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Ecological strategies of protists and their symbiotic relationships with prokaryotic microbes

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Protistan species are found in almost every environment on our planet, and have adapted in many ways to survive and thrive under dramatically different conditions. Some of the most diverse adaptations involve symbiotic relationships with prokaryotes. Described symbioses primarily involve heterotrophic protists, including ciliates, Rhizaria (amoebae, foraminifera, radiolaria) and flagellate taxa. Recently there has been an increase in reports of environmental isolates that represent novel associations, which suggest that the symbioses are probably more widespread than conventionally thought. Future work will need to explore the function, abundance and distribution of what have been considered rare or unusual interactions.

Symbiotic interactions between protists and prokaryotes

Symbioses are close, and relatively constant, associations between two or more different organisms. Examples are found in almost every taxon, and protists (single celled eukaryotic microorganisms) display several different types of symbiotic interactions. Both endosymbiotic and ectosymbiotic relationships occur between protists and unicellular algae, bacteria and archaea. The associations can be permanent or temporary, and cover a range of interdependencies from obligate to transient. These associations endow the protistan host with the ability to succeed or thrive in particular environments. One of the ways in which symbioses aid protists is to ensure the presence of food for the protist, either by cultivation of the symbiont as an actual food source, or by using products derived from the symbiont for nutrition. Another advantage that might be conferred on protists is a mechanism of defense against attack or competition. Finally, symbionts can help either to modify the environment directly around the protistan host, or to modify the host so that it can adapt to an otherwise hostile environment. Most commonly, environmental adaptation via symbiosis is observed in inhospitable conditions, such as anoxic sediments, but this does not have to be the case. It is likely that symbiotic associations with prokaryotes are widespread, but because the environmental challenges are not as dramatic in non-extreme environments, the interactions might go unobserved.

The different categories of symbiont contribution to the host listed above are certainly not definitive. In some cases, assignment to a category will be ambiguous because the function is largely unknown, there might be more than one symbiont present, or the categories might overlap in some associations (e.g. nutritive symbiosis as an adaptive trait for an extreme environment). These categories were presented by Rosati [1], and we have continued to use them in an effort to organize the process of summarizing these diverse associations (Table 1).

Symbionts as nutrition

Ciliates, amoebae and heterotrophic (and some phototrophic) flagellates all ingest prey, most commonly prokaryotes (bacteria, archaea), algae, or other protists. Phagotrophic protists might eat several different types of prey, but they tend to exhibit selectivity for particular food items [2]. They also often require a particular threshold level of prey abundance to feed [3]. The lack of preferred prey, or a low prey density, can limit the ability of a protistan population to thrive in an environment. An effective method for heterotrophic protists to ensure adequate nutrition is to cultivate their own food source. Nutrition-supplying prokaryotic symbionts of protists can occur as either ectobionts or endobionts.

Glossary

Bathyal: the region of the ocean bottom between the depths of approximately 200 to 2,000 meters.

Chemolithoautotroph: an organism that obtains energy from inorganic compounds and carbon from CO₂.

Ectosymbiont, ectobiont or epibiont: a symbiont located on the surface of the host cell, or not within the host cell.

Endosymbiont or endobiont: a symbiont located inside of the host cell.

Heterotroph: an organism that obtains energy from ingestion of organic carbon.

Kleptochloroplast: a chloroplast that has been "stolen" from another cell. An alga has been partially digested by the host cell, leaving the chloroplast intact, but the association is not permanent.

Mixotroph: an organism that obtains energy from an inorganic source (light, chemical), but obtains carbon from organic matter. For protists, an example of a mixotroph is an alga that also ingests prey.

Oligotrophic: an environment with very low levels of nutrients.

Phototroph: an organism that obtains energy from light and carbon from CO₂.

R-bodies: cylindrical coils of insoluble protein found in *Caedibacter* and some other bacteria.

Silled basin: a depression in the seafloor with restricted circulation as a result of physiographic barrier (sill).

