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BEHAVIOURAL RESPONSES TO LIGHT IN *PARAMECIUM BURSARIA* IN RELATION TO ITS SYMBIOTIC GREEN ALGA *CHLORELLA*

BY KENJI IWATSUKI* AND YUTAKA NAITOH

Institute of Biological Sciences, University of Tsukuba, Ibaraki 305, Japan

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SUMMARY

The behavioural responses to light in the ciliate *Paramecium bursaria* Focke, which normally contains hundreds of the symbiotic green alga *Chlorella* in its cytoplasm, were analysed quantitatively to clarify the mechanisms governing photoreception in the cell. *P. bursaria* was found to possess three kinds of photoreceptor systems for (1) the step-up photophobic response (system I), (2) the step-down photophobic response (system II), and (3) the photokinetic response (system III). Under the influence of light, the symbiotic algae inhibited systems I and III, but activated system II. Thus the cells showed the avoiding reaction when they encountered shade (the step-down photophobic response), and consequently gathered in the light region (photoaccumulation). Inhibition of system I and activation of system II were assumed to be mediated by products of the blue-light effect of the algae, while inhibition of system III was due to photosynthetic products of the algae. The cells whose algae were experimentally removed gathered in the shade (photodispersal) due to the avoiding reaction exhibited by them when they encountered a lighted region (the step-up photophobic response mediated by system I). Lowered swimming velocity and increased frequency of spontaneous changes in the swimming direction in the shade (photokinetic responses mediated by system III) also caused photodispersal.

INTRODUCTION

The ciliate protozoan *Paramecium bursaria* contains hundreds of green algae, *Chlorella*, in its cytoplasm. Engelmann (1882) found that when a specimen of *P. bursaria* swimming in a light region encountered shade it exhibited an avoiding reaction (step-down photophobic response; Diehn *et al.* 1977) and returned to the light region (photoaccumulation; Diehn *et al.* 1977; Diehn, 1979; Jennings, 1906).

Iwatsuki & Naitoh (1981) demonstrated that a potent inhibitor of photosynthesis, 3-(3,4-dichlorophenyl)-1′,1-dimethylurea (DCMU), in the external solution had no

*Present address: Nippon Petrochemicals Co. Ltd, Research and Development Department, Tokyo 100, Japan.

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effects on either the step-down photophobic response or photoaccumulation in *P. bursaria*. They also found that the spectral sensitivity curve for the photophobic response was different in its major peak wavelength (520 nm) from those for the photosynthetic activity of the algae (440 nm and 680 nm). The ineffectiveness of DCMU in inhibiting the photophobic response was also reported by Cronkite & Van Den Brink (1981).

In contrast, Niess, Reisser & Wiessner (1981, 1982) reported that DCMU caused a loss of the photophobic response of *P. bursaria*, and the peak wavelength of the spectral sensitivity curve for the response coincided with that of the action spectrum for photosynthesis in the green algae. However, Pado (1972) reported that blue light (450 nm) was most effective in inducing photoaccumulation in *P. bursaria*.

Photoaccumulation of microorganisms is dependent on both the phobic response exhibited by the organisms at the light–dark border and their kinetic activities, such as forward swimming velocity (orthokinesis) and the frequency of spontaneous change in swimming direction (klinokinesis). Photoaccumulation is also influenced by light-direction-oriented locomotion of the organisms (phototaxis) (Diehn, 1979).

To understand the mechanisms governing the photobehaviour of *P. bursaria* in relation to its symbiotic green algae, we examined quantitatively the photophobic responses, the photokinetic responses, and phototaxis in both *Chlorella*-containing and *Chlorella*-free specimens. Based on these results, we hypothesize that the specimens of *P. bursaria* possess at least three kinds of photoreceptor systems responsible for (1) the step-down photophobic response, (2) the step-up photophobic response and (3) the photokinetic responses, respectively. Since symbiotic *Chlorella* modify the systems through their photosynthesis and blue-light effect (non-photosynthetic carbon metabolism; Kamiya & Miyachi, 1974; Miyachi, Miyachi & Kamiya, 1978; Senger, 1980), they change the photobehaviour of their host. Some of the discrepancies between the results obtained by previous workers can then be explained.

MATERIALS AND METHODS

Chlorella-free specimens of *Paramecium bursaria* were obtained by keeping the normal *Chlorella*-containing specimens (syngen 1, mating type IV) in complete darkness for more than 60 days (Muscatine, Karakashian & Karakashian, 1967). Both *Chlorella*-containing and *Chlorella*-free specimens were cultured in a bacterized wheat straw infusion at 21 ± 1 °C under a fixed illumination cycle of 12 h dark and 12 h light (3 W m^{-2} fluorescent lamps). Specimens obtained from culture 3 h after the light had been turned on were used as the light-adapted specimens. Those obtained from culture kept in the dark for 3 h more after the end of the dark period were used as the dark-adapted specimens (Iwatsuki & Naitoh, 1978, 1979, 1981). All specimens were washed gently with a standard saline solution for 2–5 min prior to experimentation (Naitoh & Eckert, 1972).

About 2 ml of the suspension of equilibrated cells was introduced into a cylindrical Plexiglas dish (30 mm inner diameter) under illumination with a dim light (background light; 50 mW m^{-2}) for observation and videorecording of specimen locomotion. A stimulating light was applied to the specimens through the bottom of the vessel. Light intensity was suddenly increased from the background level to a higher stimulation level (step-up photostimulation), and kept at that level for 2 min. Light intensity was then suddenly decreased to the original background level (step-down photostimulation). To indicate the degree of the response, the number of specimens which showed a photophobic response to photostimulation was counted on a replayed picture, and presented as a percentage of the total number of specimens (40–60) in the dish.

Monochromatic light was obtained by putting an interference filter (half-bandwidth; less than 18 nm) and a cut-off filter (Toshiba) in front of a light source (a 650-W halogen lamp). Light intensity was controlled by neutral density filters and monitored with a photometer (Spectra PR-1000). The thermal radiation from the light source was minimized by an infrared cut-off filter. All the experiments were performed at 21 ± 2°C. Some details of the experimental methods will appear in the Results.

