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Mixotrophy in Ciliates: A Review of Chlorella Symbiosis and Chloroplast Retention [1]

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Abstract

Ciliate mixotrophy can represent a true symbiosis with an algal (*Chlorella*) cell or the retention of chloroplasts from ingested algae, a pseudosymbiosis. The two strategies differ in their inherent stability, the diversity of taxa involved, both host and symbiont, and the apparent plasticity (facultative or obligatory) of the symbiosis. In contrast to *Chlorella* symbiosis, chloroplast retention is inherently unstable, taxonomically restricted in terms of host ciliate species, involves a diversity of 'symbionts' and appears to be largely an obligatory strategy. Despite these distinctions research results on the two strategies can profitably be compared. Work on the establishment of *Chlorella* in *Paramecium bursaria* indicates that chloroplast retention in oligotrichs likely involves a membrane-membrane reaction in a food vacuole, with a relatively low success rate, which occurs early in the digestive process. Results of studies of carbon metabolism in chloroplast-retaining oligotrichs show that photosynthetic rates are variable and not easily estimated as fixed carbon is respired quickly and respired carbon is fixed, which could also occur in chlorellic ciliates. Behavioral studies with chlorellic ciliates have not uncovered any consistent "photobehaviors", some of which are only indirectly related to light, urging caution in assumptions of photobehavior in chloroplast-retaining oligotrichs. Both mixotrophic types can dominate ciliate communities in oligotrophic systems but are also common in eutrophic waters. The factors governing their relative abundances are unclear.

Key words: Ciliate, Mixotrophy, Symbiosis, *Chlorella*, Chloroplast retention.

Résumé

La mixotrophie chez les ciliés : la symbiose à chlorelles et la rétention de chloroplastes

La mixotrophie chez les ciliés peut consister soit en une vraie symbiose avec une cellule algale (*Chlorella*), soit en une pseudosymbiose grâce à la rétention de chloroplastes d'algues ingérées. La différence entre les

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deux stratégies est leur stabilité, la diversité taxonomique à la fois de l'hôte et du symbionte, ainsi que leur caractère obligatoire ou non. A la différence de la symbiose à *Chlorella*, la rétention de chloroplastes est instable et taxonomiquement limitée par le nombre d'espèces de ciliés-hôtes; elle implique une diversité des "symbiontes" et paraît une stratégie largement obligatoire. En dépit de ces distinctions, il est utile de comparer les résultats des recherches sur ces deux stratégies. Les travaux sur l'"installation" de *Chlorella* chez *Paramecium bursaria* indiquent que la rétention des plastes chez les oligotriches met vraisemblablement en jeu, tout au début du processus digestif, une réaction membrane-membrane dans une vacuole digestive, avec un faible taux de succès. Les résultats d'études sur le métabolisme carboné des oligotriches retenant des chloroplastes montrent des taux variables de photosynthèse, difficiles à estimer dans la mesure où le carbone est respiré et fixé rapidement; ce comportement a également lieu chez les ciliés à chlorelles. Il n'a pas pu être démontré de manière précise des réactions directes vis-à-vis de la lumière chez les ciliés hébergeant des chlorelles. Les deux types de mixotrophie peuvent dominer dans les communautés de ciliés des écosystèmes oligotrophes, mais ils sont également communs dans les eaux eutrophes. Les facteurs régulant leur importance relative restent cependant inconnus.

Introduction

Mixotrophy, used here to denote the use of both photosynthesis and heterotrophic feeding (Sanders, 1991), occurs in a large variety of taxa. Among organisms which have chloroplasts, such as phytoflagellates, it represents the uptake of particles; this particular phenomenon and its ecological implications has been reviewed recently (Borass *et al.*, 1988; Sanders and Porter, 1988). In taxa without chloroplasts, mixotrophy involves the acquisition and exploitation of either whole algal cells or isolated chloroplasts, two strategies which have been noted as physiologically and ecologically distinct (Stoecker, 1991). The exploitation of whole algal cells is generally thought of a symbiosis. Often, both algae and animal reproduce in coordination yielding a stable relationship. Chloroplast retention has been termed a pseudosymbiosis (Taylor, 1990). An isolated component of an ingested algae is exploited, one which does not apparently reproduce in the animal (Stoecker, 1991; Trench, 1975).

Examples of both types of animal photosynthesis can be found in protozoa and metazoa (see Reisser, 1992). The 'zooxanthellae' of corals or *Chlorella* and hydra are well-known examples of whole algal cell symbiosis while the exploitation of isolated chloroplasts has been noted in rotifers (reported in Taylor, 1970) and received a good deal of attention in sacoglossan molluscs (reviewed in Trench, 1979). Both photosynthetic strategies occur in distinct protistan groups, in foraminifera (Lopez, 1979; Lee *et al.*, 1988), heliozoans (Patterson and Dürschmidt, 1987), 'heterotrophic' dinoflagellates (Sweeney, 1971; Elbrächter, 1991; Fields and Rhodes, 1991), and ciliates (Laval-Peuto, 1991; Stoecker, 1991). The peculiar characteristics of *Mesodinium rubrum*, an obligate autotrophic ciliate, have been summarized by others (Lindholm, 1985, Crawford, 1989; Stoecker *et al.*, 1991) and will not be considered here.