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Table 1. Summary of symbiosis examples

Protistan host	Ecto- or Endobiotic	Symbiont	Function
Nutrition			
<i>Kentrophoros</i> (ciliate)	Ectobiotic	Sulfur oxidizing bacteria	Ingestion of ectobionts [4]
<i>Zoothamnium niveum</i> (ciliate)	Ectobiotic	Sulfur oxidizing bacteria	Ingestion of ectobionts [4-6]
<i>Codonella</i> (tintinnid ciliate)	Ectobiotic	Cyanobacteria (<i>Synechococcus</i> and <i>Prochlorococcus</i> sp.)	Unknown [8,9]
<i>Ornithoceros</i> (dinoflagellate)	Ectobiotic	Cyanobacteria (<i>Prochlorococcus</i> sp.)	Possible nitrogen fixation [8,9]
<i>Citharistes</i> (dinoflagellate)	Ectobiotic	Cyanobacteria (<i>Synechococcus</i> and <i>Prochlorococcus</i> sp.)	Possible nitrogen fixation [8,9]
<i>Amphisolenia</i> (dinoflagellate)	Ectobiotic	Cyanobacteria (<i>Prochlorococcus</i> sp.)	Possible nitrogen fixation [8,9]
<i>Dinophysis</i> (dinoflagellate)	Ectobiotic	Cyanobacteria (<i>Prochlorococcus</i> sp.)	Possible nitrogen fixation [8,9]
<i>Histioneis</i> (dinoflagellate)	Ectobiotic	Cyanobacteria (<i>Prochlorococcus</i> sp.)	Nitrogen fixation [8,9]
<i>Euplotes magnicirratu</i> s (ciliate)	Endobiotic	<i>Devosia</i> sp.	Prey digestion [11]
<i>Euplotes aediculatus</i> (ciliate)	Endobiotic	<i>Polynucleobacter</i>	Processing of host glycogen [10]
Trypanosomatid flagellates	Endobiotic	Beta-proteobacteria	Complements defects in host metabolism [12]
<i>Rhopalodia gibba</i> (diatom)	Endobiotic	Cyanobacteria	Nitrogen fixation [13,14]
<i>Peridinium cinctum</i> (dinoflagellate)	Endobiotic	Alpha and gamma proteobacteria	Unknown [15]
Defense			
<i>Euplotidinium</i> (ciliate)	Ectobiotic	<i>Verrucomicrobia</i> (epixenosomes)	Prevent ingestion of host by predatory ciliates [18]
<i>Paramecium</i> (ciliate)	Endobiotic	<i>Caedibacter</i> sp.	Kill <i>Paramecium</i> spp. that lack symbiont [19,20]
<i>Spirostomum</i> (ciliate)	Endobiotic	<i>Caedibacter</i> sp.	Unknown, but might be similar to killer phenomenon in <i>Paramecium</i> [21]
Environmental adaptation			
<i>Folliculinopsis</i> (ciliate)	Ectobiotic	Cocoid and rod shaped bacteria	Unknown, but might serve as food [22]
Anoxic and microoxic ciliates	Ectobiotic	Sulfur reducing bacteria, but largely unknown	Unknown [4,23]
Anoxic and microoxic flagellates	Ectobiotic	Sulfur oxidizing bacteria	Potential for detoxification of local environment, food source [23]
Termite gut flagellates	Ectobiotic	Spirochete bacteria	Movement, production of acetate [25]
	Endobiotic	Bacteroidales	Hydrogen uptake [25]
		Methanogenic bacteria	Hydrogen uptake [25]
Benthic foraminifera	Ectobiotic	Bacteroidales	Nitrogenous nutrients [25,28]
		Candidate phylum Termite Group 1	Nitrogenous nutrients [25,29]
		Unknown	Potential for detoxification of local environment [30]
Allogromiid foraminifera	Endobiotic	May be sulfur oxidizing bacteria	Potential for reducing cellular levels of hydrogen sulfide [31]
<i>Virgulinema fragilis</i> (foraminifera)	Endobiotic	Sulfur oxidizing bacteria and diatom chloroplasts	Chloroplasts potentially used for nitrogen fixation under anoxic conditions [32,34]
<i>Fursenkonia rotundata</i> (foraminifera)	Endobiotic	<i>Synechococcus</i> sp.	Potentially used for nitrogen fixation under anoxic conditions [33]
<i>Trimyema compressum</i> (ciliate)	Endobiotic	Methanogen	Close association with hydrogenosome, might increase function of the organelle [35]
<i>Strombidium purpureum</i> (ciliate)	Endobiotic	Purple non-sulfur bacteria	Photosynthetic growth under anoxic conditions [37]