RESULTS

Photophobic responses in the Chlorella*-free specimens*

Some specimens in a single population of the dark-adapted *Chlorella*-free *P. bursaria* showed the step-up photophobic response, whereas others showed the step-down photophobic response. The number of specimens that showed each response increased with increasing stimulus intensity, up to a maximum (Fig. 1A). When the intensity was more than 2 W m^{-2}, about 70 % of the specimens showed the step-up response, while the remaining 30 % showed the step-down response. The threshold intensities for the half-maximal response (35 % step-up and 15 % step-down) were about 0·20 W m^{-2} and 0·26 W m^{-2}, respectively.

Long-term (10 min) observation of a single specimen stimulated every 30 s by a white light of 2 W m^{-2} revealed that it showed both the step-up response and the step-down response. About 70 % of the responses during this time were step-up, while 30 % were step-down. The light-adapated *Chlorella*-free specimens showed photophobic responses identical with the dark-adapted ones.

Photophobic responses in the Chlorella*-containing specimens*

Photophobic responses exhibited by the dark-adapted *Chlorella*-containing specimens were identical to those that were *Chlorella*-free, i.e. 70 % of the specimens showed the step-up and 30 % the step-down response when exposed to a supramaximal photostimulation (2 W m^{-2}). In contrast, the light-adapted *Chlorella*-containing specimens showed only the step-down photophobic response. The number of specimens which showed the response increased with increasing stimulus intensity.

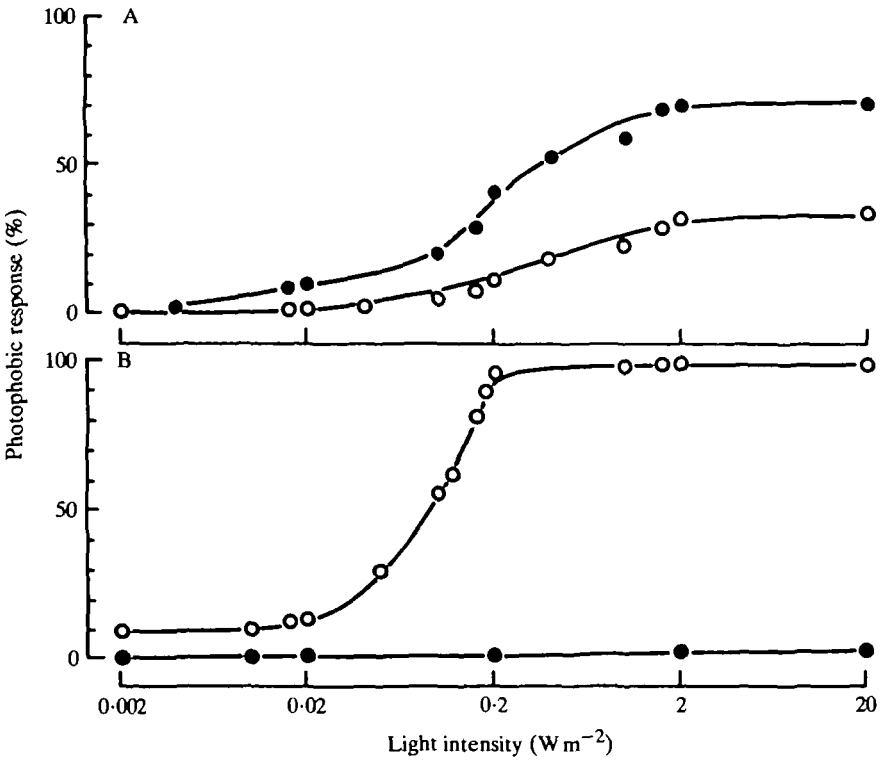

Fig. 1. Degree of photophobic response to a white light in *Paramecium bursaria* as a function of light intensity. (A) *Chlorella*-free (colourless) specimens; (B) light-adapted *Chlorella*-containing (green) specimens; ●, step-up photophobic response; ○, step-down photophobic response.

All showed the response when light intensity was over 2 W m^{-2} (Fig. 1B). The threshold for the half-maximal response (50 %) was about 0·09 W m^{-2}.

Action spectra for the photophobic response

To find the most effective light for inducing photophobic responses, we determined the relationship between the degree of response and the intensity of the stimulating light (fluence rate) using nine monochromatic lights of different wavelengths, ranging from 400 to 720 nm. Two examples (520 nm and 600 nm) of the 'fluence rate–response curves' are shown in Fig. 2. The degree of response increased almost linearly with a logarithmic increase in the fluence rate, reaching a maximum. This was about 70 % for the step-up response (Fig. 2A) and about 30 % for the step-down response (Fig. 2B) in the *Chlorella*-free specimens. It was 100 % for the step-down response in light-adapted *Chlorella*-containing specimens (Fig. 2C).

The fluence rate corresponding to the half-maximal response was estimated for each curve. The reciprocal of the rate (the relative response, which corresponds to the effectiveness of the light in inducing the response) was plotted against the

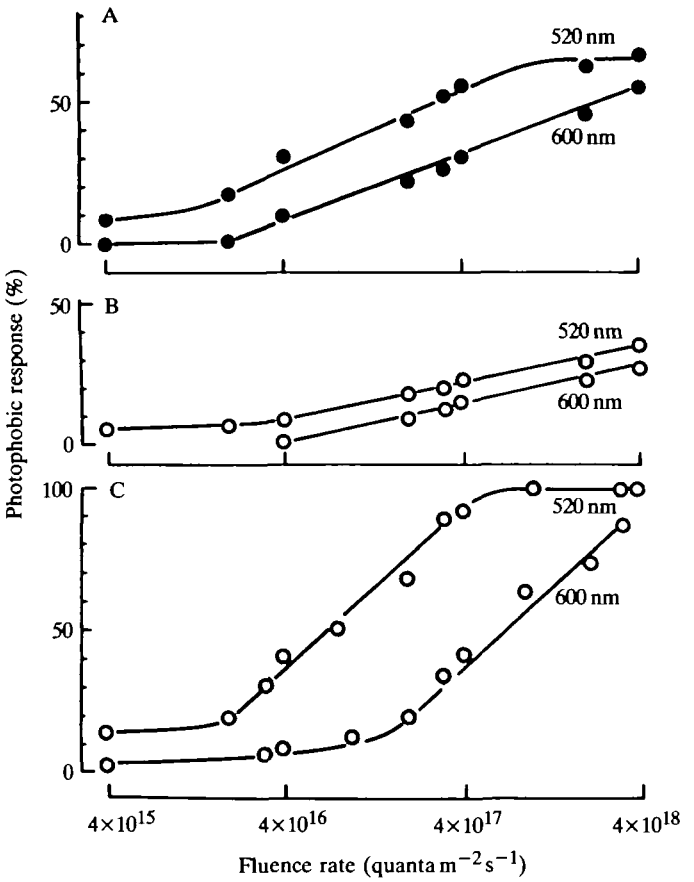

Fig. 2. Fluence rate–response curves for photophobic responses in *Paramecium bursaria*. Two examples of the curves, for 520 and 600 nm, are presented in each figure. (A,B) *Chlorella*-free specimens; (C) light-adapted *Chlorella*-containing specimens; ●, step-up photophobic response; ○, step-down photophobic response.

