Why Do so Few Animals Form Endosymbiotic Associations with Photosynthetic Microbes? [and Discussion]

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Why do so few animals form endosymbiotic associations with photosynthetic microbes?

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SUMMARY

A survey of modern associations in which protists or invertebrates are hosts shows that very few of the many species of photosynthetic microbes are adapted to an endosymbiotic existence. None occurs as intracellular symbionts in animals structurally more complex than cnidarians and platyhelminths. Photosynthetic symbionts are not usually capable of being the sole food source for hosts because they do not provide a balanced diet; most hosts therefore retain holozoic feeding. Interactions between hosts and intracellular symbionts are complex, and have to include mechanisms for inducing release of photosynthate from symbionts as well as controlling symbiont cell division. Possession of symbionts imposes a measurable cost on hosts. For the great majority of animals, the costs of adapting to herbivory or other forms of nutrition are probably less than that of hosting photosynthetic symbionts, especially when the need for exposure of a large surface area to light is borne in mind. Once hosts become multicellular, it is virtually impossible for any photosynthetic symbionts they possess to evolve into organelles because they are restricted to specific host cell types. After the evolution of the eukaryotic ancestors of plants in the late Precambrian, the major significance of endosymbiosis to the evolution of plant–animal interactions has been the development of gut symbioses in some major groups of herbivores.

1. INTRODUCTION

This paper deals with endosymbiotic associations between photosynthetic microbes and non-photosynthetic organisms. In particular, it addresses the question of why this type of symbiosis, so important in the early evolution of some major eukaryote groups in the Precambrian, has been of only very minor significance in subsequent evolution, contributing to the success of just a limited number of animals groups, mostly lower invertebrates and all aquatic.

The main types of modern associations will be surveyed, and the complexities of interactions between host and symbiont will be analysed. The term 'photosynthetic microbe' will include both cyanobacteria and unicellular algae; 'endosymbiosis' will refer to associations where the symbionts are within the body of the host, being either intracellular, extracellular between host cells, or contained in cavities. Some reference will be made to other types of symbiosis in order to reinforce the conclusions that are reached.

2. MODERN ASSOCIATIONS BETWEEN PHOTOSYNTHETIC MICROBES AND LOWER INVERTEBRATES OR PROTISTA

(*a*) *Protists as hosts*

Non-photosynthetic prokaryote symbionts occur quite widely (Lee *et al.* 1985), suggesting that symbiosis is a common strategy for protists. With regard to photosynthetic symbionts, in freshwater habitats at least 30 genera of ciliates as well as various amoebae have *Chlorella* symbionts (Reisser & Wiessner, 1984).

Ecologically, one of the most important groups is symbiotic benthic marine foraminifera which carpet extensive areas of certain shallow water habitats; they have a variety of algal symbionts, including dinoflagellates, chlorophytes and various diatoms. Radiolarians, whose symbionts are mostly dinoflagellates, are sometimes major contributors to the planktonic zone.

A variety of protists, distributed amongst at least seven genera, also form associations with cyanobacteria. The symbionts are unculturable, and are usually termed cyanelles. The cyanelles of *Cyanophora paradoxa* have a very low genome size (comparable to that of plastids), and their 16*s* rRNA sequence suggests a close alliance to plastids.

In all cases, the photosynthetic symbionts are intracellular, each symbiont cell being surrounded by a host membrane.

(*b*) *Cnidarians*

Cnidarians have only algal and not cyanobacterial symbionts, and these are normally intracellular within the digestive cells of the gastrodermis, each symbiont being surrounded by a host membrane. In a few sea anemones, symbionts may also occur in the mesoglea. The only freshwater cnidarians are *Hydra*, some species of which have *Chlorella* symbionts and are collectively known as green hydra.

Ecologically, by far the most important group are the hermatypic corals, mostly comprised of *Scleractinia*, which all have symbionts belonging to the genus *Symbiodinium*. Coral reefs cover about 2×10^8 km^2 of the earth's surface. The presence of symbionts is responsible both for the high primary productivity of reefs (about 10 to 50 times greater than that of the surrounding tropical waters), and for enhancing the rate of calcification or corals to high levels, enabling them to exploit their habitats successfully (Goreau 1961).

