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Bimodal Polarotropism of *Vaucheria* to Polarized Blue Light: Parallel Polarotropism at High Fluence Rate Corresponds to Negative Phototropism

Hironao Kataoka, Fumio Takahashi and Tamotsu Ootaki

Institute of Genetic Ecology, Tohoku University, Aoba-ku, Sendai, 980-8577 Japan

Species of *Vaucheria* (Xanthophyceae) exhibited cruciform polarotropism when they were grown under polarized white or blue light for several days. The coexistence of two groups of branches growing perpendicular and parallel to the electric vector (*E*-vector) resulted in cruciform polarotropic orientation. Such polarotropic bending was, however, not detected within 24 hr. As the fluence rate of polarized white or blue light increased, parallel orientation to the *E*-vector became dominant. Polarized red light produced exclusively perpendicular polarotropism. This shift in pattern was much obvious in *V. terrestris* sensu Götz than *V. sessilis* and *V. dichotoma*. Since the photoperception is restricted to the tip of the apical dome and since this region receives maximum photons when the *E*-vector is at a right angle to the cell axis, *Vaucheria* becomes oriented normal to the *E*-vector as far as the fluence rate is optimum. The direction of growth is expected to change into parallel to the *E*-vector when the fluence rate is supraoptimum. The perpendicular (normal) and parallel polarotropism of *Vaucheria*, thus, correspond to positive and negative phototropism, respectively. Orientation of photoreceptor molecules is suggested to be predominantly parallel to the surface of the apical dome.

Key words: Blue light — Phototropism (alga) — Positive and negative phototropism — Polarotropism — Tip growth *Vaucheria*

The bending response of plants and fungi to the direction of the *E*-vector of linear polarized light is called polarotropism (Bünning and Etzold 1958, Etzold 1965, Hartmann *et al.* 1965, Wada and Kadota 1989, Dennison 1979). Polarotropism has frequently been observed and analyzed in nonvascular plants, particularly in fern and moss protonemal cells (Bittisnich and Williamson 1985, Hartmann and Weber 1990, Jaffe and Etzold 1962, Jenkins and Cove 1983 a, b, Kadota *et al.* 1984, 1985, Wada and Kadota 1989). The tip-growing protonemal cells bend perpendicularly to the *E*-vector of blue and red light, whereas the rhizoid cells bend parallel to the *E*-vector of such lights. The polarotropic orientation of protonemata perpendicular to the *E*-vector obviously corresponds to its positive phototropic bending (Etzold 1965). The apical dome of the protonemal cell is not only the site of photoreception but also the site of tip growth and bending. When unilateral light is shone on the flank of the dome, the center of growth quickly shifts to the irradiated side. This indicates that there is a positive regulation system: i.e., the geometrical distribution of the light absorption coincides with that of growth activity. The polarotropism of the protonemal cell perpendicular to the *E*-vector can be explained, if it is assumed that the surface parallel to the direction of the *E*-vector receives more photons.

Since flavins, phytochromes and many other pigment molecules respectively have one maximum light absorption axis at a wavelength, the existence of any difference in response size between the direction of vibration of the *E*-vectors is an indication of these molecules being oriented parallel to each other in a fixed array. Since plasmalemma and cortical cytoplasmic layer are the most stable two-dimensional structures of plant cells, the existence of an action dichroism is evidence that the localization of photoreceptor molecules is either parallel or orthogonal to the plasmalemma or cortical gel layer of the protoplasm (Dennison 1979, Schmidt 1980). The perpendicular polarotropism of tip-growing cells is a good indication of the parallel orientation of absorption dipoles of the photoreceptor molecules to the apical plasmalemma.

We attempted to detect a polarotropic response in *Vaucheria*, because we had previously analyzed the phototropic response in some detail and found that *Vaucheria* apparently lacks a phytochrome system (Kataoka 1975a, b, 1977, 1979, 1981, 1987). If *Vaucheria* showed polarotropism, it would be a more suitable material than fern protonemata for studying the localization and the orientation of blue light (BL) absorbing photoreceptors. However, no sign of action dichroism was detected in the short-term (i.e., <2 hr irradiation) experiments. Why was action dichroism not detected in short-term experiments? There are two possibilities 1) Photoreceptor molecules are randomly oriented in the cell surface. 2) During active growth, the apical plasmalemma is not a smooth sheet, but very rough while fusing with exocytotic vesicles (Kataoka 1991). As Zurzycki and Lełatko (1969)

Abbreviations: BL, blue light; NPI, normal (perpendicular) polarotropic index; PPI, parallel polarotropic index; *E*-vector, electric vector.

reported the existence of an action dichroism to polarized BL in high fluence rate movement of chloroplasts in *V. sessilis,* the first possibility can be discarded. The second possibility might be the reason for the lack of clear polarotropism. Thus, we resumed searches for polarotropism under continuous polarized light for longer than several days, and found that the thalli of *V. terrestris* sensu Götz grew into a cruciform mat. In the present report, this novel cruciform polarotropism of *Vaucheria* will be correlated with the positive and negative phototropic response.

Before going into the subject, however, it is necessary to briefly summarize the characteristics of phototropic responses of *Vaucheria*. The body of xanthophycean (Stramenopile) alga *Vaucheria* is composed of a sparsely branched coenocytic tube. At the apex of each branch, active tip growth and phototropism occur. Similar to the case of fern protonemata, all steps of the phototropic response between perception of the direction of light and manifestation of bulging occur in the apical 1/3 region of the apical hemispherical dome of individual branches. It is noteworthy, however, that the alga has the ability to change the sign of phototropism in accordance with changes in the fluence rate of incident light. Namely, it bends towards BL when the fluence rate is low, but away from the light when the intensity is higher than a critical value (Oltmanns 1892, Weber 1958, Kataoka 1988, 1991; kataoka and Weisenseel 1988, Kataoka and Watanabe 1993). Such ability allows this alga to accommodate itself to its habitat and to move away from strong sunlight. We have observed that the optimum light intensity for positive phototropism of *V. terrestris* sensu Götz was 6-8 Wm^{-2}, but differed depending not only on species and strains, but also on physiological status. Such difference may thus be ecologically significant in their habitats.

