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Phototactic responses of four marine dinoflagellates with different types of eyespot and chloroplast

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SUMMARY

Great structural variety is seen in the eyespot of dinoflagellates, a structure involved in phototaxis. Although there are several works on the phototactic responses in some species of dinoflagellates, none of the dinoflagellates used in these studies possessed an eyespot and, therefore, we have no knowledge of the relationship between eyespot type and phototactic response. In this study, we determined wavelength dependency curves for phototaxis in four marine dinoflagellates that possess a different type of either eyespot or chloroplast. These include: (i) a dinoflagellate possessing a peridinin-containing chloroplast with an eyespot (*Scrippsiella hexapraecingula* Horiguchi et Chihara); (ii) a dinoflagellate containing a diatom endosymbiont and with the type B eyespot *sensu* Dodge (1984; (*Peridinium foliaceum* (Stein) Biecheler); (iii) a dinoflagellate with peridinin-containing chloroplasts, but lacking an eyespot (*Alexandrium hiranoi* Kita et Fukuyo); and (iv) a dinoflagellate with fucoxanthin, 19′-hexanoyloxyfucoxanthin and 19′-butanoyloxyfucoxanthin, but lacking an eyespot (*Gymnodinium mikimotoi* Miyabe et Kominami ex Oda). Regardless of the eyespot or the chloroplast type, all four dinoflagellates showed similar wavelength dependency curves for phototaxis, with sensitivity between 380 and 520 nm, the highest peak at approximately 440 or 460 nm and smaller peaks or shoulders at 400–420 nm and 480–500 nm. Substantial peaks have also been noted in the ultraviolet range (260–280 nm). The ultrastructural study of the eyespot of *Scrippsiella hexapraecingula* revealed that the eyespot consists of two layers of lipid globules and probably acts as a quarter-wave stack antenna.

Key words: action spectra, dinoflagellates, eyespot, phototaxis.

INTRODUCTION

The eyespot (or stigma) forms an essential part of the photoreceptive device in the phototactic responses of various algal cells. There are several different types of eyespots and Dodge (1969) classified them into four basic types: (i) type A; eyespot within a chloroplast but not obviously associated with a flagellum; (ii) type B: eyespot within a chloroplast and closely associated with a flagellum; (iii) type C: eyespot independent of a chloroplast but adjacent to a flagellum; and (iv) type D: various types of eyespot and ocellus found in the Dinophyceae. It is generally accepted that one particular phylogenetic class of algae possesses only one type of eyespot (Dodge 1984; Kawai 1992; Kreimer 1994). Members of the Chlorophyta generally have a type A eyespot, most members of Chromophyta have type B, and Euglenophyceae and Eustigmatophyceae have type C. The dinoflagellates are an exception and have multiple types of eyespots, which Dodge classified as type D, perhaps because dinoflagellate cells inherit the eyespot from various photosynthetic symbionts which have different types of eyespots. Therefore, it is interesting to study whether or not dinoflagellates also inherit other parts of the photoreceptive mechanism (e.g. photoreceptor pigments) from those endosymbionts.

However, the photoreceptor pigments have not been identified in any of the systematic groups. Therefore, to compare the photoreceptive mechanisms with morphological variations of the eyespots, action spectra of the phototactic responses are needed for basic characterization of the photoreceptors involved. The action spectra, or wavelength dependencies, have been studied in several taxa. Some dinoflagellates exhibit main action peaks in the range of blue light (Halldal 1958; Hand *et al.* 1967; Forward 1973), while others reveal action peaks at longer wave lengths [yellow light in *Prorocentrum micans* Ehrenberg (Halldal 1958) and red light in *Peridinium gatunense* Nygaard (Liu *et al.* 1990)]. The dinoflagellates in which action spectra have been studied, other than *P. micans* and *P. gatunense,* are *Gonyaulax catenella* Whedon et Kofoid [= *Alexandrium*

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catenella (Whedon et Kofoid) Balech] (Halldal 1958), *Gyrodinium dorsum* Kofoid et Swezy (Hand *et al.* 1967; Forward 1973), *Gymnodinium splendens* Lebour (= *G. sanguineum* Hirasaka; Forward 1974) and *Peridinium trochoideum* (Stein) Lemmermann [= *Scrippsiella trochoidea* (Stein) Balech; Halldal 1958]. However, it should be noted that none of them possesses any eyespot and, therefore, we have no data on the relationship between eyespot type and the phototactic response.

