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Heart-Shaped Prothallia of the Fern *Adiantum capillus-veneris* L. Develop in the Polarization Plane of White Light

Akeo Kadota and Masamitsu Wada

Department of Biology, Faculty of Science, Tokyo Metropolitan University, Fukazawa, Tokyo 158, Japan

When red light-precultured filamentous protonemata of *Adiantum capillus-veneris* were cultured under linearly polarized white light, heart-shaped prothallia developed in the plane parallel to the vibration plane of electrical vector of polarized light, and were directed toward the light source. When the polarization plane was rotated during the culture, the prothallial wings twisted correspondingly to develop in the new plane. Continual observation of the early steps of prothallial development with a time-lapse video system revealed that the apical cell of protonema after the first transverse cell division became flattened in the vibration plane of electrical vector of polarized light, and that the first longitudinal cell division, that is, the first step in the transition from one-dimensional to two-dimensional growth, as well as the subsequent cell divisions, occurred perpendicularly to the electrical vector.

Key words: *Adiantum capillus-veneris* L. — Fern gametophyte — Photocontrol (prothallia) — Polarized light — Polarotropic response (prothallia) — Prothallial development (polarized light effect).

Since the study by Castle (1934) on the effect of linearly polarized light on the phototropism of *Phycomyces* sporangiophore and the discovery of polarotropism in *Fucus* zygotes by Jaffe (1956), it is well known that linearly polarized light determines the direction of growth in tip-growing cells such as hyphae (Bünning and Etzold 1958), rhizoids and protonemata of fern gametophytes (Bünning and Etzold 1958) and protonemata of moss (Bünning and Etzold 1958) and liverwort (Steiner 1967). However, there are no reports so far which show the specific effect of polarized light on the development of two-dimensional multicellular systems.

In the present study, we found that the two-dimensional, heart-shaped prothallia of *Adiantum capillus-veneris* developed in the plane of electrical vector of polarized light irradiated.

Materials and Methods

Plant material and aseptic culture—Spores of *Adiantum capillus-veneris* L. were collected in the summer of 1983 in a greenhouse of the Botanical Gardens of the University of Tokyo at Koishikawa, Tokyo, Japan. The spores were stored dry in the dark at ca. 5°C.

The culture medium consisted of one-tenth strength modified Murashige and Skoog's mineral salt solution (Wada and Furuya 1970) supplemented with 1% sucrose, 0.05% yeast extract (Difco Lab., Detroit, U.S.A.) and 0.5% agar (Difco Lab.). Two ml of the medium were poured into a petri dish (3 cm in diameter). Spores sterilized by the technique of Ito (1969) with one-tenth strength "Purelox" (4–6% sodium hypochlorite solution, Oyalox Co., Tokyo, Japan) were sown on the medium. After sowing, an additional 2 ml of the agar medium

was poured into the dish so that the spores were sandwiched between the two layers of agar medium. For the study with time-lapse video system, spores were inoculated with a needle in the middle position of the agar medium which was filled in the polystyrene cuvette ($3 \times 3 \times 6$ mm^3). The inoculated cuvette was put into the petri dish and 4 ml of the medium were then added to the dish.

Spores were imbibed for 1 day in the dark and cultured for 6 days under red light (ca. 0.5 W m^{-2}) which was provided unilaterally by a fluorescent lamp (Toshiba FL 40 SD/NL, Tokyo Shibaura Electric Co., Ltd., Kawasaki, Japan) with a red acrylic resin sheet of 2 mm thickness (Shinkolite A, no. 102, Mitsubishi Rayon Co., Ltd., Tokyo, Japan). The resulting single-celled protonemata growing horizontally toward the red light source were used throughout the present study.

Polarized light treatment—Polarized white light was obtained by the combination of a fluorescent lamp (Toshiba FL 40 SD/NL or FL 20 SD/NL) and a polarizer (HN 22 or HN 38, Nippon Polaroid K.K., Tokyo, Japan). Red light-precultured filamentous protonemata were cultured under polarized white light for 3 weeks, and the orientation of heart-shaped prothallia was observed under a microscope.