Ectosymbiotic prokaryotes have been most commonly described for ciliates, and the ciliate *Kentrophoros* is a well-known example (Figure 1) [4]. It grows in microaerophilic sediments of aquatic environments, at the chemocline between oxidized and reduced layers. Sulfur-oxidizing bacteria are present on the surface of the ciliate, and the flat shape of the ciliate and its chemosensory behavior are adaptive traits that facilitate the bacteria's growth. The colonial ciliate *Zoothamnium niveum* is another example of a protist using sulfur-oxidizing gamma-proteobacterial symbionts as food [4-6]. Again, the behavior of the ciliate helps to cultivate the bacteria by creating a local seawater environment with the optimal mixture of sulfide and oxygen [7].

The tintinnid ciliate *Codonella* and dinoflagellates such as *Ornithoceros*, *Amphisolenia*, *Dinophysis*, *Histioneis*

and *Citharistes* possess ectobiotic cyanobacterial symbionts [8,9]. The symbionts of *Codonella* are located in the oral groove within the lorica, and those of the dinoflagellates are located within the girdle region of the cell. *Citharistes* is unique in holding its symbionts within a chamber that has a small hole that is thought to impose size-based selectivity for the symbiont partner [8]. Some host cells harbor multiple morphotypes of symbionts [8], and although the majority of the cyanobacterial symbionts have been identified molecularly as strains of *Synechococcus* or *Prochlorococcus*, sequences related to *Cyanothece* and *Prochloron* were also present, as were several sequence types that exhibited < 92% similarity to other cyanobacterial sequences in the database [9]. The cyanobacterial symbionts of *Histioneis* were shown to possess nitrogen-fixing activity by using immunolocalization to

