

Chapter 12

Evolution of Photosynthetic Eukaryotes; Current Opinion, Perplexity, and a New Perspective



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Abstract The evolution of eukaryotic photosynthesis marked a major transition for life on Earth, profoundly impacting the atmosphere of the Earth and evolutionary trajectory of an array of life forms. There are about ten lineages of photosynthetic eukaryotes, including Chloroplastida, Rhodophyta, and Cryptophyta. Mechanistically, eukaryotic photosynthesis arose via a symbiotic merger between a host eukaryote and either a cyanobacterial or eukaryotic photosymbiont. There are, however, many aspects of this major evolutionary transition that remain unsettled. The field, so far, has been dominated by proposals formulated following the principle of parsimony, such as the Archaeplastida hypothesis, in which a taxonomic lineage is often conceptually recognized as an individual cell (or a distinct entity). Such an assumption could lead to confusion or unrealistic interpretation of discordant genomic and phenotypic data. Here, we propose that the free-living ancestors to the plastids may have originated from a diversified lineage of cyanobacteria that were prone to symbioses, akin to some modern-day algae such as the Symbiodiniaceae dinoflagellates and *Chlorella*-related algae that associate with a number of unrelated host eukaryotes. This scenario, which assumes the plurality of ancestral form, better explains relatively minor but important differences that are observed in the genomes of modern-day eukaryotic algal species. Such a non-typological (or population-aware) way of thinking seems to better-model empirical data, such as discordant phylogenies between plastid and host eukaryote genes.

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12.1 Introduction

Adaptive radiations have been typically studied using two approaches. Classically, one may observe an established radiation and draw inferences about the past processes, which led to the present-day pattern of ecological and phenotypic diversity, informed by the understanding of the phylogenetic relationships among species. Alternatively, one may identify and study the evolutionary processes operating on a clade of relatively few species (or forms), which may be currently diverging with the assumption that the clade is a representative of the early stages of a forthcoming adaptive radiation. These two approaches represent either end of the process-pattern divide in adaptive radiation research. (Stroud and Losos 2020)

Although not all evolutionary outcomes are the consequences of adaptations, many evolutionary studies are designed based on the process- or pattern-centric approaches. If evolutionary biologists want to know why Roquefort cheese is so special, then they could resort to biochemistry, which helps to uncover how blue cheese fungi (e.g. *Penicillium roqueforti*) can produce a special flavor (process-centric). Alternatively, phylogeny may identify how blue cheese fungi are evolutionarily related or distant from other fungi (pattern-centric) (Dumas et al. 2020). Perhaps comparative biochemical analyses using multiple species and strains sampled from different phylogenetic branches may provide a more complete understanding of the evolutionary transition from “ordinary” to “special” blue cheese fungi, where process- and pattern-centric approaches are synthesized, but often such integration of approaches is not feasible. This is particularly so when an evolutionary event is archaic (e.g. the origins of plants, eukaryotes, or life on the Earth). Processes are often only observable in extant (i.e. modern-day) species, which are often highly diverged from their distant ancestors, thereby limiting their utility in inferring ancient events. Deep phylogenetic patterns are often difficult to reconstruct accurately because ‘transitional’ species between ‘in-group’ and ‘out-group’ taxa become scarce over time due to extinction events. For many ancient evolutionary events, especially when fossil records are scarce, the pattern-based approach using modern-day information is often seen as the sole option.

The theory of endosymbiosis explains that the plastids (chloroplasts), the organelles responsible for photosynthesis, originated via engulfment of photosynthetic microorganisms by host eukaryotic cells. The first photosynthetic eukaryotes arose via ‘primary endosymbiosis’ in which a eukaryotic host engulfed and retained a cyanobacterial endosymbiont¹ (Cavalier-Smith 1982). Primary plastid-bearing groups include green algae plus their land plant descendants, red algae, and glaucophytes, which are together classified as Archaeplastida (Adl et al. 2019). In contrast, some eukaryotes acquired their plastids via ‘secondary’ or ‘tertiary’ events that involved eukaryotic endosymbionts (McFadden 2001). Some researchers argue that the three primary plastid-bearing algal groups arose via a single endosymbiotic

¹There are more recently identified cases of cyanobacterial integration into the eukaryotic cells, such as in the case of photosynthetic *Paulinella* species (Lee et al. 2019) and rhopalodiacean diatoms (Nakayama and Inagaki 2017). It is, however, debated as to whether they should be called plastids (Keeling and Archibald 2008).

event that occurred more than a billion years ago, and therefore, they form a monophyletic taxonomic group called Archaeplastida. The proponents of this hypothesis suggest that a permanent integration between two distinct organisms, that is, between a unicellular eukaryotic host and a cyanobacterial endosymbiont, must have been an extremely fortuitous and random evolutionary event. By comparison, the proponents of alternative hypotheses, which often but not necessarily propose non-monophyletic archaeplastidans, assume that the plastid loss could be as rare or difficult as plastid acquisition founded on the observation that plastid-lacking members are scarce within a well-supported algal group whose common ancestors had a plastid (e.g. haptophytes and cryptophytes).