Observation with a time-lapse video system—Early steps of the gametophyte development under polarized white light were monitored and continually recorded with a time-lapse video system

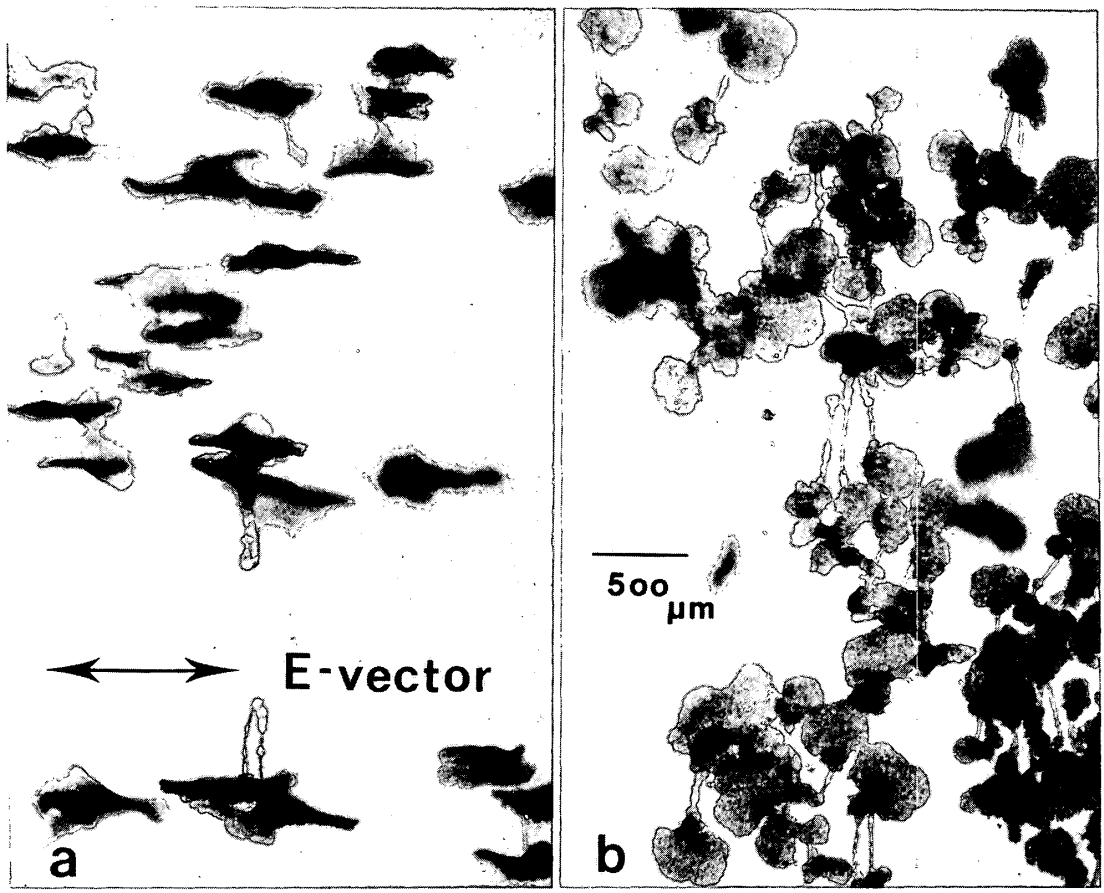

Fig. 1a, b Orientation of heart-shaped prothallia of *Adiantum capillus-veneris* under polarized (a) or non-polarized (b) white light coming from above. Red light-grown protonemata were cultured under irradiation with polarized (2.5 W m^{-2}) or non-polarized white light (10 W m^{-2}) for 3 weeks. Filamentous part of each prothallium shows a red light-grown region. Double-headed arrow indicates the vibration plane of electrical vector (E-vector) of polarized light.

