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Invited Article

Green Flagellar Autofluorescence in Brown Algal Swarmers and Their Phototactic Responses

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A flavin-like green autofluorescent substance is noticed to occur in one of the flagella of flagellated cells in the Phaeophyceae, Chrysophyceae, Synurophyceae, Xanthophyceae and Prymnesiophyceae. In the phaeophycean swarmers the autofluorescence occurs in the posterior flagellum throughout its length. It is considered to be involved in the photoreception of phototaxis, since it almost always occurs in the swarmers which have a flagellar swelling and stigma and show phototaxis. In the phaeophycean swarmers, the stigma is shown to act as a concave reflector mirror focusing the reflection light onto the flagellar swelling. In the action spectrum studies, phaeophycean swarmers showed phototaxis between 370 and 520 nm, having two major peaks at 420 or 430 nm and 450 or 460 nm. Their responses were true phototactic and not photophobic. Rotation of the swarmer was shown to be essential in the photoreception of *Ectocarpus* gametes.

Key words: Action spectrum — Brown algae — Chromophyta — Flagellar autofluorescence — Flagellar swelling — Phototaxis — Stigma

This review includes two main subjects which seem to be related to each other, although the direct evidence to connect them is yet to be found. One subject is the occurrence of flagellar autofluorescence in one flagellum of some chromophyte algae, and the other subject is about the photoreceptive mechanism of the phototaxis in the Phaeophyceae.

Recently, a green autofluorescent substance was observed to occur in the flagella of the swarmers of brown algae (the Phaeophyceae) and some chromophyte algae (Müller *et al.*, 1987; Coleman, 1988; Kawai, 1988). In order to characterize the chemical entity of the substance, Müller *et al.* (1987) studied the fluorescence emission spectra of whole cells of *Hincksia mitchelliae* [= *Giffordia mitchelliae*] (Ectocarpales, Phaeophyceae) gametes, which show flagellar autofluorescence, and reported an emission maximum at 530 nm when excited at about 450 nm. Kawai (1988) also studied the emission spectra of whole gamete cells of a brown alga *Scytosiphon lomentaria* and a golden alga *Synura petersenii*, and reported a maximum peak at 515-520 nm when excited at 440 nm. Based on the results both of the authors concluded that the substance must be a kind of flavin. However, the isolation and identification of the

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substance have not yet been achieved. In order to elucidate the function and systematic implication of this substance, a survey on the occurrence of the flagellar fluorescence in various algal taxa as well as the investigations on the mechanism of the phototaxis in the brown algae were carried out.

I. Distribution of Flagellar Autofluorescence

A. Distribution in the Phaeophyceae

Brown algae are benthic algae and they form flagellated cells as reproductive cells. These cells have two laterally inserted heterokont flagella: an anterior flagellum with hairy mastigonemes and a smooth posterior flagellum (Fig. 1) (Bold and Wynne, 1985; South and Whittick, 1987; Clayton, 1989). The anterior flagellum mainly has a locomotive function and the posterior one has a steering function. In brown algae, several types of swarmers are known depending on their nature (e.g. zoospores, isogametes, anisogametes or sperm) and systematic position (e.g. Family or Order). The primitive type of swarmers contains one chloroplast with a stigma (Fig. 1). Some swarmers contain one chloroplast but lack a stigma (e.g. zoospores of derived families of the Laminariales), and others contain several fragmented chloroplasts lacking a stigma (e.g. sperm of the Laminariales) (Table 1). Primitive types of swarmers have longer anterior flagella, and some derived types have longer posterior flagella.

The survey of approximately 40 genera in 11 orders of brown algae revealed that the flagellar autofluorescence is widely distributed in brown algae (Table 1) (Müller *et al*., 1987; Coleman, 1988; Kawai, 1988 and new data in the present paper). Within the orders Desmarestiales and Laminariales, flagellar autofluorescence was detected in the posterior flagellum of zoospores of *Desmarestia*, *Chorda* and *Pseudochorda*, in which