This review focuses on ciliates which show either *Chlorella* symbiosis or chloroplast retention. Ciliates are, in general, regarded as key organisms in planktonic

systems (Pierce and Turner, 1992) and there is good deal more data on both types of mixotrophy in ciliates than for other protistan taxa. Furthermore, the data is interestingly distributed between what is known about the largely marine chloroplast-retaining oligotrichs and the mostly freshwater ciliates that contain the eukaryotic unicellular alga *Chlorella* as a symbiont. With some notable exceptions, work on chloroplast-retaining ciliates has focused on their ecology: abundances, contribution to chlorophyll stock, primary production, and photosynthesis-irradiance relationships (see Stoecker, 1991). Studies on ciliates with *Chlorella* symbionts have been, again with notable exceptions, largely been concerned with the physiology of the symbiosis: mechanisms of symbiont uptake and differences between symbiont-containing and symbiont-free (aposymbiont) states (Berninger *et al.*, 1986).

The goal of this review is to bring together the two disparate bodies of work to clarify the distinctions between whole algal cell symbiosis (*Chlorella*) and chloroplast retention as well as pointing out possible fruitful avenues of research. I will begin by outlining the general characteristics of, and important differences between, chloroplast-retention and whole cell symbiosis in terms of host and "symbiont" taxa, and the nature of the "symbiosis". This will be followed by a comparison of the physiologies of the two types of mixotrophy: "symbiont" uptake, photosynthesis and use of photosynthate. Behavior of mixotrophic ciliates, specifically the phenomenon of photoaccumulation and the effects of light on feeding will be reviewed. Finally, the ecology of chloroplast-retaining oligotrichs will be compared to *Chlorella*-containing ciliates in terms of the trophic states of systems they inhabit.

General Characteristics of Chloroplast-Retention and *Chlorella* Symbiosis

Host Taxa

The number of species reported to exhibit chloroplast retention, compared to *Chlorella* (or *Chlorella*-like) symbiosis, differs considerably but should be viewed with caution since the two are not necessarily easily distinguished without evidence based on transmission electron micrographs (Laval-Peuto and Rassoulzadegan, 1988; Stoecker, 1991). At last count, 15 species were reported to harbor chloroplasts (Stoecker, 1991) compared to 43 from freshwaters alone with *Chlorella*-like symbionts (Christopher and Patterson, 1983). More interesting though than the difference in species numbers is a disparity in the diversity of taxa involved. Chloroplast retention appears to be largely restricted to a subset of choreotrich (tintinnid and oligotrich) taxa characteristic of estuarine and marine plankton: species of the genera *Laboea*, *Strombidium*, and *Tontonia*, all of the oligotrich family Strombidiidae (Laval-Peuto and Rassoulzadegan, 1988; Stoecker, 1991). The single documented exception is a report of a marine chloroplast-retaining *Prorodon* sp. (Blackbourn *et al.*, 1973), member of a gymnostome genus which generally feeds on

large particles, such as tissue. In contrast, ciliates which contain symbiotic *Chlorella*, while epitomized by *Paramecium bursaria*, are taxonomically very diverse. They include marine and freshwater Euplotids, freshwater peritrichs (*Vorticella*) and heterotrichs (*Stentor*). The symbiosis is quite similar across these taxa based on ultrastructural evidence (Karakashian *et al.*, 1968; Graham and Graham, 1978; Kawakami, 1984; Meier *et al.*, 1984). Within a single freshwater system, several very distinct Chorella-containing ciliate types can be found ranging from *Euplotes* to the oligotrich *Halteria grandinella* (Finlay *et al.*, 1988).

"Symbiont" Taxa

In chloroplast-retaining ciliates the plastids are separated from ingested algae and sequestered while the remainder of the algal cell is digested (Stoecker *et al.*, 1991). Some *Strombidium* species appear to contain largely cryptophyte or chlorophyte plastids (Stoecker *et al.*, 1988/89; Jonsson, 1987). However, in most species, chloroplasts are derived from a variety of algae, both chromophytic and chlorophytic taxa (Blackbourn *et al.*, 1973; Laval-Peuto *et al.*, 1986; Jonsson, 1987; Stoecker *et al.*, 1988).

Among ciliates which contain whole algal cells, symbionts are all apparently of the genus *Chlorella* (see Reisser and Wiessner, 1984; Taylor, 1984). The apparent restriction of *Chlorella* symbiosis to freshwaters has been hypothesized as simply reflecting the alga's relative abundance in freshwaters (Taylor, 1984). Based on laboratory studies, different species of *Chlorella* and different strains of the same species have distinct infectivities and aptitudes for establishing stable relationships in *Paramecium bursaria* (Hirshon, 1969; Karakashian, 1975; Weis, 1978, 1979). In general, stable relationships, *i.e.*, lasting many generations in which host and symbiont divide in coordination, are most easily formed between *Chlorella* strains derived from a symbiotic relationship but *P. bursaria* can play host to wild strains of *Chlorella* as well as yeast and bacteria (Görtz, 1982).