wavelength to obtain the action spectrum for the response. As shown in Fig. 3A, the action spectrum for the step-up response showed a major peak at 560 nm and a minor peak at 680 nm, while that for the step-down response showed a single peak at 520 nm in the *Chlorella*-free specimens. Action spectra for both step-up and step-down responses in the dark-adapted *Chlorella*-containing specimens were identical to those in the *Chlorella*-free specimens (Fig. 3B). The action spectrum for the step-down response in the light-adapted *Chlorella*-containing specimens showed a single peak at 520 nm (Fig. 3C).

The light-induced change in the photophobic responses in the dark-adapted Chlorella*-containing specimens*

As described in the previous section, about 70% of the dark-adapted *Chlorella*-containing specimens showed the step-up photophobic response, and the rest showed

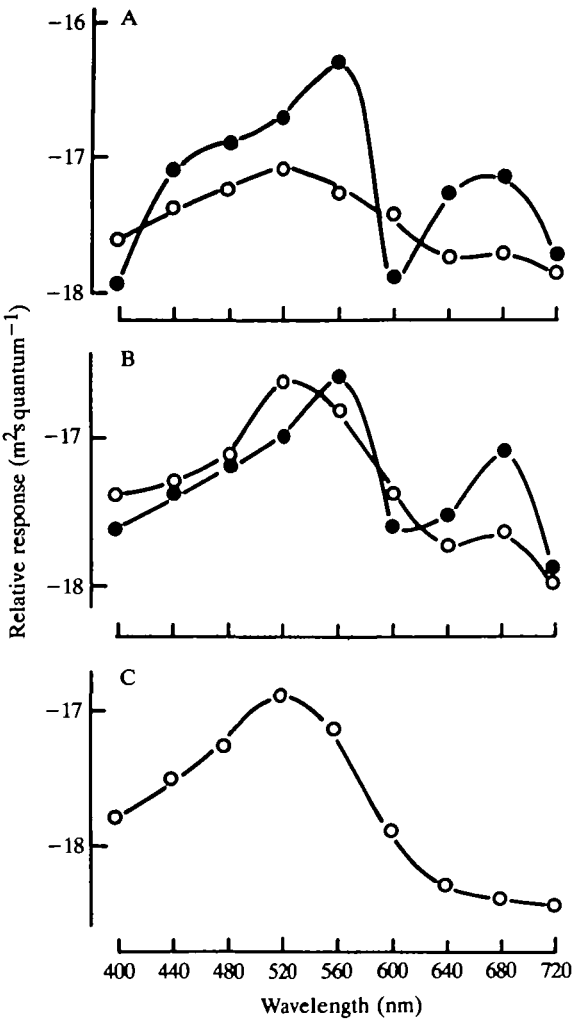

Fig. 3. Action spectra for photophobic responses in *Paramecium bursaria*. (A) *Chlorella*-free specimens; (B) dark-adapted *Chlorella*-containing specimens; (C) light-adapted *Chlorella*-containing specimens; ●, step-up photophobic response; ○, step-down photophobic response.

the step-down photophobic response. When the dark-adapted specimens were kept exposed to a white light (light adaptation), all eventually showed only a step-down response. To measure the time courses for suppression of the step-up response and enhancement of the step-down response by light, the dark-adapted *Chlorella*-containing specimens in the culture were exposed to a white light ($3\,W\,m^{-2}$) for varying periods, then the photophobic responses of the specimens were examined. A 520 nm monochromatic light with an intensity of 4×10^{18} quanta $m^{-2}s^{-1}$ was used for photostimulation. The intensity of the background light for the videorecording was 2×10^{16} quanta $m^{-2}s^{-1}$. As shown in Fig. 4, the step-up photophobic response

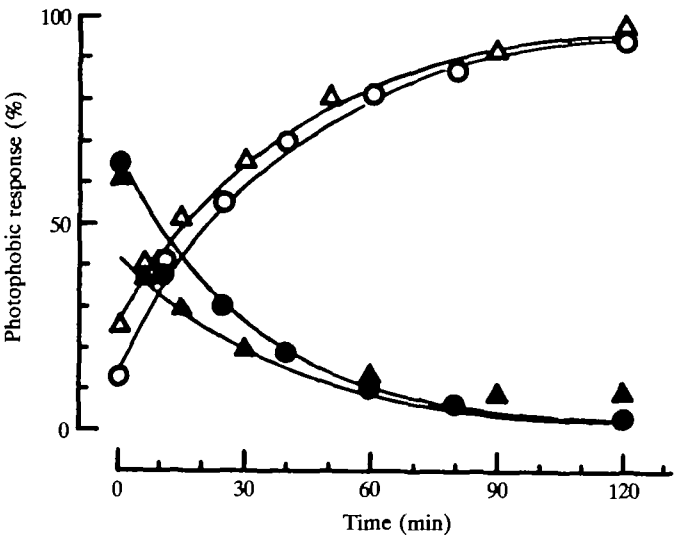

Fig. 4. Time courses of light-induced changes in photophobic responses in dark-adapted *Chlorella*-containing specimens of *Paramecium bursaria* in the absence (circles) and in the presence (triangles) of DCMU, an inhibitor of photosynthesis. Solid symbols, step-up photophobic response; open symbols, step-down photophobic response. The solid lines are best-fit exponential curves.

decreased from its original level (67 %) to zero, while that for step-down increased from its original level (13 %) to 100 % with the time of exposure.

To examine the involvement of photosynthesis by the symbiotic *Chlorella* in the light-induced changes in photophobic responses, the time courses for light-induced changes were determined in the presence of 10^{-5} mol l^{-1} DCMU, an inhibitor of photosynthesis, in the external solution. The time courses were identical with those obtained in the absence of DCMU (Fig. 4).