Therefore, in order to gain more information regarding the diversity of phototactic responses of dinoflagellates, especially the role of eyespots in the photoreceptive mechanisms as related to their evolutionary origins, we investigated the wavelength dependencies of phototaxis in four marine dinoflagellates with different types of eyespot and/or chloroplasts. These included: (i) a dinoflagellate possessing a peridinin-containing chloroplast with an eyespot (*Scrippsiella hexapraecingula* Horiguchi et Chihara); (ii) a dinoflagellate containing a diatom endosymbiont and with the type B eyespot *sensu* Dodge (1984; *Peridinium foliaceum* (Stein) Biecheler), (iii) a dinoflagellate with peridinin-containing chloroplasts, but lacking an eyespot (*Alexandrium hiranoi* Kita et Fukuyo); and (iv) a dinoflagellate with fucoxanthin, 19′-hexanoyloxyfucoxanthin and 19′-butanoyloxyfucoxanthin (Suzuki and Ishimaru 1993), but lacking an eyespot (*Gymnodinium mikimotoi* Miyabe et Kominami ex Oda). As the chloroplasts of *G. mikimotoi* do not contain peridinin, the origin of the chloroplasts is, therefore, thought to be different from those of typical dinoflagellate (peridinin containing) chloroplasts. We have studied the ultrastructure of *S. hexapraecingula* in addition to the phototactic response curves, to elucidate which type of eyespot it possesses. *Peridinium folaceum* also possesses the eyespot and, as it has been well documented (Dodge and Crawford 1969; Dodge 1984; Kreimer 1994), we have only investigated *S. hexapraecingula* in this study.

MATERIALS AND METHODS

Materials

The names and origins of the dinoflagellates used in this study are listed in Table 1. All the cultures were maintained in Provasoli's enriched seawater medium (Provasoli 1968) at 20°C, in a 16:8 LD cycle and under fluorescent lamps at a fluence rate of 30 μmol photons $m^{-2} s^{-1}$. Cells in the logarithmic growth phase were used in all experiments.

Electron microscopy

Cells of *S. hexapraecingula* were processed according to Horiguchi and Pienaar (1994b) for transmission electron microscopy.

Measurements of phototaxis

Preparation for experiments was made under cool, white fluorescent lamps (4.3 μmol $m^{-2} s^{-1}$) at 20°C. A flat glass cuvet (internal dimensions 10 × 10 × 0.5 mm) was placed on a microscope stage; the side walls of the cuvet consisted of quartz and the bottom glass was 0.86 mm thick. A cover slip was placed on top after pipeting sample cell suspension into it.

Phototaxis measurements were carried out using a computerized, motion analyzing system as described by Takahashi and Kobatake (1982), Takahashi *et al.* (1991) and Erata *et al.* (1995). Five identical sets of the system were used simultaneously at different monochromatic wavelength positions (260–740 nm) of the Okazaki Large Spectrograph (OLS; Watanabe *et al.* 1982), National Institute for Basic Biology, Okazaki, Japan. Six measurements were made at each combination of wavelength and photon fluence rate (light intensity). In the preliminary experiments, the minimum and effective light intensity range for respective species were determined. For *S. hexapraecingula* and *A. hiranoi*, 3 μmol photons $m^{-2} s^{-1}$ fluence rate was used, while for the other two species, a fluence rate of 30 μmol photons $m^{-2} s^{-1}$ was used. As a light intensity of 30 μmol photons $m^{-2} s^{-1}$ was not obtainable at 260 nm with OLS, the experiment at this wavelength was omitted for the latter two species. The fluence rates were measured with a custom-made photometer, HK-1 (Hashimoto *et al.* 1982) and adjusted by inserting neutral density filters. Appropriate stimulus times and video frame-acquisition rates were selected for each species, according to data obtained in the preliminary experiments.