Nature of the "Symbioses"

In both chloroplast retention and *Chlorella* symbiosis, the ciliates examined thus far appear to be mixotrophic. Many species with *Chlorella* symbionts have been cultured in the light in inorganic salt solutions (*e.g.* Sud, 1968), but axenic cultures have never been reported. The model chlorellic ciliate, *Paramecium bursaria*, has very long generation times (20 days) in the near absence of bacterial prey (Karakashian, 1963), and dies if no suitable prey are present (Meier *et al.*, 1980). An algivorous *Coleps* species showed poor growth when cultured without a supplementary food source (Klaveness, 1984). In chloroplast-retaining ciliates, phagotrophy is expected, since a source of chloroplasts is required.

The source of the chloroplasts is ingested algae. While chloroplasts are often considered semi-autonomous (Brandt, 1991) and reproduction outside of plant cells

has been reported (Giles and Sarafis, 1971a,b), there is no evidence that plastids reproduce inside ciliates (Laval-Peuto and Febvre, 1986; Laval-Peuto *et al.*, 1986; Stoecker *et al.*, 1988) and there are good reasons for assuming that functional plastids are incapable of such reproduction (Stoecker, 1991). Without constant replenishment, the chloroplast population would eventually decline to zero through plastid degeneration and dilution from cell division.

Although few species have been examined in detail, most chloroplast-retaining oligotrichs appear to be obligate chloroplast carriers. In wild populations, the possession of chloroplasts seems to be a consistent characteristic (Laval-Peuto and Rassoulzadegan, 1988; Stoecker, 1991) appearing in taxonomic descriptions (*e.g.*, Montagnes *et al.*, 1988), and attempts to culture chloroplast-retaining oligotrichs in the dark or on non-algal prey have failed (Stoecker *et al.*, 1988; Stoecker and Silver, 1990). A possible exception is *Strombidium reticulatum* which can be cultured in the dark, given sufficient prey (Jonsson, 1986) but it is unclear if the oligotrich harbors whole algal cells or isolated chloroplasts (Jonsson, 1987).

Relative to the plastid-oligotrich relationship, there is a large range in intimacies of the *Chlorella*-ciliate relationship. For example, in *Platyophrya chlorelligera*, *Chlorella* is present throughout the life cycle, including "resting" cysts (Kawakami, 1991) while two euplotid ciliates have been described as containing chlorellae only during the summer months (Berninger *et al.*, 1986). The symbiosis is also facultative for *Paramecium bursaria*; given sufficient bacterial prey and kept in the dark, aposymbiotic paramecia are formed which can be maintained indefinitely on a bacterial diet (Karakashian, 1963).

Comparative Physiologies

"Symbiont" uptake

The genus *Paramecium* is the best-studied of all ciliate genera in terms of the events involved in the processing of ingested items and the *Chlorella* symbiosis found in *P. bursaria*. While it is uncertain that all *Chlorella*-containing ciliates correspond in detail with *P. bursaria*, the amount of data available allows the construction of a reasonably complete model to compare with chloroplast-retaining oligotrichs for which considerably less data exists.

Early studies showed that while a variety of chlorococcales algae, described as ranging from autotrophic to heterotrophic, are ingested by *Paramecium bursaria*, a symbiosis characterized by the coordinated reproduction of the algae and the ciliate only occurs with *Chlorella* isolated from a symbiosis (*P. bursaria* or a hydra) or some free-living strains of *Chlorella vulgaris* (Hirshon, 1969; Karakashian, 1975). Symbiosis-competent strains of *Chlorella* were distinguished first by their release of large amounts of photosynthate (up to 85 %) as maltose (Muscatine *et al.*, 1967). A feature of living *Chlorella* was implicated in experiments which showed that heat

killed algae were digested unless a living *Chlorella* was also in the same food vacuole; then, like a living *Chlorella*, the heat killed algae could escape digestion (Karakashian and Karakashian, 1973). The features of a living cell which heat-killing altered, besides the excretion of maltose, were probably cell surface characteristics as later studies showed a correlation of infectivity with both sugar excretion and concanavalin-A agglutinability (Weis, 1978, 1979) and the digestion of cells treated with cellulases, pectinases or coated with antibodies (Reisser, 1981; Reisser *et al.*, 1982).

However, the fate of most symbiosis-competent *Chlorella* cells (up to 80 %) which are ingested is complete digestion, relatively few result in the formation of perialgal vacuoles and persist as symbionts (Karakashian and Karakashian, 1973; Weis and Ayala, 1979). Recent work has provided an explanation for this low rate of escape from digestion based on the model of food processing in *Paramecium*. The model, which has become fairly complete in the last ten years, is presented briefly below based on reviews by Fok and Allen (1988, 1990).