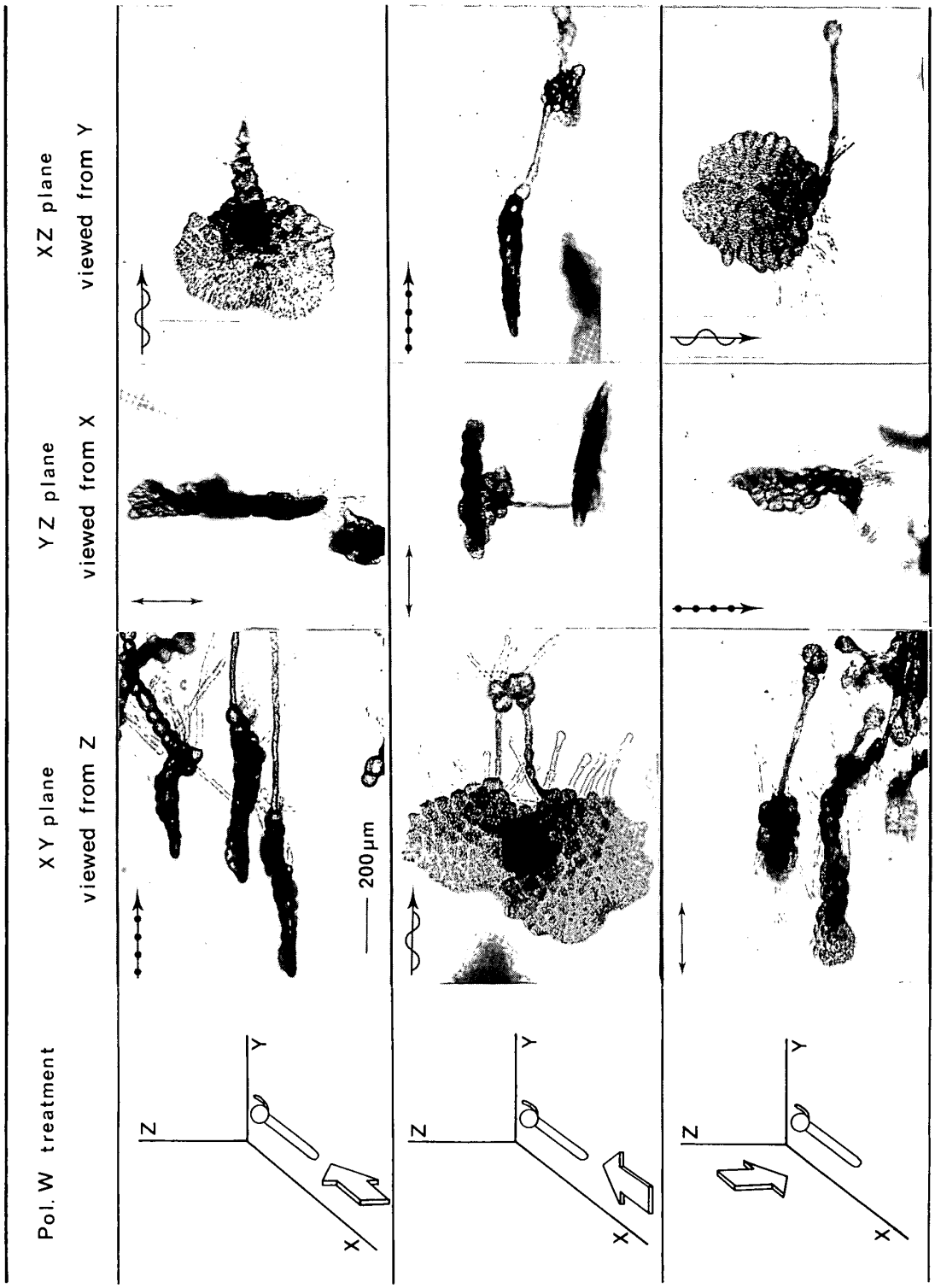

Fig. 2 Development of heart-shaped prothallia of *Adiantum capillus-veneris* under various conditions of polarized white light irradiation. Polarized white light (Pol. W) of 2.5 W m^{-2} was given to the red light-grown protonemata as indicated on the left. The arrow shows the direction of polarized light and the plane of the arrow indicates the vibration plane of electrical vector. Gametophytes were cultured for 3 weeks under each condition and the prothallia developed were viewed from the Z axis (XY plane), X axis (YZ plane) or Y axis (XZ plane). The arrow in each plate shows the direction of polarized white light with the vibration plane of electrical vector and the double-headed arrow indicates the vibration plane of electrical vector of polarized light which is coming from above.

through an inverted microscope (Nikon TMD, Nippon Kogaku K.K., Tokyo, Japan) using infrared light obtained with an infrared filter (IR 85, Hoya Corp., Akishima, Japan). A time-lapse video recorder (NV-8030, Matsushita Electric Industrial Co., Ltd., Kawasaki, Japan) was coupled with a video camera equipped with an infrared sensitive tube (Newvicon S4113, Matsushita Electric Corp., Tokyo, Japan). The video system was controlled by a time-lapse controller and the real time was recorded and shown on the monitor screen (Furuya et al. 1980).

Results

Orientation of heart-shaped prothallia under polarized white light—Filamentous protonemata elongated horizontally were irradiated continuously from above with either polarized or non-polarized white light (2.5 or 10 W m^{-2}, respectively) for 3 weeks. As shown in Fig. 1b, two-dimensional gametophytes developed in the plane which was normal to the incident