

Phagotrophy by a flagellate selects for colonial prey: A possible origin of multicellularity

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Summary

Predation was a powerful selective force promoting increased morphological complexity in a unicellular prey held in constant environmental conditions. The green alga, *Chlorella vulgaris*, is a well-studied eukaryote, which has retained its normal unicellular form in cultures in our laboratories for thousands of generations. For the experiments reported here, steady-state unicellular *C. vulgaris* continuous cultures were inoculated with the predator *Ochromonas vallescia*, a phagotrophic flagellated protist ('flagellate'). Within less than 100 generations of the prey, a multicellular *Chlorella* growth form became dominant in the culture (subsequently repeated in other cultures). The prey *Chlorella* first formed globose clusters of tens to hundreds of cells. After about 10–20 generations in the presence of the phagotroph, eight-celled colonies predominated. These colonies retained the eight-celled form indefinitely in continuous culture and when plated onto agar. These self-replicating, stable colonies were virtually immune to predation by the flagellate, but small enough that each *Chlorella* cell was exposed directly to the nutrient medium.

Keywords: algae; anti-predator adaptations; arms race; *Chlorella vulgaris*; chrysophytes; continuous culture; evolution; flagellates; multicellularity; *Ochromonas vallescia*; phagocyte; predator–prey interactions; selective pressure

Introduction

Predation may have played a pivotal role in promoting increased diversity in primordial communities. Stanley (1973) used ecological theory to address an important enigma in palaeontology: the explosive diversification of organisms at the boundary of the Cambrian, around 2×10^9 years after life originated. On the one hand, the long duration of the exclusively prokaryotic period implies that the process triggering the diversification was a unique event with an extremely low probability. On the other hand, even the scanty fossil record clearly demonstrates a rapid proliferation of new, multicellular organic forms. Stanley (1973) suggested an ecological mechanism: the origin of phagotrophy (ingestion of whole prey). He reasoned that single-celled organisms of the Precambrian were resource-limited, with a few species saturating the relatively homogeneous and limited nutrient pool available. Before phagotrophy, prevalent selection pressures among Precambrian phototrophs were for efficient nutrient competition (e.g. a high surface-to-volume ratio provided by small cell size). However, with the advent of phagotrophy, resistance from this new mortality factor became a novel selective pressure for the phototrophs, possibly selecting for multicellularity. Both herbivorous and carnivorous protists arose virtually simultaneously, which may have triggered 'self-propagating feedback systems of diversification' and the rapid proliferation of multicellular forms (Stanley, 1973). On the basis of ecological studies showing an association between 'cropping' and more diverse communities, Stanley (1973) argued that the 'sudden

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proliferation of complex food webs formed by taxa invading previously vacant adaptive zones produced an explosive diversification of life over a period of a few tens of millions of years'.

Analyses of adaptive trends in living prey populations or patterns of diversity in the fossil record are critical in proposing mechanisms for trends in early evolution (Hanson, 1977). Such analyses support an hypothesis of predation as an important factor in escalating rates of evolution (Vermeij, 1987) and in leading to multicellularity (Bonner, 1988). In addition, 'arms-race' hypotheses argue that an adaptive change by one species (usually the prey) in an interaction can select for development of a counter-adaptation by another species (usually the predator), leading to continuous evolution within the interacting species (Van Valen, 1973; Dawkins and Krebs, 1979). Data from palaeontological studies necessarily are correlative, so experiments also are needed to establish the relative selective power of predation as an evolutionary mechanism for prey morphology shifts.

In this paper, we document an experimental system where predation by a phagotrophic protist (*Ochromonas vallescia*) resulted in the rapid proliferation of a predator-resistant multicellular prey form within a population of algae (*Chlorella vulgaris*).

Materials and methods

Culture system and organisms

Organisms were maintained in continuous culture using the same chemostat systems we have used routinely in our laboratories to maintain axenic cultures of unicellular *Chlorella* since 1974 (Boraas, 1979, 1983). A single custom-blown glass culture tube (volume constant at 500 ml) was held under constant conditions of light (*c.* 100 μ Einsteins) and temperature ($25 \pm 0.5^\circ\text{C}$). The nutrient medium, which was held in a large reservoir, was deficient in nitrogen (0.5 mM nitrate), with all other essential elements in excess. Inflow from the medium reservoir into the cultures was held constant, at a flow rate of 0.035 ml h⁻¹, with a Harvard peristaltic pump.

