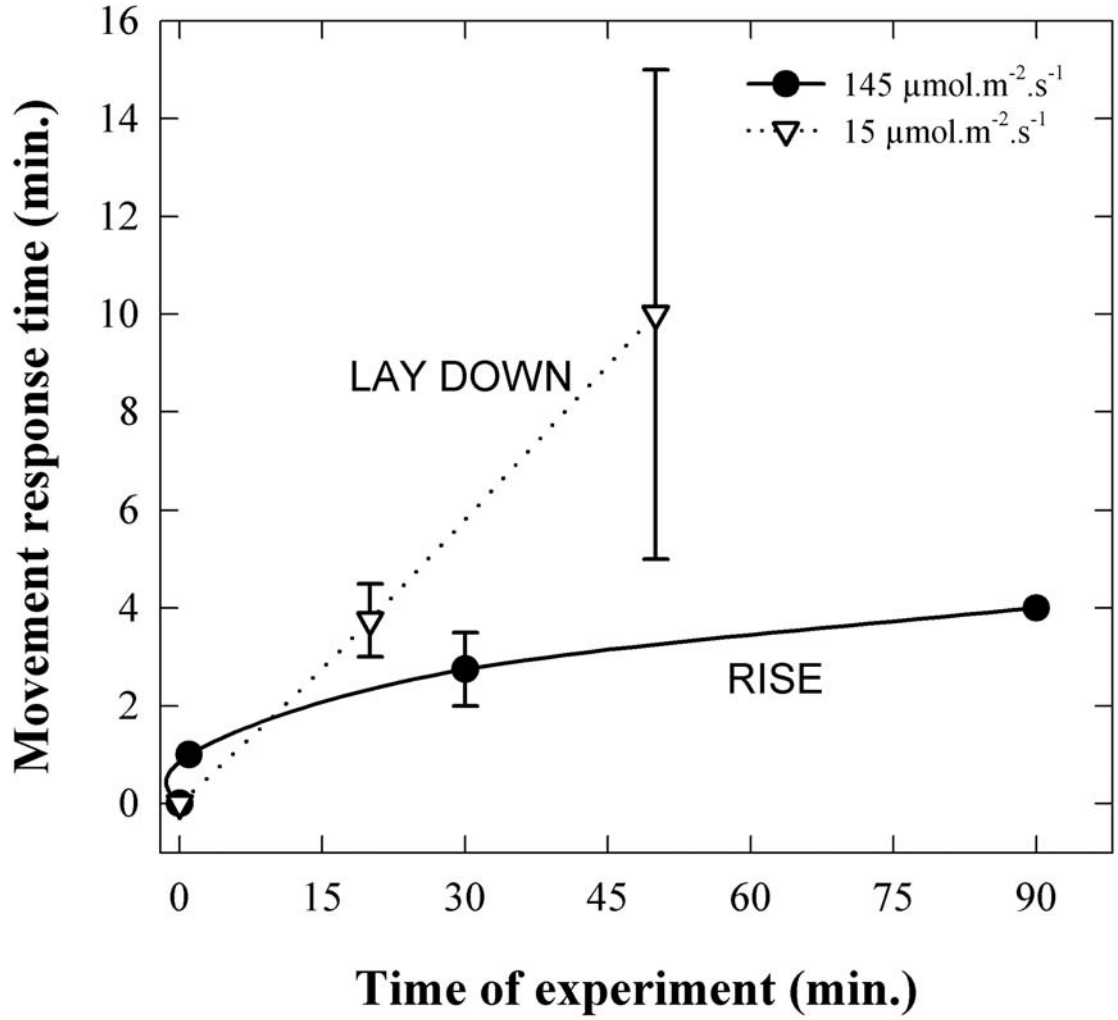

Fig. 2: The relationship between duration of experiment and time of *Micrasterias rotata* cell response to the light. Higher irradiance causes cell rising. Cells lay down repeatedly under lower irradiance. The delay of cell response is greater with time of movement experiment, especially in "laying down" reaction. Every point represents the time of experimental irradiance turnover.