In *Paramecium*, particulate material is concentrated in the single membrane limited region of the mouth cavity, the cytopharynx. Membrane is added to this region to form the food vacuole. As the vacuole grows, small vesicles, acidosomes (with an acidic content but no acid phosphatase) bind to its surface. Microfilament action is involved in the release of the food vacuole, but the precise trigger is unknown. The food vacuole with its associated acidosomes travels towards the posterior pole of the cell and the acidosomes fuse with the food vacuole, resulting in vacuole condensation and a drop in pH from 7 to about 3. This process is complete within 6 minutes of vacuole release and includes a considerable amount of membrane replacement in which food vacuole membrane is removed by vesiculation and replaced by that of the acidosomes. The condensed, acidified, vacuoles remain unchanged for 3 to 5 minutes and migrate to the cell's anterior half at which time a layer of lysosomes, small acid phosphatase containing vesicles, appear next to its surface. The lysosomes fuse with the vacuole, releasing acid hydrolases. With fusion, the vacuole increases in size and the vacuolar pH reverts from 3 back to 7. Lysosome membrane and acid phosphatase are retrieved from the vacuole as membrane tubules. The vacuole is then 'defecation-competent'; undigestible remains are ejected through the cytoproct and membrane is recycled.

An ingested *Chorella* cell which escapes digestion is found in the cytoplasm enclosed in a membrane of supposed host origin, the perialgal membrane. Both the origin of the membrane and where in the processing of food vacuoles the alga circumvents digestion were shown, following the model outlined above, by Meier and Wiessner (1989) in a series of short-term pulse-chase (30 sec pulse, 15 min chase) experiments.

Confirming earlier observations (Karakashian and Karakashian, 1973; Weis and Ayala, 1979), Meier and Wiessner found that *Chlorella* cells are ingested in groups and that most are digested. But after 3 min, individual *Chorella* cells were found surrounded by a perialgal membrane. The timing of the event, and ultrastructural characteristics of the perialgal vacuole membrane, indicated that the perialgal vacuole was not formed from membrane at the cytopharynx but formed after the food vacuole fuses with acidosomes, sometime during condensation, and before lysosomal fusion.

Meier and Wiessner proposed a sequence involving the chance occurrence of an algal cell in a portion of vacuole membrane pinched off during vacuole condensation with the long-term prevention of lysosomal fusion as likely due to *Chlorella*'s ability to alter the pH of the nascent perialgal vacuole which in turn would alter the surface characteristics of the vacuole membrane. Other workers have proposed that a 'polyanionic compound' on the surface of *Chlorella* alters the vacuole membrane preventing lysosomal fusion (Karakashian and Rudzinska, 1981).

The little data on chloroplast retention in ciliates which is available, compared to that on *Paramecium* and *Chlorella*, is based on a study of *Strombidium capitatum* by Stoecker and Silver (1990). These authors exploited the fact that, like most species, the oligotrich sequesters a variety of chloroplast types, distinguishable via pigment fluorescence and ultrastructure, in a series of pulse-chase experiments. *S. capitatum* was maintained on a diet of prymnesiophytes and then exposed to cryptophytes. After a 1 hour exposure period, isolated chloroplasts appeared in the ciliate; unfortunately, intermediate steps were not observed. Similar to findings on other species, many potential chloroplast donors undergo complete digestion (Laval-Peuto and Febvre, 1986; Stoecker *et al.*, 1988/1989). Isolated chloroplasts were apparently not surrounded by any host membrane as was found in *Laboea strobila* (Stoecker *et al.*, 1988) and in contrast to reports on *Strombidium viride* (Rogerson *et al.*, 1989) and *Tontonia appendiculariformis* (Laval-Peuto and Febvre, 1986).

Following transfer to a prymnesiophyte media, sequestered cryptophyte chloroplasts gradually declined in number to near zero levels after 30 hours. From the data presented, a cryptophyte chloroplast half-life of approximately 6 hours in feeding cells was evident (Fig. 1). In cells transfered to an algal-free media, pigment fluorescence from cryptophyte chloroplasts persisted longer (up to 50 hours) than from the prymnesiophyte chloroplasts. Thus, in *S. capitatum*, different chloroplast types have different residence times and may be turning over rapidly. Residence times of different types of chloroplast also varied in *Laboea strobila* when cells were held in darkness (Stoecker *et al.*, 1988). It should be noted that chloroplast fluorescence, used to estimate residence times, may not correspond exactly with the time a chloroplast remains functional.

We know very little about what factors influence the type of chloroplasts sequestered, the portion of chloroplasts sequestered out of those ingested, or the intracellular events involved in chloroplast enslavement. In this regard, studies of *Paramecium bursaria* which have provided a nearly complete sequence of steps in the establishment of endosymbiotic *Chlorella* populations, may prove helpful in pointing out possible research directions.

It is unlikely that all ciliates process food following the same series of events (Fischer-Defoy and Hausmann, 1982). Little data exists on digestion in oligotrichs, plastidic or heterotrophic beyond the observation of Stoecker *et al.* (1988) that in *Laboea strobila*, vacuoles of acidic pH appear only in the cytopharynx region. Further examinations of digestion in oligotrichs are needed and may show that chloroplast retention is restricted to species which process ingested items in steps which are well-separated temporally and/or spatially, which is not the case for all ciliates. For

example, in the prostome, *Pseudomicrothorax dubius*, acid phosphatases are added to forming food vacuoles in the cytopharynx (Peck and Hausmann, 1980) a manner of treating food items which would probably render plastids non-functional as they are ingested. In plastidic oligotrichs, the apparent heterogeneity of chloroplast types which are sequestered, and the fact that not all species envelope plastids in host membrane, suggests that lysosomal fusion may be a particularly precise reaction in those species, restricted to food vacuole membrane in a specific condition and/or location. Admittedly, this would not explain why other relatively undigestible algal components are not sequestered.