Initially, the culture tube was filled with medium and inoculated with *Chlorella vulgaris* Beij obtained from the University of Texas Culture Collection (UTEX #26). A steady state was established for the alga, which was then available as food to the flagellate predator. In the past two decades, except for rare anomalies (loose clusters of algae seen perhaps two or three times per year), this *Chlorella* culture has always exhibited its normal unicellular morphology in our routine microscopic screens of our continuous cultures.

We have identified the protist used in these experiments as *Ochromonas vallescia* (Boraas *et al.*, 1988). The *O. vallescia* was isolated from a rotifer-*Chlorella* culture in the laboratory and, presumably, originated from the Milwaukee Harbour (the Center for Great Lakes Studies is situated on the harbour). In culture, this almost spherical *Ochromonas* had a diameter of 8–15 μm and a minimum doubling time of 6 h.

As an obligate phagotroph (Boraas *et al.*, 1988), this *Ochromonas* requires a constant supply of food. For about 7 years, we have maintained this protist in the second stage of a two-stage chemostat culture, receiving *Chlorella* as food from the first stage (Boraas *et al.*, 1988, 1992; Holen and Boraas, 1995). In the studies reported here, the flagellate was inoculated directly into the steady-state algal culture, in the first stage. This established a predator-prey culture in which the *Chlorella* were growing on inorganic nutrients and the *Ochromonas* were feeding on the *Chlorella*.

Sampling and observation

Samples were removed by sterilized syringe through a sterilized silicone rubber port in the culture vessel tube (see Boraas, 1983). Numbers, size distributions, and biovolumes of populations of both the alga and the protist were measured in each sample with a Particle Data Celloscope. Each sample was suspended in 0.4% 0.2- μm filtered saline upon collection and processed immediately

using a 120- μm aperture at a single setting of aperture current and amplifier gain. We have found that electronic particle counting is a precise, rapid method for obtaining counts, size distributions and biomass estimates for planktonic organisms (Seale, 1980, 1982; Boraas, 1983). Since these size distributions contained both algae and protists, visual verification of the size distributions in the cultures were obtained with a light microscope.

Samples for SEM microscopic examination were preserved in 3% gluteraldehyde at room temperature. *Chlorella* samples from these vials were prepared for SEM analysis by being washed with distilled water, filtered onto a Nuclepore filter, then critical-point dried in CO_2 and sputter-coated with gold. Fresh flagellate SEM samples were fixed in 2% gluteraldehyde in 0.1 M cacodylate buffer, washed twice in distilled water with centrifugation, and then treated as for the *Chlorella*. Samples for TEM were fixed in 4% gluteraldehyde for 1 h, washed in Milliong's phosphate buffer (pH 7.2) and post-fixed in 2% osmium tetroxide for 1 h, washed twice in buffer and embedded in 1.5% agar at 45°C, dehydrated in acetone and embedded in Spur's epoxy. Sections were stained in lead and uranyl acetate immediately after sectioning. They were viewed with a Hitachi Hu 11B-2 transmission electron microscope.

Interactions between the flagellate predator and algal prey were observed with a video microscopy system (Boraas *et al.*, 1992). The microscope was a Zeiss Standard equipped with a prism trinocular and a C-mount adapter attached to a Dage-MTI model NC-65-SA video camera with a Newvicon imaging tube. Brightfield illumination was used. The output of the video scanner was fed to a Sony model VO-1600 $\frac{3}{4}$ inch (19 mm) video cassette recorder (VCR), recording at a rate of 30 frames per second.

