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Evolution: Serving Up Light

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Dinoflagellate algae form symbiotic partnerships with hosts from across a wide swath of the tree of life. New work shows that the genus *Symbiodinium* should now be considered a family, and importantly that the group is 110 million years older than previously thought. This expanded time period resolves long-standing questions about the evolution of photosymbiosis.

Photosymbiotic algae capture energy from the sun, nourishing themselves and their heterotrophic hosts in the process. Many species take advantage of this algal generosity, including corals, bivalves, foraminifera, and many other protists and marine animals. In return, the algae receive nutrients and a privileged place in the sun.

Like any relationship, photosymbiosis is more complex than it appears on the surface. Photosymbiosis between symbiotic dinoflagellates and their coral hosts, in particular, runs deeper than a mere exchange of sugars for a safe place in the sunlight. The symbionts live within the cells of the corals, and together they function to construct a well-coordinated partnership: they build cellular membranes (the symbiosome), the coral immune system is tuned to promote symbiosis, and cell cycles are synchronized to help the dinoflagellate live within the coral cells [1]. In light of this intimate cell-to-cell connection, it has always been surprising that all coral symbioses seemed to involve only one dinoflagellate genus, which furthermore also formed symbiotic relationships with a wide diversity of other host species. Evolving cell-membrane-level adaptations seems difficult when there is one evolutionary target let alone countless hosts from many animal phyla and eukaryotic kingdoms.

The first hint that the dinoflagellate *Symbiodinium* had a more complex history than it seemed became apparent when RNA sequencing exposed a

surprising diversity of nine *Symbiodinium* lineages sampled from a range of corals and other hosts [2–5]. Some closely related *Symbiodinium* lineages in clade 'A' nevertheless occurred in distantly related cnidarian and molluscan host species [2]. This along with the observation that corals gained and lost photosymbiosis repeatedly over 200 million years [6,7] indicate that there is a degree of flexibility to the range of hosts able to pair with *Symbiodinium*.

The diversity of algal lineages, the wide phylogenetic range of hosts, and the complexity of the interactions involved in photosymbiosis all imply a long and complex evolutionary history. After all, there is evidence that corals have been engaging in photosymbiosis since the Middle Triassic about 240 million years ago [8]. However, the first molecular clock analyses [9] suggested a surprisingly recent origin of *Symbiodinium*, about 50 million years ago during the Eocene. The structure and form of coral colonies reliably indicate the presence of photosymbionts in modern and extinct coral species [10], so we know that there is a long, 190-million-year history of photosymbiosis in corals predating the supposed origin of *Symbiodinium*. It was a macroevolutionary mystery filled with questions — Who were those photosymbionts? Are they extinct? How closely related were they to *Symbiodinium*? —with no obvious way to answer any of them.

Several macroevolutionary patterns present in the fossil record and apparent in molecular phylogenetic analyses hint at a shift in the nature of coral photosymbiosis sometime in the middle Jurassic, about 170–150 million years ago. Coates and Jackson [10] found that many highly integrated, colony-growth forms started to originate within the middle Jurassic. Today, these growth forms are exclusively photosymbiotic, and so their absence early in the history of corals suggests that the interaction between corals and algae was still evolving. A molecular phylogenetic analysis of coloniality and photosymbiosis [7] showed lockstep changes in the relative frequencies of these two traits beginning about 160 million years ago and continuing up to the present. Prior to this time period, the two traits had largely independent histories. Based on the patchy distributions and complex life-histories associated with colonial living, Jackson and Coates [11] predicted that colonial corals should have higher extinction rates than solitary species. Yet, much to their surprise, they found that both types of species had indistinguishable geological life spans and therefore equivalent extinction rates. Nevertheless, Jackson and Coates' prediction is observed when looking at extinction rates over time. Prior to the middle Jurassic (about 160 million years ago) colonial corals had significantly higher extinction rates than solitary corals [12]. Because photosymbiotic species become more common after 160 million years ago, this pattern suggests that photosymbiosis acts to reduce the naturally high extinction risk of colonial corals.

Extinction selectivity for more efficient photosymbiosis during the end-Triassic extinction is one hypothesis explaining these macroevolutionary changes from the Triassic to the Jurassic [13]. However, a new molecular clock analysis of *Symbiodinium* algae provides a fascinating alternative hypothesis [14]. Using a new time-calibrated molecular phylogenetic analysis, LaJeunesse and coauthors report in this issue of *Current Biology* that *Symbiodinium* is much older than previously thought, originating at about 160 million years ago coincident with the many macroevolutionary changes observed in corals. Due in part to this ancient origin, the authors also promote the genus *Symbiodinium* to the family level, the Symbiodiniaceae, and propose genus names for the nine clades.