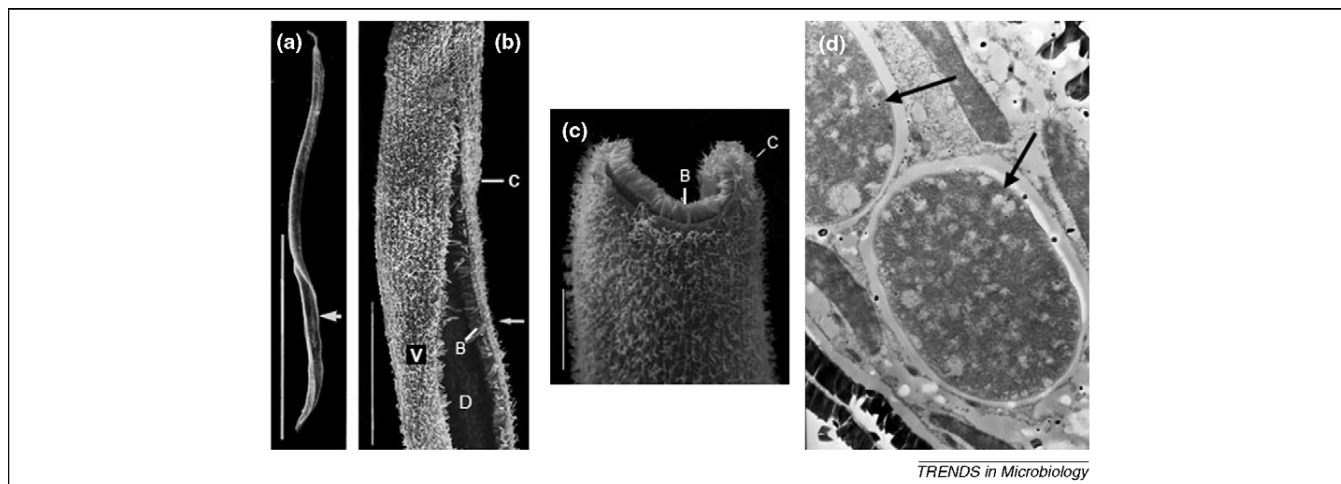


Figure 1. Nutritive symbioses: examples of ecto- and endo-symbioses. The scanning electron microscope (SEM) images in (a–c) show the ectobiotic bacteria on the ciliate *Kentrophoros*. (a) Whole ciliate (scale bar = 500 μm); (b) higher magnification image of the surface of the ciliate (scale bar = 50 μm). The body of the ciliate is flat in the environment, with the cilia on the ventral side (V) and the ectosymbionts on the dorsal side (D). Arrows point to approximately the same point on the ciliate where it is possible to observe the distribution of bacteria (B) and cilia (C). (c) Cross-section of the ciliate showing the distribution of the cilia (C) and bacteria (B). Scale bar = 20 μm . Images (a–c) are courtesy of Dr Wilhelm Foissner and were originally published in his paper [41]. (d) TEM image of the endosymbionts (spheroid bodies) of *Rhopalodia gibba* (arrows). The spheroid body is approximately 1–2 μm in width. Image courtesy of Dr Uwe Maier.

detect nitrogenase [8]. Many cyanobacterial symbioses are found in oligotrophic marine environments, and the symbionts probably fix nitrogen to help supplement nutrition obtained through heterotrophy. Further investigation is necessary to elucidate the functional relationships in most of these associations, using tools such as the transfer of labeled intermediates from autotrophic bacteria to the protists or transmission electron microscopy (TEM) to establish the state of ingestion and digestion of bacteria.

There are currently only a few reported endobiotic associations where the relationship is known to be nutrition-related. In ciliates, *Euplotes* species are found in association with bacteria that are necessary for either digestion of particular prey (*Devosia* sp.) or processing of glycogen (*Polynucleobacter*) [10,11]. In the trypanosomatid flagellates, bacterial endosymbionts appear to provide the host with essential nutrients (see references within [12]). The diatom *Rhopalodia gibba* (Figure 1) has highly integrated cyanobacterial endobionts [13]. The symbionts are referred to as spheroid bodies in the diatom, and appear to be well on their way to becoming a nitrogen-fixing organelle [14]. Bacterial endobionts have also been reported for the mixotrophic dinoflagellate *Peridinium cinctum* based upon studies using *in situ* hybridization [15]. The function of these endobionts, which were identified as members of the alpha and gamma proteobacteria, is unknown, and they might actually have been prey items. Distinguishing between ingested and symbiotic bacteria is a challenge, because many protists normally ingest bacteria.

Bacterial endobionts also are very common in environmental amoebae, for which a diverse assortment has been described [16,17]. Some are pathogens and most of the others are related to pathogenic bacterial lineages. The pathogens often induce damage to the host, whereas the others can co-exist with little apparent effect. The ability of amoebae to host such associations has led to their description as pathogen training grounds, because the pathways of destruction that the bacteria need to avoid within the protist are similar to those found in the human innate

immune system. Currently, it is unknown whether the host amoeba benefits from the association, but it is possible that they could acquire nutrients or other essential products from the bacteria, especially as some of these associations appear to be permanent.

Symbionts used for defense

A defensive role of bacterial symbionts has so far been reported only for ciliates, but that does not mean that these associations do not exist in some form for other protists. The ectobiotic extrusive *Verrucomicrobia* symbionts, termed epixenosomes, of the ciliate *Euplotidium* prevent ingestion of the protist by other predatory ciliates (Figure 2). This role is so important that *Euplotidium* has not been isolated from the environment without its epibionts, although the ciliate can be rendered symbiont-free in the laboratory without adverse effect [18]. Ciliate species of *Paramecium* that have endosymbiotic bacteria of the genus *Caedibacter* (containing R-bodies) exhibit a killing activity of ciliates that do not harbor the same symbiont [19]. The endosymbiont-containing cells grow more slowly, but they have the advantage of killing off their competitors [20]. Recently, *Caedibacter* endobionts were also discovered in another ciliate genera, *Spirostomum* [21], which suggests the association might be more widely distributed than previously believed.

