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Review paper

The Acquisition of Plastids/Phototrophy in Heterotrophic Dinoflagellates

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Abstract. Several dinoflagellates are known to practice acquired phototrophy by either hosting intact algal endosymbionts or retaining plastids. The acquisition of phototrophy in dinoflagellates appears to occur independently over a variety of orders, rather than being restricted to any specific order(s). While dinoflagellates with intact algal cells host endosymbionts of cyanobacteria, pelagophyte, prasinophyte or dictyochophyte, most organelle-retaining dinoflagellates acquire plastids from cryptophytes. In dinoflagellates with acquired phototrophy, the mechanism by which symbionts or plastids are obtained has not been well studied at sub-cellular or ultrastructural level, and thus little is known regarding their mechanism to sequester and maintain photosynthetic structures, except for three cases, *Amphidinium poecilochroum*, *Gymnodinium aeruginosum*, and *Dinophysis caudata* with peduncle feeding. Dinoflagellates with acquired phototrophy display different degrees of reduction of the retained endosymbiont and organelles, ranging from those which contain intact whole algal cells (e.g. green *Noctiluca scintillans*), to those which have retained almost a full complement of organelles (e.g., *Amphidinium poecilochroum* and *Podolampas bipes*), to those in which only the plastids remain (e.g., *Amphidinium wigrense* and *Dinophysis* spp.). A series of events leading to acquisition and subsequent degeneration of a whole-cell endosymbiont have been widely recognized as evolutionary pathway of the acquisition of plastids. However, recent work on *D. caudata* suggests that acquisition of phototrophy by predation (i.e. kleptoplastidy) may be a mechanism and evolutionary pathway through which plastids originated in dinoflagellates with 'foreign' plastids other than the 'typical' peridinin-type plastids. Most organelle-retaining dinoflagellates are facultative mixotrophs, with *Dinophysis* species and an undescribed Antarctic dinoflagellate being the only obligate mixotrophs known so far. The establishment of dinoflagellates with acquired phototrophy in cultures and careful research using the cultures would help improve our knowledge of the evolution of the dinoflagellate plastids and their ecophysiology.

Key words: Acquired phototrophy, chloroplast, endosymbiont, endosymbiosis, kleptoplastid, kleptoplasty, mixotrophy, organelle retention, photosynthesis.

INTRODUCTION

Endosymbiosis is more recognized as an important evolutionary process leading to stable plastids than is plastid retention (Keeling 2010; Nowack and Melkonian 2010). On the other hand, the temporary retention of algal organelles through predation could also yield an outcome similar to evolutionary endosymbiosis (Johnson 2011b). Whatever the mechanism by which symbionts or plastids are acquired is, in fact, we see a continuum of loss of cell organelles from completely retained cells, via exclusion of a few cell organelles and cell membrane, further reduction in most cell organelles to only the plastids (see below), but we simply

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lack terms to describe what we see for this process as functional biologists. While the symbiont is a genetically autonomous, complete organism, plastids are just organelles to perform photosynthesis. Nonetheless, retention of plastids (and sometimes additional organelles) has been often described as endosymbiosis and the retained organelles have been regarded as symbiont. In this paper, however, symbionts will refer to completely retained intact cells and all other cases will be regarded as organelle and/or plastid retention.

In this review, we employ the concept of 'acquired phototrophy' recently suggested by Stoecker *et al.* (2009) and Johnson (2011a, b), which excludes organisms with permanent plastids, but includes those retaining foreign plastids and those with intact whole algal endosymbionts. In the former case, the capture of algal prey and then temporary maintenance of one or more plastids, sometimes along with other organelles, is often called kleptoplastidy (Schnepf *et al.* 1989). In this paper, dinoflagellates with permanent plastids will refer to species which have full control of their plastids and can divide them. In this context, thus, we will not include the dinoflagellates with diatom endosymbionts (e.g. *Kryptoperidinium foliaceum*; Jeffrey and Vesk 1976) and those with plastids of haptophyte (e.g. *Karenia brevis*; Schnepf and Elbrächter 1999) or chlorophyte origins (e.g. *Lepidodinium chlorophorum*; Elbrächter and Schnepf 1996) in which the organelles are stable. Both cases would be used as examples of previous acquired phototrophy (in their ancestors) that has led to stable or permanent organelle acquisition. In this paper, we will not also include dinoflagellates with ectosymbionts (e.g. *Ornithocercus*, *Histioneis*, *Parahistioneis* and *Citharistes*). To be an acquired phototroph, dinoflagellates require some acquisition of symbionts or plastids through specific adaptations of phagotrophic pathways (Johnson 2011b), but the ectosymbiont-bearing dinoflagellates appear to grow their own 'vegetables' (symbionts) outside the cell and ingest them (Tarangkoon *et al.* 2010).

In this paper, we reviewed the occurrence of dinoflagellates with acquired phototrophy across dinoflagellate lineages, known symbionts and sources of plastids, and the acquisition and maintenance of symbionts and temporary plastids. In addition, we reviewed the degree to which retained symbionts and other organelles are reduced and discussed some evolutionary implications. We also consider the current status and limitations of ecophysiological studies of dinoflagellates with acquired phototrophy.

OCCURRENCE OF ACQUIRED PHOTOTROPHY AMONG THE DINOFLAGELLATES

Dinoflagellates with endosymbionts. So far, dinoflagellates known to practice acquired phototrophy by harboring intact algal endosymbionts are as follows (Table 1): *Amphisolenia* spp. (Lucas 1991, Daugbjerg *et al.* 2013; Fig. 1A), green *Noctiluca scintillans* (Sweeney 1976; Fig. 1B), *Podolampas bipes* (Schweiker and Elbrächter 2004), *Sinophysis canaliculata* (Escalera *et al.* 2011), *Spatulodinium* sp. 1 (Gómez and Furuya 2007), unidentified kofoidiniacean (Gómez and Furuya 2007), and *Triposolenia* spp. (Tarangkoon *et al.* 2010).