Gliding movement was exclusively phototropic negative, cells moved from the most irradiated field away only after higher irradiance treatment. On the contrary, other authors established the positive phototropic movement of various desmid species. Positive phototropic reaction of *Cosmarium cucumis* was found under less than 5 µmol.m^{-2}.s^{-1} of both blue and red light (WENDEROTH & HÄDER 1979), similarly *Micrasterias truncata* moved to the white light trap of 20 µmol.m^{-2}.s^{-1} (HÄDER & WENDEROTH 1977). This opposite findings could be explained by markedly higher irradiance we used for *Micrasterias rotata*. The usual velocity of gliding movement we found was about 1,7 µm.s^{-1} (Fig. 4a). This value was higher than described previously in *Micrasterias denticulata*: 0,5 µm.s^{-1} (NEUSCHELER 1967) and *Micrasterias rotata*: 0,8 µm.s^{-1} (BENDIX 1960).

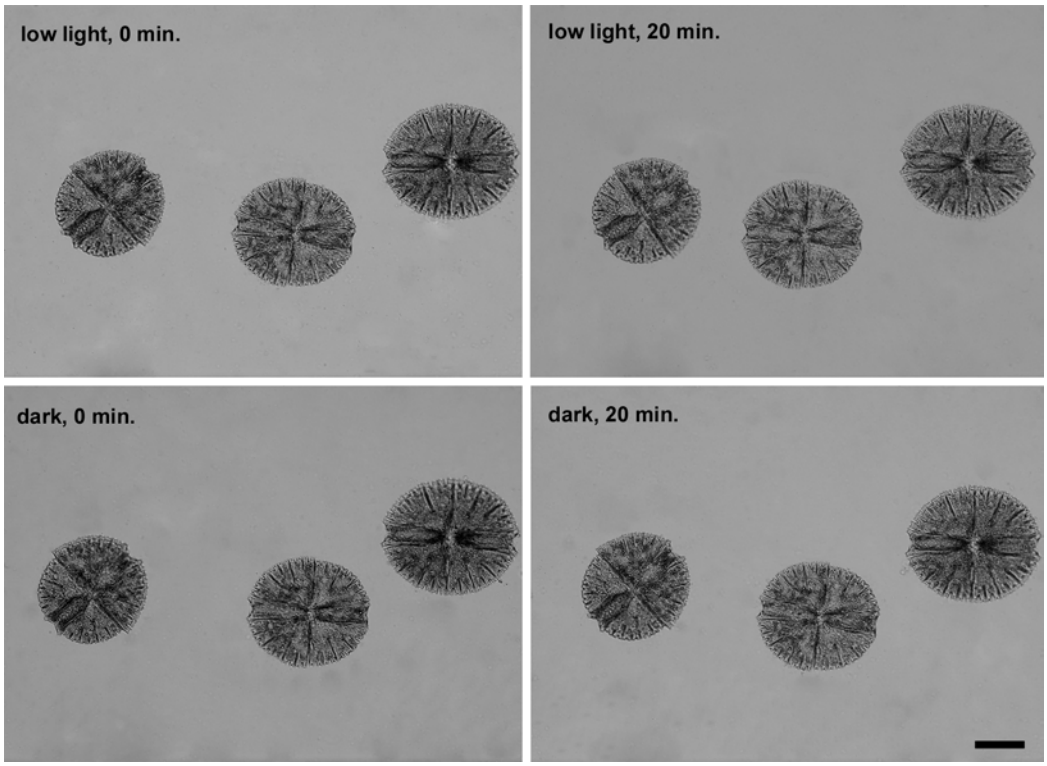

Fig. 3: Control experiment. No marked gliding movement of *Micrasterias rotata* cells occurs under lower irradiance (15 μmol.m^{-2}.s^{-1}) or in the darkness. Bar represents 100 μm.