Environmental modifications

Both nutritive and defensive symbioses involve interactions that improve the hosts' success in the environment, but other symbiotic relationships allow the host to inhabit particular environments from which it would otherwise be excluded. In some cases, it might be difficult to discriminate between these adaptations because the function in newly identified environmental associations often remains hypothetical. The detection of protist–bacterial symbioses in extreme or 'inhospitable' environments, such as anaerobic sediments or hydrothermal vents, has made considerable progress in recent years.

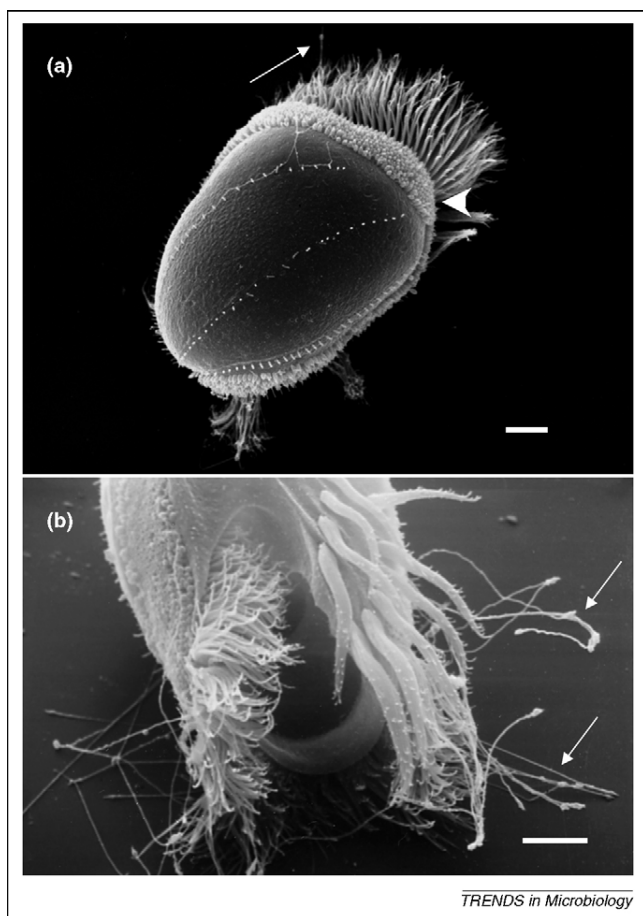


Figure 2. Defensive symbioses: the epixenosomes of the *Euplotidium* ciliate. (a) Dorsal view of *Euplotidium itoi* showing a band of epixenosomes (arrowhead) and an ejected epixenosome (arrow). (b) Partial ventral view of *Euplotidium itoi*. Ejected epixenosomes (arrows) modify its surface, thus interfering with the recognition by the predator. Scanning electron microscope (SEM) images in this figure courtesy of Dr Giovanna Rosati. Scale bars = 10 μm .

Numerous environmental isolates of ciliates and flagellates from bathyal silled basins, cold seeps and coastal sediments have been documented as having ectobiotic bacteria [4]. By contrast, to date there has been only one reported hydrothermal vent protist with symbionts. The colonial loricate ciliate, *Folliculinopsis*, has both ecto- and endo-symbiotic bacteria [22], and a chemolithoautotrophic nature of the bacteria has been suggested, but is unconfirmed. In deep-sea microoxic or anoxic sediments, the function of the ectobionts is assumed to be sulfate reduction or sulfur oxidation, either detoxifying the local environment or removing host waste products (hydrogen from the hydrogenosome). Sulfate reducing ectosymbionts can be present on ciliates that also have methanogen endosymbionts (discussed below), but tend to be more abundant on hosts without endobionts [1], potentially as a result of competition for hydrogen.