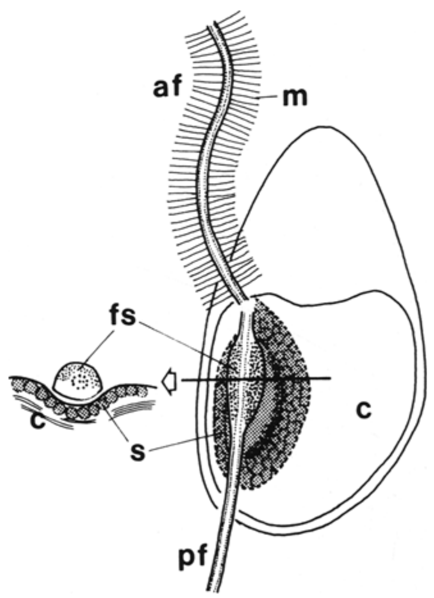

Fig. 1. Schematic representation of a typical phaeophycean swarmer provided with flagellar swelling and stigma. Left figure shows cross section through stigma and flagellar swelling. (af, anterior flagellum; c, chloroplast; fs, flagellar swelling containing electron dense materials; m, mastigonemes; pf, posterior flagellum; s, stigma)

Table 1. Presence and absence of the flagellar autofluorescence in various types of swarmers of the species in the Phaeophyceae and their cellular chracteristics

Systematic position	Species	Narure of swarmer	Fluores-cence	Stigma	Flagellar swelling	Refe-rence
Ectocarpales	*Ectocarpus confervoides*	zoospore	+	+	+	3
	Ectocarpus siliculosus	gamete	+	+	+	1
	Geminocarpus geminatus	zoospore	+	+	+	1
	Hincksia mitchelliae	gamete	+	+	+	1
	Pilayella littoralis	zoospore	+	+	+	3
		gamete	+	+	+	3
Chordariales	*Adenocystis utricularis*	gamete	+	+	+	3
	Chordaria flagelliformis	zoopsore	+	+	+	1
	Eudesme virescence	zoospore	+	+	+	3
	Leathesia difformis	gamete	+	+	+	3
	Leathesia sphaerocephala	zoospore	+	+	+	3
	Leptonematella fasciculata	zoospore	+	+	+	3
	Myelophycus simplex	zoospore	+	+	+	3
	Sphaerotrichia divaricata	zoospore	+	+	+	3
		gamete	+	+	+	1
Desmarestiales	*Desmarestia ligulata*	zoospore	+	+	+	3
		sperm	−	−	−	3
	Desmarestia munda	sperm	−	−	−	1
Dictyosiphonales	*Akkesiphycus lubricus*	zoospore	+	+	+	3
		gametes	+	+	+	3
	Asperococcus turneri	zoospore	+	+	+	3
	Delamarea attenuata	zoospore	+	+	+	3
	Dictyosiphon foeniculaceus	zoospore	+	+	+	3
	Melanosiphon intestinalis	zoospore	+	+	+	3
	Litosiphon groenlandicus	zoospore	+	+	+	3
		gamete	+	+	+	3
	Punctaria latifolia	zoospore	+	+	+	3
	Stschapovia flagellaris	sperm	+	+	+	3
Scytosiphonales	*Petalonia fascia*	zoospore	+	+	+	3
		gamete	+	+	+	3
	Petalonia zosterifolia	gamete	+	+	+	3
	Scytosiphon lomentaria	gamete	+	+	+	3
Cutleriales	*Cutleria multifida*	gametes	+	+	+	3
	Cutleria cylindrica	zoospore	+	+	+	4
		gametes	+	+	+	4
Sphacelariales	*Sphacelaria rigidula*	gamete	+	+	+	1
	Sphacelaria subfusca	zoospore	+	+	+	3
Syringodermatales	*Syringoderma abyssicola*	gamete	+	+	+	3
Sporochnales	*Peritharia caudata*	sperm	+	−	−	1
	Sporochnus scoparius	zoospore	+	+	+	4

Table 1. continued

Systematic position	Species	Narure of swarmer	Fluorescence	Stigma	Flagellar swelling	Reference
Laminariales	*Alaria marginata*	zoospore	+	+	+	2
	Alaria sp.	zoospore	−	−	−	3
	Chorda filum	zoospore	+	+	+	3
		sperm	−	−	−	1
	Chorda tomentosa	zoospore	+	+	+	1
		sperm	−	−	−	1
	Macrocystis pyrifera	zoospore	−	−	−	1
		sperm	−	−	−	1
	Laminaria angustata	zoospore	−	−	−	3
	Laminaria diabolica	zoospore	−	−	−	3
	Laminaria digitata	sperm	−	−	−	1
	Pseudochorda gracilis	zoospore	+	+	+	4
		sperm	−	−	−	4
	Pseudochorda nagaii	zoospore	+	+	+	3
		sperm	−	−	−	3
	Saccorhiza dermatodea	sperm	−	−	−	1
	Saccorhiza polyschides	sperm	−	−	−	1
	Undaria pinnatifida	zoospore	−	−	−	3
Fucales	*Fucus distichus*	sperm	+	+	+	3
	Fucus serratus	sperm	+	+	+	1
	Hormosira banskii	sperm	+	+	+	1