Spectral sensitivity curves for the light-induced changes in the photophobic responses

To find the most effective light for suppressing the step-up response and enhancing the step-down response in the dark-adapted *Chlorella*-containing specimens, we subjected them to various monochromatic lights of different wavelengths (400–720 nm; 4×10^{18} quanta $m^{-2}s^{-1}$) for 2 h, then examined their photophobic responses. A 520 nm monochromatic light with an intensity of 4×10^{18} quanta $m^{-2}s^{-1}$ was used for stimulation. The degree of suppression or enhancement was defined as the difference between the response of dark-adapted specimens and those subjected to each monochromatic light for 2 h, and was plotted against the wavelength to obtain the spectral sensitivity curve. The curves shown in Fig. 5 clearly indicate that 480 nm light was the most effective for both suppression and enhancement.

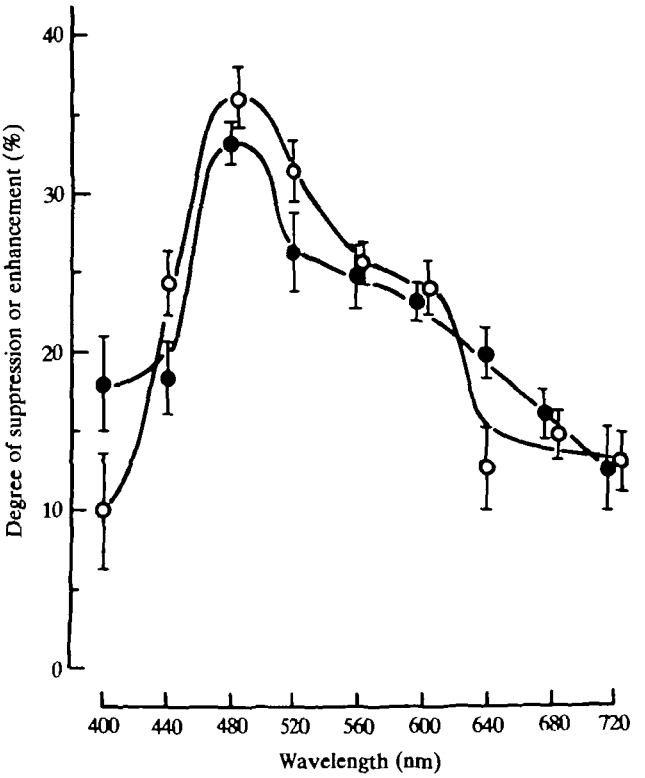

Fig. 5. Spectral sensitivity curves for light-induced suppression and step-up photophobic response (●) and enhancement of step-down photophobic response (○) in dark-adapted *Chlorella*-containing specimens of *Paramecium bursaria*. Each symbol is the mean of 10 measurements from 10 different specimens. Vertical lines, standard error of the mean.

Photokinetic responses

The photophobic response to a change in light intensity subsided in about 15 s. As that response declined, forward swimming velocity and the frequency of spontaneous changes in swimming direction (kinetic activities) reached their respective steady levels, which depended on the light intensity, in about 1 min. These levels remained unchanged for 5–30 min until the specimens showed thigmotaxis (the thigmotactic specimens did not swim but crept slowly on the bottom of their container: Iwatsuki & Naitoh, 1979). Kinetic activities were therefore measured 1 min after the light intensity had been changed.

When the light intensity was increased to more than 50 W m^{-2}, the swimming velocity of the *Chlorella*-free specimens increased (positive photo-orthokinetic response), reaching a maximum (about 1·9 mm s^{-1}; 130 % of the swimming velocity in the dark), whereas the frequency of spontaneous change in the swimming direction decreased (negative photoklinokinetic response), reaching its minimum of about 0·02 Hz (25 % of the frequency in the dark) (Fig. 6A). The threshold

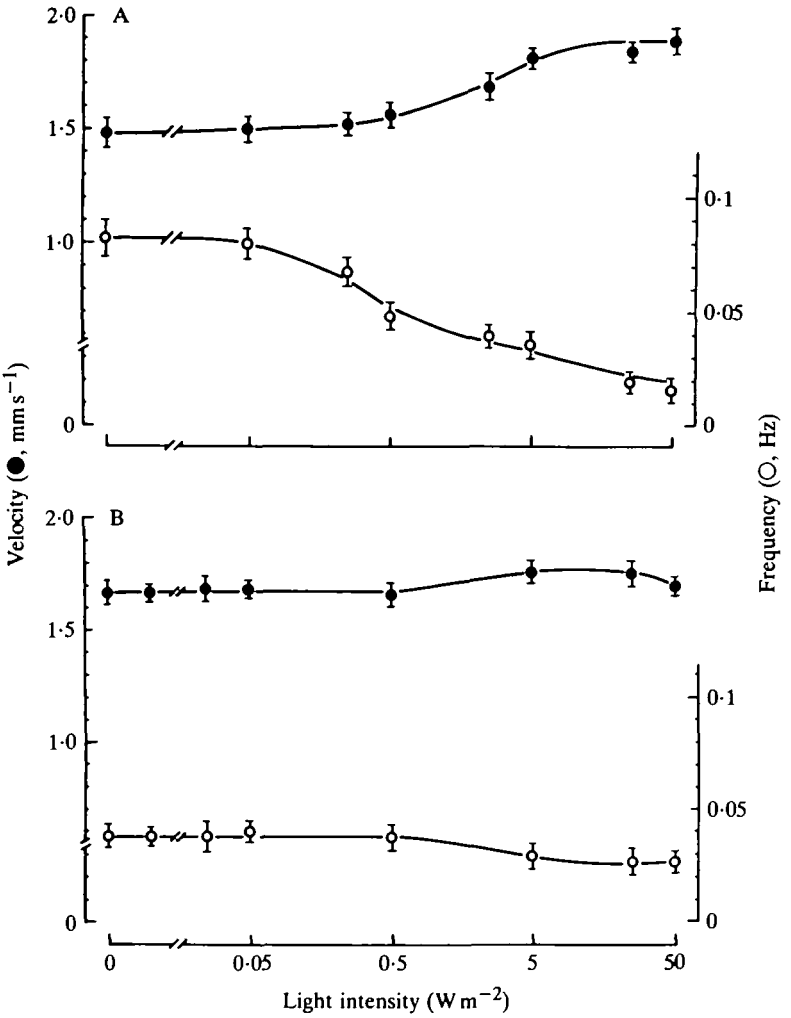

Fig. 6. Photokinetic activity of *Paramecium bursaria* as a function of intensity of white light. (A) *Chlorella*-free specimens; (B) light-adapted *Chlorella*-containing specimens; ●, forward swimming velocity (photo-orthokinesis); ○, frequency of spontaneous change in swimming direction (photoklinokinesis). Each symbol is the mean of 10 measurements from 10 different specimens. Vertical lines, standard error of the mean.