Organelle-retaining dinoflagellates. Dinoflagellates with acquired phototrophy by retaining plastids are as follows (Table 1): *Amphidinium latum* (Horiguchi and Pienaar 1992), *A. poecilochroum* (Larsen 1988; Fig. 1C), *A. wigrense* (Wilcox and Wedemayer 1985), *Amylax buxus* (Koike and Takishita 2008), *A. triacantha* (Koike and Takishita 2008, Park *et al.* 2013; Fig. 1I), *Cryptoperidiniopsis* sp. (Eriksen *et al.* 2002; Fig. 1E), *Dinophysis* spp. (e.g. Schnepf and Elbrächter 1988, Park *et al*. 2006, Kim *et al*. 2012b; Fig. 1H, J, K), *Gymnodinium acidotum* (= *G. aeruginosum*) (Wilcox and Wedemayer 1984, Schnepf *et al*. 1989, Farmer and Roberts 1990, Fields and Rhodes 1991), *G. eucyaneum* (Hu *et al.* 1980; Fig. 1D), *G. gracilentum* (Skovgaard 1998), *G. myriopyrenoides* (Yamaguchi *et al.* 2011; Fig. 1F), *Pfiesteria piscicida* (Lewitus *et al*. 1999), *Phalacroma* spp. (Hallegraeff and Lucas 1988, Koike *et al*. 2005, Nishitani *et al*. 2012), and an undescribed Antarctic dinoflagellate (Gast *et al*. 2007; Fig. 1G).

Most dinoflagellates with acquired phototrophy belong to the orders Gymnodiniales and Dinophysiales, but some belongs to the orders Gonyaulacales, Peridiniales, and Noctilucales, suggesting that acquired phototrophy in dinoflagellates occurs independently over a variety of orders, rather than being restricted to any specific order(s).

KNOWN ENDOSYMBIONTS AND SOURCES OF PLASTIDS

Dinoflagellates with endosymbionts. *Amphisolenia* species possess endosymbionts of cyanobacteria (identified as *Synechococcus carcerarius*; Lucas 1991)

Table 1. Dinoflagellates practicing acquired phototrophy (AcPh) by hosting intact algal endosymbionts (E), by possessing a reduced algal 'endosymbiont' (E*), and by retaining multiple organelles (O) or only the plastids (P) from algal prey.

Species	Theca	Habitat	Feeding mechanism	Type of AcPh	Mixotroph	Known endosymbionts or sources of plastids	References
Dinoflagellates with endosymbionts							
Amphisolenia spp.	Thecate	M		E		Cyanobacteria (identified as *Synechococcus carcerarius*); *Trichodesmium* spp. and *Nostoc* spp.[§]; Pelagophyte	Lucas (1991), Foster *et al.* (2006), Daugbjerg *et al.* (2013)
Noctiluca scintillans	Athecate	M		E	Obligate	Prasinophyte (*Pedinomonas noctilucae*)	Sweeney (1976)
Podolampas bipes	Thecate	M		E*		Dictyophyte	Schweiker and Elbracter (2004)
Sinophysis canaliculata	Thecate	M/B		E		Cyanobacteria	Escalera *et al.* (2011)
Spatulodinium sp. 1	Athecate	M		E (?)		Showing a green pigmentation	Gómez and Furuya (2007)
Unidentified kofoidiniacean	Athecate	M		E (?)		The presumed symbiotic microalgae were observed	Gómez and Furuya (2007)
Triposolenia spp.	Thecate	M		E (?)		Cyanobacteria	Tarangkoon *et al.* (2010)
Organelle-retaining dinoflagellates							
Amphidinium latum	Athecate	M/B		O		*Chroomonas* spp. (3 types)	Horiguchi and Pienaar (1992)
Amphidinium poecilochroum	Athecate	M/B	Peduncle	O	Facultative	Cryptophyte (*Chroomonas/Hemiselmis* clade)	Larsen (1988)
Amphidinium wigrense	Athecate	F		P		Cryptophyte (of freshwater)	Wilcox and Wedemayer (1985)
Amylax buxus/triacantha	Thecate	M	Engulfment	O	Facultative	Cryptophyte (*Teleaulax amphioxeia* via the ciliate *Mesodinium rubrum)*	Koike and Takishita (2008), Park *et al.* (2013)
Cryptoperidiniopsis sp.	Thecate	M	Peduncle	P	Facultative	Cryptophyte (*Storeatula major*)	Eriksen *et al.* (2002)
Dinophysis spp.	Thecate	M	Peduncle	P	Obligate	Cryptophyte (*Teleaulax amphioxeia* via the ciliate *Mesodinium rubrum)*; plastids of multiple algal origins belonging to cryptophyte, raphidophyte, and chlorophyte[§]	Park *et al.* (2006); Qiu *et al.* (2011); Kim *et al.* (2012a, b)
Gymnodinium acidotum (= *Gymnodinium aeruginosum*)	Athecate	F	Peduncle	O		Cryptophyte (*Chroomonas*)	Wilcox and Wedemayer (1984); Schnepf *et al.* (1989), Farmer and Roberts (1990), Fields and Rhodes (1991)
Gymnodinium eucyaneum	Athecate	F		?		Cryptophyte (*Chroomonas*)	Hu *et al.* (1980), Xia *et al.* (2013)
Gymnodinium gracilentum	Athecate	M	Peduncle	P (?)	Facultative	Cryptophyte (*Rhodomonas salina*)	Skovgaard (1998)
Gymnodinium myriopyrenoides	Athecate	M/B		O		Cryptophyte (*Chroomonas/Hemiselmis* clade)	Yamaguchi *et al.* (2011)
Pfiesteria piscicida	Thecate	M	Peduncle	P	Facultative	Cryptophyte (*Rhodomonas* sp.)	Lewitus *et al.* (1999), Feinstein *et al.* (2002)
Phalacroma spp. (*cuneus/rapa/favus/mitra*)	Thecate	M		P		Chrysophyte or haptophyte; plastids of multiple algal origins belonging to Bolidophyceae, Bacillariophyceae, Dictyochophyceae, Haptophyceae, Pelagophyceae, and Prasinophyceae[§]	Hallegraeff and Lucas (1988), Koike *et al.* (2005), Nishitani *et al.* (2012)
Undescribed Antarctic dinoflagellate (RS-Dino)	Athecate	M		O	Obligate	Haptophyte (*Phaeocystis antarctica*)	Gast *et al.* (2007)