Such a debate is based on an assumption that the rarity (or frequency) of a plastid's gain or loss events has been constant over time, which may not be true. In very early stages of plastid evolution, loss and regain of photosymbionts by host cells that belong to the same 'lineage' or 'population' might have occurred repeatedly (Fig. 12.1). After such associations formed and ensued over generations, variants such as those that are less competent in symbiosis could have arisen, thereby facilitating sympatric speciation (e.g. symbiont-bearing and color-less species) in a population (Fig. 12.1). A population of the phagotrophic cryptistan biflagellate protist, *Haetena arenicola*, may represent a good example of this process. *H. arenicola*, occurring on a sandy beach, internalizes and retains the green alga *Nephroselmis rotunda* in the cytoplasm (Okamoto and Inouye 2005, 2006). When the green-colored 'parent' *H. arenicola* divides into two daughter cells, only one

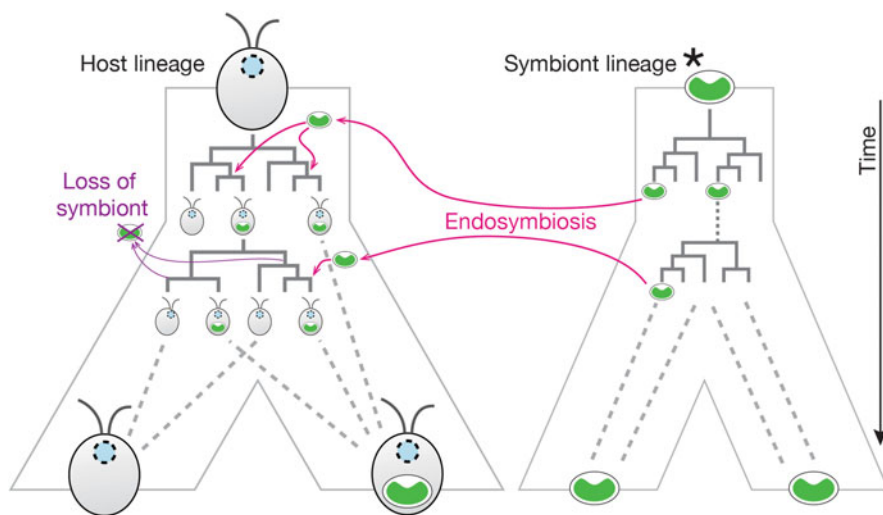


Fig. 12.1 A schematic model showing an early stage of protist–photosymbiont associations. In this model, a population of free-living phototrophs is the source for multiple endosymbioses with a host population. From such multitudinal interactions, those photosymbionts that are kept by the host population are derived from (likely many) different algal cells, but they might be seen collectively as a single entity (shown by asterisk)

inherits the endosymbiont, and the other does not and becomes colorless. What happens if the plastid-lacking cell proliferates further and regains an endosymbiont that is derived from the (ancestral) green algal population with which the parental *H. arenicola* is associated? The host and endosymbiont in the offspring would have genotypes closely related to the ones in the ancestors. In this case, the endosymbiont lineages are not continuous at the cellular level but can be genetically traced to the same green algal population. The bigger the time gap between the ancestor and the offspring generations, the bigger the genotype variations in the ‘metapopulation’ are expected to be.

Recently, a non-photosynthetic, predatory relative of red algae, named *Rhodolphis*, has been discovered (Gawryluk et al. 2019). This flagellate is the only obligate heterotrophic phagotroph known to date within Archaeplastida. Some early-diverging members of green algae are also phagotrophic, but they are pigmented and photosynthetic; thereby possessing a mixed-mode of nutrition (Maruyama and Kim 2013). Therefore, from the perspective of the trophic mode, *Rhodolphis* represents an oddity considering the Archaeplastida concept, which assumes that their common ancestor was phototrophic. Despite a lack of microscopic evidence for plastids, *Rhodolphis* is suggested to bear plastids based on an *in silico* identification of a number of putative plastid-targeted peptides. The analyses of putative protein transport machinery and transit peptide sequence motifs did not indicate that *Rhodolphis* had characteristic signals for plastids of secondary origin. The authors, therefore, suggested the plastid of this flagellate is of primary origin. However, in molecular phylogenetic analyses, the majority of *Rhodolphis*’ putative plastid-targeted proteins did not branch with red algal proteins, casting doubt on the origin of the *Rhodolphis* plastid and perhaps, even the validity of the existence of the plastid compartment. Therefore, more investigation is needed to see whether *Rhodolphis* indeed possesses plastids or not.