1, Müller *et al.* (1987); 2, Coleman (1988); 3, Kawai (1988); 4, present paper.

stigmata occur. However, it was not detected in the zoospores of *Alaria*, *Laminaria*, *Macrocystis* and *Undaria* nor in the sperm of *Desmarestia*, *Chorda*, *Saccorhiza*, *Macrocystis*, *Pseudochorda* or *Laminaria*, all of which lack a stigma and a flagellar swelling. These structures are generally considered to be related to the photoreception in phototaxis. Therefore, the fluorescent substance is also considered to be involved in the phototaxis. Exceptionally, Müller *et al.* (1987) reported the occurrence of the fluorescence in the sperm of *Perithalia caudata* (Sporochnales) and Coleman (1988) in the sperm of *Alaria marginata* (Laminariales), which lack stigmata and flagellar swellings (Table 1). The occurrence of the autofluorescence in *Perithalia* sperm may show the primitiveness of the Sporochnales sperm compared with those of the Laminariales and Desmarestiales. However, the occurrence of the autofluorescence in *Alaria marginata* zoospore is disputable since no other zoospores of the family including other species of the same genus show autofluorescence, and the species has no stigma nor flagellar swelling (Henry and Cole, 1982).

Table 2. Presence and absence of flagellar autofluorescence in various algal groups excluding the Phaeophyceae and their cellular characteristics

Systematic position	Species	Fluorescence	Stigma	Flagellar swelling	Reference
Prasinophyceae					
Pyramimonadales	*Pyramymonas* sp.	−	+	−	3
Chlorophyceae					
Volvocales	*Clamydomonas reinhardtii*	−	+	−	3
	Volvox aureus	−	+	−	6
	Volvox carteri	−	+	−	3
	Bolbocoleon piliferum	−	+	−	6
	Monostroma angicava	−	+	−	6
	Ulva pertusa	−	+	−	6
Euglenophyceae					
Euglenales	*Euglena gracilis*	(+)	+	+	1
	Euglena gracilis v. *bacillaris*	(+)	+	+	3
	Euglena sp.	(+)	+	+	6
	Phacus sp.	(+)	+	+	6
Chrysophyceae					
Ochromonadales	*Dinobryon cylinricum* (?)	+	+	+	3
	Dinobryon divergens	+	+	?	2
	Dinobryon sp.	+	+	+	5
	Ochromonas danica	+	+	+	3
	Ochromonas sp.	−	−	?	5
	Poterioochromonas malhamensis	+	+	+	3
Chromlinales	*Chromulina nebulosa*	+	+	+	5
	Microglena butcheri	+	+	+	5
	Phaeaster pascheri	+	+	+	5
Pedinemales	*Pseudopedinella* sp.	−	−	−	5
Sarcinochrysidales	*Phaeosaccion collinsii* (zoospore)	+	+	+	4
Synurophyceae					
Synurales	*Mallomonas* sp.	+	−	+	5
	Synura petersenii	+	−	+	5
	Synura uvella	+	−	+	5
Bacillariophyceae					
Centrales	*Melosira* sp. (sperm)	−	−	−	5
Xanthophyceae					
Mischococcales	*Botrydiopsis intercedens*	++	+	+	5
	Ophyocytium sp.	+	+	+	5
Vaucheriales	*Vaucheria sessilis* (sperm)	−	−	−	5
	Vaucheria sessilis (synzoospore)	−	−	−	5
Raphidophyceae					
Raphidomonadales	*Chattonella antiqua*	−	−	−	5
	Fibrocapsa japonica	−	−	−	5
	Gonyostomum semen	−	−	−	3

