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Flagellar autofluorescence in forty-four chlorophyll *c*-containing algae

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A survey of flagellar autofluorescence was made in 44 species of chlorophyll *c*-containing algae. In the five species of Chrysophyceae examined, flagellar autofluorescence was observed throughout the length of the short smooth flagellum, but only when this was associated with an eyespot and a flagellar swelling. In the three species of Synurophyceae, which lack an eyespot and have swellings in both flagella, the fluorescence was also observed in the short smooth flagellum. Among the Xanthophyceae, *Botrydiopsis intercedens* showed autofluorescence throughout the length of both flagella. It was not detected, however, in either the sperm or the synzoospore of *Vaucheria sessilis*. Prymnesiophyceae were heterogeneous. Fluorescence was observed in the proximal part of one of the isokont flagella in seven species in four genera of the Prymnesiales and the Isochrysidales, but it was not detected in the coccolithophorids and the Pavlovales examined. Flagellar autofluorescence has not been detected in the Bacillariophyceae, Raphidophyceae, Eustigmatophyceae, Dinophyceae and Cryptophyceae. In the Cryptophyceae, the so-called refractive bodies (corps de Maupas) were noted to show apparent blue autofluorescence.

INTRODUCTION

A flavin-like autofluorescent substance known to occur in the flagella of euglenoids and swarmers of brown algae has been suggested to have photoreceptive function in the phototaxis (Benedetti & Checucci 1975; Benedetti & Lenci 1977; Müller *et al* 1987; Kawai 1988). Besides brown algae, some other members of the Chromophyta are also reported to show flagellar autofluorescence (Müller *et al* 1987; Coleman 1988; Kawai 1988). To obtain further information on the distribution of this phenomenon, we have extended a survey to various classes of algae that contain chlorophyll *a* and *c*. The Phaeophyceae has been omitted from the present survey, since it was previously reported in detail (Müller *et al* 1987; Kawai 1988).

MATERIALS AND METHODS

Flagellate cells of 44 species in 34 genera (Chrysophyceae, Synurophyceae, Bacillariophyceae, Xanthophyceae, Raphidophyceae, Eustigmatophyceae, Prymnesiophyceae, Dinophyceae, Cryptophyceae) were examined (Table 1). Swimming flagellate cells were observed in living condition or after fixation on glass microscope slides by drawing 0.5% glutaraldehyde gradually in culture medium under the coverslip, to avoid damage to the alga caused by the rapid addition of the fixative. For observations of flagellar autofluorescence, a Nikon Epifluorescence Microscope EFD2 was used in the mode of Blue-Violet Excitation (Osram HBO 100 W/2 mercury lamp as the light source, 400–440 nm exciter filter, DM455 dicroic mirror, BA470 barrier filter).

RESULTS AND DISCUSSION

Results of the autofluorescence survey as well as the presence or absence of eyespots and the flagellar swelling are shown in Table 1.

CHRYSOPHYCEAE: Species in which fluorescence has been observed have eyespots and flagellar swellings (Figs 1, 2), while species in which fluorescence is absent lack these structures. The absence of the fluorescent substance (or sub-

stances) in *Pseudopedinella* is understandable, because the smooth flagellum is absent in all species of the Pedinellales so far investigated (e.g. Throndsen 1971). Although the number of species examined is restricted, the observations include representatives of various orders assigned to the Chrysophyceae. The results thus suggest that the flagellar autofluorescent substance is widely distributed and is closely correlated with the occurrence of eyespots and flagellar swellings.

SYNUROPHYCEAE: Figures 3 and 4 illustrate the autofluorescence pattern found in the three species examined. Synurophyceae differ from the Chrysophyceae that exhibit flagellar autofluorescence in that Synurophyceae lack eyespots and have flagellar swellings in both flagella (Andersen 1987). In addition to the existence of the autofluorescent substance only in the smooth flagellum an electron opaque material exists only in the swelling of the smooth flagellum in both genera (Andersen 1985, 1987, from the micrographs; Inouye, unpubl. obs.). This suggests that the two flagellar swellings have different functions and that the smooth flagellum might be responsible for photoreception in the Synurophyceae, as in the Phaeophyceae (Kawai 1988).