Table 1. List of strains used in this study

	Eyespot type	Chloroplast type	Origin or isolator
Scrippsiella hexapraecingula	C	Peridinin-type*	T. Horiguchi
Peridinium foliaceum	B	Fucoxanthin-type†	UTEX1688
Alexandrium hiranoi	None	Peridinin-type*	T. Horiguchi
Gymnodinium mikimotoi	None	‡	NIES249

Classification of the eyespot types is according to Dodge (1984). The type of chloroplast is represented by its main accessory pigments. *The peridinin-type chloroplast is typical of dinoflagellates, which is enclosed by three membranes and contains peridinin as a major photosynthetic pigment. †The dinoflagellate contains a diatom endosymbiont (Chesnick *et al.* 1996) and thus the chloroplast structure is similar to those of diatoms. ‡The chloroplast contains fucoxanthin, 19′-hexanoyloxyfucoxanthin and 19′-butanoyloxyfucoxanthin (Suzuki and Ishimaru 1993). UTEX, University of Texas, USA; NIES, National Institute for Environmental Studies, Japan.

The phototactic index used in this study was calculated as follows (modified from Takahashi *et al.* 1992). The two-dimensional displacement vectors of swimming cells calculated from the two captured consecutive video frames (fields) were classified into four categories according to their direction (shown as hatched areas A to D in Fig. 1). For positively and negatively phototactic cells, phototactic indices were defined as

$$[(a)/((a) + (b) + (c) + (d)) - 1/4] \times 4/3 \ldots \quad (1)$$

and

$$[(c)/((a) + (b) + (c) + (d)) - 1/4\ ] \times 4/3, \ldots \quad (2)$$

respectively, where a, b, c and d are the numbers of swimming tracks whose displacement vectors belong to categories A, B, C and D, respectively. The numerical factor of 4/3 in equations (1,2) was introduced to adjust the positive value of the indices to between 0 and 1.

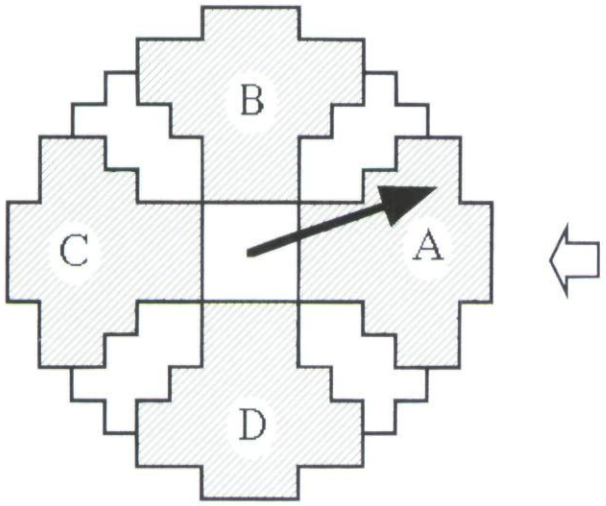
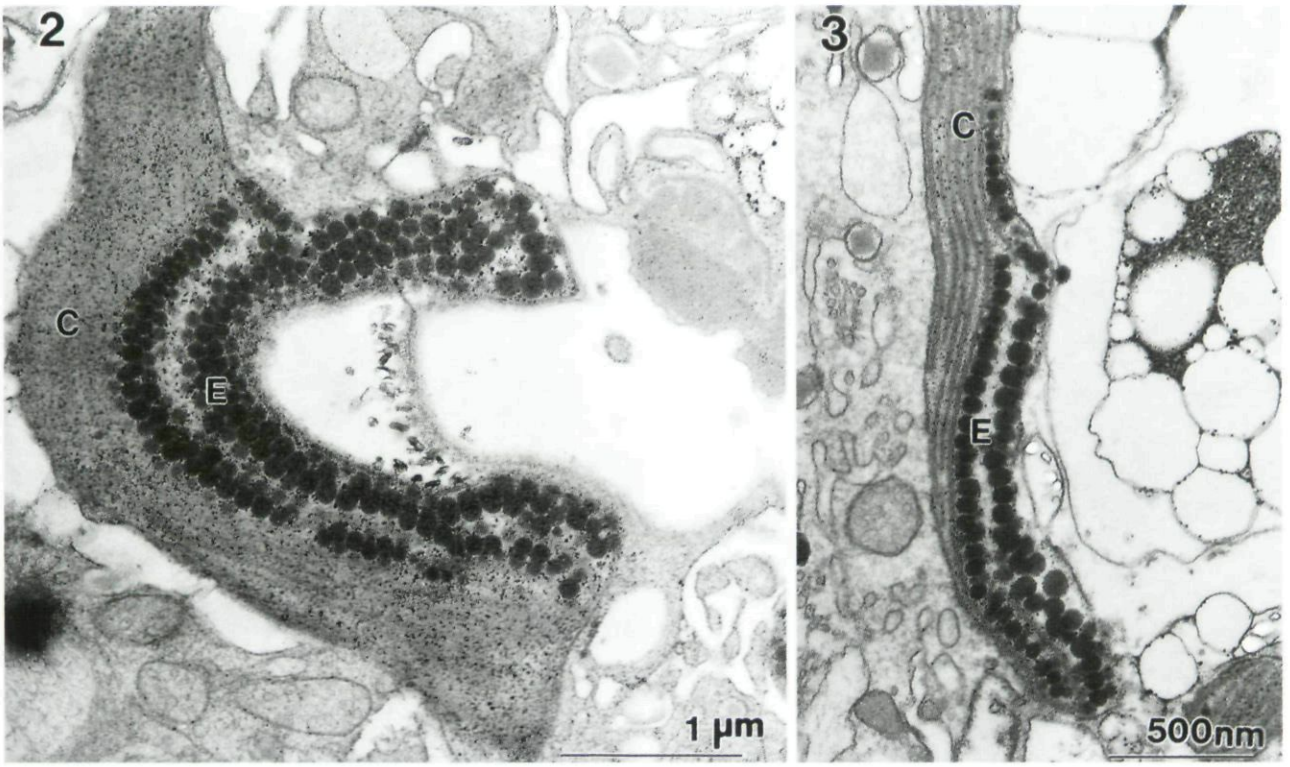