Table 2. continued

Systematic position	Species	Fluorescence	Stigma	Flagellar swelling	Reference
	Heterosigma akasiwo	−	−	−	5
	Olisthodiscus luteus	−	−	−	3, 5
Eustigmatophyceae					
Pleurochloridales	*Pseudocharaciopsis minuta*	−	+	+	5
Prymnesiophyceae					
Prymnesiales	*Chrysochromulina ericina*	+	−	−	5
	Chrysochromulina hirta	+	−	−	5
	Chrysochromulina spinifera	−	−	−	5
	Chrysochromulina strobilus	−	−	−	5
	Platychrysis pienaari	+	−	−	5
	Prymnesium parvum	+	−	−	3, 5
	Prymnesium sp.	+	−	−	5
Isochrysidales	*Isochrysis* sp. 1	+	−	−	5
	Isochrysis sp. 2	+	−	−	5
Coccosphaerales	*Coccolithus neohelis*	−	−	−	5
	Jomonlithus littoralis	−	−	−	5
	Ochrochrysis verrucosa	−	−	−	5
	Pleurochrysis roscoffensis	−	−	−	5
Pavlovales	*Pavlova* sp. 1	−	−	−	5
	Pavlova sp. 2	−	+	−	5
Dinophyceae					
Gymnodiniales	*Amphidinium* sp.	−	−	−	5
	Gymnodinium sanguineum	−	−	−	5
	Gyrodinium sp. 1	−	−	−	5
	Gyrodinium sp. 2	−	−	−	3
	Woloszynskia limnetica	−	?	−	3
	Woloszynskia sp.	−	+	−	5
Peridiniales	*Heterocapsa triquetra*	−	−	−	5
	Peridinium volzii	−	?	−	3
	Peridinium sp.	−	−	−	5
Cryptophyceae					
Cryptomonadales	*Chroomonas caudata*	−	−	−	5
	Chroomonas coerulea	−	+	−	5
	Chroomonas placoidea	−	−	−	5
	Cryptomonas acuta	−	−	−	5
	Cryptomonas ovata	−	−	−	5
	Cryptomonas sp.	−	−	−	3
	Rhodomonas sp.	−	−	−	3

1, Benedetti and Checucci (1975); 2, Müller *et al.* (1987); 3, Coleman (1988); 4, Kawai (1988); 5, Kawai and Inouye (1989); 6, present paper.

B. Distribution in other classes of algae

A survey of flagellar autofluorescence in various algal classes excluding the Phaeophyceae revealed the following distribution in each class [based on Coleman (1988), Kawai and Inouye (1990) and original data added in the present paper if not specially referred] (Table 2, Fig. 2).

Prasinophyceae and Chlorophyceae: No flagellar autofluorescence is noted in the flagellated species nor the swarmers of benthic green algae in these groups. Those flagellated cells generally have a prominent stigma in the chloroplast and show obvious phototaxis. However, there is no spatial association between the flagella and stigma.

Euglenophyceae: *Euglena* has been known to have green autofluorescence in the paraflagellar body on the basal part of the hairy locomotive flagellum (Benedetti and Checucci, 1975). The autofluorescent substance was suggested to be a flavin from the fluorescence spectrum study (Benedetti and Lenci, 1977). Recently, Schmidt *et al.*