BACILLARIOPHYCEAE: The only flagellate cells known to occur in diatoms are the spermatozoids of members of the Centrales. Those spermatozoids lack an eyespot and have only one hairy flagellum (Manton & von Stosch 1966). Flagellar autofluorescence has not been detected (Figs 5, 6). This compares favourably with other chromophytes that lack a smooth flagellum and an eyespot.

XANTHOPHYCEAE: Three different types of flagellate cells (heterokont zoospores of *Botrydiopsis intercedens* and *Ophiocytium* sp., sperm and synzoospores of *Vaucheria sessilis*) were examined. In zoospores of *B. intercedens,* autofluorescence was observed in both flagella (Figs 7–9). The flagellated cells in the class usually have both an eyespot and a flagellar swelling in the posterior, smooth flagellum and generally are thought to have substantially the same organization as the Chrysophyceae. However, the distribution of the autofluorescent substance in both flagella in this xanthophyte suggests that the anterior hairy flagellum might be also involved in photoreception. Observations of flagellar autofluorescence 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 eyespots and flagellar swelling (Figs 10, 11).

RAPHIDOPHYCEAE: Flagellar autofluorescence was not observed (Figs 12, 13). Species of Raphidophyceae lack eyespots and flagellar swellings.

EUSTIGMATOPHYCEAE: Flagellar autofluorescence has not been detected (Figs 14, 15). The genus *Pseudocharaciopsis* has, in addition to the extraplastidial eyespot, two emergent flagella, the anterior one hairy and possessing a basal swelling and the posterior one smooth (Hibberd 1972). The absence of the autofluorescent substance in the anterior flagellum, in spite of the presence of the swelling associated with an eyespot, suggests that the Eustigmatophyceae may have a chemically different system of photoreception. However, more species of the class need to be examined.

PRYMNESIOPHYCEAE: In about half of the species examined, flagellar autofluorescence was observed in one of the two flagella (Figs 16, 17). There seems to be a correlation between the occurrence of the autofluorescent substance(s) and various taxonomic entities such as species, genera and orders. In the Prymnesiales, the fluorescence was observed except in *Chrysochromulina spinifera* and *C. strobirus. C. strobirus* differs from the other species by having a very long haptonema, and both flagella and haptonema emerge from the lateral portion of the cell. *C. spinifera* was originally described as a species of *Chrysocampanula* because its haptonema is very short and incapable of coiling (Fournier 1971). All species bearing coccolith coverings so far examined have not shown autofluorescence (Figs 18, 19). The coccolithophorids have been placed in two different orders, the Isochrysidales and the Coccosphaerales. Species having no haptonema or a rudimentary one are assigned to the former order, while those for which there is no information on haptonema presence or absence are referred to the latter. The presence of the fluorescent substance in two species of *Isochrysis* and the absence in all the coccolithophorids so far examined support this classification to place them in separate orders. 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 the Chrysophyceae, the Prymnesiophyceae consists

of diverse groups as far as the photoreception system is concerned. The 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 eyespots. However, no fluorescence has been detected in the smooth flagellum even in a species having an eyespot (Figs 20, 21). This is hard to interpret and requires further investigation.

DINOPHYCEAE: Some species of the Dinophyceae have elaborate eyespots, which are at times associated with a flagellum. However, all the species so far investigated have no flagellar autofluorescence (Figs 22, 23). In the Dinophyceae, several investigations have been made on phototaxis and the photoreception system, mainly by measuring the phototactic action spectrum (Halldal 1958; Forward 1973, 1974). Forward (1974) suggested that the photoreceptive pigment in the phototaxis of *Gymnodinium splendens* may be a protein bonded carotenoid, not a flavin as presumed in the euglenoids. Therefore, it is to be expected that they have a different system of photoreception.