Fig. 1. Schematic illustration of the phototactic indices. Open arrow indicates the direction of actinic light. Filled arrow shows an example of displacement vectors calculated from digitized images of cells between two consecutive frames. Hatched areas A to D define the categories to which the vectors were classified according to their direction. Relative distributions of the numbers of the vectors within categories A and C to that of the total vectors were evaluated for positive and negative phototactic indices, using the equations described in the text.

RESULTS

Eyespot ultrastructure of *Scrippsiella hexapraecingula*

The eyespot of *Scrippsiella hexapraecingula* is rectangular and located in the ventral side of the cell, adjacent to the sulcus (Horiguchi and Chihara 1983). Transmission electron microscopy revealed that it is the type A eyespot and the osmiophilic globules are located within the chloroplast (Figs 2,3). The osmiophilic globules are arranged in two rows (Fig. 3), just beneath the chloroplast envelope. No thylakoids have been observed between the rows of globules in most parts of the



Figs 2,3. *Scrippsiella hexapraecingula* Horiguchi et Chihara, transmission electron micrographs of the eyespot. 2. Transverse section (slightly oblique) through the eyespot. 3. Longitudinal section through the eyespot, showing two-layer lipid globules. C, chloroplast; E, eyespot.

eyespot. However, a single thylakoid has invaded, to a limited extent, between the two globular layers in the lower portion of the eyespot (Fig. 3). The distance between the two layers is approximately 50 nm. The size of the globules is relatively constant and is 70–80 nm in diameter.

General features of phototactic responses

Two of the dinoflagellates, *A. hiranoi* and *G. mikimotoi*, showed positive phototaxis, while the other two, *S. hexapraecingula* and *P. foliaceum*, revealed negative phototaxis, at least under the conditions used for the experiments. The same reactions have been observed under different light intensities during preliminary experiments. The phototactic response of *P. foliaceum* was apparently weaker than the other three species.