Euglenoid flagellates from Monterey Bay cold seeps and Santa Barbara Basin sediments harbor potentially chemoautotrophic bacteria (Figure 3a,b), most probably sulfur oxidizers, that could be involved in local detoxification, or potentially used as food [4]. Santa Barbara Basin has yielded a diverse collection of epibiotic arrangements [23], and although the hosts and symbionts are currently

unidentified, the overall abundance of associations suggests that they are essential for the persistence of some protists in that environment. The diversity of attachment arrangements suggests that many different bacterial species could be involved, but this remains to be determined as cells could undergo morphologic modification as they become active symbionts. This was shown by Embley *et al.* [24] to occur for intracellular methanogens of ciliates, and by Rinke *et al.* [6] for the ectobiotic gamma-proteobacteria of *Zoothamnium niveum*.

In addition to being found as free-living cells, anaerobic flagellates and ciliates are widespread in insect and animal guts. These protists often form associations with bacterial ecto- and endobionts. The most extensively studied flagellate ecto- and endobionts are those found in termite guts. Termite symbioses were recently reviewed by Ohkuma [25], so their presentation here will be extremely condensed. The different flagellates associate with several different bacterial types, including endobiotic methanogens, ectobiotic spirochetes, ecto- and endobiotic Bacteroidales, and endobiotic bacteria of the candidate phylum Termite Group 1 (TG1) [25]. Regarding function, only the movement associated with a few spirochete ectobionts is confirmed. Other hypothesized functions include the maintenance of extracellular cytoskeletal structure [26], support of the low oxygen environment for the anaerobic flagellate [27], production of acetate [25], and production of nitrogenous compounds for the protist [25]. Support for an endosymbiotic supply of nitrogen-related nutrients came from the complete genomes of the Bacteroidales and TG1 endobionts. The symbiotic Bacteroidales has the ability to fix nitrogen, and the TG1 bacterium is redundant in some of the genes for the production of amino acids and cofactors, suggesting the potential for these cells to produce nitrogenous material for export [25,28,29].

In contrast to ciliates and flagellates, amoebae are rarely found with epibiotic bacteria, but they have been described for benthic foraminifera [30]. The symbionts occur in the pores of the foraminiferan shell, and although their actual function is unknown, they might help in detoxification of the local environment. With regard to endobiotic associations, allogromiid foraminifera (Figure 3c) from low oxygen sediments appear to have made use of symbionts to expand their environmental range or to deal with changing microenvironments. The endobionts are thought to be sulfur oxidizers [31], and they could help to keep cellular hydrogen sulfide levels low in addition to providing chemoautotrophic carbon to the host.

Foraminifera also foster associations with plastids and cyanobacteria. The foraminiferan *Virgulinea fragilis* contains both rod-shaped tentative sulfur-oxidizing prokaryotic endobionts (along the cell cortex) and sequestered diatom kleptochloroplasts (towards the host cell interior) [32], whereas *Fursenkonia rotundata* from Santa Barbara Basin has *Synechococcus* symbionts [33]. It is unlikely that these plastid associations are maintained for photosynthesis, as the hosts are found at a depth of 600 m. Whereas the diatom plastid is not an example of a prokaryote–protist interaction, studies on it in the foraminiferan *Nonionella* have suggested the plastids were used to meet nitrogen requirements of the hosts [34] in anoxic environments that might experience