CRYPTOPHYCEAE: Some species of this class have an eyespot which is often associated with the pyrenoid rather than the flagella, and is located deep inside the cell. Regarding the phototaxis in the Cryptophyceae, Watanabe & Furuya (1974, 1978) reported a unique action spectrum in a species of *Cryptomonas.* Therefore, it might be expected, that they have a different system of photoreception. We have not detected any autofluorescent substance associated with the flagella in any of the species examined (Figs 24–29), even in a species having an eyespot. In some species (*Chroomonas caudata, Ch. coerulea, Ch. placoidea* and *Cryptomonas ovata*), however, refractive cytoplasmic bodies [corps de Maupas Hollande (1942)] are noted to show blue autofluorescence (Figs 26–29). They are cask-shaped and usually two in number. When the cell is damaged by irradiation of the fluorescence microscope, they become dissipated in the medium immediately. This phenomenon suggests that the bodies contain some water-soluble substance.

The results of this survey can be summarized as follows:

1 Flagellar autofluorescence is observed only among algae whose storage products are β-glucans. They have never been observed in algal groups storing α-linked glucans (starch).

2 Among the groups of organisms with β-glucan storage, the autofluorescence, when present, is found mostly in the smooth flagellum. Exceptions to this rule are euglenoids and the xanthophyte *Botrydiopsis.*

3 The flagellar autofluorescence is often absent in specialized cells with extremely degenerated flagella, chloroplasts or eyespots, even within a group where fluorescence is usually observed.

Further phylogenetic conclusions cannot be made yet, as too few organisms have been examined, patterns are not completely clear among those organisms that have been studied, and not enough information on the biochemistry of the autofluorescent substance and its precise location in the flagellum is available.

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Figs 1–29. Micrographs of flagellate cells of chlorophyll *c*-containing algae. Scale bars = 5 μm.

Figs 1, 2. Fluorescence and normal optical micrographs of *Microglena butcheri* (Chrysophyceae) showing flagellar autofluorescence in short flagellum (arrowhead).

Figs 3, 4. *Mallomonas* sp. (Synurophyceae) showing flagellar autofluorescence in short flagellum (arrowhead). Fluorescence is not observed in long hairy flagellum (arrow).

Figs 5, 6. Sperm of *Melosira* sp. (Bacillariophyceae). Fluorescence not observed in anterior flagellum.

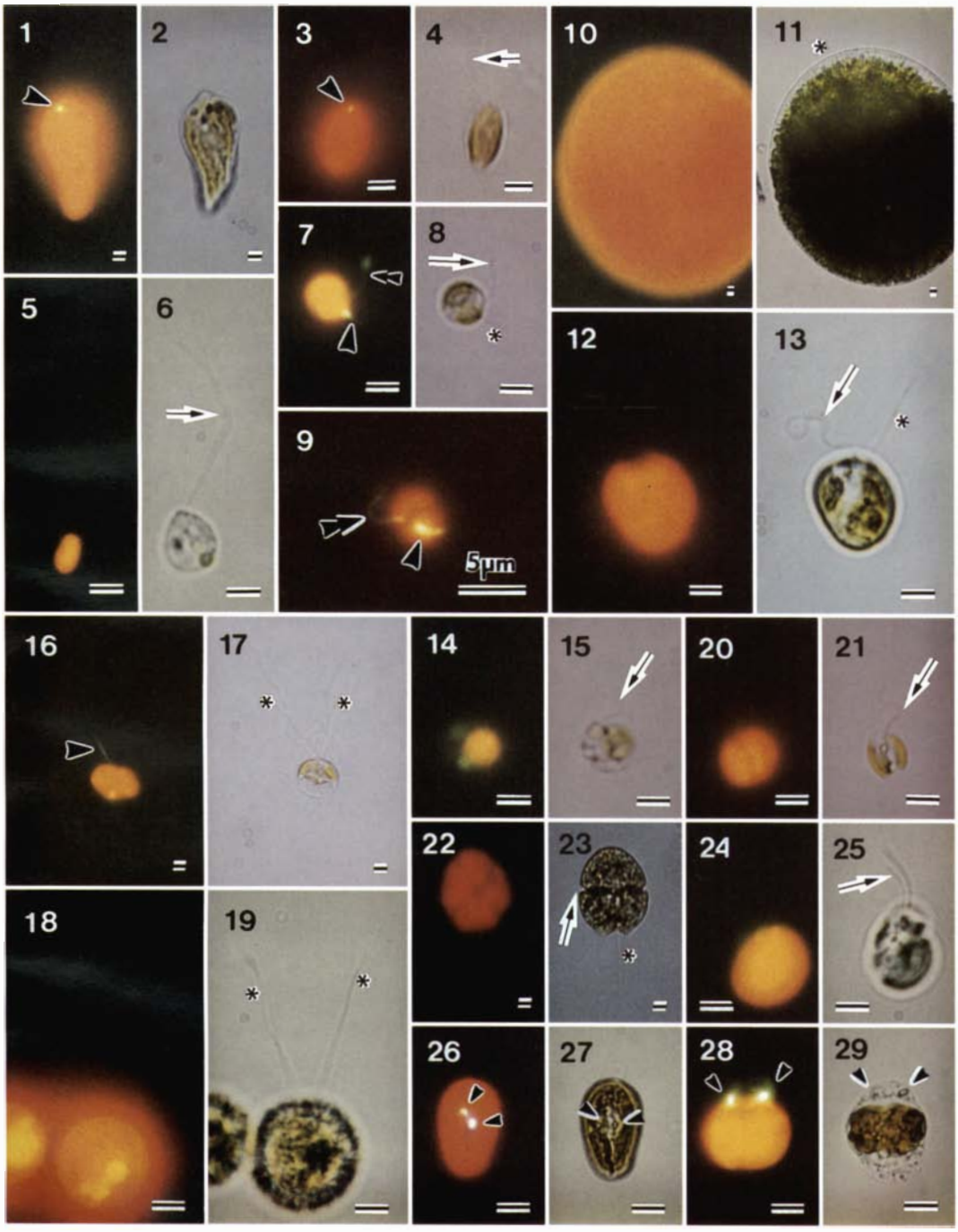
Figs 7–9. Zoospore of *Botrydiopsis intercedens* (Xanthophyceae) showing flagellar autofluorescence in long hairy (double arrowheads, arrow) and short posterior (arrowhead, asterisk) flagellum.