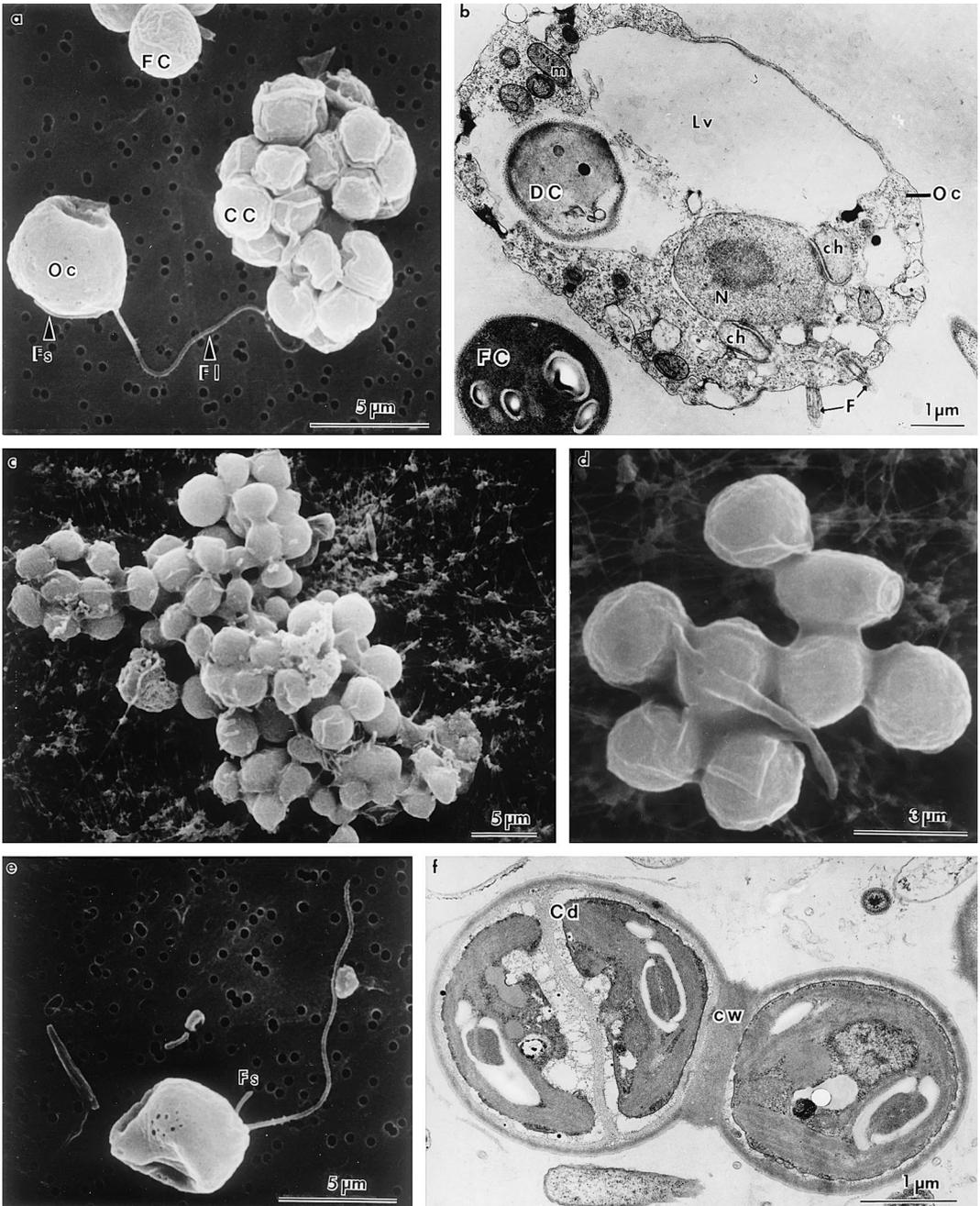
Results

Morphology and ecology of the predator

We did not detect any obvious changes in either the morphology or the feeding ecology of the predator during this study. The *Ochromonas* used in this study has two flagella (Fig. 1a,e) and a chloroplast (Fig. 1b-d), typical of the Class Chrysophyceae, genus *Ochromonas* (Boraas *et al.*, 1988). Chrysophytes, although algae, are often mixotrophic, capable of both photosynthesis and phagotrophy (Holen and Boraas, 1995). Although *Ochromonas* spp. are usually phototrophic, *O. vallescia*, despite the chloroplast, is incapable of photosynthesis (Boraas *et al.*, 1988). The long, hispid flagellum is used primarily for locomotion and food-cell capture, whereas the short, naked flagellum is used to manipulate the captured food cell during ingestion, based on our video microscopy studies (Boraas *et al.*, 1992). The flagellates used in this study retained these characteristics.