The digestive process may differ among chloroplast-retaining oligotrichs; few species have been examined in detail. In *Tontonia appendiculariformis*, digestive vacuoles are surrounded and joined together by a network of dense endoplasmic reticulum (Laval-Peuto *et al.*, 1986) and it is one of the species in which chloroplasts are enveloped in a membrane of apparent host origin (Laval-Peuto and Febvre, 1986).

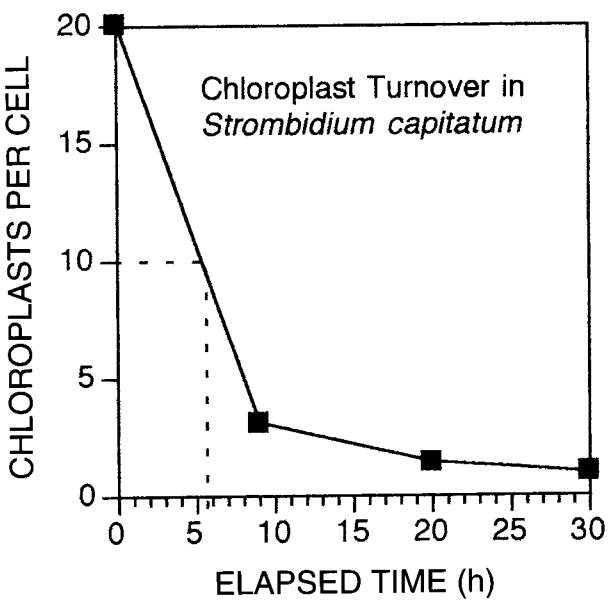

Fig. 1. Decline in the average number of cryptophyte chloroplasts per cell in *Strombidium capitatum* exposed to cryptophytes for 4 h and then held in a prymnesiophyte/prasinophyte media. Data from Stoecker and Silver (1990), table 1.

Photosynthesis and the Use of Photosynthate

In contrast to "symbiont" uptake, there is more data on photosynthesis and the metabolism of photosynthate in plastidic oligotrichs (see Stoecker, 1991; Stoecker and Michaels, 1991) than for chlorellic ciliates. For instance, while *Chlorella*-containing *Paramecium bursaria* show changes in growth rate with illumination (Karakashian,

1963) studies have thus far have not considered quantitative relationships between photosynthetically active radiation and growth rates. There is little data on either laboratory or wild populations in terms of chlorophyll per ciliate. Large differences in ciliate volume occupied by *Chlorella* cells (10-56 % of ciliate cell volume) have been found when comparing ciliates from different systems (Sud, 1968) and considerable variability between specimens from a single system has often been noted (*e.g.*, Berninger *et al.*, 1986). While such diversity could result from differences in environmental conditions, in *P. bursaria*, different ciliate strains, in combination with different strains of *Chlorella*, yield cells with markedly different numbers of algae per ciliate (Karakashian, 1975).

Little is known concerning the metabolism of photosynthate, other than most appears in the form of maltose and is transfered from the algal cell to the ciliate (Brown and Nielsen, 1974). However, in qualitative terms, the value of photosynthate to the ciliate appears to be similar based on studies of *Chlorella*-containing and plastid-sequestering ciliates, *P. bursaria* and *Laboea strobila*, respectively. When cultures of either ciliate are deprived of food, few cells survive past a few days in continous darkness, while those exposed to light for 10 or 14 h d^{-1} show either no mortality or slow growth (Karakashian, 1963; Stoecker *et al.*, 1988). The explanation for this phenomenon in *L. strobila*, in terms of the quantitative value of photosynthesis and the metabolism of photosynthate, have been revealed in recent studies.

The first investigations of chloroplast-containing oligotrichs documented uptake of ^{14}C-labeled inorganic carbon but found photosynthetic rates sufficent only to supply basal metabolism (Jonsson, 1987; Stoecker *et al.*, 1987). However, it was recognized that internal cycling of labeled carbon or preferential fixation of unlabeled, respired ciliate carbon could occur so that apparent uptake rates could be off by as much as 50 % (Jonsson, 1987; Stoecker *et al.*, 1987). The importance of these potential problems were underlined when it was shown that, while quite variable both within and between species, chlorophyll content and light-saturated rates of photosynthesis in plastidic ciliates were comparable to microphytoplankton, but considerably lower than the donating algae (Stoecker *et al.*, 1988). The question then arose: is internal cycling or rapid respiration of labeled carbon plausible, what are the ciliates doing with the photosynthate? If ^{14}C uptake underestimates the benefit of photosynthesis to the ciliate, what is the actual value?

Putt (1990) examined the metabolism of ^{14}C-labeled photosynthate in *Laboea strobila*, providing estimates of partitioning and turnover among low molecular weight compounds, polysaccharides, lipids and proteins. Most photosynthate, usually over 50 %, appeared in the polysaccharide fraction (determined as hot TCA soluable) and relatively little was found in lipid (chloroform soluable) or protein (hot TCA insoluable) fractions. The majority of lipid and protein synthesis, needed for cell reproduction, had to be based on ingested material. Photosynthate was found to turnover rapidly, with about half the photosynthate acumulated in 3 h respired within 18 h, the majority of which was lost from the polysaccharide fraction. The findings indicated that ^{14}C incubations over short time periods (few hours) likely underestimated carbon fixation due to the rapid respiration of fixed carbon.