intensities for half-maximal responses were 2·1 W m^{-2} for the orthokinetic response and 0·75 W m^{-2} for the klinokinetic response. The dark-adapted *Chlorella*-containing specimens showed photokinetic responses identical to those of the *Chlorella*-free specimens.

In light-adapted *Chlorella*-containing specimens, however, photokinetic responses were inconspicuous. That is, in light of intensity greater than 40 W m^{-2}, swimming velocity increased by less than 5 % and the frequency of spontaneous changes in the swimming direction decreased by less than 20 % of each value in the dark (Fig. 6B).

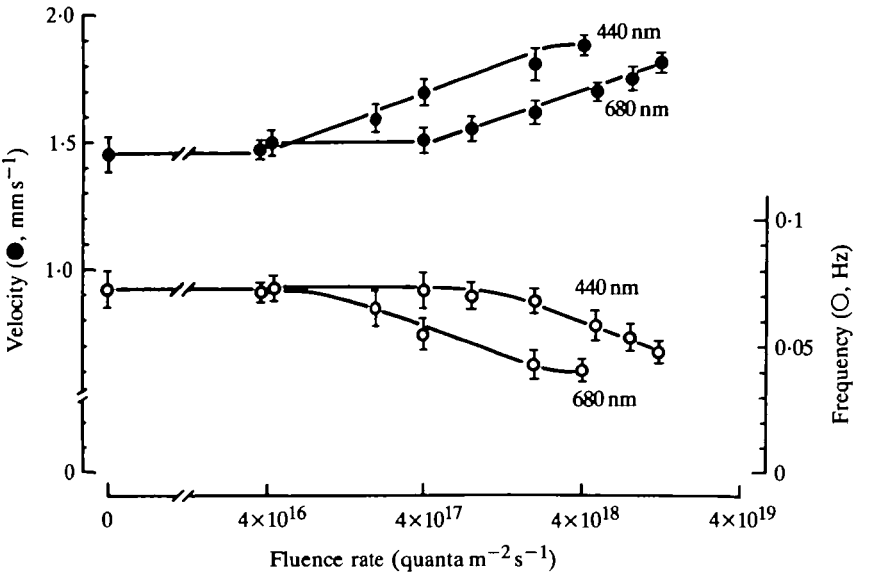

Fig. 7. Fluence rate–response curves for two kinds of photokinesis in *Chlorella*-free specimens of *Paramecium bursaria*. Typical curves for two monochromatic lights (440 and 680 nm) are presented; ●, forward swimming velocity; ○, frequency of spontaneous change in swimming direction. Each symbol is the mean of 10 measurements from 10 different specimens. Vertical lines, standard error of the mean.

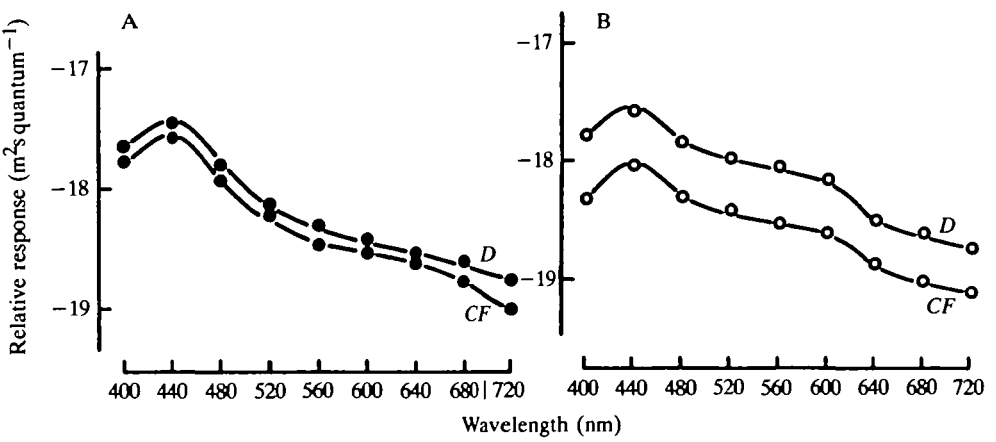

Fig. 8. Action spectra for the photo-orthokinetic response (an increase in forward swimming velocity induced by light) (A) and for the photokinokinetic response (a decrease in frequency of spontaneous change in swimming direction by light) (B) in *Chlorella*-free (*CF*) and in dark-adapted *Chlorella*-containing (*D*) specimens of *Paramecium bursaria*.

The velocity was always higher and the frequency always lower in light-adapted *Chlorella*-containing specimens than in those that were dark-adapted (or *Chlorella*-free) when they were put in the dark (0–0·05 W m^{-2}). In the light (50 W m^{-2}), however, maximum velocity was significantly higher in light-adapted specimens than in dark-adapted ones.

Action spectra for the photokinetic responses

To find the most effective light for inducing photokinetic responses in *Chlorella*-free specimens, the fluence rate–response curves for photokinetic responses were determined with nine different monochromatic lights (400–720 nm). Two examples of the curves (for 440 and 680 nm) are shown in Fig. 7. Each kinetic activity changed quasilinearly with a logarithmic increase in the fluence rate to reach its maximum. The fluence rate of each monochromatic light corresponding to the half-maximal kinetic response (the orthokinesis, 1·7 mm; the klinokinesis, 0·049 Hz) was determined. The reciprocal of the fluence rate (the relative response) was plotted against the wavelength to obtain the action spectrum. The action spectrum for the orthokinetic response and that for the klinokinetic response showed a single peak at 440 nm (Fig. 8). The action spectra obtained for *Chlorella*-free and for dark-adapted *Chlorella*-containing specimens were similar.