It is also worth pointing out that the Archaeplastida hypothesis is perhaps too dependent on prior knowledge of eukaryotic phylogeny (Baldauf et al. 2000; Moreira et al. 2000; Rodríguez-Ezpeleta et al. 2005). With an increase in taxon sampling and the use of updated phylogenomic matrices, many recent phylogenomic analyses do not support the monophyly of Archaeplastida (Strasser et al. 2019). In particular, Cryptista—comprising cryptophytes, goniomonads, katablepharids, and *Palpitomonas* (Adl et al. 2019)—often branches within Archaeplastida, thereby disrupting its monophyly (Burki et al. 2016; Cenci et al. 2018; Strasser et al. 2019; Gawryluk et al. 2019). If this topology correctly reflects the species’ relationships, this may provide evidence against the hypothesis on single plastid-generating event at the ancestry of Chloroplastida, Rhodophyta, and Glaucophyta. Alternatively, some argue that the Archaeplastida hypothesis still holds if we expand the Archaeplastida concept, such as by including Cryptista. Under this scenario, there was a single primary plastid-generating event at the ancestry of Chloroplastida, Rhodophyta, Glaucophyta, and Cryptista, but the complete loss of plastids happened before the common ancestor of Cryptista diverged. This illustrates how the pattern-based (phylogenetic) approach could be limiting in addressing an archaic evolutionary event, such as the origin of plastids.

Here, we propose that ‘modern’ symbiotic associations may be sources of insight for ancient processes of plastid acquisition. The way by which plastid evolution progressed from free-living bacteria through obligate endosymbionts to permanent cellular organelles remains mysterious. Considering the spectrum of the strength in host–symbiont relationships, facultative associations found in modern-day environments may provide clues about the early stages of plastid evolution. Symbioses also span a spectrum in partner specificity between ‘specialists’ and ‘generalists,’ the latter defined here as having a broad host range. While generalist algal symbionts are notable in marine and freshwater environments, this concept has not been taken into account in modeling plastid evolution. In this chapter, we survey those modern associations that involve photosynthetic symbionts, especially those that are generalists in host specificity. Relationship dynamics seen in such modern symbioses may be parallel to the ancient associations that eventually led to the evolution of eukaryotic photosynthesis.

12.2 Generalist Photosymbionts in Modern Aquatic Environments

There are a great variety of photosymbionts in nature, and their diversity has been reviewed extensively elsewhere (Martin et al. 2016). Here, we focus on the three groups of eukaryotic algae that associate with a broad range of host taxa and hence, could be considered as generalist symbiont lineages. Of these, the Symbiodiniaceae dinoflagellates and the *Chlorella*-related algae are symbiotic champions in marine and freshwater ecosystems, respectively.

12.2.1 Symbiodiniaceae Dinoflagellates

Dinoflagellate algae belonging to the family Symbiodiniaceae are known to form stable endosymbiotic relationships with a number of marine eukaryotic hosts, including cnidarians (e.g. coral, sea anemone, and jellyfish), ciliates, and foraminiferans. One of the most ecologically relevant examples is coral–algal symbiosis, which sustains the primary production of coral reefs in oligotrophic oceans.

Based upon the most up-to-date classification proposed by LaJeunesse et al. (2018), Symbiodiniaceae forms a monophyletic taxon in the dinoflagellate phylogeny. Members of the Symbiodiniaceae either (1) associate with only a single host group (e.g. clade I only found in foraminiferans), (2) appear as exclusively free-living in nature (e.g. *Effrenium voratum*), or (3) associate with multiple hosts, which are often colonized by multiple distinct Symbiodiniaceae species/genera. For example, a single species of coral can host a number of symbiont genera, and the composition can vary depending on geographic location, environmental condition,

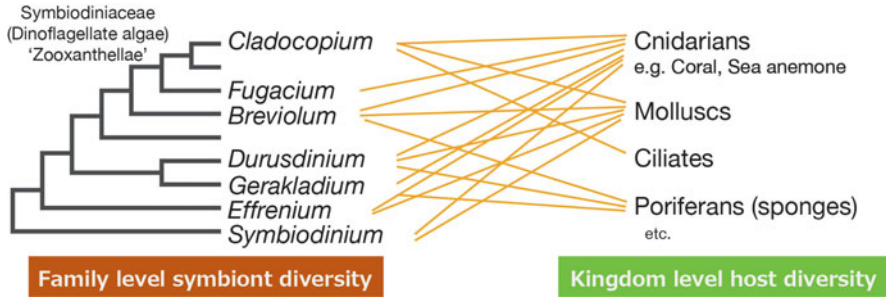


Fig. 12.2 Taxonomic relationships between Symbiodiniaceae dinoflagellates and their various hosts (LaJeunesse et al. 2018; Mies et al. 2017)

and developmental stage (Pochon et al. 2014; Mies et al. 2017). There seems to be no general trend of lineage-specificity in host–dinoflagellate relationships, suggesting that a wide host-range is an ancestral characteristic of the Symbiodiniaceae algae (Fig. 12.2). This ‘many-to-many’ partnership is likely a factor providing flexibility in the face of changing physiological, developmental, and environmental circumstances.

Flexible ‘many-to-many’ partnerships appear stable and may be advantageous to both hosts and dinoflagellate symbionts as long as the symbiont population size is large enough to sustain the host population. A disadvantage to the host in such a flexible partnership is the potential for host gastrodermis cells to become occupied by less-beneficial symbiont algae unless a mechanism to selectively recruit the optimally mutualistic algae is already set in place. Similarly, a disadvantage to the endosymbiont is that if a stronger competitor co-occupies the residential space within the host, then it may be forced outside to inhabit the oligotrophic ocean where it could starve due to low nutrient availability.