We have succeeded in analyzing the negative phototropism of *Vaucheria terrestris* sensu Götz under laboratory conditions within a short period of time (<15 min) by developing a "simultaneous background illumination method" (Kataoka 1988). Using this method we found that a BL-induced Ca^{2+} influx at the cell apex and the eventual (transient) increase in the cytoplasmic level of Ca^{2+} in the apical region were necessary for negative phototropism (Kataoka 1988, 1990). If the intensity of unilateral BL was higher than 200 Wm^{-2}, the supporting "background BL" was not necessary. In the presence of 4 mM Ca^{2+}, the negative bending was elicited by a 10-s laser pulse (457.9 nm) (Kataoka and Watanabe 1992, 1993). It is not known, however, whether the rise of the intracellular level of Ca^{2+} is also the cause of the negative phototropic bending observed in the growth cabinet after several days of irradiation. The cruciform polarotropism observed after prolonged irradiation appeared to be similar to the positive and negative phototropism observed in long-term experiments.

Materials and Methods

Plant material and culture conditions

Vaucheria terrestris sensu Götz var. *terrestris* [= *V. frigida* (Vauch.) C.A. Agardh (Christensen 1969, personal communication)], used in previous studies (Kataoka 1981, 1988, 1990), was also mainly used in the present study. The brackish species, *V. dichotoma* (L.) MARTIUS, collected from Kuhgraben See, near Bremen, Germany (Henschel et al. 1991, Henschel 1992), and a strain of *V. sessilis* isolated from a wet rock wall at Yamadera, Yamagata was also used for comparison.

The composition of the culture solution was the same as that previously described (Kataoka 1990). The algae had been subcultured aseptically in Erlenmeyer's flasks by periodic renewal of the culture solution for several years. The culture was made in a growth cabinet (NK-LP-300-S; Nihon Ikakikai, Osaka, Japan) at 20±1C under white fluorescent lamps (FL20SS-W/18; National, Osaka, Japan; ca. 8 Wm^{-2}) with a 14-hr light/10-hr dark regime (the dark period commenced at 06 : 00 J.S.T.).

Polarotropism

Polarotropic responses were usually observed and analyzed after culturing algae on agar plates, except for the preliminary short-term irradiation experiments where the alga growing in a Petri dish was irradiated and observed with an inverted microscope (see Kataoka 1975a). The culture medium was solidified with 1% agar (Bacto Agar, Difco Lab., Detroit, MI, USA) in either Pyrex glass dishes or sterile polystyrene dishes (SH 90-20, Iwaki Glass, Tokyo, Japan). A pinch of thalli was placed in the center of the agar plate, a few drops of culture solution was poured onto the thalli, and the gap between dish and lid was sealed with a layer of Parafilm (American National Can, Chicago, IL, U.S.A.). The dishes were transferred to a custom-made temperature-controlled cabinet. White or blue fluorescent tubes were placed about 10 cm above the glass ceiling of the cabinet. White light was provided from white fluorescent lamps (FL20SS-W/18, Toshiba, Tokyo, Japan) and BL, from "pure blue" fluorescent lamps (FL21S-B-F, National, Osaka, Japan).

Inside the cabinet, the dishes were covered with a bottomless paper box (18×13 cm, 3-6 cm height) to shade them from the oblique light. The top of the box was a window made of a sheet of plastic polarizing filter (for illumination, Kenko, Tokyo, Japan). The distance between the polarizer and the dish was 0-3 cm. One layer of this polarizing filter transmits about 26% and 28% of white light and BL, respectively. With this setup, polarized light shone on alga from above. The desired fluence rate was obtained by changing the distance from the lamp. Fluence rate at the specimen level was measured with a custom-made radiometer equipped with a miniaturized thermopile (MIR-100Q, Mitsubishi Yuka, Tokyo, Japan) and expressed in Wm^{-2}. To avoid change in direction of growth during day and night cycles, polarized light was continued until the end of the experiment. The vibration plane of the *E*-vector lying in the 0°-180° direction was fixed during the experiment.

Most experiments were conducted using glass dishes, to avoid optical complexities, which otherwise might have affected the results. Since the lid of the polystyrene dish was birefringent (estimated maximum retardation after passing 0.9

mm thickness being about 600 nm), linearly polarized light was converted into elliptically polarized light of various directions and magnitudes when it reached the specimen. Nonetheless, some experiments conducted to compare glass and polystyrene dishes did not produce any significant differences.

Data analysis

After the irradiation experiment, dishes were taken out of the cabinet and photographed. The angles of the apical 5 mm of growing branches with the vibration plane of the *E*-vector were measured on photographic prints and recorded on circular histograms. The percent angular distributions of orientations were plotted on 12 sectors of the histogram. Figure 1 is a diagram showing the calculation of the polarotropic indices. The sum of percentages of branch distributions in the 0° and 180° sectors (hatched) were defined as the parallel polarotropic index (PPI or //), and that in the 90° and 270° sectors (black), as the normal (=perpendicular) polarotropic index (NPI or ⊥). (Normal means right angle; used only for practical purposes of avoiding confusion with PPI.) If the orientation is completely equal in all directions, NPI=PPI=16.7%.

The thalli often curved greatly and/or produced new branches during the experimental period. Since the extreme tip regions and the short, actively growing branches are the most recently grown, the apices oriented different directions from those of the proximal parts indicate that the direction of growth had changed several days before. Thus, in some figures, angular distributions determined from approximately 10 mm basal regions of the thalli (E, =early; white areas) are superimposed on those of the extreme tips (L,=late; black areas). Also, early, late and total PPIs are designated in figures as E//, L// and T//, respectively. Early, late and total NPIs are quite similar; namely, E⊥, L⊥, and T⊥. They are also expressed in the text as E-PPI, L-PPI, or T-PPI.