Sequence of development of multicellular Chlorella

The *Chlorella* seen in the culture before predation was the typical unicell (5–6 μm diameter), with numerous empty mother cells interspersed (see Discussion). As expected from previous studies (Boraas, 1980), in the predator-prey culture, this *Chlorella* population declined immediately from an initial density of 2×10^6 cells ml^{-1} , and the predator population increased to a maximum density. The combined suspended particle size distribution throughout this initial growth phase (0 days in Fig. 2a) showed two distinct peaks: *Chlorella* unicells with a mean diameter of about 6 μm and *O. vallescia* with an equivalent spherical diameter of 12 μm (Fig. 2a). After their consumption had reduced the *Chlorella*, the flagellate population also declined, due to starvation and washout of cells from the culture (5 days, Fig. 2a). This reduction in predation pressure allowed the *Chlorella* population to recover and increase rapidly, in the manner of a classical predator-prey oscillation. During the recovery of the algal population, an unexpected result was observed: the prey *Chlorella*



now included colonial growth forms as well as unicells (10 days, Fig. 2a). The number of cells per colony ranged from four to hundreds (Fig. 1c), bracketing and masking the flagellate size distribution (Fig. 2a). During a second cycle of flagellate growth and *Chlorella* decline (16 days, Fig. 2a), the *Chlorella* colonies persisted while the *Chlorella* unicells declined to < 1% of the total cells in the culture.

After 20 days of mixed-species culture, the *Chlorella* population had many colonies with more than 20 cells (Figs 1c, 2b). The number of cells per colony then gradually declined to a mode of eight and the system entered a steady state (Fig. 2a,b). Flagellates and *Chlorella* unicells in this steady state had population densities reduced to about 0.1% of their maximum numbers during the transient phases. The bulk of the biomass in this steady state was in eight-celled *Chlorella* colonies (Fig. 1d), with a mean colony diameter of approximately 17 μm . The cells in these stable colonies of predated cultures were enclosed within an envelope (Fig. 1d), apparently the mother cell wall of the neonatal cells (see Discussion). Empty mother cell walls were virtually absent from the culture.

The *Chlorella* colonies were stable and self-replicating. Colonies had variable morphologies, even during the steady-state phase (Fig. 1a,d), but the majority were roughly spherical, usually with eight (but occasionally four) cells per colony. The colonies were photosynthetically active, and reproduced indefinitely as colonies in the light in either chemostat culture or on agar. When placed into the darkened second stage of a two-stage chemostat, the *Chlorella* colonies rapidly washed out of the culture, leaving only the unicellular *Chlorella* supplied from the primary chemostat.

Colony growth was a 'budding' process, based on visual observations. Individual cells of the colony grew in size while dividing into daughter cells; the new colony then separated from the original colony by tearing or breaking the original mother cell wall. We have replicated this experiment many times, and have observed the formation of *Chlorella* multicells in about 70% of the replicates.

Observations of predation events

In our video microscopy observations of predation events between *Ochromonas* and *Chlorella*, unicells and 'neonatal' colonies were eaten. Although the typical feeding behaviours were expressed on unicells and small colonies (Boraas *et al.*, 1992), the *O. vallescia* failed to ingest fully grown colonies larger than about 15 μm . The smaller of the young or 'neonatal' colonies (10–12 μm),

Figure 1. (a) The predatory flagellate (Oc), with long (Fl) and short (Fs) flagella, and a *Chlorella* colony (CC) sampled together from a culture 240 days after inoculation. There was a 25–50% shrinkage of the flagellate diameter during SEM preparation. The colony apparently is breaking apart due to growth of component eight-celled colonies. Note the 'ropy' appearance of the enclosing membrane. A single *Chlorella* cell (FC) is shown for comparison. (b) Thin section of *Ochromonas* (Oc) with an ingested and partially digested *Chlorella* cell (DC) and a free, undigested *Chlorella* cell (FC). The nucleus (N), chloroplasts (ch), flagella (F), a mitochondrion (m) and a large vacuole (Lv) are shown. (c) A large, multicellular *Chlorella* colony subjected to 8 days of grazing by *Ochromonas*. All cells in the colony appear to adhere by membranes. (d) An eight-celled *Chlorella* colony taken from a culture 12 days after inoculation of the flagellate with an enclosing membrane, apparently the old mother cell wall, which envelopes all the cells in the colony. (e) The predatory flagellate showing one short, naked flagellum (Fs) and one long, hispid flagellum (F1) with an apparent 'oral pore' about 90° from the flagellar base and an 'anal pore' directly opposite the two flagella. (f) Thin section of colonial *Chlorella* from an 18-month-old mixed-species *Chlorella*–*Ochromonas* culture. Two cells, one dividing (Cd), are joined by a thick section of cell wall (CW). There is a clear demarcation between old and new cell wall in the dividing cell, the old wall being contiguous with the material joining the two cells.