In the family Symbiodiniaceae, the genus *Cladocopium* (formerly called ‘clade C *Symbiodinium*’) is known to include generalist members that thrive inside a number of host eukaryotes (e.g. cnidarians, foraminiferans, and acoels) and have an ectosymbiotic partnership with molluscan hosts (LaJeunesse et al. 2018). Conversely, a single host species can accommodate multiple *Cladocopium* species or subspecies. Under a condition where endosymbiosis is destabilized by environmental cues (e.g. thermal stress), host corals expel a portion of residing symbionts (e.g. *Cladocopium*) and take up new symbionts from surrounding environments, including the genus *Durusdinium*, which is also a generalist in host choice (Boulotte et al. 2016). *Durusdinium trenchii* is an opportunistic symbiont of the model sea anemone *Exaiptasia diaphana* (formerly *E. pallida* or *Aiptasia* sp.), but studies based on the transcriptome, proteome, and metabolome suggest that the alga may not be as beneficial as native *Breviolum* spp. symbionts (Matthews et al. 2017, 2018; Sproles et al. 2019). Such versatile, yet selective, many-to-many partnerships are the basis for the stability in cnidarian–dinoflagellate symbioses.

12.2.2 *Oophila*: Amphibian Egg-Loving Green Algae

Symbioses between amphibian embryos and green algae, the latter referred to as *Oophila*, have been known for more than a century (Orr 1888). Symbiont algae live and bloom inside the intracapsular region, rendering a conspicuous green hue to the eggs (Kerney et al. 2011). Previous studies suggest the symbiont algae benefit the host animals by increasing the concentration of oxygen via photosynthesis activity and possibly by producing additional molecules that promote embryonic development (Bachmann et al. 1985; Desnitskiy 2017). Conversely, the algae may flourish living inside the eggs by utilizing ammonia excreted by the embryos (Small et al. 2014). While most of the amphibian embryo–green algal associations appear ectosymbiotic in nature, at least one host salamander (i.e. the spotted salamander *Ambystoma maculatum*) also “allows” algal cells to penetrate into its own tissues and even cells, forming endosymbioses (Kerney et al. 2011, 2019). Globally, amphibian embryo–green algal symbioses have been noted from North America (the USA and Canada), Europe, Russia, and Japan (Desnitskiy 2017). Yet, molecular sequence data for the green algal symbionts are currently limited to those associated with four North American salamander and frog species (Kim et al. 2014; Kerney et al. 2019) and the Japanese black salamander (Muto et al. 2017). The phylogenetic analyses of 18S rDNA sequences suggest the algae that associate with these five amphibian embryos are closely related to each other, forming a clade (together with a few free-living *Chlamydomonas* strains) within the Chlamydomonadales (Kim et al. 2014). While Nema et al. (2019) suggested a polyphyly of *Oophila* species by showing several sequences that fall outside of the *Oophila* clade proposed by Kim et al. (2014), all of those sequences were generated from the isolated and cultured algae and not directly from field materials. Laboratory culturing of algae is typically highly selective, such that there is a good chance of minor, non-symbiont algal species outcompeting *Oophila* algae during the isolation process, as noted previously (Kim et al. 2014). Therefore, it is prudent, and perhaps even necessary, to compare the isolates to the source material(s) by sequencing both.

Within the *Oophila* clade, five subclades have been recognized (Kim et al. 2014; Muto et al. 2017). *Oophila* subclade III has been reported from the eggs of three North American amphibians (Fig. 12.3). Conversely, two *Ambystoma* species were shown to associate with at least two *Oophila* subclades (Fig. 12.3). Even a single amphibian embryo could bear multiple *Oophila* subclades (Kim et al. 2014). Together, these observations suggest that the algal switching of amphibian hosts is (and likely has been) occurring whilst at the same time, *Oophila* has been diversifying into genetically discernable subgroups, without developing host specificity. While *Oophila* has gotten the most attention in the context of their associations with amphibian eggs, they also occur in the water outside the amphibian hosts (Lin and Bishop 2015). This suggests that those free-living chlamydomonad taxa branching within the *Oophila* clade (Kim et al. 2014) may have the capacity to colonize amphibian eggs if given the opportunity. Finally, despite *Oophila*’s apparent loving of amphibian eggs, only a small number of unrelated amphibian taxa are colonized



Fig. 12.3 Taxonomic relationships between *Oophila* algae and their amphibian hosts (Kim et al. 2014; Muto et al. 2017; Kerney et al. 2019). Given a limited number of field samples analyzed to date, the actual picture is likely to be more complex than the version presented here

by *Oophila*. To summarize, the amphibian–algal symbioses are affected by the ecology and life history of host animals, whereas the algae appear to have developed lineage-specific traits that enable their proliferation inside the embryos of diverse amphibians.