(*c*) *Porifera*

Eight of the 18 genera of freshwater sponges which have been examined possess *Chlorella* symbionts. Symbiotic sponges are more prominent in shallow-water marine habitats, where many species contain an abundant population of both intracellular and extracellular prokaryotic symbionts, including cyanobacteria as well as bacteria.

(*d*) *Molluscs*

Few genera of molluscs possess photosynthetic symbionts. Ecologically the most prominent are giant clams, which can occur abundantly on indo-pacific reefs, and which all possess *Symbiodinium* symbionts located extracellularly within the blood vessels of the mantles. The two freshwater genera that are symbiotic, *Anodonta* and *Unio*, have *Chlorella* symbionts, also extracellular within the body of the host.

Some nudibranchs such as *Elysia viridis* sequester chloroplasts from the seaweeds (usually members of the Siphonales) upon which they feed. These chloroplasts continue photosynthesis for prolonged periods after entering host digestive cells (Hinde & Smith 1975) and this phenomenon has been termed 'chloroplast symbiosis'. In an analogous way, some aeolian opisthobranch molluscs acquire *Symbiodinium* from feeding on symbiotic alcyonaria (Rudman 1981).

(*e*) *Platyhelminths*

Examples of symbiosis are scarce. A few freshwater neorhabdocoel turbellarians have *Chlorella* endosymbionts. Amongst marine forms, only the acoel turbellarian *Convoluta roscoffensis* is of sufficient ecological prominence to occur abundantly in certain habitats. Its endosymbiont, which is intracellular, is *Tetraselmis*.

(*f*) *Ascidians*

Some marine ascidians have *Prochloron* (now considered to belong to the cyanobacteria) as extracellular symbionts, but these are rarely so common as to dominate habitats. *Prochloron* occurs in the cloaca and test in members of at least five genera of the family Didemnidae, and also on the surface of certain members of this family and three others (Polyclinidae, Polycitoridae and Styelidae). Some ascidians have a variety of other cyanobacteria, particularly *Synechocystis* on their surface as well as prochlorons.

3. CONCLUSIONS FROM MODERN ASSOCIATIONS

The following conclusions can be drawn from the survey of modern associations.

(*a*) *Few photosynthetic microbes successfully adapt to endosymbiosis*

There are very many species of photosynthetic microbes in aquatic habitats, but very few live endosymbiotically in animals or protists. The only eukaryotic unicellular algae that exist as intracellular or extracellular endosymbionts in a range of different types of freshwater hosts can all be assigned to the *Chlorella vulgaris* group (comprising *C. vulgaris*, *C. sorokiniana* and *C. saccarophila*) (Douglas & Huss 1986). In marine habitats, the range is a little wider, extending to a number of *Symbiodinium* spp. (Trench & Blank 1987) and one or two other dinoflagellate genera. Apart from the cyanelles of protists and the symbionts of some marine sponges, cyanobacteria do not occur as intracellular symbionts in animals.

The fact that very few photosynthetic microbes are adapted to an intracellular existence agrees closely with observations on symbiosis in general, where the range of endosymbionts which are extracellular is greater than those which are intracellular (Smith & Douglas 1987).

In marine habitats where several different types of photosynthetic symbiont occur, it is nevertheless rare for host cells – and indeed host animals – permanently to accommodate several different types of photosynthetic symbiont at the same time. The only exceptions are foraminifera in general, and the flatworm *Haplodiscus* (Trench & Winsor 1987) in whose cells the two dinoflagellates *Symbiodinium* and *Amphidinium* can coexist. Muscatine (1971) reported the presence of both *Symbiodinium* and an unidentified green algal symbiont in some specimens of sea anemones of the genus *Anthopleura* (although he speculated that the green algae might be a 'parasitic' infection).

In the case of foraminifera, Lee (1974) and Lee *et al.* (1979) speculate that the simultaneous presence of different symbiont types within a single host may have evolved from their unusual habit of gathering food and storing it for later digestion, so that digestion-resistant organisms would have a greater chance of survival in the host.