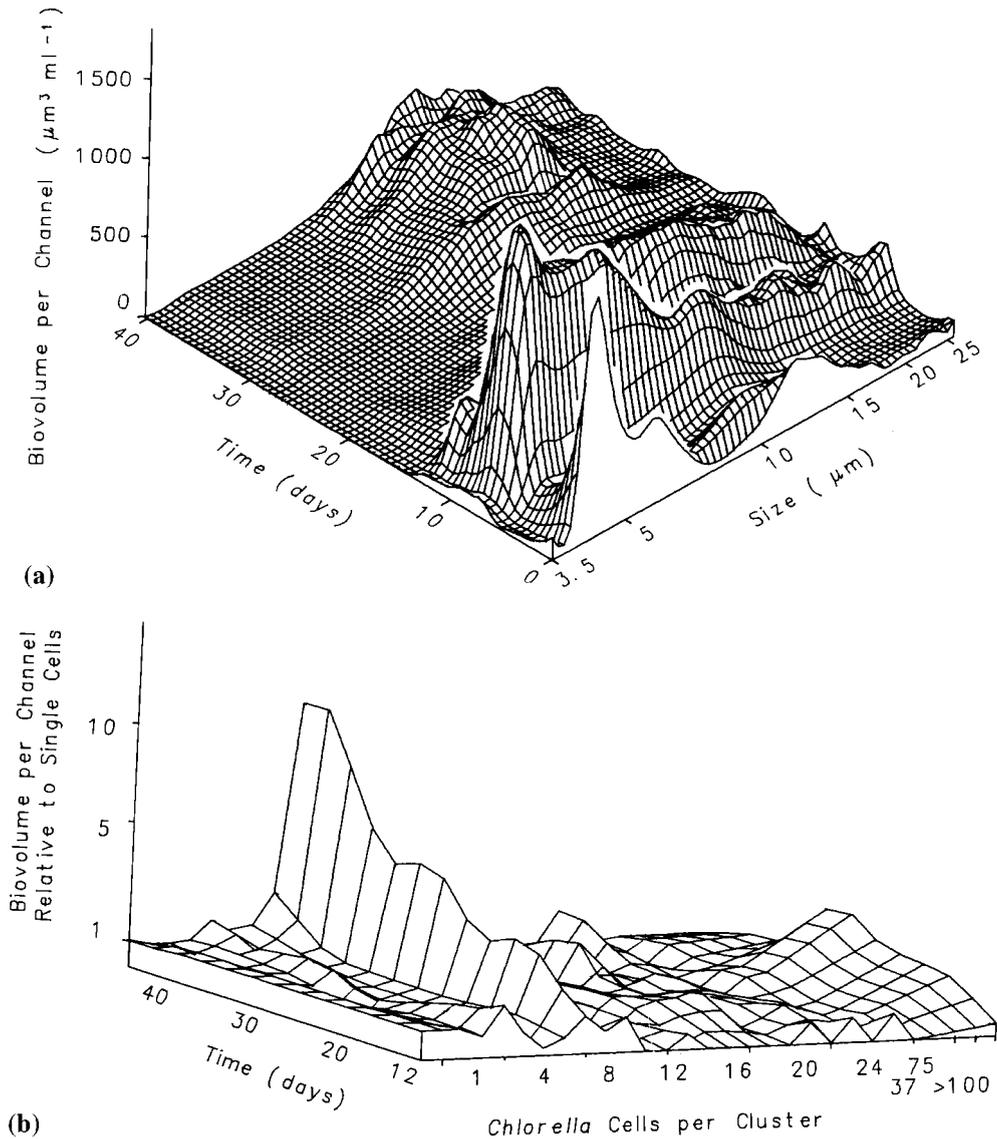


Figure 2. (a) Time course of size distributions of a mixed-species continuous culture of *Chlorella* and *Ochromonas*. The flagellate population is presented by the region between 10–15 μm and the *Chlorella* population is represented by the bimodal distribution between 3.5 and 7.5 μm . Flagellates persisted throughout the experiment but, after about 5 days, the flagellate size distributions were obscured by *Chlorella* colonies. (b) Mean *Chlorella* colony size vs time. The numbers of cells per colony were counted microscopically from glutaraldehyde-preserved samples. The majority of *Chlorella* cells were in colonies with more than 24 cells per colony for the first month of culture. The large colonies then disappeared from the culture and the number of cells per colony stabilized at eight.

about the size of the eight-celled 'buds' in Fig. 1a, could be ingested. Flagellates with food vacuoles containing a single 'neonatal' colony were seen on occasion, but this vulnerable size class was rare in the cultures. The 'young' colonies rapidly grew to full size by increasing individual cell volumes.