Light-induced suppression of the photokinetic responses in dark-adapted Chlorella*-containing specimens*

As described above, the photokinetic responses in the dark-adapted *Chlorella*-containing specimens became inconspicuous after light exposure (Fig. 6B). To determine the time course for light-induced suppression of photokinetic responses, the degree of each photokinetic response of dark-adapted specimens was determined after exposing them to white light (5 W m^{-2}) for various times (0–120 min). The degree of the response, defined here as the difference between the kinetic activity of dark-adapted specimens in background light (520 nm: 2×10^{17} quanta m^{-2} s^{-1}) and stimulating light (520 nm: 4×10^{18} quanta m^{-2} s^{-1}, strong enough to produce the maximal response), is presented as a percentage of the maximum degree seen in the specimens before their exposure to white light (at 0 exposure time). The photokinetic response decreased exponentially with the exposure time, reaching a minimum in about 120 min (Fig. 9).

To examine whether the photosynthetic activity of the symbiotic green algae is involved in the mechanism of light-induced suppression, the time course for suppression was determined in the presence of 10^{-5} mol l^{-1} DCMU in the external solution. The suppression at 12 min was less than 10 %, in contrast to more than 90 % in the absence of DCMU (Fig. 9).

Spectral sensitivity curves for light-induced suppression of the photokinetic responses

To find the most effective light for suppressing photokinetic responses, the dark-adapted *Chlorella*-containing specimens were exposed to various monochromatic

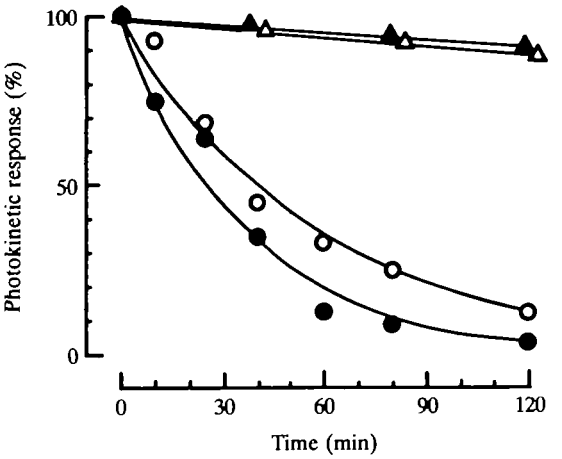

Fig. 9. Time courses of light-induced suppression of photokinetic responses in dark-adapted *Chlorella*-containing specimens of *Paramecium bursaria* in both the absence (circles) and the presence (triangles) of an inhibitor of photosynthesis, DCMU. Open symbols, klinokinetic response; solid symbols, orthokinetic response.

lights (4×10^{18} quanta $m^{-2}s^{-1}$) with different wavelengths (400–720 nm) for 120 min, then the percentage of each photokinetic response was determined. The difference between this value and 100% (an indication of the degree of the suppression) was plotted against each corresponding wavelength to obtain the spectral sensitivity curve for suppression. Suppression of the orthokinetic response and the klinokinetic response occurred most effectively at 440 and 680 nm (Fig. 10).

Examination of the phototaxis

To determine whether specimens of *P. bursaria* show phototaxis, we examined the swimming direction of about 400 specimens in an experimental vessel subjected to both collimated and diffused white light (10 W m^{-2}).

The swimming direction of a specimen was defined as the angle between the direction of the collimated light and the direction to which the anterior end of the specimen pointed. There was no significant directional difference in movement between specimens in collimated light and diffused light, or between *Chlorella*-free specimens and light-adapted *Chlorella*-containing specimens (Fig. 11).

DISCUSSION

Behavioural responses to light in dark-adapted *Chlorella*-containing specimens of *P. bursaria* were identical with those that were *Chlorella*-free. All showed the avoiding reaction to an increase in light intensity (step-up photophobic response) and accumulated in the dark region (photodispersal). Forward swimming velocity (orthokinesis) was higher and the frequency of spontaneous changes in swimming direction (klinokinesis) was lower when the specimens were in the lighter region.

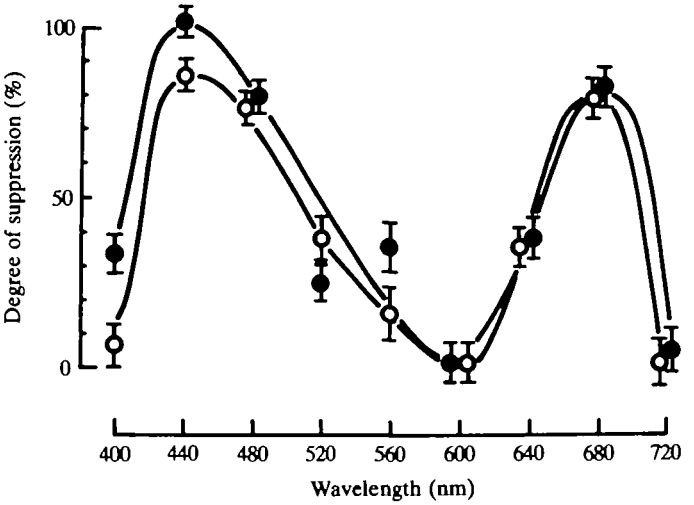

Fig. 10. Spectral sensitivity curves for light-induced suppression of photokinetic responses in dark-adapted *Chlorella*-containing specimens of *Paramecium bursaria*. ○, klinokinetic response; ●, orthokinetic response. Each symbol is the mean of 10 measurements from 10 different specimens. Vertical lines, standard error of the mean.

Higher swimming velocity and a lower frequency of changes in swimming direction in the lighter region decrease the probability that the specimens will stay in the region. Therefore, photokinetic responses (changes in the kinetic activities) also cause photodispersal.

However, light-adapted *Chlorella*-containing specimens showed an avoiding reaction to a decrease in light intensity (step-down photophobic response), and thus they accumulated in the light region (photoaccumulation). The kinetic responses in the light-adapted specimens were weak, and thus the responses did not interfere with the photoaccumulation mediated by the step-down photophobic response.