Wavelength dependency curves for phototaxis

Figure 4 shows phototactic wavelength dependency curves for the four dinoflagellates, all of which possess main peaks in the same range between 260 and 500 nm and curves of similar shape, although minor discrepancies exist in peak positions. The wavelength dependency curves of *A. hiranoi* and *S. hexapraecingula* are almost identical, with a main peak at 460 nm and smaller peaks at 420 and 500 nm. The shape of the wavelength dependency curve of *G. mikimotoi* is basically the same as those of the former two species but each peak is shifted 20 nm towards shorter wavelength (i.e. main peak at 440 nm, smaller peaks at 480 and 400 nm). The main peak in *P. foliaceum* was at 440 nm, as was that of *G. mikimotoi*, while other smaller peaks were not present, probably due to its weaker responses. In all these dinoflagellates, strong

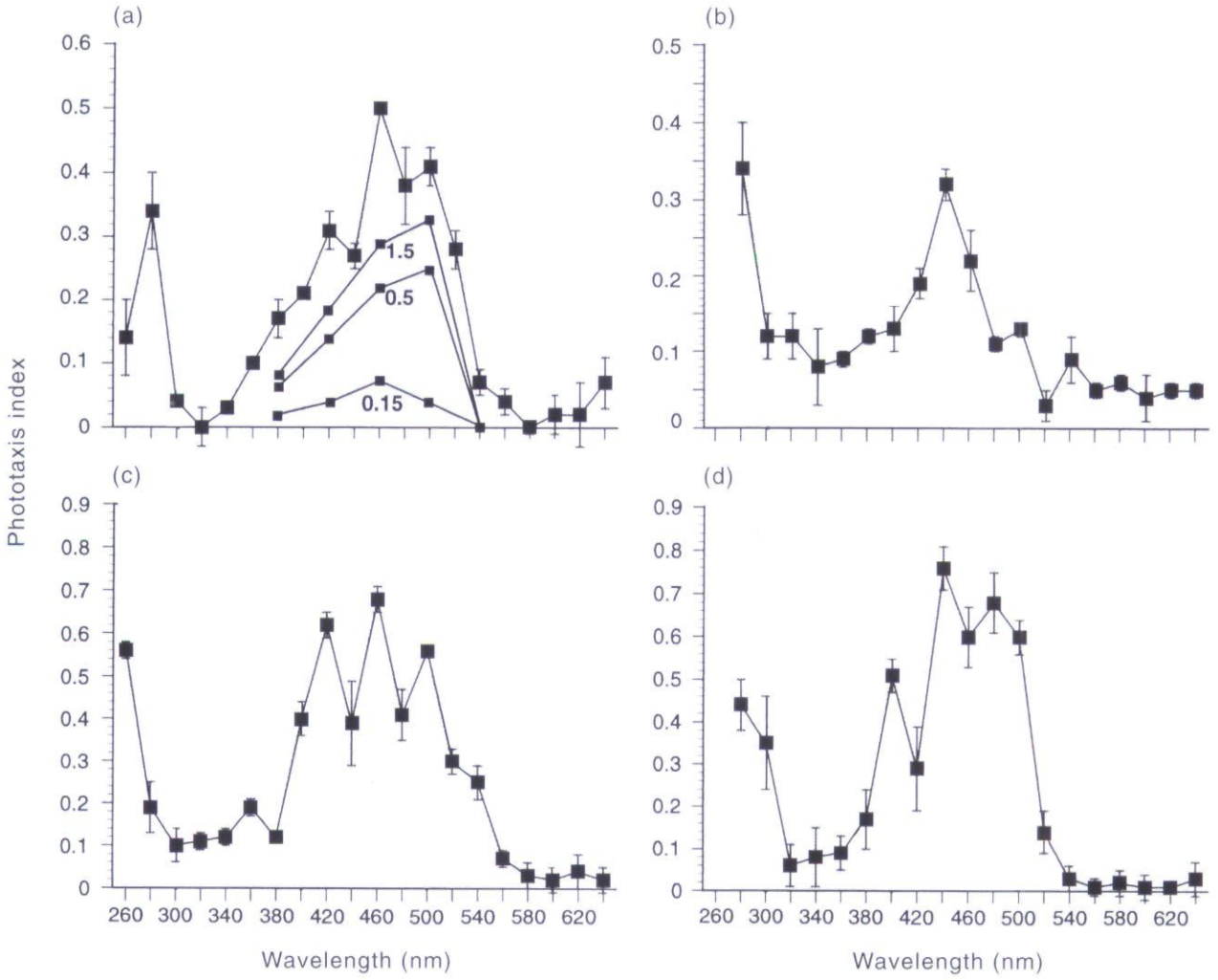


Fig. 4. Phototactic wavelength dependency curves for four dinoflagellates. (a) *Scrippsiella hexapraecingula*; (b) *Peridinium foliaceum*; (c) *Alexandrium hiranoi*; (d) *Gymnodinium mikimotoi*. Fluence rate used for (a) and (c) was 3 μmol photons $m^{-2}s^{-1}$, while 30 μmol photons $m^{-2}s^{-1}$ was used for (b) and (d). (a) Additional wavelength dependency curves for lower fluence rates are also presented. Note that the peaks at 500 nm are comparable to those at 460 nm even under the low fluence rate. Vertical bars represent standard error.

phototactic responses have been observed in the ultraviolet range (260–300 nm); however, no peaks have been observed at wavelengths longer than 560 nm. In Fig. 4a, additional wavelength dependency curves for lower fluence rates (0.15, 0.5 and 1.5 μmol photons $m^{-2} s^{-1}$) are presented. These curves demonstrate that the peaks at 500 nm are comparable with those at 460 nm, even under such fluence rates.

DISCUSSION

Dinoflagellates have all four types of the eyespots in Dodge's original classification (Dodge 1969). For this reason, Dodge (1984) reclassified the eyespots types of Dinophyceae as follows: type A, eyespot is an independent structure not membrane bound; type B, eyespot is an independent membrane-bound structure; type C, eyespot is part of a chloroplast. However, this classification does not cover all of the eyespots found in Dinophyceae. A complex eyespot called ocellus, is not included in the classification and, furthermore, a novel type of eyespot has been recently described in *Gymnodinum natalense* Horiguchi et Pienaar (Horiguchi and Pienaar 1994b). More recently, a similar eyespot has been observed in a heterotrophic dinoflagellate, *Amphidinium lacustre* Stein (Calado *et al.* 1998). In the following discussion, the revised eyespot types of Dodge (1984) will be adopted, rather than those of the original classification Dodge (1969).