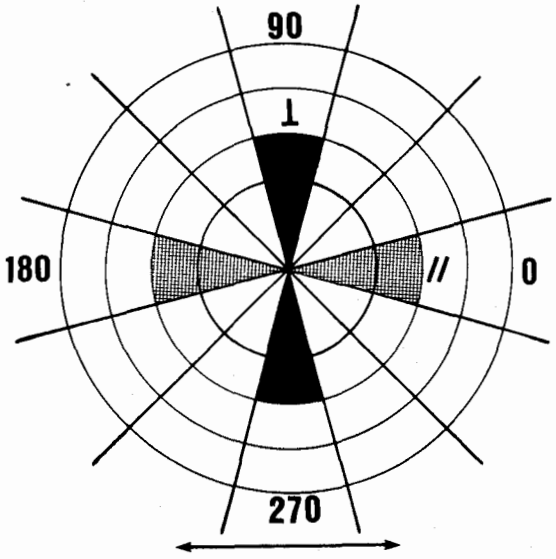


Fig. 1. Data analysis and histogram of polarotropic orientation. The percent angular distributions of orientations were plotted on 12 sectors of the histogram. The sum of percentages in the 0° and 180° sectors (dotted) were defined as the parallel polarotropic index (PPI or //), and that in the 90° and 270° sectors (black), as the normal (=perpendicular) polarotropic index (NPI or ⊥). If the orientation is completely equal in all directions, NPI=PPI=16.7%.

Results

Preliminary short-term experiments with polarized BL on a microscope stage.

The polarotropic response in *Vaucheria geminata* and *V. terrestris* sensu Götz has not heretofore been detected in short-term experiments with polarized light on a microscope stage. Figure 2 shows schematic sketches of the irradiation protocols and the results. The localization of BL photorece-

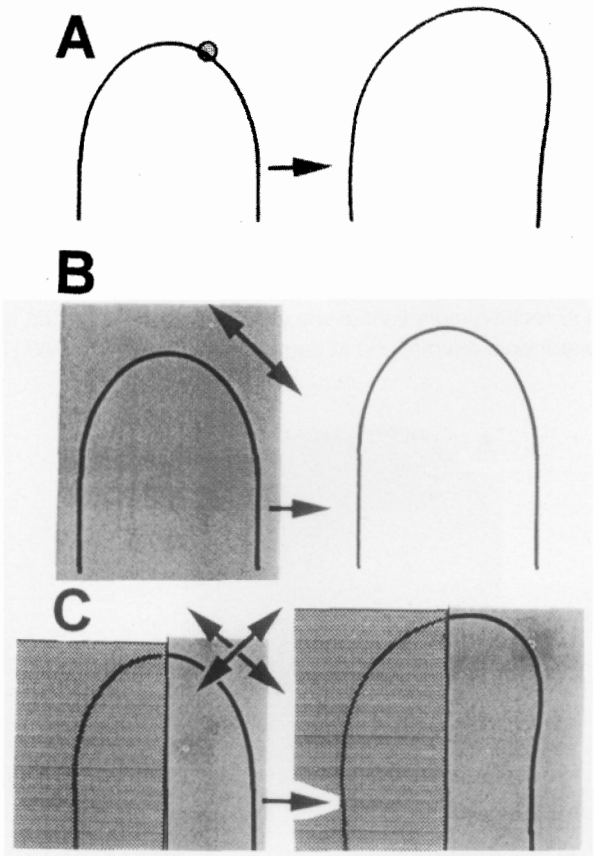


Fig. 2. Polarotropism of *Vaucheria* was not detected in short-term experiments. A: Investigation of phototropism. A BL-beam of moderate fluence rate skimming through the apical surface of *Vaucheria* induced a bending towards the lighted spot, indicating that the photoreceptor(s) resides at, or just beneath, the apical plasmalemma. B: Absence of any polarotropic curvature by a short (<20 min) pulse of polarized BL (<6 Wm^{-2}) whose *E*-vector rotated either +45° or −45°. C: Failure of polarotropic response by split-field illumination with a short pulse (<20 min) of polarized BL. Pale gray area: BL-irradiated region; Hatched area: shaded region. Double arrows represent direction of *E*-vectors.

ptors for phototropism in the outermost layer of the cytoplasm of the apical hemispherical dome has been suggested by microbeam irradiation experiments (Fig. 2A, Kataoka 1975a, 1980). Irradiation of the apex of *V. terrestris* sensu Götz with polarized BL (ca. 4 Wm^{-2}) of *E*-vectors orienting + 45° or −45° to the cell axis did not show any bending, even when the irradiation lasted for 2 hr (Fig. 2B). We then irradiated a longitudinal half of the growing apex of *V. terrestris* sensu Götz with polarized BL of two different *E*-vector directions (see Fig. 2C). In this setup the alga always bent towards the irradiated side (Kataoka 1975a). If the BL photoreceptors were oriented parallel to the cell surface, polarized BL, whose *E*-vector was parallel to the irradiated flank, would produce a larger curvature. However, no significant difference in curvature was detected.

Polarotropic orientation of several Vaucheria species under white light

In contrast to the short-term irradiation, prolonged polarized light irradiation for several days induced polarotropism. Thalli of *Vaucheria dichotoma* predominantly grew perpendicular to the *E*-vector when they were grown in a shallow layer of culture solution. Fig. 3 shows examples of the clear polarotropism of *V. dichotoma*. The growth of a few branches changed direction from parallel to perpendicular (Fig. 3A photograph, e: early, and l: later orientation). This may indicate that this alga adapted to strong light and changed its polarotropism to a perpendicular orientation so as to receive more light at the apex. These earlier and later polarotropic orientations in terms of the *E*-vector (0-180°) are demonstrated in the circular histogram. The inner white areas represent the earlier orientation and the outer black areas are for the later orientations; hence, the outer profile of the histogram indicates the total (i.e., sum of E and L) orientations. The total NPI (T⊥) and total PPI (T //) shown under the histogram indicate that as many as 70% of the total branches grew perpendicular to the *E*-vector of continuous polarized white light at a fluence rate of 4 Wm^{-2}.