light under non-polarized light. Under polarized white light, however, prothallia developed in the plane which was parallel to the electrical vector of polarized light, and were directed toward the light source (Fig. 1a). The difference in the plane of prothallial orientation was not due to the difference of fluence rates of the white lights because prothallia cultured under non-polarized white light of 2.5 W m^{-2} showed the same orientation as that under 10 W m^{-2} white light (data not shown).

Next, protonemata were irradiated horizontally or vertically with polarized white light (2.5 W m^{-2}) and after 3 weeks the prothallia developed were observed from 3 different directions (Fig. 2). The results indicate that heart-shaped gametophytes were oriented in the plane of electrical vector of polarized light in each of the light treatments. The orientation of prothallia under polarized white light is schematically shown in Fig. 3 a–d.

Influence of the change of vibration plane of polarized white light during prothallial development—Protonemata were first irradiated from above with polarized white light (2.5 W m^{-2}) for 17 days and the polarization plane of white light was then rotated 90°. Prothallia developed were observed from above 4 days after the change of vibration plane. As shown in Fig. 4, the prothallial wing twisted correspondingly to be parallel to the new polarization plane.

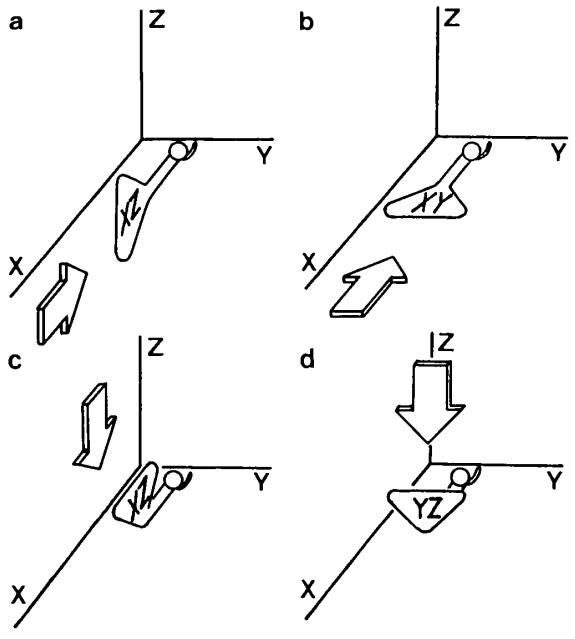

Fig. 3a–d Diagrams showing the prothallial development of *Adiantum capillus-veneris* in relation to the direction and the polarization plane of polarized white light. The arrow shows the direction of polarized light and the plane of the arrow indicates the vibration plane of electrical vector.