Of the eyespot types, type B has so far been found in dinoflagellates with a chromophyte endosymbiont (Dodge 1984; Horiguchi and Pienaar 1994a; Chesnick *et al.* 1996, 1997). There is an hypothesis regarding the origin of the type B eyespot, which is that the dinoflagellate originally possessed the type C eyespot, engulfed a diatom cell and eventually the original chloroplast was replaced with the endosymbiont chloroplast, but that the eyespot portion of the original chloroplast was retained (Dodge 1984). It was reasoned that the eyespot was essential for the photoreceptive mechanism in the dinoflagellates and, thus, the structure was maintained in the descendants (Cavalier-Smith 1992). As the type B eyespot is surrounded by three membranes, like a typical dinoflagellate chloroplast, the above hypothesis is plausible. It is, then, interesting to see whether the dinoflagellates with the type B eyespot do have the same phototactic response to those of dinoflagellates with type C eyespots. The response curves obtained in this study have demonstrated that *P. foliaceum* (type B) and *S. hexapraecingula* (type C) acted in similar manner and it can be concluded that they probably possess the same photoreceptive mechanism.

The eyespot of *S. hexapraecingula* was demonstrated to consist of two rows of lipid globule layers within the chloroplast. Therefore, it is the type C eyespot as defined by Dodge (1984). It is, however, different from other type C eyespots hitherto described, which are composed of a single layer of lipid globules (Kreimer 1994). However, the endosymbiotic dinoflagellate *Scrippsiella velellae* Banaszak *et al.* also probably possesses double-layered eyespot (Banaszak *et al.* 1993). This type of structural variation (number of lipid layers) can be seen in eyespots of chlorophyte algae (Dodge 1969; Melkonian and Robenek 1984; Kreimer 1994) and, thus, the presence of a double-layered eyespot indicates that similar types of variation also exist in type C eyespots. To elucidate the range in variation, the ultrastructure of more dinoflagellate eyespots needs to be investigated.