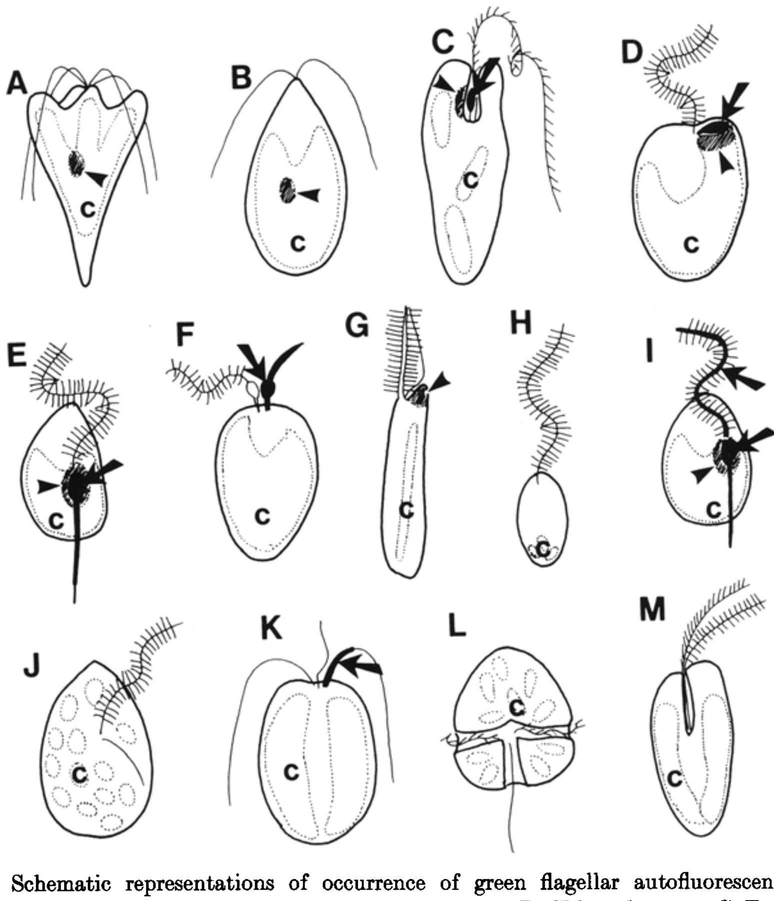

Fig. 2. Schematic representations of occurrence of green flagellar autofluorescence (if present) in various algal groups. A, Prasinophyceae; B, Chlorophyceae; C, Eugleno-phyceae; D, Chrysophyceae; E, Phaeophyceae; F, Synurophyceae; G, Eustigmato-phyceae; H, Bacillariophyceae (Centrales, sperm); I, Xanthophyceae; J, Raphido-phyceae; K, Prymnesiophyceae; L, Dinophyceae; M, Cryptophyceae. (c, chloroplast; arrow, fluorescent flagellum or paraflagellar body; arrowhead, stigma)

(1990) microspectro-photometrically confirmed the presence of a flavin-like fluorescent substance in the paraflagellar bodies of isolated flagella in *Euglena gracilis*. They also suggested the presence of pterine-like autofluorescent substance in the paraflagellar bodies. There is no autofluorescence in the flagella themselves.

Chrysophyceae: The fluorescence has been observed in the species which have stigmata and flagellar swellings, whereas the species which lack these structures showed no autofluorescence. These distributional patterns agree with the situation in the Phaeophyceae and suggest that they have a common photoreceptive system. However, in some genera such as *Chromulina* and *Phaeaster*, secondary (smooth) flagella are considerably short and located in the flagellar pocket (Rouller and Faure-Fremiet, 1958; Belcher and Swale, 1967; Inouye *et al*., 1990), and they may not actually have the function as a rudder as in the Phaeophycean swarmers.

Synurophyceae: All of the species in the Synurophyceae examined to date showed flagellar autofluorescence in the short (second) flagellum throughout its length. Synurophyceae differ from the Chrysophyceae that also exhibit flagellar autofluorescence in lacking stigmata but having flagellar swellings in both flagella (Andersen, 1987). However, the electron-dense material which is considered to be involved in the photoreception exists only in the swelling of the smooth flagellum which shows autofluorescence (Andersen, 1985, 1987). This suggests that the two flagellar swellings have different functions and that the smooth flagellum might be responsible for the actual photoreception in the Synurophyceae.

Bacillariophyceae: The only flagellate cells known to occur in the Bacillariophyceae (diatoms) are the spermatozoids of members of the Centrales. Those spermatozoids lack a stigma and have only one hairy flagellum (Manton and von Stosch, 1966). Flagellar autofluorescence has not been detected in the sperm of *Melosira*.

Xanthophyceae: Three different types of flagellate cells (heterokont zoospores of *Botrydiopsis intercedens* and *Ophiocytium* sp. sperm and synzoospores of *Vaucheria sessilis*) were examined in the Xanthophyceae. In zoospores of *B. intercedens*, autofluorescence was observed in both flagella. The flagellated cells in the class usually have both a stigma and a flagellar swelling in the posterior, smooth flagellum and generally are thought to have substantially the same organization as the Phaeophyceae and Chrysophyceae. Observations in *Ophiocytium* have been made only on the zoospores in the sporangium before release. In these cells, the short flagellum but not the long one shows autofluorescence. Flagellar autofluorescence was not observed in either the sperm or the synzoospores of *Vaucheria*, both of which lack stigmata and flagellar swellings.

Raphidophyceae: In the Raphidophyceae, flagellar autofluorescence was not observed. Species of the Raphidophyceae lack stigmata and flagellar swellings.

Eustigmatophyceae: Flagellar autofluorescence was not detected in *Pseudocharaciopsis texensis* [= *P. minuta*] which has an extraplastidial stigma and two emergent flagella. The anterior flagellum is hairy and possesses a basal swelling, while the posterior one is smooth (Hibberd, 1972).