M – marine, B – benthic, F – freshwater
[§] detected by molecular techniques

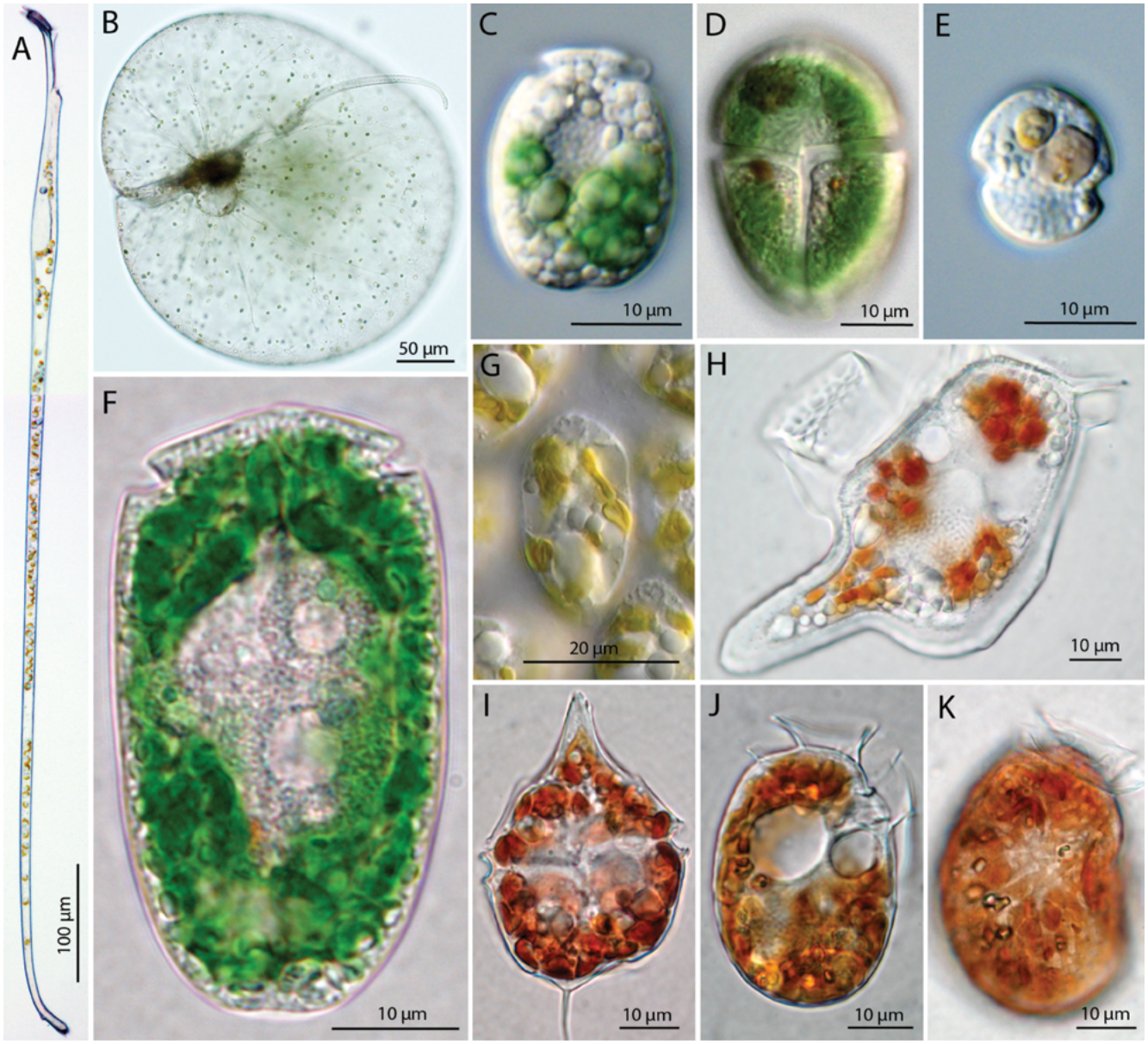

Fig. 1. Light micrographs of some dinoflagellates with acquired phototrophy. **A** – *Amphisolenia bidentata* (micrograph provided by Niels Daugbjerg); **B** – green *Noctiluca scintillans* (micrograph provided by Ken Furuya); **C** – *Amphidinium poecilochroum*; **D** – *Gymnodinium eucyaneum* (micrograph provided by Guoxiang Liu); **E** – *Cryptoperidiniopsis* sp.; **F** – *Gymnodinium myriopyrenoides*; **G** – undescribed Antarctic dinoflagellate (micrograph provided by C. Grier Sellers); **H** – *Dinophysis caudata*; **I** – *Amylax triacantha*; **J** – *Dinophysis acuminata*; **K** – *Dinophysis fortii*.