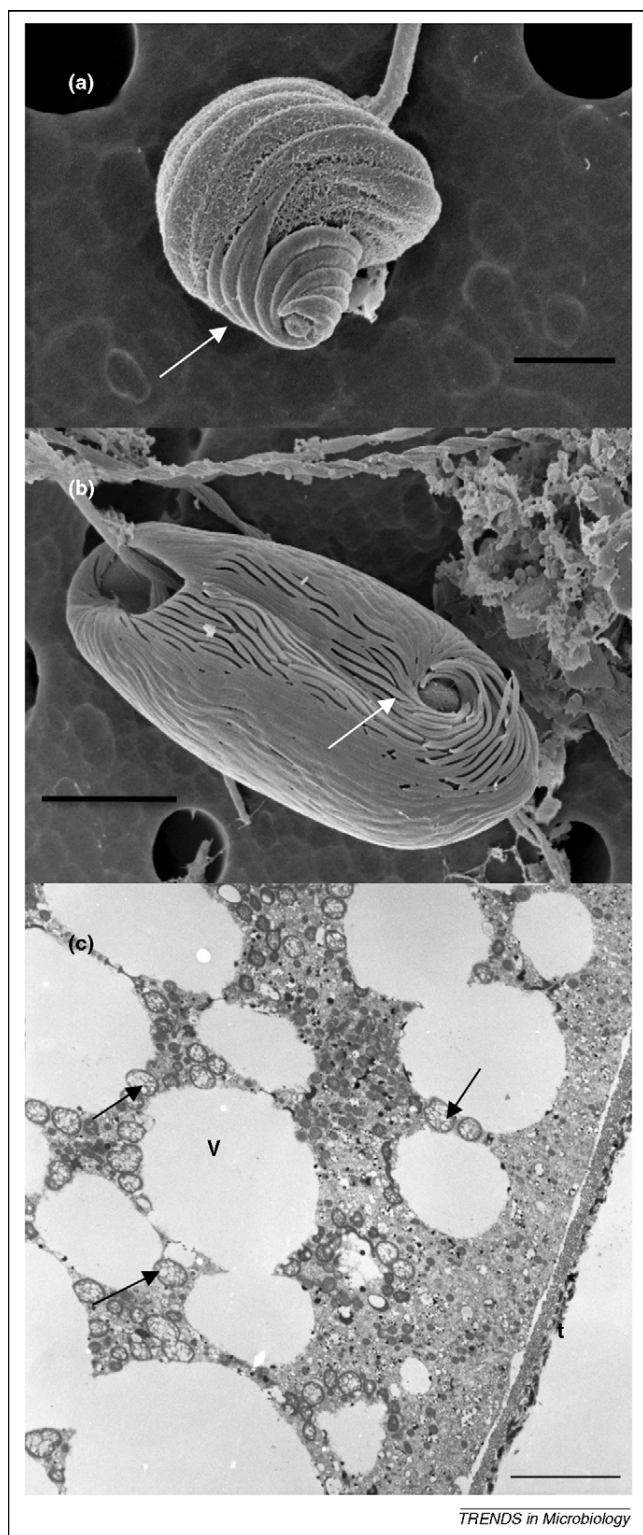


Figure 3. Environmental isolates from sediments. Scanning electron microscope (SEM) images in (a) and (b) are from bathyal silled-basin sediments, and illustrate some of the different patterns that epibiotic bacteria form (arrows). (a) Euglenoid flagellate with rod-shaped ectobionts at its posterior [23]. Scale bar = 2 μm . (b) The flagellate *Postgaardia mariagerensis* veiled with rod-shaped ectobionts. Scale bar = 5 μm . (c) TEM image of the endobiont (arrows) in an allogromiid foraminiferan [31]. t, foraminiferan test; v, vacuoles. Scale bar = 5 μm . All images courtesy of Dr Joan Bernhard.

periods of denitrification in surface sediments. A similar function might be speculated for the *Synechococcus* symbionts as well.

Many anaerobic ciliates, both gut-associated and free-living, exhibit endosymbiotic associations with methanogens. The bacteria are often found in close association with the hydrogenosomes, and are thought to increase the function of the organelle in the anaerobic host. Some of these associations might not be obligate, but their loss does affect the fitness of the host. A study that eliminated a methanogen from its ciliate, *Trimyema compressum*, documented a decrease in growth by the host, along with a change in the fermentation end products [35]. Although eradication of the methanogen symbiont did not prevent the ciliate from growing in laboratory culture [35], it might result in a significant decrease in environmental fitness. Anaerobic ciliates in different environments appear to have acquired symbionts from those environments, but they have been inherited vertically over time so that they are no longer identical to the environmental types [36].

Another interesting example of adaptation is found in the anaerobic marine ciliate *Strombidium purpureum*. The ciliate harbors endosymbiotic purple non-sulfur bacteria that contain both bacteriochlorophyll *a* and spirilloxanthin [37]. The endosymbionts are photosynthetically active, as the ciliates require light for survival and growth under anaerobic conditions. This interesting symbiosis represents an evolutionary transition of an aerobic organism to an anaerobic one, as well as steps in the evolution of organelles.