Culturing in liquid medium is, however, very inconvenient to manage, especially when the specimens are taken out of the growth cabinet, because they are easily dislodged in the water. Therefore, experiments were mainly conducted on agar plates. As shown in Fig. 3B, *Vaucheria dichotoma* grew in a bimodal, cruciform orientation on the agar plate, even at the same fluence rate. Since perpendicular and parallel orientations were almost equal and there were fewer intermediate orientations, the shape of the colony and histogram became cruciform. The increase in parallel orientation was probably due to changes in optical conditions within the cell.

Ratios of perpendicular to parallel orientation were very different among species and strains. Fig. 4 presents examples of the polarotropic orientation pattern of three different species, *Vaucheria sessilis, V. terrestris* sensu Götz, and *V. dichotoma*, grown on agar plates under continuous white light of 2.9-3.6 Wm^{-2}. Obviously, *V. sessilis* collected in Yamagata, exhibits a very perpendicular polarotropic pattern. A different strain of *V. sessilis* (LB146) exhibited a similar orientation pattern, albeit quantitatively slightly different (data not shown). *V. terrestris* sensu Götz, and *V. dichotoma* showed cruciform patterns.

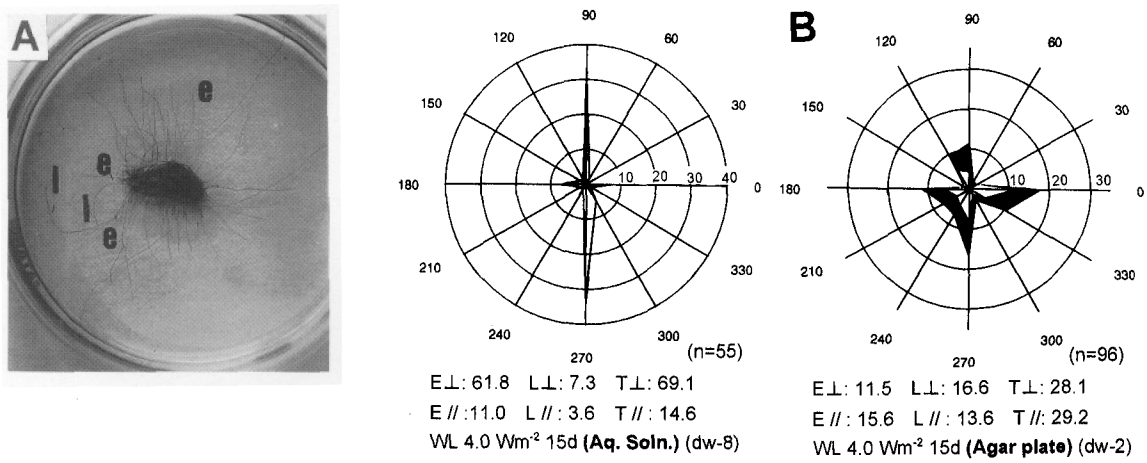


Fig. 3. Predominate perpendicular polarotropism of *Vaucheria dichotoma* in culture solution and on agar plate. A: *V. dichotoma* grown in a culture solution under polarized white light for 15 days. Perpendicular orientation is predominant. Most filaments grew fairly straight throughout the experimental period. Some other filaments changed their directions of growth during the experiment; in such cases, the orientations were counted as early (e) and late (l) orientations. Circular histogram of this particular example is shown on the right. B: Polarotropic orientation of *V. dichotoma* on agar plate. The fluence rate and duration of the experiment are the same as in A. Concentric circles represents 10, 20, 30 and 40%. Early and total (early+late) orientations are shown in white and black areas, respectively. Polarotropic indices and other data are described below each histogram. Numbers of branches used for the calculations are indicated in parentheses. E⊥: early NPI; L⊥: late NPI; T⊥: total NPI (=E⊥+L⊥). E//: early PPI; L//: late PPI; T//: total PPI (=E//+L//). WL: white light. Data identification codes are also represented in parentheses (e. g., d: *V. dichotoma*; w: white light).