and pelagophyte origin (Daugbjerg *et al*. 2013). *Triposolenia* spp. also possess endosymbionts of cyanobacterial origin (Tarangkoon *et al.* 2010) but the identity of the symbionts was not investigated yet. The benthic dinophysioid dinoflagellate *Sinophysis canaliculata* contains cyanobacterial endosymbionts (Escalera *et al.* 2011). A certain kofoidiniaceans have been reported to show a green pigmentation (*Spatulodinium* sp.) and contain symbiotic microalgae (unidentified kofoidiniacean) (Gómez and Furuya 2007), but their symbionts were not identified in detail. Unlike the red heterotrophic form, green *Noctiluca scintillans*, which is commonly found in Southeast Asian waters, harbors large numbers of free-swimming cells of the prasinophyte

Pedinomonas noctilucae within its buoyancy vacuole (Sweeney 1976, Hansen *et al.* 2004). *Podolampas bipes* contains endocytobionts of dictyochophyte origin (Schweiker and Elbrächter 2004). However, acquired phototrophy in *Podolampas bipes* seems to be more or less variable because different authors have reported conflicting results on the presence or absence of chloroplasts. In plankton samples obtained from different oceans during several cruises, Schweiker and Elbrächter (2004) observed several hundred *P. bipes* cells, all containing the same kind of endocytobionts of dictyochophyte origin, and also reported that all daughter cells produced over four cell division contained apparently the same number of chloroplasts. By contrast, Hallegraeff and Jeffrey (1984) classified *P. bipes* as a heterotrophic species, based on observation with epifluorescence microscopy. On the other hand, Lessard and Swift (1986) observed that all specimens of *P. bipes* were either completely devoid of chloroplasts or were filled with red-fluorescing spherical bodies, depending on the sampling locations. Thus, acquired phototrophy in *Noctiluca* and *Podolampas* appears to be variable among populations.

Organelle-retaining dinoflagellates. *Phalacroma* spp. have been reported to possess plastids of chrysophyte or haptophyte origin (Hallegraeff and Lucas 1988, Koike *et al.* 2005). Undescribed Antarctic dinoflagellate is known to acquire haptophyte plastids from *Phaeocystis antarctica* (Gast *et al*. 2007).

Except for the above cases, all other organelle-retaining dinoflagellates acquire plastids from cryptophytes (Table 1). These cryptophyte kleptoplastids represent three clades (*Teleaulax*/*Geminigera*/*Plagioselmis*, *Chroomonas*/*Hemiselmis*/*Komma*, and *Rhodomonas*/*Rhinomonas*/*Storeatula*) of the seven major clades of plastid-containing cryptomonad genera identified by Deane *et al*. (2002); kleptoplastids in the freshwater dinoflagellates *Amphidinium wigrense*, *Gymnodinium acidotum* (= *G. aeruginosum*) and *G. eucyaneum* originate only from cryptophyte species belonging to the *Chroomonas*/*Hemiselmis*/*Komma* clade, while those in marine species originate from each of the three clades mentioned above. Most known organelle-retaining dinoflagellates sequester plastids by feeding directly on cryptophyte prey, but *Amylax triacantha* (Park *et al.* 2013) and *Dinophysis* spp. (Park *et al.* 2006, Kim *et al.* 2012b) are exceptions to this trend, as they sequester plastids from the mixotrophic ciliate *Mesodinium rubrum* (= *Myrionecta rubra*), which feeds on members of the *Teleaulax*/*Geminigera*/*Plagioselmis* clade.

Interestingly, recent molecular studies (Qiu *et al*. 2011, Kim *et al.* 2012a) have revealed individual *Dinophysis* spp. cells to simultaneously contain the well-known 'common' plastids of the cryptophyte origin along with multiple plastids originating from other algal groups. For example, Kim *et al*. (2012a) isolated a total of 66 *Dinophysis* cells representing *D. acuminata*, *D. caudata*, *D. fortii*, and *D. infundibulus* from the western and southern coasts of Korea and investigated plastid diversity using light and epifluorescence microscopy, single-cell PCR technique, and restriction fragment length polymorphism (RFLP) analysis. They found that approximately two-thirds of the analyzed *Dinophysis* cells contained two types of cryptophyte plastids (*Teleaulax amphioxeia* and *T. acuta*). Surprisingly, some *Dinophysis* cells contained three (i.e. cryptophytes *T. amphioxeia* and *T. acuta* and raphidophyte *Heterosigma akashiwo*) or even four (i.e. cryptophytes *T. amphioxeia* and *T. acuta*, raphidophyte *H. akashiwo*, and chlorophyte *Pyramimonas* sp.) different types of plastid. Similarly, Qiu *et al*. (2011) determined plastid SSU rDNA sequences from four eight-cell *D. miles* colonies isolated in the South China Sea and detected three distinct types of sequences, belonging to plastids of a cryptophyte, a haptophyte and a cyanobacterium. They thought that the cyanobacterial sequences may represent an ectosymbiont of the *D. miles* cells and thus, their result indicates that natural assemblage of *D. miles* was likely containing at least two different types of plastids. Like *Dinophysis* spp., *Phalacroma mitra* can also have multiple types of plastids of several algal origins. In 14 *P. mitra* cells, Nishitani *et al.* (2012) detected more than 100 different plastid *rbc*L gene sequences representing the Bolidophyceae, Bacillariophyceae, Dictyochophyceae, Haptophyceae, Pelagophyceae, and Prasinophyceae. Similarly, multiple types of plastids of several cyanobacterial (filamentous *Trichodesmium* spp. and heterocystous *Nostoc* spp.) origins have also been detected in endosymbiont-bearing *Amphisolenia* spp. (Foster *et al.* 2006). The results from these molecular studies raise a question as to whether all plastids 'detected' by the molecular techniques are indeed used for photosynthesis. We should be very careful in interpretation of molecular data on cells collected from the field as whether all the 'detected' plastids are photosynthetically functional or mainly serve as food source is not clearly determined yet. In order to answer this question, experiments (perhaps, using the culture materials) need to be carried out to actually document this.