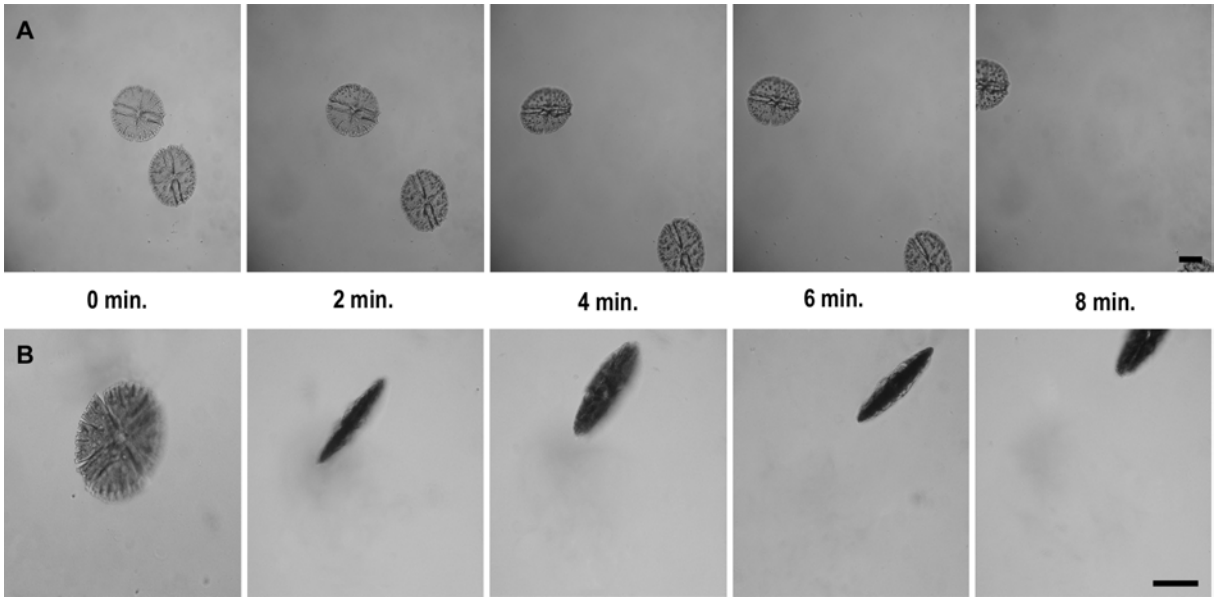

Fig. 4: Time sequence of *Micrasterias rotata* cells gliding movement as a result of irradiance of 145 μmol.m^{-2}.s^{-1}. Cells usually move from the most irradiated field (center of photographs) away in the horizontal orientation (a), more rarely in the risen position (b). Bars represent 100 μm.

It was possibly caused by sensitivity enhancement as a result of long pretreatment in the darkness. Moreover, we observed gliding movement at the risen position of cells also as described above (Fig. 4b). In this case, both of these movement types occurred simultaneously. The control of these movements (gliding or positioning) remains still unclear. Similarly, various angles to the substratum were ascertained during gliding movement (NEUSCHELER 1967).

We observed neither directed gliding nor rising movement when using lower irradiance (15 μmol.m^{-2}.s^{-1}) within 20 minutes; cells just slightly oscillated on their places. As found in *Micrasterias thomasiana* cells, a minimal irradiance required for recognizable rising movement was 10 μmol.m^{-2}.s^{-1}; this differential sensitivity could be species and/or strain dependent or due to experimental conditions (NOSSAG & KASPRIK 1984). Control experiment in the darkness revealed no remarkable movement reactions, cells did not rise and no gliding movement of cells was observed within 20 minutes or longer (Fig. 3).

We described two types of photomovement, positioning (rising) and gliding. Both movements seem to be an ecological strategy of strong light avoidance. It is evident that sphagnophil species *Micrasterias rotata* occupying commonly metaphyton desmid communities prefers low light conditions and moves in accordance to its demands. Although the gliding movement of *Micrasterias* cells caused by slime excretion is well described, detailed mechanisms of positioning movements remain still unclear and need some onward studies.

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