12.2.3 *Chlorella*-Related Symbionts

The *Chlorella* clade (Trebouxiophyceae) is well known for its propensity to form symbioses with a wide range of freshwater organisms, including ciliates, (polyphyletic) testate amoebae, the centroheliozoan *Acanthocystis turfacea*, and invertebrates, such as *Hydra* (Pröschold et al. 2011; Pitsch et al. 2017). While there are several genera, including *Chlorella*, *Micractinium*, *Didymogenes*, and *Meyerella*, that have been described for the *Chlorella* clade, their SSU rRNA gene sequences are very similar (on average >98%) (Hoshina et al. 2010), suggesting relatively recent divergence times. In contrast, host eukaryotes that associate with members of the *Chlorella* clade are distributed widely and patchily across the eukaryotic tree of life.

The well-studied freshwater ciliate *Paramecium bursaria* associates primarily with *Chlorella variabilis* or *Micractinium reisseri* (Hoshina et al. 2010; Pröschold et al. 2011). Less frequently, *P. bursaria* has been found to form symbioses with *Chlorella vulgaris* or the chlorophycean green alga *Scenedesmus* sp. (Pröschold et al. 2011). Under laboratory conditions, *P. bursaria* can be induced to associate with non-native *Chlorella*-related algae (Summerer et al. 2007). While the algal–*P. bursaria* associations are mutualistic and stable over generations, aposymbiotic *P. bursaria* occurs in nature (Tonooka and Watanabe 2002), and it is possible to experimentally create symbiont-free *P. bursaria* by growing them in the dark (Summerer et al. 2007). To summarize, *P. bursaria* prefers associating with specific *Chlorella*-clade algae, but, in principle, it has not closed its door to other green algae as symbiotic partners.

In oligotrophic *Sphagnum* peatlands of the Northern Hemisphere, green-pigmented testate amoebae are abundant (Jassey et al. 2015). Referred to as the mixotrophic testate amoebae (MTA), they are a polyphyletic assemblage of microbial protists sharing convergently evolved morphological traits, such as the presence of a shell (or test) (Lara and Gomaa 2017). Taxonomically, MTA are distributed in three eukaryotic supergroups, including Amoebozoa, Rhizaria, and Stramenopiles (Lara and Gomaa 2017). All the surveyed MTA cells by Gomaa et al. (2014) harbored the symbionts of the *Chlorella* clade, with the vast majority of them having nearly identical *rbcL* gene sequences, likely representing a single species. This alga, TACS (Testae Amoeba *Chlorella* Symbiont), is most closely related to the *P. bursaria* symbiont *Chlorella variabilis* and appears exceptionally well adapted for living inside (polyphyletic) testate amoebae (Gomaa et al. 2014).

The *Chlorella*-related algae are also found living inside cells of the *viridissima* group of the freshwater cnidarian *Hydra* (Kobayakawa 2017). Their associations are mutualistic; the algae provide photosynthates to the host and in return receive amino acids and possibly additional growth factors that are synthesized by the host (Hamada et al. 2018). Molecular phylogenetic analyses of several strains of *Hydra viridissima* and their respective algal symbionts suggest an intriguing possibility of cospeciation as the host and algal phylogenies largely mirror each other despite the symbiont algae not being monophyletic within the *Chlorella* clade (Kawaida et al. 2013). Nonetheless, aposymbiotic *Hydra* strains can be colonized by non-native *Chlorella*-related algae under laboratory conditions, although questions remain concerning the long-term stability of such non-natural associations (Kessler et al. 1988; Kawaida et al. 2013).

Three algal groups—the *Symbiodiniaceae* dinoflagellates, *Oophila* clade, and *Chlorella* clade—reviewed here are characterized by their apparent propensity to associate with non-photosynthetic organisms of diverse taxonomic origins. A recent study suggests that *Symbiodiniaceae* originated ~160 mya and since has diversified (LaJeunesse et al. 2018), whereas their hosts are distributed patchily across different eukaryotic supergroups. While comparable molecular clock data are not currently available for *Oophila* and *Chlorella* clades, their respective hosts are also similarly patchy in taxonomic distribution. Such a pattern indicates that the eco-physiological context is a major driving force in host selection, although host–algal cospeciation may be occurring in some sub-lineages. Host switching is common among some members of these generalist algal groups, and laboratory rearing experiments showed host eukaryotes can often be induced to accommodate non-native relatives of algal symbionts. It is also noteworthy that some algal species within these generalist symbiont lineages are found free-living in nature. Overall, these algae appear to be undergoing abundant symbiosis experiments in nature (and experimentally), exemplified by their variability in host choices and stability in associations. The versatile nature seen in modern-day symbioses might also have been a feature of the protist–algal associations that gave rise to eukaryotic photosynthesis.

12.3 Population Thinking for Symbiosis

Considering the pattern of symbiont inheritance in the phagotrophic protist *Hatena*, not a single cell, but a ‘population’ (an ecological group of individuals) of algal cells is a more realistic representation of an ancestor of the symbionts found in modern-day *Hatena* cells. Here, a ‘population’ does not necessarily signify the capacity to interbreed with each other within a group of organisms, but it means that all the members share a geological and ecological niche. In this light, the cyanobacterial ancestor of the plastid was not necessarily a distinct individual, but is rather a conceptual framework encompassing a spatiotemporal continuum.