The extreme paucity of photosynthetic microbes capable of a permanent intracellular symbiotic existence agrees with the belief that despite the wide variety of algal and plant groups, modern plastids are derived from very few ancestral lines.

(*b*) *Photosynthetic endosymbioses are restricted to aquatic habitats*

The symbioses only become ecologically important (i.e. as dominant organisms in significant communities) in marine habitats, especially tropical. Douglas (1991), reviewing the evolutionary significance of endosymbioses, points out that hosts may be able to extend their ecological range by acquiring endosymbionts with novel metabolic capabilities. Thus, the success of hermatypic corals is due to the acquisition through their symbionts both of a supply of photosynthetically fixed carbon and of processes which lead to a very substantial enhancement of calcium carbonate deposition.

(*c*) ***Intracellular photosynthetic symbionts are restricted to relatively few animal groups***

Intracellular photosynthetic symbionts are only at all common in certain groups of protists and cnidarians. Some of the cyanobacterial symbionts of sponges are intracellular, as are probably the few algal symbionts of platyhelminths which are symbiotic. Even when non-photosynthetic microbes are considered, they are commonly intracellular only in the lower animal groups with the exception of arthropods – especially mycetocyte-bearing insects. The general absence of intracellular symbionts from most other evolutionarily advanced groups may well reflect that they have reached a stage of biochemical and morphological differentiation where little or no net selective advantage would be gained from the possession of intracellular symbionts.

(*d*) ***Photosynthetic endosymbionts usually supplement rather than replace holozoic feeding***

Hardly any hosts lose the capacity for holozoic feeding, so that photosynthetic symbionts usually supplement rather than replace conventional sources of food. This is not surprising as photosynthate is released from symbionts to hosts as only one or a few types of molecule (see below), so providing an incomplete diet and therefore being unsatisfactory as a sole food source. For example, *Chlorella* symbionts release only maltose. Although this sugar might seem little better than 'junk food', it enables the host to divert amino acids from respiration and gluconeogenesis to growth processes, and this can be particularly valuable in habitats where nitrogen sources are scarce (Rees & Ellard 1989).

The few associations in which adult hosts cease feeding holozoically include adult *Convoluta roscoffensis* which lives in nutrient-rich habitats, *Aiptasia* and xeniid corals. However, in the symbioses between chemoautotrophic bacteria and marine organisms such as the pogonophorans of deep-sea hydrothermal vents, the digestive systems of most types of adult host become non-functional, although juveniles feed.

4. INTERACTIONS BETWEEN INTRACELLULAR SYMBIONTS AND HOST CELLS ARE COMPLEX

For a photosynthetic symbiont to exist permanently inside a host cell, a variety of complex interactions must occur between the partners, and this may help to explain why so few photosynthetic microbes have become successful symbionts. These interactions may be summarized as follows.

(*a*) ***Living cells of the symbiont are induced to release photosynthate in substantial quantities***

Host cells do not normally acquire organic nutrients from intracellular symbionts by digesting them. Hence, mechanisms must develop whereby symbionts release photosynthate to a much greater extent than free-living algae; release in symbiosis may be substantial, and in some corals may constitute over 90% of total carbon fixed in photosynthesis (Muscatine *et al.* 1984). Although photosynthate release from symbionts has been one of the most studied aspects of symbiosis, the phenomenon is still not fully understood. Factors inducing release have been partly identified, but the mechanisms remain obscure. Furthermore, these factors differ with different types of symbiont. Cells of isolated symbiotic *Chlorella* release 50–60% of their photosynthetically fixed carbon when maintained at pH 4.0; but at pH 7.0, release is negligible (Douglas & Smith 1984). By contrast, release from isolated *Symbiodinium* symbionts varies little with pH but is markedly stimulated by thermolabile, water-soluble 'factors' present in homogenates of host tissue (see, for example, Sutton & Hoegh-Guldberg (1990)); 'factors' from one type of host can induce release from the symbionts of another type (Muscatine, 1967). These 'factors', which have not been characterized, have no effect on free-living algae, nor on *Chlorella* nor even on the photosynthetic symbionts of lichens; in addition, 'factors' cannot be isolated from non-symbiotic hosts. In lichens, the fact that symbionts are extracellular should make them easier to study experimentally, but even so, the process remains poorly understood and release appears to depend on some aspect of physical contact between symbiont and host.