With the new timescale of symbiodinian evolution [14], we can begin to address questions about the evolution of photosymbiosis that we could not before. Some questions are as simple as identifying the ancestral host for symbiodinian algae. With the compressed timescale of our prior understanding, any of the potential ancestral hosts we could identify by comparative phylogenetic methods would have seemed nonsensical as they suggest considerable missing evolutionary history. This new phylogenetic framework also opens the door to ask more detailed evolutionary questions; for example, how much of the genetic toolkit for use in photosymbiosis preexisted prior to its origin? Are there any novelties in the *Symbiodinium* lineages that lead to more significant innovations and an expansion of the hosts they can utilize? An intriguing possibility is that the evolution of symbiodinian algae to live photosymbiotically with corals also permitted it to live with other hosts. For example, the Jurassic origin of the symbiodinian algae makes it more likely that putatively photosymbiotic bivalve mollusks, such as *Lithiotis*, the reef-building rudists that became common in the Cretaceous and Jurassic [15], were in fact photosymbiotic. These questions are just the beginning. The paper by LaJeunesse and coauthors [14] gives us all permission to rethink the evolution of photosymbiosis in light of the long timescale and high diversity of symbiodinian evolution.

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Eukaryotic Evolution: An Ancient Breath of Nitrate

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Unicellular foraminifera that thrive in oxygen-depleted marine sediments respire nitrate when oxygen is scarce, explaining their early success. A new study shows that some foraminifera express denitrification genes acquired from prokaryotic ancestors.

The diversity of mitochondrial respiration has fascinated biologists for generations. Although mitochondria generally produce ATP by respiring oxygen, mitochondria of anaerobic eukaryotes use other terminal electron acceptors, such as organic molecules from central carbon metabolism [1]. Fungi in the *Fusarium* genus respire nitrate and oxygen simultaneously in their mitochondria in a process termed 'hybrid respiration' [2]. Remarkably, these fungi can also use elemental sulfur as a terminal electron acceptor and produce hydrogen sulfide [3]. In 2006, a group of unicellular protists called foraminifera were the first reported eukaryotes to reduce nitrate to dinitrogen — a complete denitrifying pathway — in oxygen-depleted ecosystems [4,5], likely explaining their success as early as the Cambrian era [5]. This finding ignited some debate as to whether their activity was a genomic trait acquired by ancient foraminifera from prokaryotes or whether denitrification was actually catalyzed by symbiotic denitrifying bacteria. A new study published in this issue of *Current Biology* by Woehle *et al.* [6] now provides the first genomic and transcriptomic evidence from two *Globobulimina* foraminifera species that their ability to denitrify is indeed an ancient inherited trait originally acquired by lateral gene transfer from prokaryotic ancestors.

Complete denitrification in prokaryotes requires the combined activity of four enzymes: nitrate reductase, nitrite reductase, nitric oxide reductase and nitrous oxide reductase (Figure 1A). Prokaryotes have evolved a diverse array of denitrifying enzymes to return reactive nitrogen to the atmosphere as inert N_2, thus completing the nitrogen cycle [7]. Bacteria with complete denitrifying pathways thrive in oxygen-limited ecosystems where nitrogen oxides are readily available as electron acceptors. Foraminifera are abundant in the fossil record as far back as the Neoproterozoic to Early Cambrian era, when it is speculated that their symbiotic relationships with denitrifying bacteria were of utmost importance, as oxygen availability was quite low in marine ecosystems [1,8]. Studies of modern unilocular foraminifera (also known as Allogromiida), which are considered more primitive than multilocular calcareous species, were shown to accumulate nitrate and harbor bacterial endobionts that express a gene, *nirK*, that encodes nitrite reductase [8]. The calculated abundance of endobionts per allogromiid could, in theory, account for previously measured rates of denitrification in environments where the organisms grow and thrive. Multilocular foraminifera also accumulate nitrate and denitrify in benthic sediments [5]. However, these species, as typified by the *Globobulimina* genus, harbor few endobionts (<100 per individual) [5], leading Woehle and colleagues to question whether these foraminifera themselves encode and express genes required for complete denitrification.

The challenge for the authors was to find the full complement of genes in foraminifera genomes and transcriptomes to demonstrate their potential to completely denitrify nitrate to dinitrogen. Several thousand individuals of viable *Globobulimina* representing two species were manually picked from sediments for genomic and transcriptomic analysis along with measurements of their denitrification rates. The readily identified genes of prokaryotic ancestry included the copper-containing nitrite reductase (*nirK*) and a nitric oxide reductase with high similarity to the nitric oxide dismutase (*norZ*/*nod*) of the bacterium *Methylomirabilis oxyfera* [9]. This finding was intriguing, as Nod enzymes produce N_2 and O_2 from the dismutation of two