Given the examples of modern-day symbioses as discussed above, the capacity to endocytose symbiont cells was likely a characteristic of the ancestral hosts and their relatives; the algal symbiont, likewise, had the capacity to invade and stay inside a range of host cells. If the cyanobacterial or eukaryotic algal progenitor of the plastid originated from such a population, having a general characteristic of forming plural host–symbiont partnerships—whether the ancestral algal symbiont was a single cell or a group of plural cells (e.g. a few, hundreds, or millions)—would not make a significant impact on our ability to infer its origin. The cumulative effect of mutations and natural selection processes, genome rearrangements, and so on would ‘dilute’ signals of genetic variation originally present in algal symbionts over time. If, for example, the algal symbiont experienced a reduction in ploidy, the plurality of the allele information would be concealed.

Another challenge of phylogeny is the stability of the out-group, which serves as a reference point for in-group relationships. In a phylogenetic tree, some might see an out-group taxon (taxa) as a ‘static’ reference in inferring the internal relationships among in-group taxa of interest. However, the out-group is also dynamically evolving and changing over time. If one discusses the origin of plastids, modern cyanobacteria are often seen as out-group taxa, which have evolved independently of the plastids for more than a billion years. Therefore, one needs to be cautious when inferring evolutionary transitions from cyanobacteria to plastids as modern cyanobacteria are likely very different from their >1 billion-year-old ancestors in their genomes.

Cnidarian–algal relationships suggest that genotypic and phenotypic variations of the host animals and algae could provide more stability and flexibility by the formation of consortia in the face of fluctuating environmental conditions, thereby conferring an evolutionary advantage to the combined unit. A flexible many-to-many partnership may allow the cnidarian animals to find their optimally compatible symbiont algae (and vice versa) under a given condition. In an evolutionary time-scale, some characteristics are beneficial and others are not in one environmental context, while they may be opposite in another. A subpopulation may evolve through exclusive partnership into an obligate one, similar to the plastids in plant cells. Hundreds of millions of years in the future, if some corals and algae evolve to form an inseparable unit like modern plants with their distinct protist and cyanobacterial ancestors, it may be difficult to imagine their ancestors were a bit loose in their associations and had sustained a variety of many-to-many relationships in nature.

A classical typological view on an ancestor of the plastid does not seem to fit this flexible host–symbiont partnership accommodating substantial genomic variations. Even if a host cell successfully acquires a symbiont, the offspring generation may lose it, but then the next generation may acquire another symbiont related to the one that their parental lineages once had (Fig. 12.1). In a typological view, the ‘lost generation’ and the discontinuity in the symbiont pedigree might be a problem, but it would be no surprise if such a scenario indeed played out in the early stages of plastid evolution. Rather, in this case, the hosts and the symbionts may better be described as closely associating populations of different organisms.

Population thinking implies the symbiotic spectrum is applicable even within a single population; some members in a symbiont population may be more beneficial to hosts (mutualistic), while others may be less productive or more parasitic (Rueckert et al. 2019). There is merely a conceptual distinction between mutualists and parasites, and it is often extremely difficult to apply this concept to organisms in nature where the boundary is diffusing and changing. The cost–benefit balance in two associating organisms should be condition-dependent, and the degree of their dependence should differ among individuals. Under each distinct environmental or seasonal condition, symbionts can move along an axis of the continuum between mutualism and parasitism. Consequently, the symbionts behave like a population with variations.

12.4 Conclusion

Free-living ancestors of plastids may have been a diversified population of generalist cyanobacteria that include multiple individuals (i.e. cells, for we envisage the ancestors as unicellular organisms) (Fig. 12.4). This hypothesis suggests that the plastids of eukaryotic algae may be traced back to multiple ancestral cyanobacterial cells, which constituted together with their free-living relatives, a coherent ancestral lineage. Over a long period of time, information on the ancestral population and resolution to distinguish between individuals or subpopulations within the population gets inherently lost. Consequently, a phylogenetic lineage is informed from a single or a few sampled individuals, which do not accurately represent the original population structure. Examples of modern-day host–symbiont relationships suggest that ecological, environmental, and developmental conditions, in addition to phylogenetic constraints, have forged various platforms for endosymbioses. Such population thinking may be helpful in resolving issues surrounding the early evolution of plastids. For example, from the perspective of typological thinking, the presence of proteobacterium-type RuBisCO subunits in red algal plastid genomes creates a perplexing situation as green algae and glaucophytes instead have cyanobacterium-type RuBisCO subunits (Delwiche and Palmer 1996). A usual solution to signal conflict such as this is to invoke a lateral gene transfer event, which is based on typological thinking whereby the common ancestor should have a single distinct genotype. Population thinking, however, does not require such a strict