It is interesting to note that both *Chlorella*-free and dark-adapted *Chlorella*-containing specimens showed not only step-up photophobic but also step-down photophobic responses, though the former was predominant (Iwatsuki & Naitoh, 1981). The peak wavelengths of the action spectrum differed between the two photophobic responses (560 nm and 680 nm for the step-up, 520 nm for the step-down; Fig. 3A). This suggests that the two photophobic responses are under the control of two different photoreceptor systems. They will be termed 'photoreceptor system I' for the step-up response and 'photoreceptor system II' for the step-down response. Nevertheless, the action spectra for the two kinetic responses showed a single peak at a common wavelength of 440 nm (Fig. 8). This implies that the two photokinetic responses are under the control of a single photoreceptor system, 'photoreceptor system III'.

Under the influence of light, the symbiotic *Chlorella* inhibits activities of systems I and III, but enhances that of system II (Figs 3, 6). Thus the specimens show

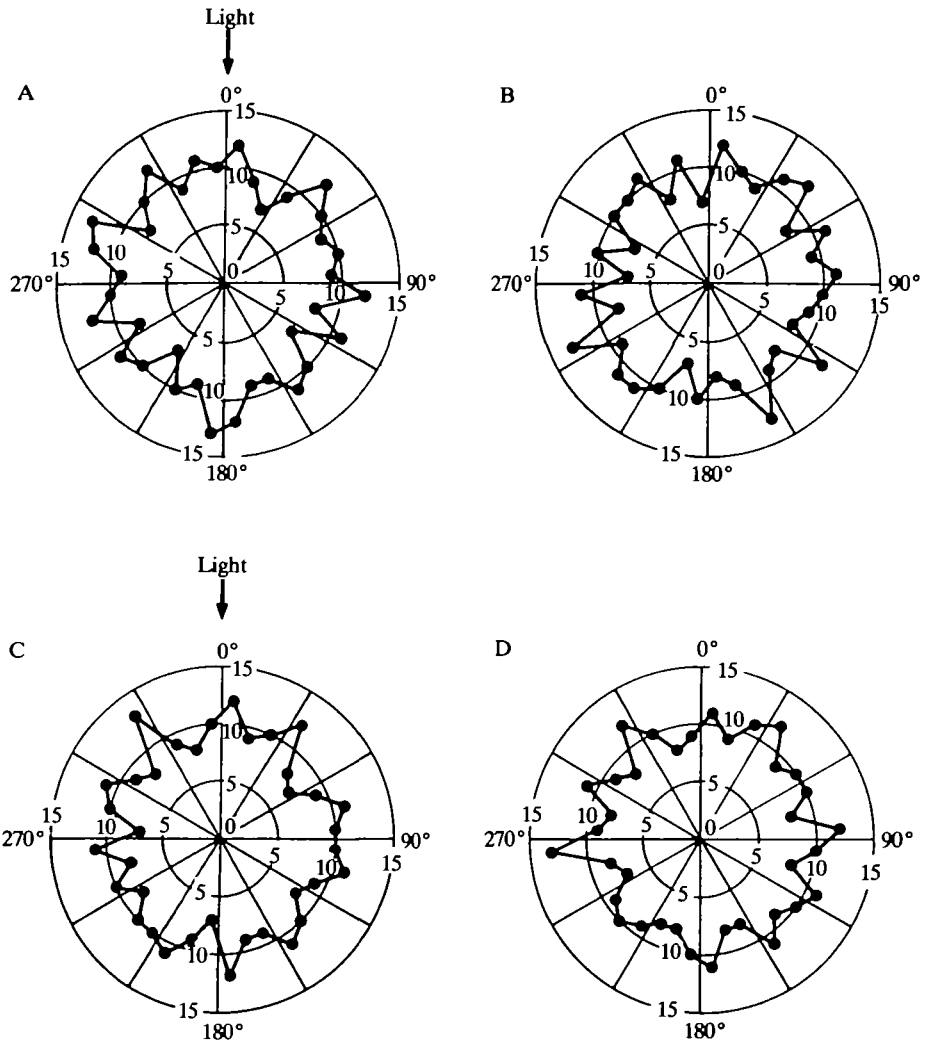

Fig. 11. Swimming direction of specimens of *Paramecium bursaria* in a cuvette under the influence of both collimated (A,C) and diffused (B,D) light. The numerals on each concentric circle show the number of specimens that swam into each directional division of 10° width. A,B, *Chlorella*-free specimens; C,D, light-adapted *Chlorella*-containing specimens.

photoaccumulation. Clearly, photoaccumulation of the host is essential for survival of the photosynthetic symbionts. Some of the photosynthetic products are known to benefit the host (Brown & Nielsen, 1974).

The symbiont-mediated, light-induced suppression of system I and the enhancement of system II were not inhibited by DCMU, an inhibitor of photosynthesis (Fig. 4). Moreover, the spectral sensitivity curves for both suppression and enhancement showed a single peak at a common wavelength, 480 nm. Interestingly,

this peak does not correspond to any of the photosynthetic action spectrum peaks but does correspond to the blue-light effect of the symbionts (Kamiya & Miyachi, 1974; Miyachi *et al.* 1978). These results suggest that both suppression and enhancement are mediated by the blue-light effect of the symbionts. However, DCMU inhibited the suppression of system III (Fig. 9). Moreover, the two peaks of the spectral sensitivity curves for the suppression coincided with those of the action spectrum for the photosynthesis of symbionts (440 and 680 nm; Fig. 10) (Haxo, 1960). This may mean that the suppression is mediated by the photosynthesis of symbionts. The long lag period for suppression or enhancement (Figs 4, 9; 60–90 min) suggests that at least some products of either the blue-light effect or photosynthesis must accumulate before such responses occur.

The photophobic response is caused by a transient reversal in the beat direction of cilia (ciliary reversal). This reversal is due to an inflow of Ca^{2+} into the cilia through the depolarization-activated Ca^{2+} channels in the ciliary membrane (Eckert, Naitoh & Machemer, 1976; Naitoh, 1982). It is conceivable, therefore, that light stimulation of photoreceptor systems I and II produces a transient depolarizing receptor potential, which spreads electrotonically to the ciliary membrane to activate the Ca^{2+} channels. A sustained membrane hyperpolarization causes a sustained increase in the forward swimming velocity, due primarily to an increase in ciliary beat frequency, and it suppresses the spontaneous avoiding reaction in *Paramecium* (Machemer, 1976; Naitoh, 1982). Photoreceptor system III, therefore, may produce prolonged membrane hyperpolarization in response to a sustained increase in light intensity. Our preliminary (unpublished) examinations of *Chlorella*-free specimens reveal that a transient membrane depolarization followed by a sustained hyperpolarization occurred during increasing light intensity. These hypothetical receptor systems of *P. bursaria* and their modifications by the symbiotic *Chlorella* are presented schematically in Fig. 12.