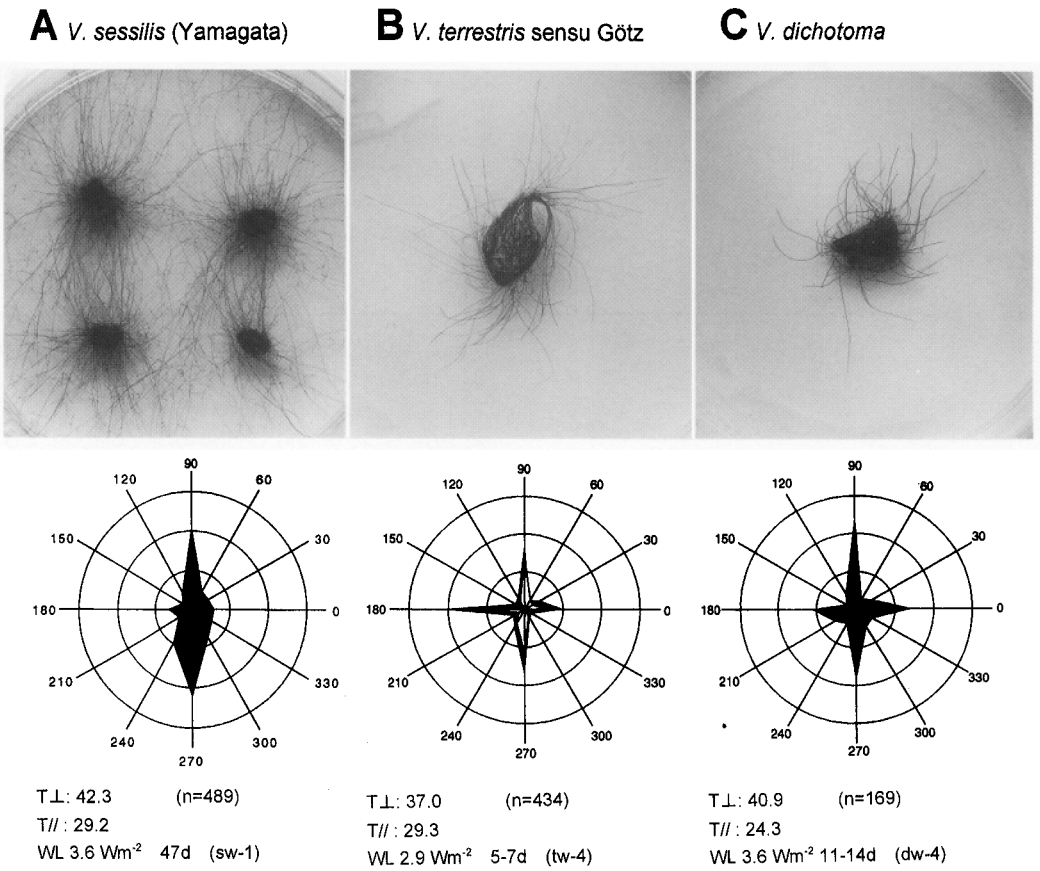


Fig. 4. Polarotropism of three different species of *Vaucheria*. A: *V. sessilis* (Yamagata strain); B: *V. terrestris* sensu Götz; C: *V. dichotoma*. Upper row: representative photographs of *Vaucheria* species grown on agar plates under polarized white light. *E*-vectors are in the horizontal direction (0°-180°). Lower row: histograms showing the polarotropic orientations under the conditions described below the data of NPIs and PPIs. Data are taken from up to 10 plates placed under the same light conditions. T⊥: total NPI; T//: total PPI. Other descriptions are same as in Fig. 3. *V. sessilis* shows very predominate perpendicular polarotropism, while the other two species exhibit a clear cruciform ("+"-shaped) polarotropic pattern.

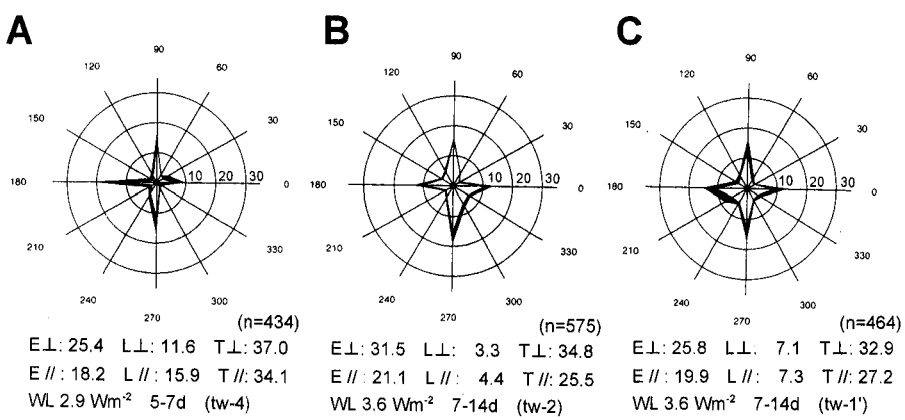
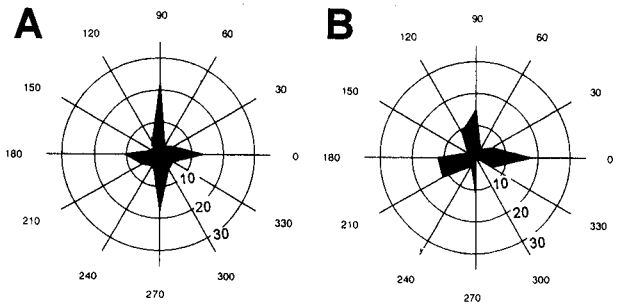


Fig. 5. Fluence rate-dependent increases in parallel polarotropic cells of *Vaucheria terrestris* sensu Götz to white light. Polarized white light irradiations at 2.9 Wm^{-2}(A), 3.6 Wm^{-2} (B) and 6 Wm^{-2} (C). Compare histograms and polarotropic indices (T⊥ and T//) between the three fluence rates. At 3.6 Wm^{-2} and 6 Wm^{-2}, the cruciform pattern becomes less clear due to the increased population of thalli that point in the intermediate direction. Explanations of figures hereafter are the same as in Figs. 3 and 4.

V. dichotoma



T⊥: 40.9 (n=169)
T// : 29.2
WL 3.6Wm^{-2} 11-14d (dw-4)

T⊥: 27.3 (n=33)
T// : 30.3
WL 6.0 Wm^{-2} 11d (dw-1)

Fig. 6. Fluence rate-dependent increases in parallel polarotropic cells of *V. dichotoma* to white light. Polarized white light irradiation at 3.6 Wm^{-2} for 11-14 days (A), and at 6 Wm^{-2} for 11 days(B). Parallel polarotropism is predominant in B.

The total perpendicular polarotropic index (T⊥) of *Vaucheria terrestris* sensu Götz significantly decreased with increasing intensity of polarized white light, as shown in Fig. 5. The total parallel polarotropic index (T//) was the largest at 2.9 Wm^{-2}; it slightly increased when the fluence rate increased from 3.6 Wm^{-2} to 6 Wm^{-2}. As a result, the sharp cruciform pattern displayed at the low fluence rate leveled off at 6 Wm^{-2}. However, the early polarotropic indices (E//) were almost the same at all intensities. The large difference between E// and T// seen at 2.9 Wm^{-2} was mainly due to the stimulated branch induction several days after the beginning of continuous irradiation. The induction of branches and their subsequent growth were a slightly predominate in the direction parallel to the *E*-vector. Tip growth, initially perpendicular to the polarized light, often changed direction, becoming parallel to the *E*-vector after several days of irradiation. In contrast to *V. dichotoma* (c.f. Fig. 3A), none of apices of *V. terrestris* sensu Götz changed their direction of growth from parallel to perpendicular. This also contributed to the increase in parallel polarotropic orientation at the higher fluence rate. The decrease in NPI and the increase in PPI with increasing intensity of polarized white light were more clearly demonstrated in *V. dichotoma* (Fig. 6).