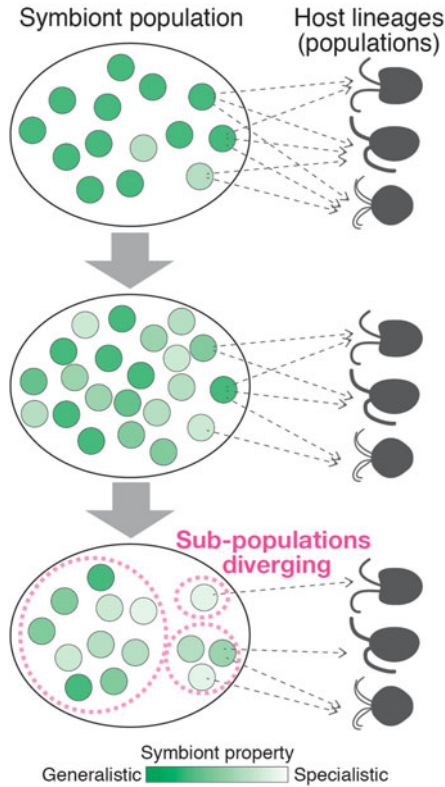


Fig. 12.4 A cartoon illustrating a scenario of plastid evolution. Under this scenario, the plastids originated from a population of a generalist algal symbiont. We hypothesize that such an ancestral generalist symbiont had the capacity to establish partnerships with multiple host lineages. Generalist symbionts are expected to be more stable and persistent through a long time period than specialist symbionts, which could lose their ecological niches more easily (unless their partners proliferate and become abundant). Over time, information on genetic variation within the ancient generalist population is diluted, potentially leading to the fallacy of oversimplification of evolutionary relationships by the observer

assumption, rather it envisages variation and diversity in the genotypes of the common ancestral population. As the research community gathers large-scale genomic and molecular biological data from diverse algal species, we likely face more perplexing cases with our usual pattern-centric approaches. Population thinking may open up a way to change our perspective on how to analyze and interpret data and allow us to draw a more realistic picture of ancient evolutionary processes.

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References

- Adl SM, Bass D, Lane CE et al (2019) Revisions to the classification, nomenclature, and diversity of eukaryotes. *J Eukaryot Microbiol* 66:4–119
- Bachmann A, Beard VL, McCarty PL (1985) Performance characteristics of the anaerobic baffled reactor. *Water Res* 19:99–106
- Baldauf SL, Roger AJ, Wenk-Siefert I, Doolittle WF (2000) A kingdom-level phylogeny of eukaryotes based on combined protein data. *Science* 290:972–977
- Boulotte NM, Dalton SJ, Carroll AG et al (2016) Exploring the *Symbiodinium* rare biosphere provides evidence for symbiont switching in reef-building corals. *ISME J* 10:2693–2701
- Burki F, Kaplan M, Tikhonenkov DV et al (2016) Untangling the early diversification of eukaryotes: a phylogenomic study of the evolutionary origins of Centrohelida, Haptophyta and Cryptista. *Proc R Soc B Biol Sci* 283:20152802
- Cavalier-Smith T (1982) The origins of plastids. *Biol J Linn Soc* 17:289–306
- Cenci U, Sibbald SJ, Curtis BA et al (2018) Nuclear genome sequence of the plastid-lacking cryptomonad *Goniomonas avonlea* provides insights into the evolution of secondary plastids. *BMC Biol* 16:137
- Delwiche CF, Palmer JD (1996) Rampant horizontal transfer and duplication of rubisco genes in eubacteria and plastids. *Mol Biol Evol* 13:873–882
- Desnitskiy A (2017) A symbiosis of amphibian embryos and larvae with unicellular green algae. *Russ J Herpetol* 24:223–227
- Dumas E, Feurtey A, Rodríguez de la Vega RC et al (2020) Independent domestication events in the blue-cheese fungus *Penicillium roqueforti*. *Mol Ecol* (Early View online)
- Gawryluk RMR, Tikhonenkov DV, Hehenberger E et al (2019) Non-photosynthetic predators are sister to red algae. *Nature* 572:240–243
- Gomaa F, Kosakyana A, Heger TJ et al (2014) One alga to rule them all: unrelated mixotrophic testate amoebae (Amoebozoa, Rhizaria and Stramenopiles) share the same symbiont (Trebouxiophyceae). *Protist* 165:161–176
- Hamada M, Schröder K, Bathia J et al (2018) Metabolic co-dependence drives the evolutionarily ancient *Hydra–Chlorella* symbiosis. *elife* 7:e35122
- Hoshina R, Iwataki M, Imamura N (2010) *Chlorella variabilis* and *Micractinium reisseri* sp. nov. (Chlorellaceae, Trebouxiophyceae): redescription of the endosymbiotic green algae of *Paramecium bursaria* (Peniculia, Oligohymenophorea) in the 120th year. *Phycol Res* 58:188–201
- Jassey VEJ, Signarbieux C, Hättenschwiler S et al (2015) An unexpected role for mixotrophs in the response of peatland carbon cycling to climate warming. *Sci Rep* 5:1–10
- Kawaida H, Ohba K, Koutake Y et al (2013) Symbiosis between hydra and chlorella: molecular phylogenetic analysis and experimental study provide insight into its origin and evolution. *Mol Phylogenet Evol* 66:906–914
- Keeling PJ, Archibald JM (2008) Organelle evolution: What's in a name? *Curr Biol* 18:R345–R347