An accurate assessment of the value of photosynthesis was made using uniformly-^{14}C-labeled ciliates and examining respiration in the dark and in the light among chloroplast-containing and heterotrophic oligotrichs (Stoecker and Michaels, 1991). The results indicated that ^{14}C uptake underestimated carbon fixation by 56-70 %. For *Laboea strobila* under light-limited conditions (50 μE m^{-2} s^{-1} = approx. Secchi depth illumination), the difference between light and dark respiration was estimated to be 1.4 % of body carbon h^{-1}; this, in addition to the carbon gain estimate from ^{14}C uptake in the light (1.1 % body carbon h^{-1}), yielded an estimate of 2.5 % body carbon h^{-1}. Similar calculations made for *Strombidium conicum* and *S. capitatum*, under light-saturating conditions, gave results of 7.5 and 2.7 % body carbon h^{-1}, respectively, as estimates of the benefits from photosynthesis. These rates are in the same range as recent estimates for the autotrophic ciliate, *Mesodinium rubrum*, 2-14 % body carbon h^{-1}, under light-saturated conditions (Stoecker *et al.*, 1991).

It is perhaps noteworthy that the parameters of photosynthesis-irradiance relationship are quite variable in chloroplast-containing oligotrichs. For instance, saturating irradiances vary considerably between species, *i.e.* 850 *vs.* 200 μE m^{-2} s^{-1} for *L. strobila* (Stoecker *et al.*, 1988) and a *Strombidium* species from the Nordic Sea (Putt, 1991). Experiments on different populations of the Nordic Sea *Strombidium*, yielded large variations in α values, slopes of the light-limited portion of the photosynthesis-irradiance relationship, ranging from 0.01-0.07 pg C pg Chl^{-1} h^{-1} μE m^{-2} s^{-1}. Similar variability between populations in terms of α (0.004-0.030 pg C pg Chl^{-1} h^{-1} μE m^{-2} s^{-1}) were found for *Mesodinium rubrum* (Stoecker *et al.*, 1991). These data indicate that further work concerning the physiology of photosynthetic ciliates is needed before estimates of photosynthetic rates can be made based on a bulk property such as cell numbers and physical parameters.

There is very little data for either chloroplast-retaining or chlorellic ciliates on nutrient sources or fluxes. It is a temptation to assume that in mixotrophs, nitrogen and phosphorus are supplied through ingestion. In the chloroplast-retaining *Strombidium viride*, phosphorus is apparently supplied through the ingestion of bacteria-sized particles (Taylor and Lean, 1981). However, in *Paramecium bursaria*, ciliates with *Chlorella* take up ammonia while aposymbiotic cells excrete ammonia (Albers *et al.*, 1982). It has been suggested that plastidic oligotrichs may take up dissolved nitrogen (Stoecker, 1991). Clearly this is an area which deserves attention since at present there is no way of predicting if mixotrophic ciliates are net nutrient consumers or producers.

Behavior

Photoaccumulation

Defined as the formation of aggregates in a light trap (Cronkite and Van Der Brink, 1981), photoaccumulation has been studied in *Paramecium bursaria* for over a century (*i.e.* Engelman, 1882). Two distinct phenomena are involved in photoaccumulations of *P. bursaria*. The ability to find a lighted area is mediated by an avoidance reaction, a

change in swimming direction, to decreases in light intensity (a "step down" response), (Saji and Oosawa, 1974), while remaining in a lighted area results from decreasing swimming speed to near zero (Cronkite and Van Der Brink, 1981). Neither behavior occurs in aposymbiotic *P. bursaria* (Neiss *et al.*, 1982; Reisser and Häder, 1984). Treatment of *Chlorella*-containing paramecia with DCMU, an inhibitor of photosystem II and oxygen evolution, blocks the cessation of swimming in light (Cronkite and Van Der Brink, 1981; Niess *et al.*, 1981), but does not alter the avoidance behavior. The step down response involves a blue light receptor and is not dependent on actively photosynthesising, and oxygen evolving, *Chlorella* (Cronkite and Van Der Brink, 1981). Interestingly, both reactions are dependent not on stable symbioses, but rather on the possession of an apparent minimum number of *Chlorella* cells, 50 ciliate^{-1} (Niess *et al.*, 1982).

Photoaccumulation, however, does not involve the same two mechanisms in all *Chlorella*-containing ciliates, nor does it even occur in all species. *Climacostomum virens*, examined both with and without symbionts, showed no "photoresponses" (Reisser and Häder, 1984). Finlay *et al.* (1987) showed that in some species, apparent photoaccumulations were probably responses to oxygen concentrations and were light-related only in so far as photosynthesis altered local oxygen concentrations. The same stimulus is apparently involved in photoaccumulation of pigmented, non-photosynthetic ciliates (Colombetti, 1990).