Prymnesiophyceae: In the Prymnesiophyceae, in about half of the species examined, flagellar autofluorescence was observed in one of the two flagella. There seems to be a correlation between the occurrence of the autofluorescent substance(s) and various taxonomic categories such as species, genera and orders. In the Prymnesiales, the fluorescence was observed except in *Chrysochromulina spinifera* and *C. strobirus*, which are considered to have rather distant systematic positions in the genus. All species bearing coccolith coverings so far examined have not shown autofluorescence. Species belonging to the three orders of the Prymnesiophyceae are similar in cellular organization except for the features of the haptonema and cell covering. This suggests that, in contrast to the Phaeophyceae and Chrysophyceae, the Prymnesiophyceae consists of diverse groups as far as the photoreception system is concerned. Pavlovales is generally thought to be the most primitive in the class and related to other classes of chlorophyll *c*-containing plants because many species of this order have heterokont flagella and stigmata. However, no fluorescence has been detected in the smooth flagellum, even in a species having a stigma.

Dinophyceae : Some species of the Dinophyceae have elaborate stigmata, which are at times associated with a flagellum. However, all the species so far investigated showed no flagellar autofluorescence.

Cryptophyceae : Some species of the Cryptophyceae have a stigma which is often associated with the pyrenoid rather than the flagella, and is located deep inside the cell. No flagellar autofluorescence was detected in any of the species examined, even in a species having a stigma.

II. Photoreceptive Mechanism of Phototaxis in Phaeophycean Swarmers

Most flagellated algae, including the swarmers of benthic algae, have some means to detect the orientation of light and swim towards or escape from it (i.e. phototaxis). Although many of the phaeophycean swarmers are known to show strong positive or negative phototaxis, there had been no detailed studies on this phenomenon. Two different types of responses are known in the phototaxis in many organisms (i.e. true phototaxis and photophobic response) ; however, phaeophycean zoospores of *Pseudochorda gracilis* (Laminariales) were found to show only true phototaxis (Kawai *et al.*, 1991).

A. Structural background

In the phototaxis of the phaeophycean swarmers, the flagellar swelling and stigma (eyespot) have generally been thought to be involved in photoreception (Fig. 1). Among them, the flagellar swelling has been considered to be the actual photoreceptive site and the electron-dense granular materials contained in the swelling to be the photoreceptive pigments (Dodge, 1973 ; Moestrup, 1982 ; Clayton, 1989). However, there has been no convincing evidence confirming this hypothesis, although the fine structures of them were studied by some authors in some species of the Chrysophyceae and Xanthophyceae (Kristiansen and Walne, 1976, 1977 ; Moestrup, 1982).

Concerning the function of the stigmata, those of euglenoids are generally believed to have a shading function to the true photoreceptive site on the paraflagellar body (rod) of the longer locomotive flagellum (Mast, 1911). On the other hand, in the green algae, the stigmata were known to reflect blue and green light (Mast, 1927). Foster and Smyth (1980) provided theoretical explanation on the reflective function of the green algal stigmata by proposing that light is reflected and concentrated at the photoreceptive site by interference reflection from a quarter-wave stack of different refractive index layers of stigma globules. Recently, Kreimer and Melkonian (1990) experimentally proved that multi-layered stigmata of green algal flagellated cells actually acted as interference reflectors.

Regarding the function of the stigma in the Phaeophyceae and Chrysophyceae, because of the resemblance of their spatial arrangement of stigma and flagellar swelling to euglenoids, the phaeophycean stigma was also considered to have a shading function to the flagellar swelling (Foster and Smyth, 1980; Kristiansen, 1990; Clayton, 1989). Recently, Kawai *et al.* (1990) noticed that the stigma of *Ectocarpus* gamete strongly reflected blue epi-illumination light. The reflection occurred in swimming cells as well as in settled cells. Kreimer *et al.* (1991) confirmed the reflective properties of the phaeophycean stigma by an investigation using confocal laser scanning microscopy in the epireflection contrast mode. The complex reflection pattern obtained after optical xy (horizontal) and xz (vertical) sectioning was consistent with stigma ultrastructure as revealed by serial thin sections. The intensity and pattern of the reflection signal varied with the orientation of the cell/stigma to the incident laser light. Maximal reflection occurred only in approximately normal orientation of the stigma to the light source. Focusing of reflected light from an elongated concave depression of the stigma on the region of the flagellar swelling was observed in xy and xz sections of living and fixed gametes.