For all types of photosynthetic symbiont, only one or a very few products of photosynthesis are released to the host cell: maltose from *Chlorella* symbionts; glycerol, alanine and fatty acids from *Symbiodinium*; glucose from 'symbiotic chloroplasts'; and either glucose or a polyol from symbionts of lichens.

(*b*) ***Host cell and intracellular symbiont have to compete with each other for scarce essential nutrients***

Some of the key nutrients essential for the growth of host and symbiont are often in restricted supply in aquatic habitats; indeed, nutrient shortage is often regarded as encouraging colonization by symbiotic associations. At the cell level, mechanisms therefore have to develop so that competition for scarce nutrients such as phosphorus is sufficiently balanced to enable both partners to continue growing. Thus, when green hydra is starved, both hosts and symbiont stop growing (Ellard 1986). The need to acquire scarce nutrients may also help explain why so few animal hosts forsake holozoic feeding mechanisms. The value of the symbionts to the host in situations where nitrogen is limiting is that, as noted above, provision of ample fixed carbon through symbiont photosynthesis 'spares' amino acids from having to be used as substrates for respiration or for gluconeogenesis (Rees & Ellard 1989).

(*c*) ***Cell division of symbionts must be controlled***

The growth rate of photosynthetic symbionts within host cells is usually slower than growth rates in culture by one or more factors of ten (Smith & Douglas 1987). For example, symbiotic *Chlorella* increase at a rate of 2.3 cells cell^{-1} day^{-1} in culture, but only 0.1 cells cell^{-1} day^{-1} in symbiosis with green hydra (Douglas & Huss 1986). A variety of hypothetical explanations have been offered on the basis of experimental observations. They include: restriction of nutrient supply to symbionts by host cell (Rees 1986); restriction in the supply of some 'factor' essential to symbiont cell division (McAuley 1985); diversion of fixed carbon from growth to translocation to host (Douglas & Smith 1984); and variation in factors known to affect symbiont cell-division such as pH in the case of *Chlorella* symbionts (Douglas & Smith 1984).

(*d*) *Size and location of the symbiont population must be regulated*

As well as controlling symbiont cell-division, mechanisms are needed to regulate both the overall size of the symbiont population and its location (not only to specific host cell types or morphological regions, but also specific regions of the host cell). Again, such mechanisms are not understood.

(*e*) *Mechanisms are required for symbionts to be transmitted to succeeding host generations*

In the case of protist hosts, transmission of intracellular symbionts to succeeding generations routinely occurs during host cell division. Allocation of symbionts to host daughter cells may be precise when the number per cell is small (for example, as in *Paulinella*), or more randomly when symbionts are numerous and the probability of transfer to a daughter cell very high (as in, for example, *Paramecium*).

In metazoan hosts with sexual reproduction, mechanisms are required which ensure either that symbionts are transmitted with the egg, or that they are acquired afresh from the environment at each generation. In the latter case, it must be remembered that few if any of the common intracellular photosynthetic symbionts can exist permanently and abundantly independent of the host (perhaps not surprising, because the intracellular environment to which they are so successfully adapted is presumably very different from that external to the host).

In green hydra, symbionts may become exocytosed from gastrodermal cells into the mesoglea and then phagocytosed by the epidermis-derived oocytes (Campbell 1990). In corals, some species rely on acquisition from the environment whereas others (which may even be from the same genus) can transmit symbionts directly to eggs (see review by Fitt (1984)). On coral reefs, most hosts release *Symbiodinium* symbionts into the surrounding seawater; possibly either as a mechanism for regulating symbiont population size, or providing a supply of symbionts for those animals who have to acquire symbionts from the environment each sexual generation. Giant clams rely entirely on acquisition from the environment: because of their growth habit, embedded in crevices on reefs, it would be a considerable advantage if they could acquire symbionts released from corals, implying that symbionts could be adapted to existence in a variety of different host types. In general, specificity of some strains of *Symbiodinium* symbionts for their hosts seems relatively low (Trench & Schoenberg 1976; Trench *et al.* 1981).