Photoaccumulation has never been noted in laboratory cultures of plastidic oligotrichs (Stoecker, 1991). Reports of diurnal distributions of wild populations, while documenting non-random distributions, have not provided much evidence of any phototaxis. For example, three different diurnal distribution patterns have been reported for *Laboea strobila*, chloroplast-retaining oligotrich. All three studies examined vertical distributions in spring or early summer in waters where chlorophyll *a* concentrations averaged about 1 μg l^{-1}. In Long Island Sound, *L. strobila* concentrations at 3 m depth showed maximum numbers in the early afternoon and minima at night (McManus and Fuhrman, 1986) while maximal concentrations were found at night (4:00) in surface waters on Georges Bank by Stoecker *et al.* (1989). In the Lindaspollene, *L. strobila* population maxima, along with a tintinnid (non-mixotrophic), were found at 15 m during the day and at 10 m at night (Dale, 1987). These patterns clearly must be interpreted with caution as distributions are affected by many factors (concentrations of food, competitors, predators, hydrodynamics, etc.). In addition, oligotrich behavior can be complex as shown by reports of changes in geotaxis with nutritional states (Fenchel and Jonsson, 1988). A modeling effort accounting for these factors, as well as motility and growth might be useful in determining the dominant factors involved in the observed field distributions.

Given the heterogeneity in the photobehavior of chlorellic ciliates, and recent evidence that the supposed phototactic *Mesodinium rubrum* may actually be more rheotactic than phototactic (Crawford and Purdie, 1992), it would seem advisable to avoid the assumption that plastid-containing ciliates, as photosynthetic ciliates, are capable of accumulating at certain light levels. Even if distributions vary with light, the precise stimuli may be only indirectly related to light.

Feeding

An interesting relationship between light and feeding rate in *Paramecium bursaria* was described recently by Berk *et al.* (1991). Light had no effect on feeding rates in paramecia without *Chlorella*. However, in chlorellic *P. bursaria*, they found increases in feeding rate associated with exposure to increases in light intensity. Short-term changes in illumination had no effect on the feeding rates. Changes in feeding rate were apparent in ciliates which had been habituated over a period of days to a given light intensity; the habituation corresponded with differences in the size of the *Chlorella* population in the ciliate. Higher light intensities over a range of 1 to 90 μE s^{-1} m^{-2} yielded a higher average number of *Chlorella* per cell and roughly proportional increases in feeding rates (Fig. 2). Thus the larger the photosynthetic component harbored by the ciliate, the higher the feeding rate. While aposymbiotic *P. bursaria* showed no significant differences in feeding rates with light, rates were consistently higher than *P. bursaria* with *Chlorella*.

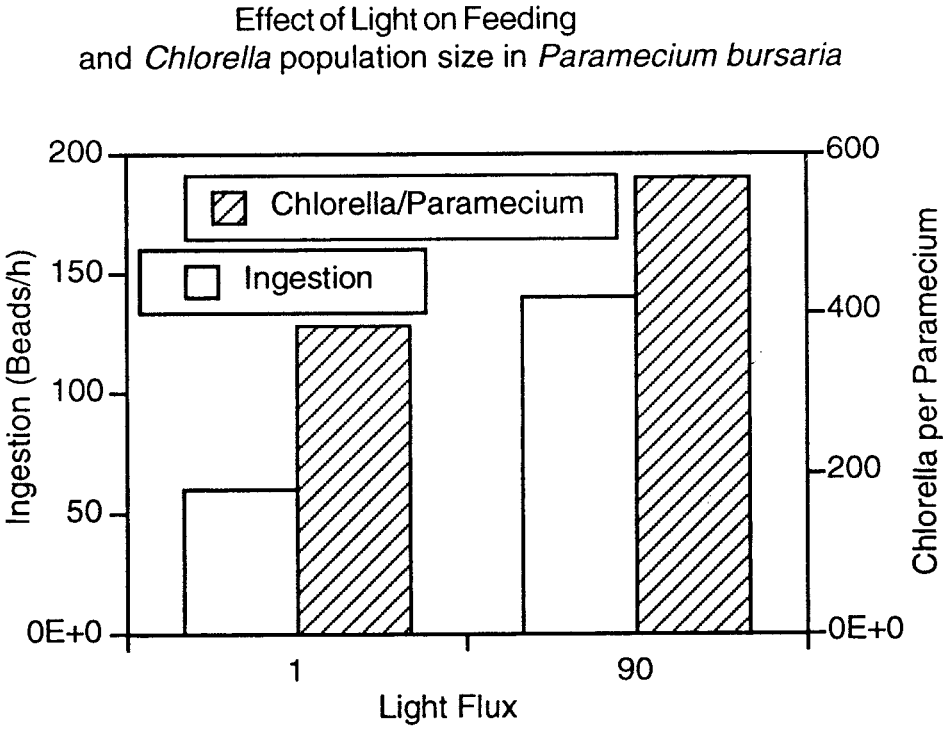

Fig. 2. Changes in numbers of *Chlorella* per *Paramecium bursaria* and ingestion rate with light flux (μE m^2 s^{-1}). Data from Berk *et al.* (1991), figures 1 and 3.

The only data available on the effect of light on feeding in plastidic oligotrichs is based on Stoecker *et al.*'s study (1988) of *Laboea strobila.* No significant difference in feeding in light or darkness over a 14 h period was found. However, it is not known if plastidic oligotrichs habituated to different light fluxes harbor different chloroplast "loads" and perhaps show correspondingly different feeding rates.