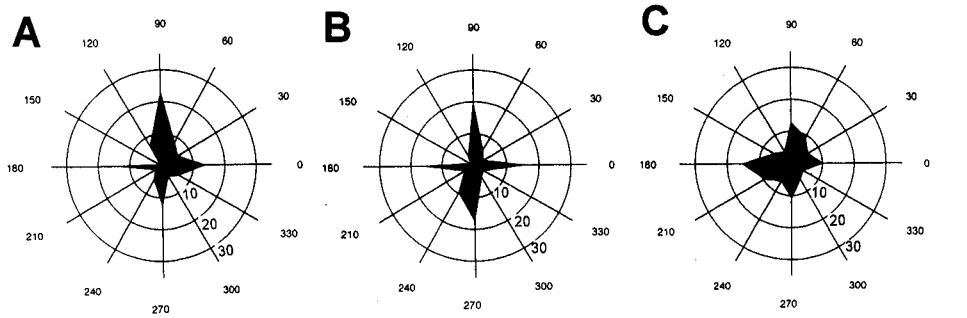
Cruciform polarotropic orientation to polarized BL

Increased parallel polarotropic orientation was more evident under continuous polarized BL. As shown in Fig. 7, PPI (T//) increased with either an increase in the fluence rate or an extension of the period of irradiation. Comparing histograms A and B, PPI (T//) can be seen to increase from 26.5 to 30.1, although the difference in NPI (T⊥) was insignificant. After 39 days of illumination with BL at 2.3-3.2 Wm^{-2}, PPI slightly superceded NPI. In *Vaucheria dichotoma*, however, prolonged irradiation did not change the NPI predominance over PPI, but increased the population of thalli that grew in intermediate directions (Fig. 8). Obviously from Figs. 7 and 8, prolonged irradiation destroyed the clear cruciform pattern of polarotropic orientation.

In contrast to BL, red polarized light always produced predominate perpendicular polarotropism in both *Vaucheria terrestris* sensu Götz and *V. dichotoma* (Fig. 9). This was also the case with very dim white light. If the fluence rate of red light was higher than 10 Wm^{-2}, the effect became similar to that with white light, but was evidently different from that with BL (data not shown). Red light only serves as dim white light. Longer incubation under red light led to a decrease in growth activity and in cell diameter, and consequently death after several weeks.

It can be concluded from the present results that the cruciform polarotropism of *Vaucheria* species observed after

V. terrestris sensu Götz

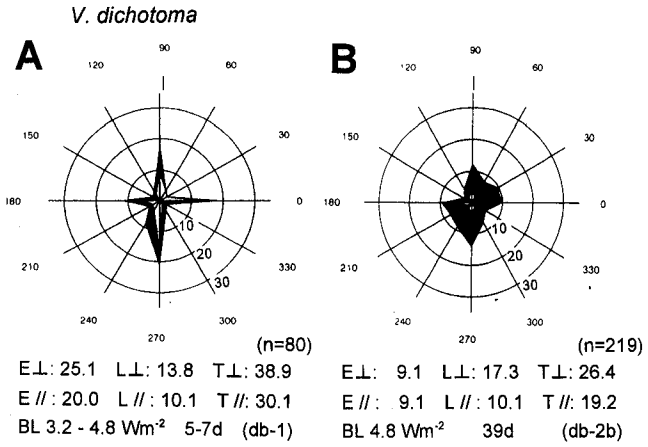


T⊥: 35.1 (n=185)
T// : 26.5
BL 3.2 Wm^{-2} 5-7d (tb-1)

T⊥: 36.6 (n=93)
T// : 30.1
BL 4.8 Wm^{-2} 5-7d (tb-3)

T⊥: 23.6 (n=441)
T// : 25.2
BL 2.3-3.2 Wm^{-2} 39d (tb-2)

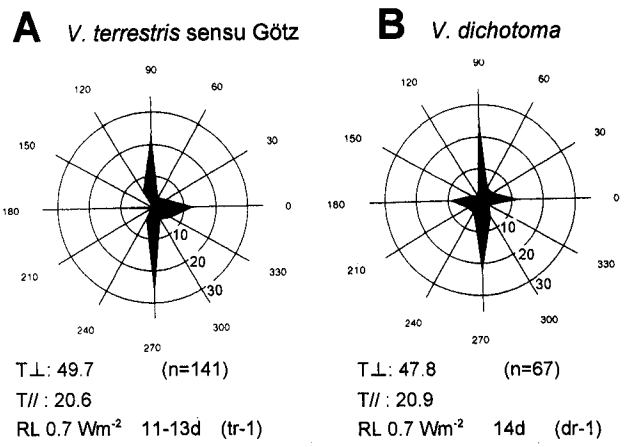
Fig. 7. Fluence rate- and exposure time-dependent increase in parallel polarotropic cells of *Vaucheria terrestris* sensu Götz to BL. Polarized BL irradiations at 3.2 Wm^{-2} for 5-7 days (A), at 4.8 Wm^{-2} for 5-7 days (B), and 2.3-3.2 Wm^{-2} for 39 days (C). Compare with Fig. 5. Total PPIs (T//) increased, but not Total NPI (T⊥), with increasing BL fluence rates. When irradiation periods were extended (A to C), total NPI decreased, leaving total PPI constant, while the population showing the intermediate direction increased.



E⊥: 25.1 L⊥: 13.8 T⊥: 38.9
E //: 20.0 L //: 10.1 T //: 30.1
BL 3.2 - 4.8 Wm^{-2} 5-7d (db-1)

E⊥: 9.1 L⊥: 17.3 T⊥: 26.4
E //: 9.1 L //: 10.1 T //: 19.2
BL 4.8 Wm^{-2} 39d (db-2b)

Fig. 8. Polarotropism of *Vaucheria dichotoma* induced by prolonged exposure to polarized BL. Polarized BL irradiation at 3.2-4.8 Wm^{-2} for 5-7 days (A) and for 39 days (B). Compare with Figs. 3 and 6. Orientation becomes random, and both total NPI and total PPI decrease.



T⊥: 49.7
T// : 20.6
RL 0.7 Wm^{-2} 11-13d (tr-1)

T⊥: 47.8
T// : 20.9
RL 0.7 Wm^{-2} 14d (dr-1)

Fig. 9. Growth orientation with red polarized light. A: *Vaucheria terrestris* sensu Götz; B: *V. dichotoma*. The effect of polarized dim red light is similar to that of very dim white light. Even at a much higher fluence rate, the NPI-predominate pattern was preserved in both species.