ACQUISITION AND MAINTENANCE OF SYMBIONT OR PLASTID

In dinoflagellates with acquired phototrophy, the mechanism by which symbionts or plastids are acquired has not been well studied at sub-cellular or ultrastructural level, and thus little is known regarding their mechanism to sequester and maintain them. Thus far, the mechanism for acquisition of plastids is known only for three species, *Amphidinium poecilochroum*, *Gymnodinium aeruginosum* and *Dinophysis caudata*. In *A*. *poecilochroum*, the periplast of the cryptophyte prey is pierced by the dinoflagellate's peduncle (Larsen 1988), and the prey cytoplasm and organelles are subsequently ingested into the dinoflagellate cytoplasm, not into a phagocytotic vacuole (= digestive vacuole) (Onuma and Horiguchi 2013; Fig. 2B). The ingested organelles, including chloroplasts, are encircled by a single membrane of unknown origin (but, perhaps formed by the dinoflagellate; Larsen 1988). Then, *A*. *poecilochroum* forms a digestive vacuole rapidly and removes the cryptophyte cytoplasm together with its organelles in the order of mitochondria, ejectosomes and nucleus within a few hours, by actively transferring them into a digestive vacuole (Onuma and Horiguchi 2013; Fig. 2B). *A*. *poecilochroum* retains the plastids for about 3 days (Onuma and Horiguchi 2013), but the duration during which the retained plastids are photosynthetically functional remains unknown. As in *A*. *poecilochroum*, the ingested cryptophyte organelles are encircled by a single membrane of unknown origin in the cytoplasm of *G*. *aeruginosum* (Onuma and Horiguchi 2013; Fig. 2C). Unlike *A*. *poecilochroum*, however, *G*. *aeruginosum* does not form a digestive vacuole directly after ingestion of the prey. In *G*. *aeruginosum*, the cryptophyte organelles together with its cytoplasm are retained relatively for longer time (up to 24 hours after ingestion). Interestingly, the ingested plastids in *G*. *aeruginosum* are substantially enlarged, with the volume being increased up to 10 times compared to that of the plastids shortly after ingestion. *G*. *aeruginosum* can retain the plastids for more than 1 month, but its functional retention time remains unknown. By contrast, *Dinophysis* spp. acquire plastids of cryptophyte origin in a unique way by feeding on the mixotrophic ciliate *Mesodinium rubrum*, which in turn feeds on cryptophyte (Park *et al*. 2006). Very recently, Kim *et al.* (2012b) demonstrated the detailed sequestration and retention mechanism of plastids in *D*. *caudata* using light microscopy, time-lapse videography, and single-cell TEM. Chloroplasts and other organelles of the *M*. *rubrum* prey are transported through the peduncle into a central food vacuole. Prey chloroplasts ingested by *D*. *caudata* escape from the food vacuole, perhaps with the aid of membrane vesicles, and enter the dinoflagellate cytoplasm (Fig. 2D). After entering the cytoplasm of *D. caudata*, the sequestered prey plastids undergo considerable ultrastructural modifications (e.g. change in pyrenoid position from a lateral position to a terminal position and shift in thylakoid arrangement from predominately stacks of 3, to a mix of stacks of 3 and stacks of 2, and eventually to predominately stacks of 2) to form the stellate compound chloroplast typically reported for plastid-retaining *Dinophysis* species. In the cytoplasm of *D*. *caudata*, the retained plastids remain photosynthetically active for up to 2 months (Park *et al.* 2008).

It is interesting to note that *A*. *poecilochroum* and *Dinophysis* show large differences in the degree of the cryptophyte reduction, as well as retention time of the chloroplasts, although they use the same feeding mechanism (myzocytosis through the peduncle). In case of *A*. *poecilochroum*, it is not clear whether the cryptophyte chloroplasts serve as food only, or whether they remain photosynthetically functional for sufficient time for the delayed digestion of chloroplasts to be considered kleptoplastidy in the strict sense (Kim *et al.* 2012b). These differences in handling of prey plastids suggest that *A*. *poecilochroum* may be in the earliest stage of the chloroplast acquisition, while *D*. *caudata* appears to have achieved a more advanced state of plastid retention.

VARIATION IN THE DEGREE OF REDUCTION AND EVOLUTIONARY IMPLICATIONS

The degree of reduction of the retained symbionts and/or organelles greatly differs depending on the host species (Fig. 2).

Dinoflagellates with endosymbionts. *Amphisolenia* spp. and *Sinophysis canaliculata* contain complete endosymbionts of either prokaryotic or eukaryotic origin (Lucas 1991, Escalera *et al.* 2011). Green *Noctiluca scintillans* contains intact whole cells of the prasinophyte *Pedinomonas noctilucae* within the vacuole (Sweeney 1976). *Podolampas bipes* retains all cell