Figs 10, 11. Synzoospore of *Vaucheria sessilis* (Xanthophyceae). Fluorescence not observed in flagella (asterisk).

Figs 12, 13. *Olisthodiscus luteus* (Raphidophyceae). Fluorescence not observed in hairy (arrow) and smooth (asterisk) flagella.

Figs 14, 15. Zoospore of *Pseudocharaciopsis texensis.* Fluorescence not observed in anterior flagellum which bears an eyespot.

Figs 16, 17. *Chrysochromulina ericina* (Prymnesiophyceae) showing flagellar autofluorescence (arrowhead) in the proximal part of one of the two isokont flagella (asterisks).



Figs 18, 19. *Pleurochrysis roscoffensis* (Prymnesiophyceae). Fluorescence not observed in two isokont flagella (asterisks).
Figs 20, 21. *Pavlova* sp. 2 (Prymnesiophyceae). Fluorescence not observed in anterior flagellum (arrow).
Figs 22, 23. *Gymnodinium sanguineum* (Dinophyceae). Fluorescence not observed in anterior (arrow) and posterior (asterisk) flagella.
Figs 24, 25. *Chroomonas caudata* (Cryptophyceae). Fluorescence not observed in two heterokont flagella (arrow).
Figs 26–29. *Cryptomonas ovata* (Cryptophyceae) showing cellular autofluorescent bodies (arrowheads). Figs 28, 29 show damaged cell by irradiation.

Table 1. Presence and absence of the flagellar autofluorescence in the species examined and their cellular characteristics