Photoaccumulation is dependent on both the step-down photophobic response exhibited by the specimens at the light–dark border and on the photokinetic responses. If the rate of decrease in light intensity experienced by specimens at a light–dark border is below a certain threshold (Saji & Oosawa, 1974) the specimens do not show the photophobic response at the border. Their accumulation, therefore, depends mostly on kinetic responses. However, if the rate of decrease is large enough to produce a photophobic response, accumulation may depend primarily on the phobic response. The contribution from each response to photoaccumulation may vary according to light intensity, light intensity gradient at the light–dark border, shape and size of the experimental vessel, ionic composition of the experimental solution, temperature, culture age, circadian time, nutritive condition, etc. We controlled carefully for these factors. In our experimental system, photoaccumulation was dependent almost exclusively on the photophobic response (Iwatsuki & Naitoh, 1981, 1983). The administration of DCMU did not affect photoaccumulation, since DCMU did not affect the photophobic response (Fig. 4). If, for some reason, the phobic response at the border is weak, suppression of kinetic responses is indispensable for accumulation that is mediated by the phobic response. This is

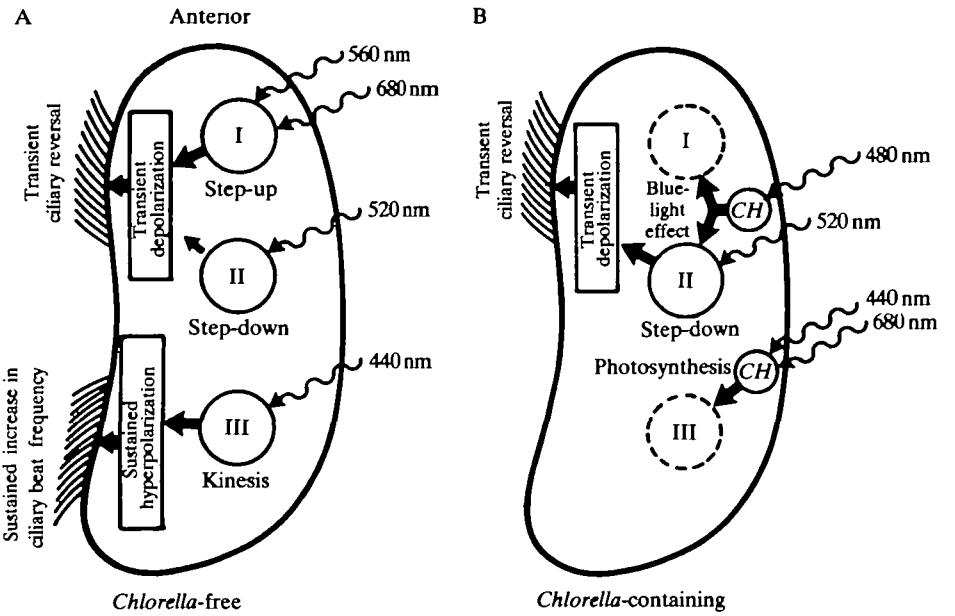

Fig. 12. Schematic representation of three hypothetical photoreceptor systems (I, II and III) in both *Chlorella*-free (A) and *Chlorella*-containing (B) specimens of *Paramecium bursaria* and modification of their activities by symbiotic *Chlorella* (*CH*) under the influence of light (wavy lines with arrowheads). The numerals beside the wavy lines indicate wavelength (nm) of the most effective light for activating the receptor systems of *Chlorella*. Smaller arrow beside II in A indicates that photoactivated system II is less effective than system I in producing a transient membrane depolarization. The transient depolarization produces a transient ciliary reversal, which causes a phobic response (avoiding reaction) of the specimen. Photoactivated system III produces a sustained hyperpolarization, which causes a sustained increase in ciliary beat frequency or an increase in the forward swimming velocity. In response to a change in light intensity, ciliary reversal takes place first, and is followed by an increase in the ciliary beat frequency. Systems I and III enclosed by dotted circles in B indicate that the systems are inactivated by photoactivated *Chlorella*. Only system II, therefore, is effective in producing the photophobic response of the specimens. See the text for more details.

because such responses (i.e. increased forward swimming velocity and decreased frequency of the spontaneous light-avoiding reaction) antagonize photoaccumulation. Since suppression of the photokinetic response was photosynthesis-dependent, inhibition of the symbiont's photosynthesis by DCMU abolished photoaccumulation, as in the cases reported by Niess *et al.* (1981, 1982). Interaction between the photophobic response and motile activity of the cells whilst accumulating in the light was clearly demonstrated by Cronkite & Van Den Brink (1981). They found that light-induced (supposedly O_2-mediated) suppression of motile activity in creeping *Chlorella*-containing specimens of *P. bursaria* was a cause of photoaccumulation of the specimens. Administration of DCMU removed the suppression, and the specimens became motile. However, the motile specimens stayed in a light spot,

because they showed the step-down photophobic response at the light–dark border even in the presence of DCMU.

Our finding, that blue light inhibited the step-up photophobic response and enhanced the step-down photophobic response, is consistent with that of Pado (1972), i.e. dark-adapted specimens accumulated in the blue-light region of spectral components of a white light. The specimens that entered the blue-light region showed a step-down photophobic response and thus stayed in the region. Further detailed examinations of the photoresponses are needed to explain some discrepancies between our results and those of Reisser & Haeder (1984).

Common colourless *Paramecium*, such as *P. caudatum*, *P. tetraurelia* (Iwatsuki & Naitoh, 1982) and *P. multimicronucleatum* (Iwatsuki & Naitoh, 1983) showed a step-up photophobic response and resulting photodispersal in a manner similar to *Chlorella*-free *P. bursaria*. However, these colourless *Paramecium*, which have never established a symbiotic relationship with *Chlorella*, did not show the step-down photophobic response seen in *Chlorella*-free *P. bursaria*. It is conceivable that the establishment of the symbiotic relationship of *P. bursaria* with the green algae is dependent on their possession of photoreceptor system II, which mediates the step-down photophobic response indispensable for photoaccumulation.

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