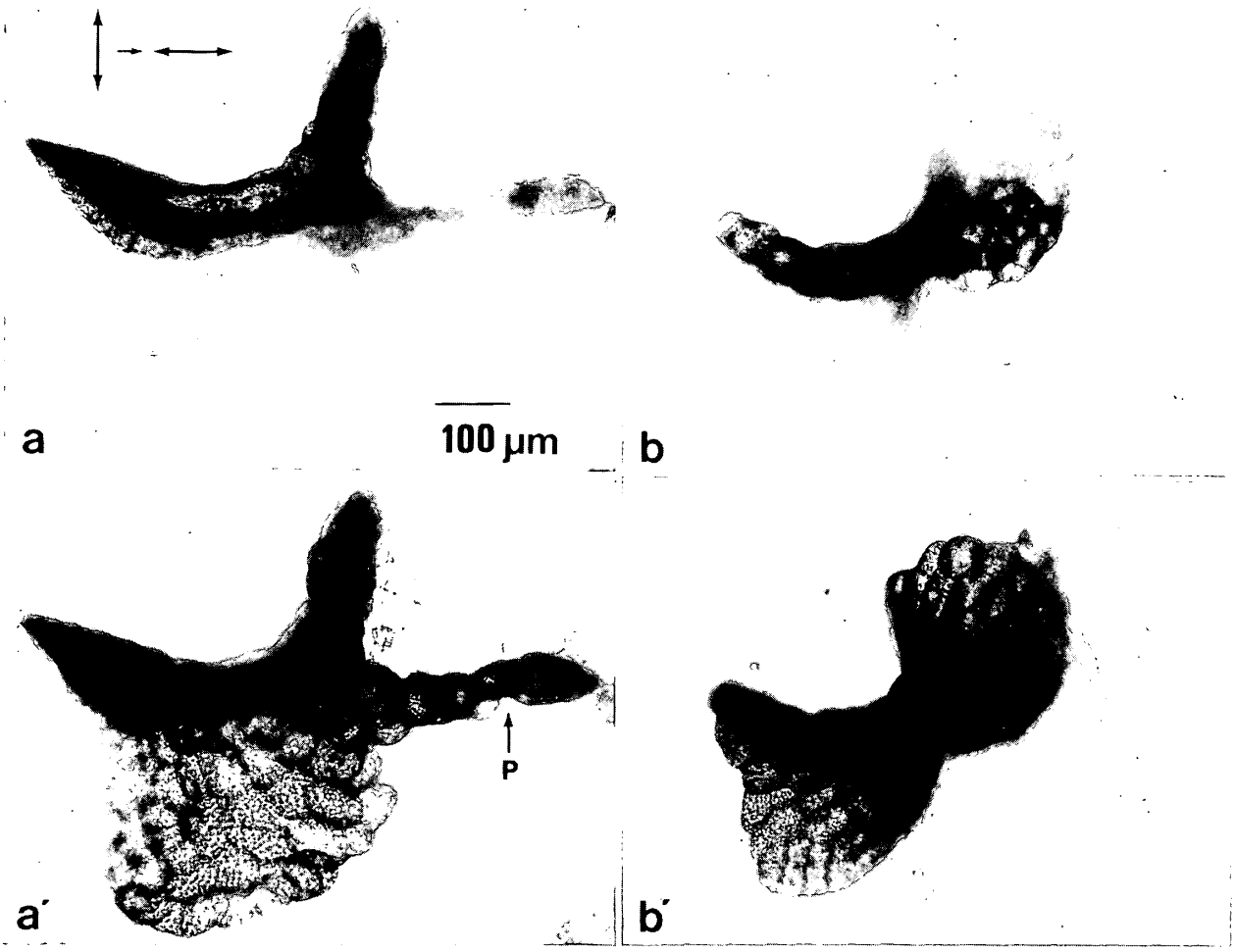

Fig. 4a, b Effect of the directional change of polarization plane of polarized white light on the development of prothallia in *Adiantum capillus-veneris*. Protonemata were irradiated from above with polarized white light (2.5 W m^{-2}) for 17 days and then the vibration plane of electrical vector was rotated 90°. Prothallia were observed after 4 days. a and b show two individual prothallia. a′ and b′ are the same prothallia as a and b, respectively and focused on the basal part of each prothallium. Filamentous part (P) in a′ shows the region which was elongated under red light. Double-headed arrow indicates the vibration plane of electrical vector.

Observation of the early steps in prothallial orientation—Protonemata were horizontally irradiated from their growing direction with polarized white light (3.8 W m^{-2}) and the early steps of prothallial development were recorded with time-lapse video system. The side view of the gametophyte was also recorded at appropriate intervals during the experiments by turning the cuvette in which gametophytes were cultured. In Fig. 5, traces of the apical parts of 3 gametophytes are shown with the time of incubation under polarized white light. These gametophytes represent typical early steps in prothallial development. The results showed that the apical cell of protonema after the first transverse cell division became flattened parallel to the electrical vector of polarized light. The width of the apical cell (top view) was 5–10% longer than the thickness of the cell (side view). The first longitudinal cell division (the third division in each of the three gametophytes shown in Fig. 5) as well as the subsequent cell divisions (data not shown) occurred perpendicularly to the vibration plane of electrical vector so that the gametophytes developed parallel to the polarization plane.