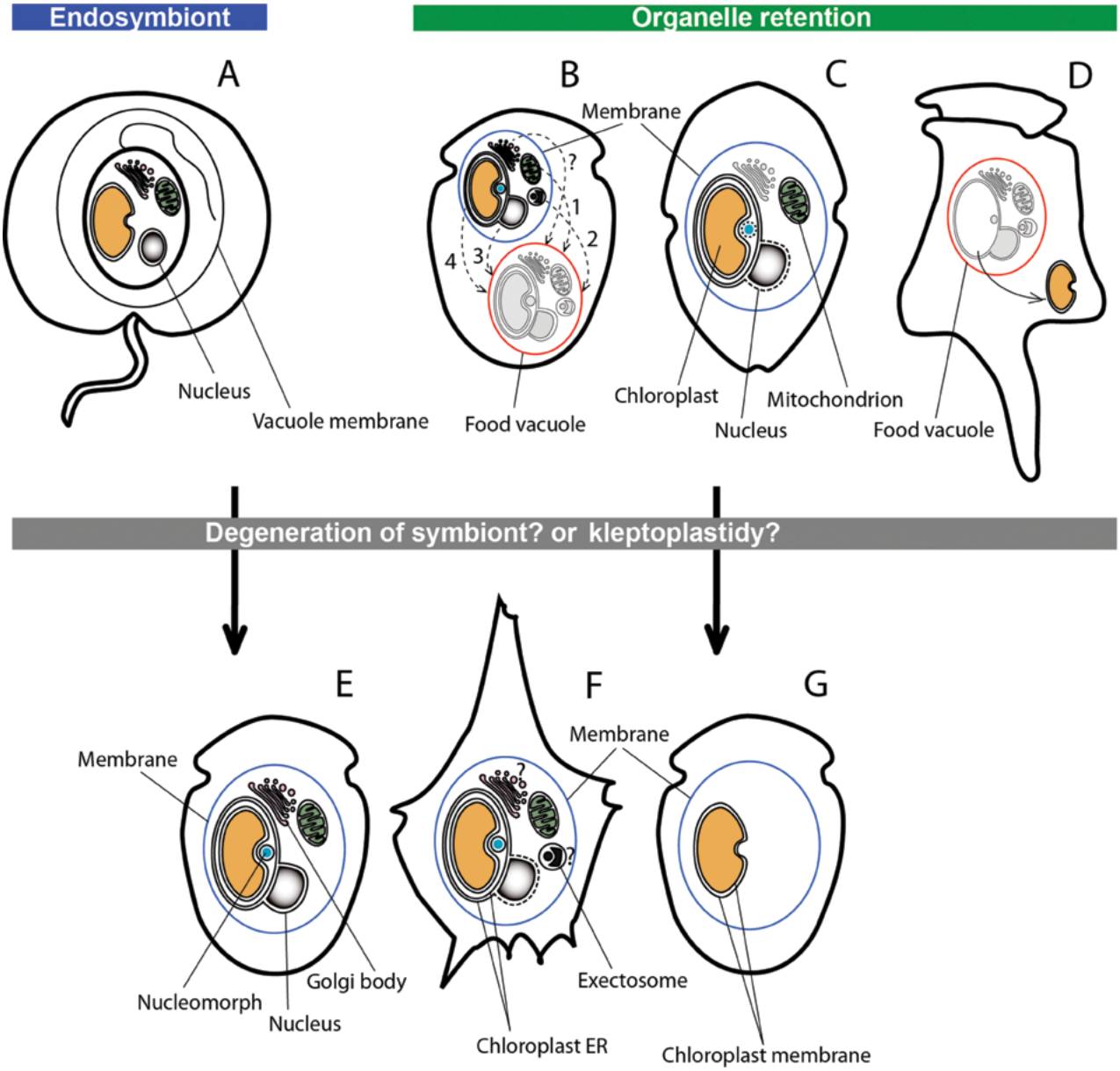

Fig. 2. Variation in degree of reduction of the retained endosymbiont or organelles in dinoflagellates with acquired phototrophy. Black thick lines: plasma membrane; Blue circles: a single membrane of unknown origin that separates the cryptophyte cytoplasm from the dinoflagellate cytoplasm; Red circles: digestive vacuole (= food vacuole). **A** – green *Noctiluca scintillans* harboring an intact cell of the prasinophyte *Pedinomonas noctilucae*; **B–D** – the three cases where the mechanism for acquisition of organelles by dinoflagellates is known; **B** – *Amphidinium poecilochroum*. The ingested cryptophyte organelles are encircled by a single membrane of unknown origin, and then are actively transferred to and digested in a digestive vacuole in the order of the numbers indicated; **C** – *Gymnodinium acidotum* (= *G. aeruginosum*). In the dinoflagellate, the cryptophyte's Golgi body (indicated in grey color) was degenerated. The cryptophyte nucleus and nucleomorph (indicated by dotted lines) were present in some cells, but not in other cells. In the dinoflagellate, a peduncle has been identified (Wilcox and Wedemayer 1984, Farmer and Roberts 1990), but it is not clear whether the peduncle feeding is actually involved in the ingestion process (Fields and Rhodes 1991). As in *A. poecilochroum*, the ingested cryptophyte organelles are encircled by a single membrane; **D** – *Dinophysis* spp. The arrow means that the plastids escape from the food vacuole and move to the cytoplasm of the dinoflagellate; **E–G** – cases where the mechanism for acquisition of cryptophyte organelles remains unknown. The plastids and other organelles may originate from a series of events leading to acquisition and subsequent degeneration of a whole-cell endosymbiont (i.e., an intact cryptophyte symbiont – **E, F** or **G**), or may be acquired as organelles via predation (i.e., kleptoplastidy; an ingested cryptophyte partially digested to give states shown in **E, F** or **G**); **E** – *Amphidinium latum* and *Gymnodinium myriopyrenoides*; **F** – *Amylax triacantha*. According to Koike and Takishita (2008), a single *Amylax* cell had 14 cryptophyte vestiges, of which only one was found to contain a cryptophyte nucleus (indicated by dotted line). The presence of a Golgi body and exectosome was not confirmed (indicated by question marks); **G** – *Amphidinium wigrense* retaining only plastids of cryptophyte origin.

content of the endosymbiont of dictyochophyte origin, except for the loss of its flagella (Schweiker and Elbrächter 2004). No ultrastructural data are at present available for *Spatulodinium* sp., unidentified kofoidiniacean and *Triposolenia* spp. to examine the degree of reduction of the symbionts.