In *Convoluta roscoffensis*, symbionts are deposited onto the surface of the egg capsule, and so are available for young larvae when they hatch.

(*f*) *Hosts must afford the cost of sustaining their symbionts*

The variety of interactions listed previously impose an undoubted cost upon hosts, which can be shown by comparing the growth of hosts in the dark with and without symbionts (see, for example, Douglas & Smith (1983) for green hydra). The cost of bearing symbionts has to be set against the benefits they bring to the host. For example, nitrogen-fixing organelles probably never evolved because of the high energy costs of nitrogen fixation (and would have been particularly high for an ancestral host which was a unicell and therefore already had a relatively high specific metabolic rate), coupled with the fact that the nitrogenase enzyme is inactivated by exposure to molecular oxygen.

The cost of maintaining photosynthetic endosymbionts may be greater for most animals than the costs of adapting to the digestion of plants or the capture of herbivores. This may be a reason why so few have adopted this symbiosis as an evolutionary strategy. Indeed, the animal group in which photosynthetic symbionts are most widespread, the cnidarians, already have a plant-like sessile habit, often exposing relatively large surface areas because they are also filter feeders.

5. THE REQUIREMENT FOR EXPOSURE OF PHOTOSYNTHETIC SYMBIONTS TO LIGHT

Dependence upon photosynthetic symbionts carries with it requirement for prolonged periods of exposure to light of a relatively large surface area of host. For unicellular or cnidarian hosts, which naturally have large surface area to volume ratios, this presents few problems. Structurally more complex organisms have to develop adaptations both exposing large surface areas, and to reducing the risk this brings of attack by predators. Thus, a variety of adaptations can be seen in molluscs. The symbiont-containing mantle of giant clams is extremely sensitive to shading, and is withdrawn inside the shell within seconds of a sudden reduction in light intensity. The freshwater bivalve *Anodonta* makes use of the fact that damage to the shell causes transparent areas to develop, which then allow light through to its *Chlorella* symbionts while still retaining the defensive advantage of a shell. The nudibranch *Elysia viridis*, which acquires 'symbiotic chloroplasts' from its food plant, is believed to benefit from the camouflage of its consequential deep green colour when it is feeding on its host seaweed. Its tropical relative, *Placobranchus ianthobapsus* can either expose its plastid containing tissues, or fold them up so they are not visible (Trench 1975 and personal communication).

6. EVOLUTION OF PHOTOSYNTHETIC SYMBIONTS INTO ORGANELLES IS PROBABLY IMPOSSIBLE IN MULTICELLULAR ORGANISMS

Douglas (1991) states that a symbiont can be considered to have evolved into an organelle if genes essential to its functioning are transferred to the host nucleus. The host nuclei most likely initially to acquire such DNA are those of the cells containing the symbionts. However, the transferred sequence of symbiont DNA will only persist in succeeding generations if the recipient nuclei also contribute to the reproductive propagules of the host. Douglas points out this is the norm in unicellular protists, but virtually unknown in sexually reproducing multicellular hosts. Hence, once multicellular organisms evolved, it became most unlikely that any further new organelles could evolve from symbionts within them.

Incidentally, Douglas' statement clarifies the confusing situation concerning the extent to which the cyanelles of certain protists can be considered organelles. In *Cyanophora paradoxa*, the cyanelles have a genome size of 127000 base pairs, comparable to that of plastids (Wassman *et al.* 1987), and this has led to questions of the extent to which they differ from plastids. However, gene transfer from cyanelles to host nucleus has not been shown; a key feature is that the small subunits of ribulose bisphosphate carboxylase are coded in the nucleus of chlorophytes and plants, but are coded in the cyanelles of *C. paradoxa* (Heinhorst & Shively 1983).