Discussion

Our experiments demonstrate the power of predation as a single selective force in promoting increased morphological complexity, within a short time frame. In a system where environmental conditions were held constant, a multicellular organic form evolved from a unicellular one within 10–20 generations. Based on our data and observations, the multicellular *Chlorella*, a rare genetic mutant in unicellular culture, was selected over unicells by *Ochromonas* predation. In the presence of phagotrophs, there was a clear selection mechanism for colony formation: unicells and colonies with small, young cells could be eaten, whereas colonies with larger cells could not. Since *Chlorella* is obligately asexual, sexual recombination does not cloud the issue.

Mechanisms for formation of multicellular Chlorella and regulation of colony size

During normal unicellular cell division and growth, *Chlorella* divide into 2–16 daughter cells, followed by a split of the mother cell wall and subsequent dispersal of the neonatal cells; empty mother cell walls are interspersed with whole cells at a ratio of about 1:4. That is, about 75% of *Chlorella* divisions are into two daughter cells and 25% into four daughter cells (Fott and Novakova, 1969; Williams, 1971). We have repeatedly observed the same pattern in our *Chlorella* continuous cultures since 1974 (Boraas, 1979).

In continuous cultures with the predator, the rapid appearance of very large multicellular *Chlorella* (Fig. 1c) apparently resulted from incomplete division: an initial loss of the mechanism for separating successive generations. The most probable initial mechanism for colony formation, adhesion of the daughter cells to the mother cell wall, is suggested by two observations: the membrane that surrounds the colonies (Fig. 1d) and the absence of cast-off mother cell walls in cultures dominated by colonial morphs (personal observation). Incomplete cell division has also been proposed as a mechanism for selection of elongated bacteria in the presence of protozoan predation (Shikano *et al.*, 1990; Gillott *et al.*, 1993).

Two potential mechanisms for reducing colony size were suggested from electron micrographs of multicellular *Chlorella*. First, the original mother cell wall became increasingly fragile in multicells. The enclosing membrane of colonies from cultures 20–100 days old had a ‘ropy’ appearance (Fig. 1a). Such ‘ropes’ could form if the mother cell wall split and curled on itself, unlike wild-type cell walls. As the daughter cells grew, the ‘ropy’ form may have broken more readily than the earlier ‘sheet’ form of the mother cell wall (Fig. 1d). Secondly, colonial cells shared cell walls (Fig. 1f: two cells, from a culture 540 days old). One of the cells (Fig. 1f) was dividing, implying division may not have been synchronous within colonies. As the cells grew and their radii increased, the cell walls would slowly tear apart, with separation occurring at some critical radius.

Alternative hypotheses

Our results could be interpreted as evidence that the flagellates released a substance inducing colony formation in *Chlorella*, similar to predator-induced morphological changes in zooplankton (Dodson, 1989) and in coccoid green algae (Hesssen and van Donk, 1993). We discount this alternative hypothesis, based on four observations. First, colonies did not become apparent for about 20 *Chlorella* generations after inoculation of the flagellates. An ‘induction’ should have been expressed as soon as the inducing substance produced by the flagellates had reached some critical concentration. Secondly, once it has appeared, the colonial growth form has been maintained in mixed-species cultures for over 2 years, even at low flagellate densities. Induction should vary with flagellate density. Thirdly, when the colonies are cultured in the absence of any source of an inducing substance, the colonies ‘breed true’. The colonial *Chlorella* morph remains colonial both on agar and in monospecific liquid culture, including chemostats where steady states have been

maintained for several months. Finally, in nitrogen-limited, two-stage chemostats where both stages are illuminated, with unispecific *Chlorella* in the first stage and mixed *Chlorella*–*Ochromonas* in the second stage, we see colonial *Chlorella*. These *Chlorella* must be growing on the inorganic nitrogen excreted by *Ochromonas*. When the second stage is darkened, the *Chlorella* colonies rapidly wash out of the culture, leaving only the unicellular *Chlorella* supplied from the first stage and, of course, their flagellate predators. This shows that *active photosynthesis* by the algae and *continued interaction with the predator* are essential to maintain the colonial algae in continuous culture. When cultured in the absence of the predator, the cell size of the unicells in the second stage declines, showing that cell division and associated morphogenetic processes are taking place, but the colonies are not formed in the absence of the predator.