The stigmata of the Chrysophyceae and Xanthophyceae have very similar structure as the phaeophycean stigma (e.g. a single layer of isodiametric carotenoid-rich globules situated in a concave area of the chloroplast facing the flagellar swelling of the second smooth flagellum, and contains autofluorescent substance in the smooth flagellum and some electron-dense materials in the swelling). Therefore, their stigmata are also considered to have the same reflective function as those of the Phaeophyceae. Most of the Prymnesiophyceae have no stigma except for some species of the Pavlovales (Green, 1980). Among them, *Diacronema vlkianum* has a similar structure as the phaeophycean stigma (concave stigma associated with a flagellar swelling containing electron-dense materials), although flagellar autofluorescence is not observed in the Pavlovales to date (Kawai and Inouye, 1989). The occurrence of a concave stigma (probably have concave reflector function), as well as the occurrences of flagellar autofluorescence in a flagellum in some taxa of the class, suggest the systematic affinity between the Prymnesiophyceae and the Phaeophyceae, Chrysophyceae and Xanthophyceae. The occurrence of similar green autofluorescence in the paraflagellar body of the Euglenophyceae is considered to be a result of evolutionary convergence because they differ in many principle characteristics (i.e. on hairy locomotive flagellum vs. on smooth steering flagellum ; non-reflective stigma vs. reflective stigma ; in paraflagellar body vs. flagellum itself) aside from their distant phylogenetic positions.

B. Spectroscopical studies

In order to identify the photoreceptive pigment of phototaxis, action spectrum studies provide useful information especially when the actual pigments are not isolated. In the chlorophyll *a*- and *b*-containing groups (Chlorophyta), the action spectra have been reported in *Platymonas* (Halldal, 1961) of the Prasinophyceae, *Dunaliella* and *Ulva* (Halldal, 1958) and *Clamydomonas* (Nultsch *et al.*, 1971) of the Chlorophyceae and *Euglena* (Diehn, 1969) of the Euglenophyceae (Fig. 3). On the other hand, in the chlorophyll *a*- and *c*-containing groups (Chromophyta), *Gyrodinium* (Forward, 1973), *Gymnodinium* (Forward, 1974) and *Peridinium* and *Gonyaulax* (Halldal, 1958) of the Dinophyceae and *Cryptomonas* (Watanabe and Furuya, 1974) of the Cryptophyceae have been studied (Fig. 3). However, in the recent concept, the Dinophyceae are not included in the Chromophyta and the Cryptophyceae are considered to have rather distant phylogenetic position from other members of the Chromophyta (Cavalier-Smith, 1986, 1989). Therefore, there have been no detailed investigations on the action spectra or phototaxis in the Phaeophyceae and related groups (Chrysophyceae, Xanthophyceae and probably Prymnesiophyceae) which seem to have the same photoreceptive mechanisms (see above).

Concerning the phototactic responses of the phaeophycean swarmers, Müller *et al.* (1987) studied the spectral sensitivity curve for photoaccumulation of *Ectocarpus* male

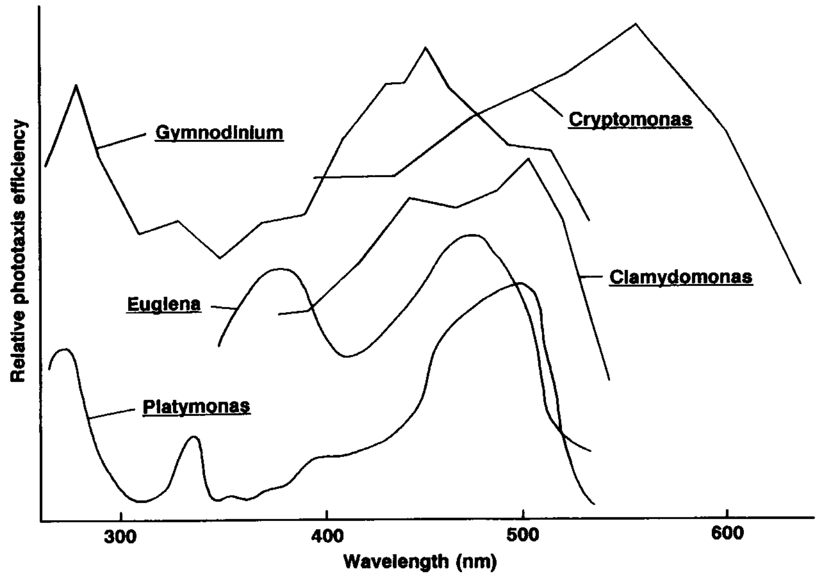

Fig. 3. Action spectra of phototaxis in various algal groups based on previous reports. *Clamydomonas* (Chlorophyceae) by Nultsch *et al.* (1971); *Cryptomonas* (Cryptophyceae) by Watanabe and Furuya (1974); *Euglena* (Euglenophyceae) by Diehn (1969); *Gymnodinium* (Dinophyceae) by Forward (1974); *Platymonas* (Prasinophyceae) by Halldal (1961).