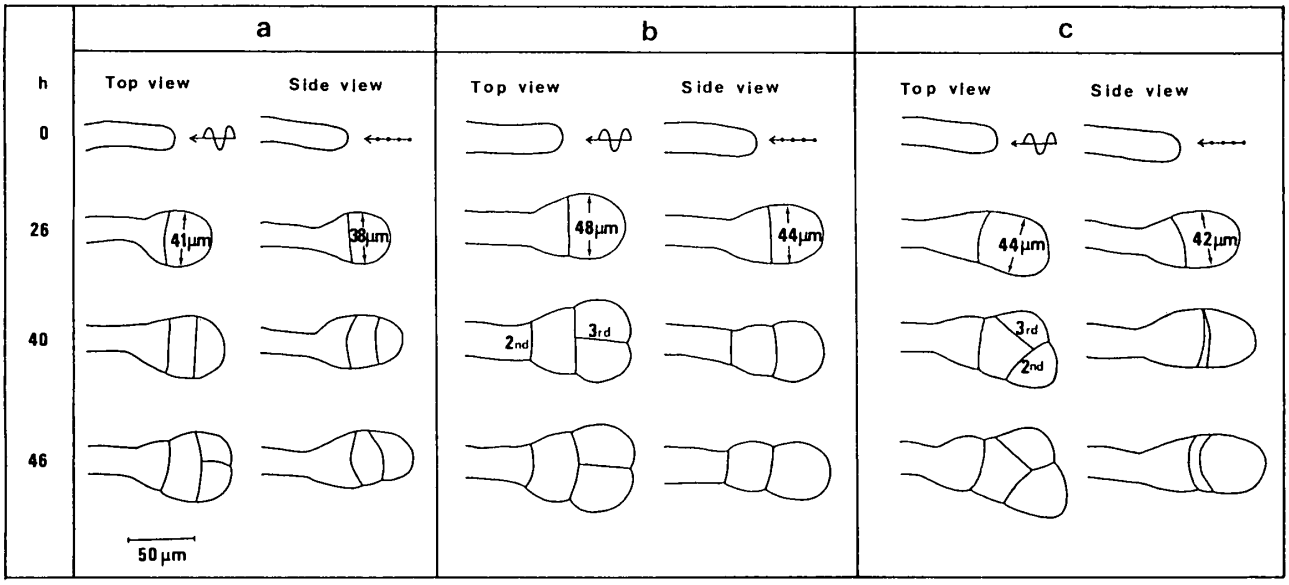

Fig. 5a–c Traces of the apical parts of protonemata during early steps of prothallial development in *Adiantum capillus-veneris* under irradiation of polarized white light vibrating horizontally. Early steps of prothallial orientation were recorded continually with time-lapse video system and the recorded images were traced on the monitor screen. a–c show the top and side views of three individual gametophytes with the time in hours under polarized white light (3.8 W m^{-2}). At the 2-celled stage (26 h), the width and thickness of the apical cell were shown in the traces. The "2nd" and "3rd" denote the sequence of cell division.

Discussion

It has long been established that two-dimensional prothallia of various fern species develop in the plane which is at right angle to the direction of incident non-polarized light (Leitgeb 1877, Prantl 1879). Prothallia of *Adiantum* also show the same orientation to non-polarized white light (Wada and Furuya 1971, Fig. 1b). In addition, we found here that gametophytes of *Adiantum* developed heart-shaped prothallia in the plane of electrical vector of polarized white light and were directed toward the light source (Fig. 1a, 2,). Thus, the orientations of prothallia under non-polarized and polarized white light irradiation are quite different from each other (compare Fig. 3 with Fig. 1 of Wada and Furuya 1971). But the situation resembles the relation between phototropism and polarotropism of filamentous protonemata; that is, protonemata grow towards the light source under non-polarized light while under polarized light irradiation they grow not toward the light source but perpendicularly to the electrical vector of polarized light given (Bünning and Etzold 1958).

The polarotropism of protonemata has been considered to be the result of the dichroic orientation of photoreceptor pigments in the cell (Etzold 1965, Wada et al. 1981, Kadota et al. 1982, 1985). The same consideration may be proposed for the "polarotropic" orientation of prothallia under polarized light irradiation. Namely, the polarized light effect may indicate the intracellular dichroic orientation of photoreceptor pigment regulating prothallial development. At present, we can say nothing conclusive about the nature of