Significance of this experimental study to evolutionary hypotheses

Our data demonstrate that predation, as a single selective factor, can select for multicellularity in a prey organism. The *Chlorella* had maintained the normal unicellular body shape for thousands of generations in the same laboratory culture conditions. After about 10–20 generations in the presence of the phagotroph, an eight-celled *Chlorella* ‘colony’ became the dominant phototroph in all replicates of this experiment. The data support Stanley’s (1973) hypothesis for the origin of multicellular organisms: the emergence of phagotrophic unicells in the Precambrian could have rapidly selected for colonial prey, eventually resulting in the Metaphyta and Metazoa.

After a long Precambrian history of slow, unicellular evolution, multicellular life forms abruptly appeared in the late Precambrian. Stanley (1973) proposed that the ‘invention’ of unicellular predation was the cause of the origin of multicellular phototrophs or chemotrophs in the late Precambrian. The appearance of phagotrophs or predators probably first increased potential species diversity by recycling resources (Tilman, 1982) such as space (Paine, 1966) or chemical elements (Seale, 1980). Then prey with increased body size would have a selective advantage, since such organisms could escape predation. The combination of the two processes could have contributed to the observed increase in species and structural diversity (Stanley, 1973).

Stanley’s (1973) argument hinges on the observation that small-celled organisms usually have a competitive advantage for limiting mineral nutrients. In experiments where the unicells and colonies were placed in competition in the absence of the phagotroph in the light, the multicellular form was slowly displaced by unicells (data not shown). Hence, without the predator, unicells are superior competitors in our continuous culture system.

There are many examples in the literature describing adaptive trade-offs in related groups or species (Townsend and Calow, 1981; Stephens and Krebs, 1986; Bennett and Boraas, 1989; Bennett *et al.*, 1993). The eight-celled, stable colony in our system probably expresses an adaptive ‘trade-off’ between the two main selective pressures in our experimental system: (1) predation selected for more cells per colony, minimizing death by phagotrophy, and (2) competition for nutrients selected for fewer cells per colony, maximizing nutrient uptake per cell. In the absence of predation, *Chlorella* maintain a unicellular state, despite occasional mutational ‘mistakes’. With predation, these ‘mistakes’ allow survival in the face of almost certain death. The single cells in the new ‘colony’ may be at a disadvantage in a straightforward ‘scramble’ for inorganic nutrients. However, small differences in the ability to take up nutrients would be more than offset by the large selective advantage in the ability to avoid immediate death from predation. One way to avoid such mortality is to be too large to be eaten, and hence live to reproduce rather than serve as a food source for the new predator. As long as no larger, more voracious predators enter the system (through evolution or immigration), the multicellular form can proliferate, even if at a probable competitive disadvantage.

The 'invention' of predation in the Precambrian itself may have led to the evolution of eukaryotic cells from prokaryotes by a process of ingestion and symbiosis (Margulis, 1981). This hypothesis is supported by extensive evaluation of contemporary genomes and other characteristics of plastids (Gray and Doolittle, 1982). Apparently, the development of a cell nucleus was a necessary prerequisite for the first predators. There are no known true phagotrophic prokaryotes, either extant or in the fossil record (Margulis, 1981). Consequently, prokaryote communities without phagotrophic nutrition (but possibly with osmotrophic parasitism) would be structured by competitive interactions, particularly differential uptake of soluble nutrients. As discussed above, osmotrophic organisms with a large surface-to-volume ratio (i.e. small cell size) can often grow more rapidly than larger competitors. The argument can be extended to communities without phagotrophs with eukaryotes and symbionts. Thus, in the absence of phagotrophy, natural selection should favour small, free-living cells, which have the greatest access to nutrients and the most efficient mechanisms for their uptake and conversion. The appearance of phagotrophy would reverse this advantage, favouring larger prey.

An 'arms race' between predator and prey?