There is some reason to suspect that plastidic oligotrichs differ in their feeding behavior relative to strictly heterotrophic oligotrichs and tintinnids. In a study of aggregation behavior in response to phytoplankton prey, Verity (1991) found that plastidic oligotrichs showed a consistent response to dinoflagellate prey; in contrast, aplastidic oligotrichs and tintinnids were most consistently attracted to prymnesiophyte prey. Given that plastidic oligotrichs harbor chloroplasts from a variety of phytoplankters (*op. cit.*), and that thus far they have been cultured on non-dinoflagellate prey (Stoecker *et al.*, 1988, 1988/1989; Stoecker and Silver, 1990), it is unclear if aggregation toward dinoflagellates is a simple response to a preferred food category. The responses reported may be, like *Didinium* attracted to bacterial exudates (Antipa *et al.*, 1983), an indirect mechanism of finding prey items.

Habitats

It is an appealing notion that mixotrophy, either *Chlorella* symbiosis or chloroplast retention, is an adaptation to food-poor environments. Mixotrophic ciliates can dominate the ciliate community in oligotrophic systems, either as a chloroplast-retaining oligotrich in an Antarctic lake (Laybourn-Parry *et al.*, 1991) or a chlorellic *Stentor* in an acidic subtropical lake (Bienert *et al.*, 1991). However, mixotrophic ciliates are common in eutrophic waters as well. The same plastid-retaining oligotrich, *Strombidium viride*, which dominates the ciliate community (80 % of cell numbers) in Crooked Lake, Antarctica (Laybourn-Parry *et al.*, 1991) also forms a consistent component (avg. 13 % of cell numbers) of the ciliate community in eutrophic Lake Olgelthorpe (Pace, 1982). A diverse community of *Chlorella*-containing ciliates was found in Priest Pot, a eutrophic pond (Finlay *et al.*, 1988). Thus, it is not surprising that mixotrophic ciliates (chlorellic *Stentor* or plastidic *S. viride*) make poor indicator species relative to bacteriovorous or algivorous ciliates (Beaver and Crisman, 1989a).

At present it is not clear which factors regulate the relative contribution of mixotrophic forms to the ciliate community. There was no trend in the contribution of mixotrophic ciliates to total ciliates in a set of colored Florida lakes of different trophic states; for example, in two lakes with chlorophyll concentrations of 3.6 and 43 μg chlorophyll *a* l^{-1}, mixotrophs represented 54 and 58 % of the total ciliate biomass, respectively (Beaver *et al.*, 1989). Similarly, plastidic ciliates are a major component of the ciliate community in a variety of marine waters, ranging from 22 to 65 % of the ciliate fauna (Stoecker *et al.*, 1989).

Mixotrophy in ciliates, especially with regard to the *Chlorella* symbiosis, may be an adaptation for opportunism as well as a specialization allowing exploitation of food-poor environments (Beaver and Crisman, 1989b). Evidence for this view can be found

in a study of chlorellic ciliates from a stratified eutrophic pond (Berninger *et al.*, 1986) which revealed distinct differences between species. Some ciliates apparently used endosymbionts to allow the exploitation of low oxygen waters and were found to contain large amounts of ingested food while other species appeared to substantially rely on their endosymbionts, based on a lack of apparent ingested food. This finding is not unexpected, given the plasticity of the ciliate-*Chlorella* relationship. *Chlorella* symbiosis may provide benefits unrelated to the photosynthetic activity of the symbiont. *Paramecium bursaria* without *Chlorella* are apparently ingested much more readily than chlorellic paramecia by *Didinium nasutum* (Berger, 1980).

The factors regulating the relative and absolute abundances of chloroplast-retaining oligotrichs may be consistent, given the consistency of the chloroplast-ciliate relationship, but at present are unknown. Regulatory factors will perhaps become clearer with knowledge of the "costs" involved with chloroplast retention. The oligotrichs may be limited by the abundance of chloroplast source taxa. However, a variety of phytoplankters are apparently exploited. Chloroplast retention could translate into a higher metabolic cost in processing food items in mixotrophs relative to heterotrophs, leading to a lower maximum growth rates. Alternatively, plastid retention may not impose a metabolic disadvantage but could indirectly yield higher mortality rates though increased predation pressure as mixotrophs are largely restricted to surface layers.

Conclusion

The two types of ciliate mixotrophy are distinct but the techniques which have been used to investigate one type could be profitably applied to fill lacks in our knowledge of the other type. For example, modeled on *Paramecium* studies, investigations of digestion in oligotrichs followed by short-term pulse-chase experiments may elucidate the mechanisms involved in chloroplast retention. Similar to work on oligotrichs, carbon uptake studies which account for internal carbon cycling are needed to make estimates of primary production for *Chlorella*-containing ciliates.

For both mixotrophic strategies significant questions remain. What factors govern the relative abundances of mixotrophs? Or turning the question around, why are not all ciliates mixotrophs? What are the differences in growth or mortality rates between mixotrophs and heterotrophs? Do mixotrophs compete with autotrophs for nutrients? Hopefully, some of these questions will be addressed in future studies.

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