Systematic position	Species	Fluorescence	Eye-spot	Flagellar swelling	Source of strains
Chrysophyceae					
Ochromonadales	*Dinobryon* sp.	+	+	+	Zhang X. (TKB)[1]
	Ochromonas sp.	−	−	?	Zhang X. (TKB)
Chromulinales	*Chromulina nebulosa* Cienkowsky	+	+	+	Inouye I. (TKB)
	Microglena butcheri Belcher	+	+	+	Zhang X. (TKB)
	Phaeaster pascheri Scherf	+	+	+	Enomoto M. (TKB)
Pedinemales	*Pseudopedinella* sp.	−	−	−	Suda S. (NIES)[2]
Sarcinochrysidales	*Phaeosaccion collinsii* Farlow (zoosp.)	+	+	+	Kawai H. (HKD)
Synurophyceae					
Synurales	*Mallomonas* sp.	+	−	+	Zhang X (TKB)
	Synura petersenii Korschikov	+	−	+	Kawai H. (HKD)[3]
	S. uvella Ehrenberg	+	−	+	Inouye I. (TKB)
Bacillariophyceae					
Centrales	*Melosira* sp. (sperm)	−	−	−	Idei M. (TKB)
Xanthophyceae					
Mischococcales	*Botrydiopsis intercedens* Vischer et Pascher (zoosp.)	++	+	+	UTEX[4] 296
	Ophiocytium sp.	*	+	+	Inouye I. (TKB)
Vaucheriales	*Vaucheria sessilis* (Vauch.) de Candolle (sperm)	−	−	−	UTEX LB 745/1b
	(synzoospore)	−	−	−	UTEX LB 745/1b
Raphidophyceae					
Raphidomonadales	*Chattonella antiqua* (Hada) Ono	−	−	−	NIES 161
	Heterosigma akashiwo (Hada) Hada	−	−	−	NIES 5
	Fibrocapsa japonica Toriumi et Takano	−	−	−	NIES 136
	Olisthodiscus luteus Carter	−	−	−	NIES 15
Eustigmatophyceae					
Pleurochloridales	*Pseudocharaciopsis texensis* Lee et Bold (z.)	−	+	+	UTEX 2113
Prymnesiophyceae					
Prymnesiales	*Chrysochromulina ericina* Parke & Manton	+	−	−	Kawachi M. (TKB)
	Ch. hirta Manton	+	−	−	Kawachi M. (TKB)
	Ch. spinifera Pienaar & Norris	−	−	−	Kawachi M. (TKB)
	Ch. strobirus Parke & Manton	−	−	−	Kawachi M. (TKB)
	Platychrysis pienaari Gayral et Fresnel	+	−	−	Inouye I. (TKB)
	Prymnesium parvum Carter	+	−	−	Inouye I. (TKB)
	Pr. sp. 1	+	−	−	Kogame K. (HKD)
Isochrysidales	*Isochrysis* sp. 1	+	−	−	Inouye I. (TKB)
	I. sp. 2	+	−	−	Okauchi M. (NRIA)[5]
Coccosphaerales	*Coccolithus neohelis* McInntyre et Bé	−	−	−	Kawachi M. (TKB)
	Jomonlithus littoralis Inouye et Chihara	−	−	−	Inouye I. (TKB)
	Ochrosphaera verrucosa Schussnig	−	−	−	Inouye I. (TKB)
	Pleurochrysis roscoffensis (D.) Inouye & Chihara	−	−	−	Tenjin M. (FMS)[6]
Pavlovales	*Pavlova* sp. 1	−	−	−	Inouye I. (TKB)
	P. sp. 2	−	+	−	Inouye I. (TKB)
Dinophyceae					
Gymnodiniales	*Gymnodinium sanguineum* Hirasaka	−	−	−	NIES 11
	Gyrodinium sp.	−	−	−	Sawaguchi (NIES)
	Woloszynskia sp.	−	+	−	Sawaguchi (NIES)
Peridiniales	*Heterocapsa triquetra* Stein	−	−	−	NIES 7
	Peridinium sp.	−	−	−	Kawai H. (HKD)

Table 1. Continued

Systematic position	Species	Fluorescence	Eyespot	Flagellar swelling	Source of strains
Cryptophyceae					
Cryptomonadales	*Chroomonas caudata* Geitler	−	−	−	Erata M. (TKB)
	Ch. coerulea (Geitler) Skuja	−	+	−	Erata M. (TKB)
	Ch. placoidea Butcher	−	−	−	Erata M. (TKB)
	Cryptomonas acuta Butcher	−	−	−	Erata M. (TKB)
	Cr. ovata Ehrenberg	−	−	−	Erata M. (TKB)

+, flagellar autofluorescence in smooth flagellum. ++, flagellar autofluorescence in hairy and smooth flagellum. *, flagellar autofluorescence at least in one smooth flagella. −, flagellar autofluorescence absent.

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