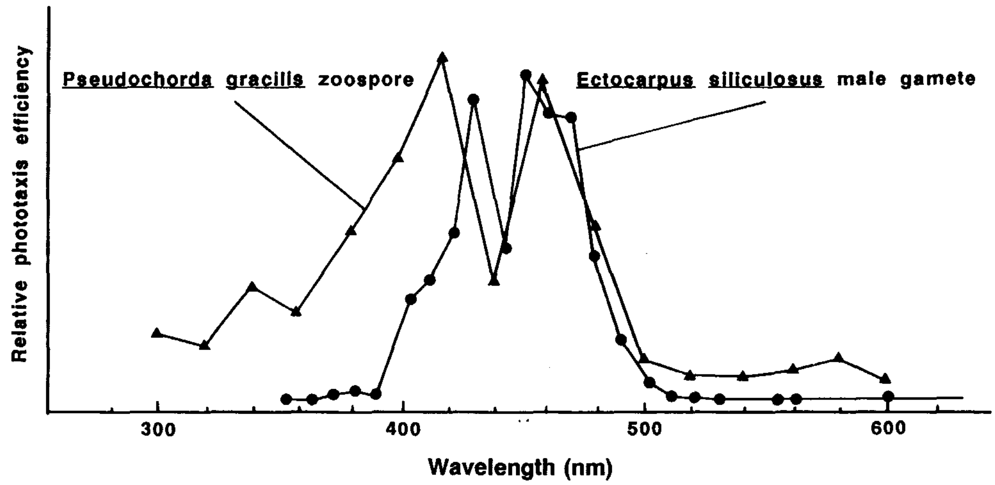

Fig. 4. Action spectra of phototaxis in two species of the Phaeophyceae, *Ectocarpus siliculosus* male gamete (Ectocarpales) (Kawai *et al.*, 1990) and *Pseudochorda gracilis* zoospore (Laminariales) (Kawai *et al.*, 1991).

gametes, using the light beam of a spectrophotometer. In the experiment they obtained a single maximum in the blue range of the spectrum centered around 430-450 nm. They detected no activity with wavelengths below 380 down to 300 nm or above 540 nm. Kawai *et al.* (1990) studied the action spectra of male gametes of the brown alga *Ectocarpus* (Ectocarpales) using a computerized cell-tracking system. Under monochromatic light stimulations, *Ectocarpus* gametes showed mainly positive phototaxis between 370 and 520 nm (Fig. 4). The action spectrum had a minor peak near 380 nm, and two major peaks at 430 nm and 450-460 nm and a shoulder at 470 nm adjoining a remarkable depression near 440 nm. Kawai *et al.* (1991) also studied the action spectrum in zoospores of a brown alga *Pseudochorda gracilis* (Laminariales). The action spectra had two peaks at 420 and 460 nm, while light below 360 nm and above 500 nm was not effective (Fig. 4). These two action spectra investigated on two different kinds of swarmers (isogamete and zoospore) of systematically distant taxa agreed in general and suggest that a common photoreceptive system exists in the Phaeophyceae. However, in order to speculate about the identity of the photoreceptive pigments based on the action spectrum data, the reflection spectrum of the stigma should be studied because the presumptive photoreceptor is considered to receive the reflection light from the stigma.

Kawai *et al.* (1990) showed that the rotation of the cell was essential for the photoreception in the *Ectocarpus* male gamete phototaxis by an investigation using stroboscopic illumination. Under unilateral stroboscopic illumination with more than four pulses per second, the gametes showed clear phototaxis. However, the response was disturbed at lower frequencies. Addition of methyl cellulose, which increases the viscosity of the medium and slows down gamete rotation, deceased the threshold frequency.

In *Ectocarpus* male gametes, under equal intensities of bilateral illumination at an angle of 90°, most of the gametes swam on the resultant between the two light beams (Kawai *et al.*, 1990). This result agreed with that of negative phototaxis of *Euglena gracilis* under strong light irradiance and contrasted with that of positive phototaxis under low illuminance in which the populations of *Euglena* split into two components swimming towards either light source (Häder *et al.*, 1986).

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