Concluding remarks and future directions

This review has presented basic information on a range of protist–prokaryote symbioses that are important for the survival of the hosts, and these and other associations are likely to be common strategies for environmental adaptation. Despite the potential for these interactions to be widespread, there remain significant difficulties in their identification and description. A fundamental barrier to the description of symbioses is that most environmental microbes are still intractable with regard to laboratory culture. Studies to identify the function often rely on cultures and microcosms for experimental manipulation.

Extreme, or unusual, environments have allowed researchers to survey for adaptations to unique ecophysiological conditions. Symbioses in these environments might be abundant, and microscopical surveys can be effectively carried out, but access to the sites is often limited or difficult. Non-extreme environments also yield interesting associations, such as defensive symbioses, but these relationships are potentially less abundant and require more labor-intensive methods to uncover their presence. So, in instances where environmental conditions are less extreme, how can potential symbioses be detected? A full-cycle approach based upon ribosomal DNA or RNA can be applied. Nucleic acids are extracted directly from an environmental sample, or from cells of interest recovered from the sample, and the small subunit ribosomal sequences amplified, cloned and sequenced. Phylogenetic analyses identify the taxonomic affiliations of the sequences recovered, and subsequently probes can be made and applied using *in situ* hybridization of the

environmental sample or cells to confirm identity of the symbiotic partners. Isolated host cells can also be examined by TEM to determine the intracellular location and the status of digestion or degradation of symbionts.

Function is ultimately difficult to assess. We still understand very little about the metabolic requirements of symbiotic associations, and so we look to the ecophysiological conditions under which the association occurs to generate hypotheses about function. This has certainly been useful in many of the cases presented above. In particular, the presence of plastids and cyanobacteria in deep-sea foraminifera led to hypotheses of alternative plastid functions that were eventually tested [34]. *In situ* studies of function are difficult, but are certainly possible if hypotheses can be made based upon information about organism identification and environment. Probes for mRNA could be useful in looking for expression of genes associated with particular functions, as could the addition of metabolic tracers to follow the incorporation of symbiont generated products into the host. Metagenomics and whole genome sequencing of bacterial symbionts are likely to play a large role in future efforts at prediction of function. These methods have already proven useful for the termite gut symbionts mentioned previously, and for the epsilon proteobacteria on the surface of the vent polychaete *Alvinella* [38].

In the future, environmental surveys, either whole-cell or molecular, are essential not only to help identify interactions but to examine their distribution and abundances. Just a small number of recent environmental surveys have revealed that a large portion of the protistan populations carry ectobionts. This strongly suggests that symbioses can be abundant in particular environments, and are potentially widespread in all environments. A cautionary note regarding environmental surveys is necessary. Even though endobiotic bacteria can be shown by *in situ* hybridization to be present, the ability of most protists to consume particles suggests that the detected bacterial cells might only be ingested prey, so although these methods are valuable for identifying potential interactions, additional work will be required to confirm the relationship.

Beyond the identification of host and symbiont, molecular tools can allow researchers to address questions regarding co-evolution of the partners and similarity between interactions that occur in discontinuous, but physiologically similar, environments. An example of such a study is the methanogenic symbioses of anaerobic ciliates. Molecular studies have shown that although the symbionts were vertically inherited within a particular ciliate host genera, they were probably acquired several different times from different environments, as the symbionts from a monophyletic group of ciliate hosts belong to different orders of methanogens [39]. The endosymbionts were related to free-living methanogens from the same type of environment as the host (freshwater, marine, intestinal), although they were not identical, indicating that the acquisition of new symbionts from the environment is rare. Similar work has been accomplished for termite gut symbionts [40], and future studies will be valuable for understanding how these interactions become established and evolve.

Molecular methods can also help to streamline analyses for distribution and abundance, especially for endosymbiotic interactions that are more difficult to detect with traditional microscopical methods, or for samples that are difficult to acquire. Quantitative PCR can be used to target both DNA and RNA, allowing quantification of partners and gene products rapidly from large numbers of samples. This will help to assess both abundance and activity in natural samples, so not only would it be possible to determine whether these interactions really are as widespread as we think they might be, but to begin to examine how they function under real environmental conditions.

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