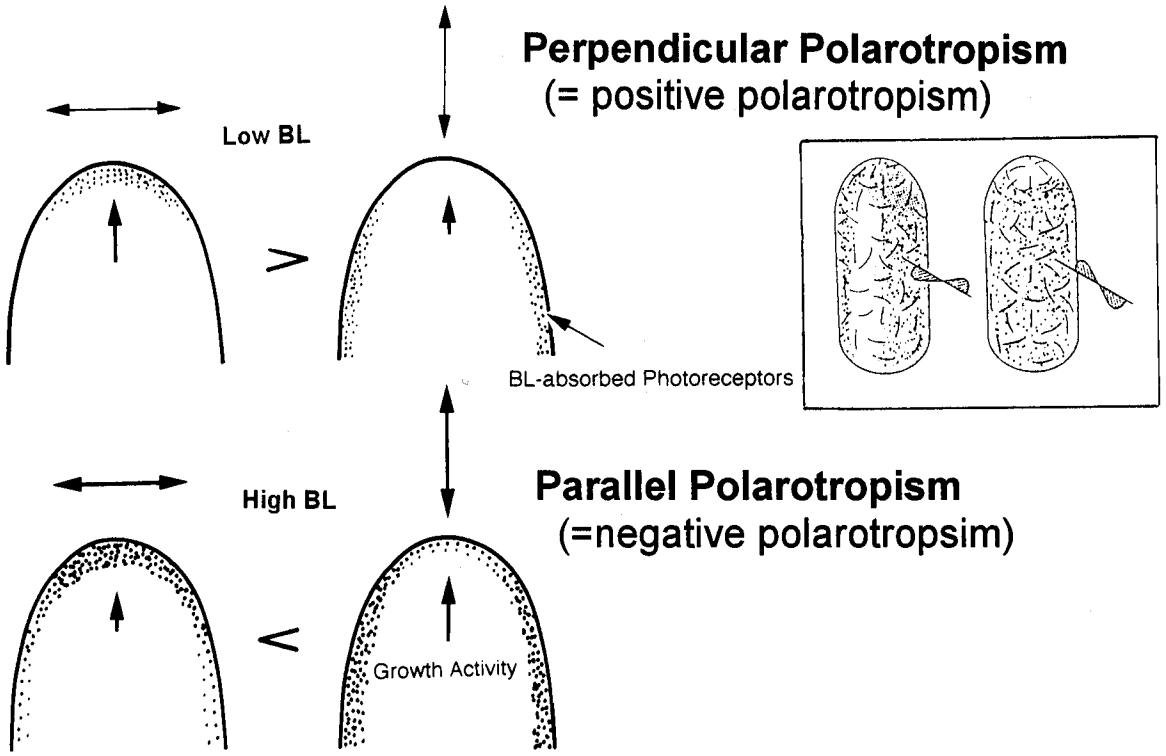


Fig. 10. Schematic diagram explaining the cause of cruciform polarotropism of *Vaucheria* in relation to the distribution of photoabsorption and growth activity in the apical dome. Tip growth is due to localized exocytosis at the apex. If BL-absorbing photoreceptor molecules are oriented more or less parallel to the cell surface, maximum light absorption must be at the surface parallel to the *E*-vector of polarized BL. Since tip growth is activated by a low fluence rate of BL and inhibited by a high fluence rate of BL, the perpendicular and parallel polarotropism can be explained simply by the hypothetical absorption gradient. The degree of photoabsorption and activity of tip growth are indicated by the density of dots and the lengths of arrows, respectively. Double arrows: vibrating planes of *E*-vectors. Thickness of the double arrows reflects the fluence rates of BL. Inset: light absorption profiles of *Vaucheria* cell apex with perpendicular (left) or parallel (right) *E*-vector orientation to the cell axis, estimating the orientation of photoreceptor molecules (short bars) estimated to be parallel to the cell surface.

several days of continuous irradiation reflects the variation in positive to negative switchover points of individual apices of these alga species in a given physiological status. The shift from perpendicular to parallel polarotropism seems to correspond to positive and negative phototropic bending, respectively, because, 1) the alga can switch the phototropic response from positive to negative when the light intensity increases, becomes, 2) BL is the most effective light for phototropism.

Discussion

The bimodal polarotropism of *Vaucheria* spp. seems to be closely connected with this algae's ability of positive/negative phototropic inversion. Both positive and negative phototropism are induced by BL, although it is still not known whether they are mediated by a single BL photosystem or by two different BL photosystems. Red light neither induces phototropic bending nor modifies phototropic sensitivity to BL when given previously, simultaneously or afterward (Kataoka 1988, Kataoka and Weisenseel 1988). The localization of BL photoreceptor molecules for positive and negative phototropism has been assumed to be equal at the outermost layer of the apical hemisphere, as proved by microbeam irradiation experiments (Kataoka 1975b, 1988, 1991). Positive phototropism of *Vaucheria* requires a positive regulation mechanism between light absorption and the induction of positive curvature. In the apical 1/3 area of the hemispherical apical dome, all processes of phototropic response between photoreception and manifestation of bulging are completed. If BL photoreceptor molecules are oriented parallel to the cell surface, polarized BL whose *E*-vector is normal (90°) to the cell axis will be maximally absorbed by the photoreceptor molecules at the extreme apical region. The absorption of polarized light at the tip and the mechanism that changes perpendicular- to parallel-polarotropic bending is schematically shown in Fig. 10. The light absorption profiles of a cylindrical plant cell irradiated with polarized light schematically shown in the inset are adopted from Haupt (see e.g. 1960, 1983). The optimum fluence rate for positive phototropism varies species to species and/or depending on the physiological status. If the fluence rate is supraoptimum, tip growth is inhibited as proved by short pulse (< 20 min) experiments (Kataoka 1988), and the apex eventually bends until the cell axis becomes parallel to the *E*-vector. Namely, the negative phototropism and the parallel polarotropism equally require a negative regulation mechanism.