Organelle-retaining dinoflagellates. *Amphidinium poecilochroum* retains almost all cryptophyte organelles except for the periplast and the flagellar apparatus and the retained plastids are surrounded by five membranes (i.e., the double membrane chloroplast envelope, the double membrane of the chloroplast endoplasmic reticulum, and the outmost membrane of unknown origin) (Larsen 1988, Onuma and Horiguchi 2013). *Amphidinium latum* also retains most cryptophyte organelles, except for the periplast, the flagellar basal bodies, and the ejectosomes (Horiguchi and Pienaar 1992). The remnant condition of the cryptophyte organelles in *Gymnodinium myriopyrenoides* is similar to that of *A. latum*, but the cryptophyte nucleus is usually deformed and the Golgi body is degenerated (Yamaguchi *et al.* 2011). *Amylax* spp. and *G. acidotum* (= *G. aeruginosum*) also retain multiple organelles, but in these species the cryptophyte nucleus and/or nucleomorph are sometimes lost. An *A. buxus* cell had 14 cryptophyte vestiges, of which only one was found to contain a cryptophyte nucleus (Koike and Takishita 2008). In *G. acidotum* (= *G. aeruginosum*), only 10–57% of the cells examined possessed a cryptophyte nucleus (Schnepf *et al.* 1989, Farmer and Roberts 1990, Fields and Rhodes 1991). In addition, *G. acidotum* (reported as *G. aeruginosum*) has no nucleomorph (Schnepf *et al.* 1989). The cryptophyte chloroplasts in *A. latum*, *G. myriopyrenoides*, *Amylax* spp. and *G. acidotum* are all surrounded by 5 membranes. By comparison, *A. wigrense* and *Dinophysis* spp. retain only chloroplasts and lack remnants of other cryptophyte organelles (Wilcox and Wedemayer 1985, Schnepf and Elbrächter 1988, Lucas and Vesk 1990, Garcia-Cuetos *et al*. 2010, Kim *et al.* 2012b). Further, the chloroplasts in the former species are surrounded by only 3 membranes (Wilcox and Wedemayer 1985) and those in the latter species are surrounded by only 2 membranes (Schnepf and Elbrächter 1988, Lucas and Vesk 1990, Garcia-Cuetos *et al*. 2010, Kim *et al*. 2012b).

As noted above, dinoflagellates with acquired phototrophy display different degrees of reduction of the retained endosymbiont or organelles, ranging from those which contain intact whole algal cells (e.g. green *Noctiluca scintillans*; Fig. 2A), to those which have retained almost a full complement of organelles (e.g., *Amphidinium poecilochroum* and *Podolampas bipes*; Fig. 2B), to those in which only the plastids remain (e.g., *Amphidinium wigrense* and *Dinophysis* spp.; Fig. 2D and G). The variation in degree of reduction, sometimes along with symbiont (and/or plastid) specificity and synchronization between dinoflagellate host and symbiont (and/or plastid), have been widely recognized as circumstantial evidence in supporting the theory of an endosymbiotic origin of plastids (e.g. Wilcox and Wedemayer 1985, Schnepf and Elbrächter 1988, Yamaguchi *et al.* 2011). However, recent work on *Dinophysis caudata* (Kim *et al.* 2012b) suggests that plastids can be acquired as isolated chloroplasts via predation, not through a series of events leading to acquisition and subsequent degeneration of a whole-cell endosymbiont. In *D. caudata*, the plastids seem to be 'selectively' recognized and extracted from other cryptophyte organelles inside the central food vacuole, although the mechanism for this remains unknown. As already noted above, the acquisition mechanism of cryptophyte organelles, including plastids, through kleptoplastidy was also recently revealed in *A. poecilochroum* and *G. aeruginosum* (Onuma and Horiguchi 2013). Recently, *Amylax triacantha* was reported to ingest the mixotrophic ciliate *M. rubrum* by myzocytosis (Park *et al.* 2013), and thus it is more likely that the dinoflagellate retains plastids of cryptophyte origin by kleptoplastidy rather than by an endosymbiotic origin of plastids. Therefore, acquisition of phototrophy by predation (i.e. kleptoplastidy) may shed light on the mechanisms and evolutionary pathways through which plastids originated in dinoflagellates with 'foreign' plastids other than the 'typical' peridinin-type plastids.

ECOPHYSIOLOGY

Dinoflagellates with endosymbionts. Despite several previous reports on dinoflagellates with acquired phototrophy, little is known about their ecophysiology, as most of them has not been established in culture. Food requirement of the green *Noctiluca scintillans* seems to be strain-specific (Hansen *et al.* 2004, Furuya *et al.* 2006, Saito *et al.* 2006). While some strains can grow photoautotropically for generations although they also have an ability to feed on the prey (i.e. facultative phagotrophy), other strains require food supply (i.e. obligatory phagotrophy). Phagotrophy does promote faster growth of the green *N. scintillans* (Hansen *et al.*

2004, Furuya *et al.* 2006). Photosynthesis facilitates survival of the host during food limitation rather than enhancing growth of the host (Saito *et al.* 2006). Green *N. scintillans* lose their endosymbionts after some time in laboratory cultures under all culture conditions (e.g., prey type and concentration and light intensity) tested so far, perhaps due to the shortage of unknown growth factor derived from food ingested by the host (Sweeney 1971, Hansen *et al.* 2004). Thus, green *N. scintillans* does not survive beyond one month without a food supply (Sweeney 1971, Hansen *et al*. 2004). By comparison, Furuya *et al*. (2006) have reported that non-feeding strains can grow by asexual binary fission in the absence of the prey for at least two years, although any available information about the change in number of the symbionts was not provided in their study. At present, there is no available information about ecophysiology of other dinoflagellates with endosymbionts discussed in this paper.