Can an adaptive change by one species in an interaction select for the development of a counter-adaptation by the other species? This 'arms-race hypothesis' has been proposed as a mechanism for continuous evolution within the interacting species (Van Valen, 1973; Dawkins and Krebs, 1979). Testing arms-race hypotheses is difficult. Some existing fossil data sets have been analysed for changes over time for a specific morphological feature such as brain size (Jerison, 1970, 1973) or leg morphology (Bakker, 1983). Obviously, this approach can miss adaptational shifts in unmeasured morphological or physiological characteristics. In addition, the coarse temporal resolution of the fossil record has not allowed us to determine whether an improvement in one species followed a change in another or occurred simultaneously (Abrams, 1986). Thus, we cannot distinguish between a change being a response to interactions among species or to some other selective factor affecting all the species involved. The validity of Jerison's conclusions have been questioned due to challenges to the evidence and challenges to the underlying assumptions (Gould, 1975; Radinsky, 1978).

It can be difficult to cut through the ambiguity surrounding determination of causal factors in a field setting. Some field data hint at arms races (Brodie and Brodie, 1990, 1991), but involve complicating factors such as learning (Hori, 1993) or phenotypic plasticity (Weis *et al.*, 1989) in another species. In the laboratory, Chao *et al.* (1977) appear to have obtained an arms race of short duration between *Escherichia coli* and T7 phage in continuous culture. Though these workers have interpreted their results as models for predator-prey systems, they probably have more in common with parasite-host systems (see definitions in Thompson, 1982). With the possible exception of these, there are no laboratory studies of arms races in predator-prey systems.

These experimental predation-induced morphology changes may provide a system for testing some hypotheses of arms races in predator-prey evolution. In this study, we observed that the prey evolved much more rapidly than the predator, rather than having similar evolutionary rates for both forms as implied by some theoretical studies (Schaffer and Rosenzweig, 1978). Perhaps the most surprising aspect of our study was the rapidity with which the colonies appeared in the presence of predation. This supports the notion that predation is a far more powerful evolutionary force than competition and, even, symbiosis. Considering the rapidity with which our simple cultures shifted from unicellular to multicellular dominance, the forces of selection and counter-selection would be more than adequate for accelerating the rate of morphological differentiation in the prey.

We did not see any obvious changes in the predatory protozoan with respect to morphology or feeding behaviour. As seen with our *Ochromonas*, some modern planktonic predators may have become 'fixed' with respect to size preferences. *Ochromonas* has some capacity for active selection, at least for size (Boraas *et al.*, 1988, 1992; Seale *et al.*, 1990). As summarized in Boraas *et al.* (1992), it readily ingests plastic beads $>3 \mu\text{m}$, but usually rejects smaller ones. Most prey 4–15 μm in size are readily ingested, but bacteria 0.5–3 μm in their longest dimension are usually rejected and cannot serve as the sole food source. Hence, the unicellular *Chlorella* are within the preferred size range and mature *Chlorella* colonies are just outside the range ingested by *O. vallescia*. We did not detect any obvious shift in size of prey captured by these *Ochromonas*. It is likely that we would have not been able to detect shifts in prey morphology without a relentless, unshifting predation pressure on a particular prey size. Counter-shifts in the predator might well have eliminated the multicells before they could proliferate.

Flagellate predation in modern ecosystems

In today's aquatic environment, it is unlikely that flagellate predation would select for multicellular prey, because such organisms would be subject both to predation by larger predators and to competition with pre-existing multicellular species. Mean cell sizes of natural populations of bacteria in natural ecosystems can be quite small (i.e. 0.2–0.3 μm), which may be a consequence of flagellates' preference for larger bacteria (Holen and Boraas, 1991). These very small bacteria are captured by *O. vallescia* much less readily than bacteria in the size range 1–3 μm (Holen and Boraas, 1991; Boraas *et al.*, 1992). Therefore, while phagotrophy may have resulted in the origin of multicellularity, it may also be responsible for maintaining some of the smallest free-living organisms in today's marine and freshwater ecosystems.

Acknowledgements

We wish to thank C.C. Remsen, P. Anderson, P. Bizub and M. Schaller for help with the electron microscopy; L. Buchholz and S. Piacentine for laboratory assistance; and R. Wassersug, T. Powell, K. Nealon and M. Koehl for critical reviews of the manuscript. This work was supported by Sea Grant R1MW-32 and EPA grant R-810871-OL-O.

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