receptor pigment and further studies on the effects of colored lights may be warranted. There should be at least two candidates for the photoreceptor, that is, phytochrome and blue light-absorbing pigment(s), both of which were shown to be oriented in the protonemal cell of *Adiantum* for other photoresponses (Wada et al. 1978, Kadota et al. 1982, 1985, Yatsuhashi et al. 1985).

Wada and Furuya (1971) studied the early steps of prothallial orientation to non-polarized

light at the cellular level in *Adiantum* gametophytes. They showed that the first longitudinal cell division (the first step of the transition from one- to two-dimensional growth) as well as the subsequent divisions were always parallel to the direction of the incident light. On the contrary, the present observation on the early steps in the prothallial orientation to polarized light revealed that the apical cell of protonema after the first transverse cell division became somewhat flattened in the plane of electrical vector and that the first longitudinal cell division and the subsequent cell divisions occurred perpendicularly to the plane of electrical vector, leading to the flattening of apical part of the gametophyte (Fig. 5). Thus, it seems likely that the directions of both cell growth and cell division may be regulated by the vibration plane of polarized light.

The observation on the early stages of prothallial development also indicated that the orientation of gametophytes could be detected even at a very early stage such as the 4 cell stage (Fig. 5). This is consistent with the case of orientation to non-polarized light, in which the orientation could be also detected in the 4-celled gametophytes (Wada and Furuya 1971). However, the plane of prothallial development may not be fixed at the early stage of development because the change of direction of electrical vector induced a twisting of prothallia so that the prothallial wings came to be parallel to the new polarization plane (Fig. 4).

Together with the orientation under non-polarized light, the present photoresponse under polarized light may provide a suitable system for the analysis of the regulation of the directions of cell growth and cell division in the multicellular organism.

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