The eyespot acts as either a light-shading or light-reflecting device or a mixture of both (Kreimer 1994). The combined thickness of the one globular layer and the space in the eyespot of *S. hexapraecingula* is approximately 130 nm. This value is similar to those of green algal eyespots, which have been proposed to act as quarter-wave stack antennae (Foster and Smyth 1980). Although we have not studied whether the eyespot reflects light or not, based on the structural similarities with eyespots of chlorophytes, it is highly probable that the eyespot of *S. hexapraecingula* is also a quarter-wave stack antenna. The eyespot of *P. foliaceum* is also thought to act as quarter-wave stack antenna (Foster and Smyth 1980; Kreimer 1994) and these similarities support the idea that the type B eyespot has been derived from the multi-layered type C eyespot.

Withers and Haxo (1978) reported that *P. foliaceum* strain UTEX 1688 (as IUCC 1688) showed no phototactic response. We used the same strain and found that, although the response was weaker compared with those of other species, it did show a negative phototactic response. The reason for this discrepancy is not clear at this stage.

Our present results demonstrated that, irrespective of the eyespot types and chloroplast origin, the action spectra of the four marine dinoflagellates are almost identical. This suggests that the photoreceptive machinery in these species originated from the host (dinoflagellate) cells rather than the endosymbionts. Although wavelength dependency curves for phototaxis are almost the same in the four dinoflagellates, the two dinoflagellates with an eyespot showed negative phototaxis, while the two without an eyespot revealed positive phototaxis under the experimental conditions used. We do not know the reason for these differences at present.

The wavelength dependency curves for phototaxis obtained here are in general accord with the blue-light type action spectra reported previously for other dinoflagellates, *Gyrodinium dorsum* (Hand *et al.* 1967) and *Gymnodinium splendens* (Forward 1974). The present results also demonstrated that all four dinoflagellates responded to UV light (260–300 nm). This is also consistent with the previous report (Forward 1974; stop response and phototaxis of *G. splendens*). It is note-

worthy that in the curves a, b and d in Fig. 4, the extents of the phototactic responses are comparable at 500 nm (blue green) and at 440 and 460 nm (blue). This fact is in some contrast with typical blue-light action spectra (Watanabe 1995); for example, *Euglena* photophobic responses (Matsunaga *et al.* 1998) and phototaxis in brown algal zoids (Kawai *et al.* 1990, 1991) in which the effectiveness is considerably lower at 500 than at 440 and 460 nm.

We still do not know the nature of the photoreceptor pigments or even their location in dinoflagellates. Although Liu and Häder (1994) isolated putative photoreceptor pigments in the dinoflagellate *Peridinium gatunense* which responds to red light, the nature of the substance is still unclear. Autofluorescent substances are found in the swelling of the posterior flagellum of many of the chromophytic algae (Kawai 1988; Kawai and Inouye 1989) and flagellar swelling (paraflagellar rod) of the emergent flagellum of *Euglena* (Brodhun and Häder 1990). These substances probably contain a flavin and a pterin and are thought to act as photoreceptive pigments (Brodhun and Häder 1990; Kawai *et al.* 1996; Yamano *et al.* 1996). No such autofluorescent substances have been identified in dinoflagellate flagella (Kawai and Inouye 1989). Foster and Smyth (1980) have suggested that a rhodopsin may be responsible for positive phototaxis at least in species which use quarter-wave stack antennae. Our current results and previous reports (Forward 1973, 1974) revealed presence of small peaks at approximately 500 nm in dinoflagellate phototactic action spectra. This peak in the green region, which is not seen in other blue-light sensitive organisms (flavin–pterin type), may suggest the involvement of rhodopsin in the dinoflagellate photoreceptive mechanism. According to Kreimer (1994), observed differences in the wavelengths triggering maximal phototactic responses of dinoflagellates could theoretically be explained by small differences in the protein moiety, affecting charge delocalization on photoexcitation, which typically leads to red or blue shifts in retinal-based photoreceptors. In any case, these are still only hypotheses and have to be proved.

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