Organelle-retaining dinoflagellates. *Amphidinium poecilochroum*, *Amylax triacantha*, *Cryptoperidiniopsis* sp., and *Gymnodinium gracilentum* can grow heterotrophically in the dark if supplied with sufficient prey, but their growth rates increase in the light (Skovgaard 1998, Jakobsen *et al.* 2000, Eriksen *et al*. 2002, Park *et al*. 2013). By comparison, *Pfiesteria piscicida* can also grow heterotrophically in the dark if supplied with sufficient prey, but light effect on its growth seems to be different depending on the strains (Eriksen *et al*. 2002, Feinstein *et al*. 2002); while growth of the two strains, SCHABP 9701 and 113-3, was less influenced by light intensity (Eriksen *et al.* 2002), strain CCMP-1831 showed the enhanced growth with increasing light intensity (Feinstein *et al*. 2002). Thus, the five dinoflagellates mentioned above may be considered as facultative mixotrophs. In *A. poecilochroum*, *A. triacantha* and *G. gracilentum*, ingestion rates increased with increasing light intensity, and thus their increased growth rates seemed to be accompanied by the correspondingly increased ingestion rates (Skovgaard 1998, Jakobsen *et al*. 2000, Park *et al*. 2013); the growth efficiencies in the latter two species did not change with light intensity. Thus, it seems that photosynthesis by kleptoplastids does not contribute to increased growth of plastid-retaining dinoflagellates in food-replete conditions. In those dinoflagellates, we cannot exclude the possibility that the enhanced growth under light could result from light-stimulated digestion (e.g. Strom 2001). On the contrary, *Pfiesteria piscicida* strain CCMP-1831 showed enhanced growth rates and growth efficiencies with increasing light intensity without any significant effect on grazing (Feinstein *et al.* 2002), indicating that kleptoplastidy may enhance growth of the dinoflagellate, perhaps through active photosynthetic activity for a short time until plastids become digested, and then the rapid incorporation of the photosynthetic products into biomass (Jakobsen *et al*. 2000). On the other hand, *Cryptoperidiniopsis* sp. showed an increase in growth rates but decrease in ingestion rates and growth efficiencies with increasing light intensity (Eriksen *et al*. 2002). Unlike light intensity, however, little data are available concerning the effect of prey concentration on growth efficiency, with such relationships addressed only in *A. triacantha* so far (Park *et al*. 2013). For *A. triacantha*, growth efficiencies (36–43%) at high prey concentrations were within the range (12–64%) reported for heterotrophic and kleptoplastidic dinoflagellates (Hansen 1992, Buskey *et al.* 1994, Skovgaard 1998, Kim *et al.* 2008), while those at low prey concentrations were erroneously high (81–179%). This result indicates that growth at low prey concentrations was substantially supplemented by photosynthesis from retained plastids, with photosynthesis possibly playing a more important role for growth and/or survival of the plastid-retaining dinoflagellates during food limitation and starvation.

The undescribed Antarctic dinoflagellate and *Dinophysis* species are the only obligate mixotrophs known so far among the organelle-retaining dinoflagellates, as they require both light and food for growth in the long run (Gast *et al.* 2007, Kim *et al.* 2008). Much more work on the undescribed Antarctic dinoflagellate is required in the future to understand its ecophysiology. In *Dinophysis*, the effects of light intensity and prey concentration on growth and ingestion rates have so far been studied only in *D. acuminata* (Kim *et al.* 2008, Riisgaard and Hansen 2009). Growth and ingestion rates of *D. acuimata* increase with increasing light intensity, with higher growth efficiencies (40–54%) observed at intermediate light levels rather than at low or high light levels (Kim *et al*. 2008). At high prey concentrations, *D. acuminata* acquires most (70–90%) of its carbon requirements from food uptake, while at low prey concentrations like natural environments, the dinoflagellate appears to receive a large fraction of its carbon requirement from photosynthesis (Riisgaard and Hansen 2009).

CONCLUSIONS AND PERSPECTIVES

Most dinoflagellates with acquired phototrophy have been generally considered mixotrophs (Stoecker *et al.* 2009, Johnson 2011b). In cases of dinoflagellates with organelle retention, however, whether all of these dinoflagellates should be indeed regarded as mixotrophs seems to be often doubtful because some dinoflagellates (e.g. *Pfiesteria piscicida*) retain the plastids within a phagocytotic vacuolar membrane, where the retained plastids are slowly digested. In addition, the time that the retained plastids remain photosynthetically active is relatively short (usually 2 to 14 days) in dinoflagellates, except for *Dinophysis caudata* with longest functional retention time (2 months) known so far among the plastid-retaining dinoflagellates. Thus, quantitative measurements of photosynthesis over the same time frame as the retention time are highly encouraged in future studies to better understand if the delayed digestion of plastids acts as food only or whether they remain photosynthetically functional long enough to provide significant benefit.

The extensive use of a variety of microscopic techniques (e.g., transmission electron microscopy, epifluorescence microscopy and time-lapse videography) has led to carry out the investigation of dinoflagellates with acquired phototrophy. On the other hand, recent subsequent applications of a variety of molecular techniques (e.g., sequencing and RFLP) to dinoflagellates practicing acquired phototrophy of interest isolated from the field samples also led to much progress in better understanding of these dinoflagellates (in particular, the presence of the plastids of multiple algal origins), as seen in examples of *Dinophysis* spp. and *Phalacroma mitra*. Nonetheless, molecular studies raise several questions that need to be addressed in the future: e.g., how the dinoflagellate hosts acquire such diverse plastids and whether the 'detected' plastids play an important role in photosynthesis or simply serve as food only are poorly understood.

So far, a few dinoflagellates with acquired phototrophy have been established in laboratory cultures and recently have started to unveil their secrets, driven by progress in culture experiments. However, most dinoflagellates with acquired phototrophy have still failed to be established in cultures, thereby inhibiting the in-depth research of these dinoflagellates, including the acquisition mechanism and maintenance of the plastid, the exact relationship between the dinoflagellate host and its symbiont/plastid, and genetic integration between them.

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