There is a very steep gradient of phototropic sensitivity along the surface of the apical dome (Kataoka 1975a). This seems to be the main reason that the final growth direction is limited to being either perpendicular or parallel to the *E*-vector. As expected, in such *Vaucheria* species as *V. sessilis* or *V. dichotoma* which change to negative phototropism at much higher fluence rates, the parallel polarotropic orientations predominate at higher fluence rates than in *V. terrestris* sensu Götz..

A similar cruciform pattern of polarotropism has been reported in primary chloronemata of a moss, *Physcomitrella patens*, when they were irradiated with polarized red light at high fluence rate (Jenkins and Cove 1983a, b). At low fluence rates the primary chloronemata grew positive phototropically in response to unilateral red light and perpendicular to the *E*-vector in polarized red light. At high fluence rates, it became laterally phototropic in unilateral light and parallel polarotropic in polarized light. The colony consequently showed cruciform polarotropism under polarized red light of intermediate fluence rates. Although the shapes of the histograms look similar to those of *Vaucheria*, the underlying mechanisms between the two systems are completely different. First, the photoreceptor system is quite different; i.e., phototropism of *Vaucheria* is not induced by red light. Second, the moss chloronemata show lateral phototropism (diaphototropism) at a high fluence rate. The lateral phototropism of these moss chloronemata can be explained by their inability to switch the sign of phototropism. Namely, when the apices are at a ±90° angle to strong unilateral light source, they receive the least optimum light for their growth, while they still receive supra-optimum light when they are irradiated from behind. On the other hand, *Vaucheria* shows negative orthotropic curvatures (−180°) at high fluence rates, although in a restricted fluence rate range, especially on agar plates, similar lateral phototropism was observed. The light intensity gradient within the apical photoreceptive region is probably the cause of the lateral phototropism, although the true mechanism is not known. Nevertheless, we can conclude that the coexistence of phototropically sensitive and less-sensitive branches in a colony produce the cruciform polarotropic pattern of *Vaucheria*.

Action dichroism to BL has been observed in many plants and fungal photosystems. One of the most closely related phenomena to parallel polarotropism is the polarity induction in fucacean zygotes and spores of the fern *Osmunda cinnamomea*. They form rhizoids from their darker side when they are unilaterally irradiated with BL, and if they are under polarized BL, rhizoids grow in parallel to the *E*-vector of the polarized light (Jaffe 1958). Contrary to *Osmunda*, however, spores of the fungus *Botritis cinerea* germinate towards a unilateral BL source, although they also germinate parallel to the *E*-vector of the polarized BL (Bunning and Etzold 1958). Jaffe and Etzold (1962) compared the apparently opposite behaviors of *Osmunda* and *Botritis* and concluded that their polarity induction mechanisms are different. They hypothesized that the absorption dipoles of BL receptor molecules are oriented in an orthogonal fashion in *Botritis* but parallel to the cell surface in *Osmunda*. If this is true, BL is absorbed most by a *Botritis* spore in 0° and 180° regions of the vibration plane of the *E*-vector, and from this sites germination occurs. On the other hand, in an *Osmunda* spore, BL is least absorbed in these same regions from which the rhizoid is induced. This requires a mechanism that converts the intracellular light gradient to a morphological axis, and resulting in germination from the darkest region. Bergers and Brownlee (1994) disputed this view and suggested the involvement of lens effect by showing much light scattered from rear side of the unilaterally irradiated *Fucus* zygote.

However, Robinson (1996) has recently argued against the existence of such a lens effect in *Pelvetia* zygotes. He directly measured how much BL was transmitted to a zygote, and proved that only 1-2% of incidental BL reached the rear side. Using half-side illumination, he also proved that the *Pelvetia* zygote formed a rhizoid from dark side.

Very clear polarotropism has been well documented in fern protonemata (Bunning and Etzold 1958, Etzold 1965 ; Kadota et al. 1984, 1985). Protonemal cells of *Dryoptelis filix-mas* were found to grow perpendicular to the *E*-vector of red light. If the polarizer was turned 50° and the irradiation was continued for another 8 hr, the direction of growth rotated by 50° and became oriented perpendicular to the new direction of the *E*-vector during the following 16 hr in darkness (Etzold 1965). The red light signal was found to be mediated by phytochrome. However, the action spectrum of the *Dryopteris* polarotropism (Steiner 1969 a, b) revealed that the effectiveness of BL was 100 times higher than that of red light. This finding clearly indicates the presence of separate dichroic BL photoreceptors oriented parallel to the cell surface. Phytochrome and BL-photoreceptors are not always both involved in polarotropic responses of fern protonemal cells. Kadota *et al.* (1989) found that both phytochrome and blue light absorbing pigments were dichroic in *Adiantum*, whereas only BL-absorbing pigment was dichroic in *Pteris*. Namely, *Pteris* lacks phytochrome(s) specific to polarotropism and chloroplast photomovement. This does not mean, however, the absence of phytochrome in *Pteris*, because the phytochrome was active in other photoresponses. This was evidence for multiple phytochrome photosystems. Evidence for the presence of different molecular species of phytochromes in ferns and their different expression timing and localization have recently been yielded by molecular biological studies (Furuya 1993, Wada *et al.* 1997, Okamoto *et al.* 1997).

In contrast, *Vaucheria* apparently lacks a phytochrome-mediated system. Red light seems to be used as the energy source. *Vaucheria* is therefore advantageous for studies of BL responses. For further kinetical analysis of polarotropism, however, *Vaucheria* may not be useful, because the polarotropic response of *Vaucheria* can be detected only after at least several days. This may indicate, as considered above, that the BL-photoreceptors are oriented more or less randomly or that the undulated plasma membrane at the growing apex may be the main cause of slowly developing polarotropism. However, a photoreceptor(s) for phototropism and/or polarotropism of *Vaucheria* is not necessarily a membrane protein, as Christie *et al.* (1998) showed that NPH1, the recently identified photoreceptor protein for higher plant phototropism, is not a membrane protein.

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