7. DISCUSSION

There are various reasons why symbiosis between animals and photosynthetic microbes is not widespread. Very few of the many species of photosynthetic unicells can exist as successful intracellular symbionts, and the choice of those that can associate with a range of organisms is virtually restricted to *Chlorella* in freshwater habitats, and to a small number in marine habitats, especially the dinoflagellates *Symbiodinium* and *Amphidinium*. Further, reliance on photosynthetically fixed carbon from symbionts as a food source means that hosts have to expose a relatively large surface area to light for long periods.

Interactions between intracellular photosynthetic symbionts and their hosts are complex. They impose costs upon the hosts, including those of mechanisms for control of symbiont cell division, and regulation of symbiont population size and location. However, once organisms became multicellular, it was more or less impossible for any symbionts they possessed to evolve into organelles unless symbionts were present in all host cells. Photosynthetic symbionts are invariably restricted to specific cell types in multicellular hosts.

Symbiosis with photosynthetic microbes has therefore been of very limited importance in animal evolution. However, quite another type of symbiosis, involving non-photosynthetic microbes, has been of major significance to evolutionary interactions between plants and animals. These are the associations with cellulose-degrading endosymbionts which occur in the guts of certain major groups of herbivores. Much of the fixed carbon which accumulates in plants is in the form of structural polysaccharides, especially cellulose. Successful vertebrate and some insect herbivores do not themselves produce cellulases, and depend to a greater or lesser extent on populations of digestive tract symbionts to break down the refractory plant material that comprises the bulk of their food.

Several authors (Bauchop 1977; McBee 1977; Douglas 1991) have noted that cellulolytic gut symbionts are more advantageous to large rather than small animals. Cellulose is degraded slowly, and the host has to allocate considerable space to the fermentation chamber containing symbionts and decomposing plant material (Alexander, this symposium). Small mammals such as rabbits and voles must pass food through the gut more rapidly and select more digestible food than large herbivores. For relatively very small herbivores such as insects, the cost of cellulolytic symbionts is much greater than for vertebrates. Hence, many insect herbivores produce their own cellulases, whereas others develop alternative strategies for exploiting microbial cellulases. For example, an ecologically important strategy is the degradation of plant material externally to insects, as in 'fungus gardening' by ambrosia beetles and by Macrotermitidae. The latter, together with various insects such as siricid wasps, scolytid and cerambycid beetles, not only feed on the fungi and bacteria which have degraded the cellulose, but also utilize free microbial cellulases in their guts. Such microbial cellulases are stable and contribute to the degradation of dietary cellulose for extended periods (Martin 1987, and this symposium).

Thus, symbiosis has had important evolutionary consequences in both the Precambrian and Phanerozoic eras, but the symbiotic acquisition of the capacity for photosynthesis ceased to be an important strategy after the rise of photosynthetic eukaryotes in the Precambrian era. Thereafter, herbivory, whether or not involving gut symbioses, was the major route for gaining access to photosynthetically fixed carbon.

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Discussion

E. A. Bernays (*Department of Entomology, University of Arizona, U.S.A.*). What is special about *Chlorella* that enables it to form most of the symbiotic interactions?

D. C. Smith. A very recent paper by Kessler *et al.* (1991) examined the excretion of sugar (maltose, glucose or glucose-6-phosphate) by cultures of 38 strains of *Chlorella* belonging to 15 species of which seven are capable and eight incapable of symbiosis with green hydra. All excreted some sugar, but only in trace amounts by 24 strains, and relatively small amounts by a further eight strains. However, one non-symbiotic strain (*C. mirabilis*) excreted large amounts of maltose below pH 4, and several non-symbiotic strains of *C. vulgaris* had maximum rates of maltose excretion below pH 4. Kessler *et al.* therefore concluded that there was no obvious correlation between sugar excretion and the ability or inability of the *Chlorella* species to form stable symbioses with green hydra. All the species that can form a stable symbiosis are those that are capable of growing in acid media at or below pH 4.0. Hence, acid tolerance seems likely to be an important factor.

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