- Kerney R, Kim E, Hangarter RP et al (2011) Intracellular invasion of green algae in a salamander host. *Proc Natl Acad Sci* 108:6497–6502
- Kerney R, Leavitt J, Hill E et al (2019) Co-cultures of *Oophila amblystomatis* between *Ambystoma maculatum* and *Ambystoma gracile* hosts show host-symbiont fidelity. *Symbiosis* 78:73–85
- Kessler E, Huss VAR, Rahat M (1988) Species-specific ability of *Chlorella* strains (Chlorophyceae) to form stable symbioses with *Hydra viridis*. *Plant Syst Evol* 160:241–246
- Kim E, Lin Y, Kerney R et al (2014) Phylogenetic analysis of algal symbionts associated with four North American amphibian egg masses. *PLoS One* 9:e108915
- Kobayakawa Y (2017) Symbiosis between green algae and hydra. In: Grube M, Muggia L, Seckenbach J (eds) *Algal and cyanobacteria symbioses*. World Scientific, London, pp 347–369
- LaJeunesse TC, Parkinson JE, Gabrielson PW et al (2018) Systematic revision of Symbiodiniaceae highlights the antiquity and diversity of coral endosymbionts. *Curr Biol* 28:2570–2580
- Lara E, Gomaa F (2017) Symbiosis between testate amoebae and photosynthetic organisms. In: Grube M, Muggia L, Seckenbach J (eds) *Algal and cyanobacteria symbioses*. World Scientific, London, pp 399–419
- Lhee D, Ha J-S, Kim S et al (2019) Evolutionary dynamics of the chromatophore genome in three photosynthetic *Paulinella* species. *Sci Rep* 9:1–11
- Lin Y, Bishop CD (2015) Identification of free-living *Oophila amblystomatis* (Chlorophyceae) from yellow spotted salamander and wood frog breeding habitat. *Phycologia* 54:183–191
- Martin G, Joseph S, Lucia M (2016) *Algal and cyanobacteria symbioses*. World Scientific, London
- Maruyama S, Kim E (2013) A modern descendant of early green algal phagotrophs. *Curr Biol* 23:1081–1084
- Matthews JL, Crowder CM, Oakley CA et al (2017) Optimal nutrient exchange and immune responses operate in partner specificity in the cnidarian-dinoflagellate symbiosis. *Proc Natl Acad Sci* 114:13194–13199
- Matthews JL, Oakley CA, Lutz A et al (2018) Partner switching and metabolic flux in a model cnidarian–dinoflagellate symbiosis. *Proc R Soc B Biol Sci* 285:20182336
- McFadden GI (2001) Primary and secondary endosymbiosis and the origin of plastids. *J Phycol* 37:951–959
- Mies M, Sumida PYG, Rädecker N, Voolstra CR (2017) Marine invertebrate larvae associated with *Symbiodinium*: a mutualism from the start? *Front Ecol Evol* 5:56
- Moreira D, Le Guyader H, Philippe H (2000) The origin of red algae and the evolution of chloroplasts. *Nature* 405:69–72
- Muto K, Nishikawa K, Kamikawa R, Miyashita H (2017) Symbiotic green algae in eggs of *Hynobius nigrescens*, an amphibian endemic to Japan. *Phycol Res* 65:171–174
- Nakayama T, Inagaki Y (2017) Genomic divergence within non-photosynthetic cyanobacterial endosymbionts in rhopalodiacean diatoms. *Sci Rep* 7:1–8
- Nema M, Hanson ML, Müller KM (2019) Phylogeny of the egg-loving green alga *Oophila amblystomatis* (Chlamydomonadales) and its response to the herbicides atrazine and 2,4-D. *Symbiosis* 77:23–39
- Okamoto N, Inouye I (2005) A secondary symbiosis in progress? *Science* 310:287–287
- Okamoto N, Inouye I (2006) *Hatena arenicola* gen. et sp. nov., a katablepharid undergoing probable plastid acquisition. *Protist* 157:401–419
- Orr H (1888) Note on the development of amphibians, chiefly concerning the central nervous system; with additional observations on the hypophysis, mouth, and the appendages and skeleton of the head. *Q J Microsc Sci New Ser* 29:295–324
- Pitsch G, Adamec L, Dirren S et al (2017) The green *Tetrahymina utriculariae* n. sp. (Ciliophora, Oligohymenophorea) with its endosymbiotic algae (*Micractinium* sp.), living in traps of a carnivorous aquatic plant. *J Eukaryot Microbiol* 64:322–335
- Pochon X, Putnam HM, Gates RD (2014) Multi-gene analysis of *Symbiodinium* dinoflagellates: a perspective on rarity, symbiosis, and evolution. *PeerJ* 2:e394
- Pröschold T, Darienko T, Silva PC et al (2011) The systematics of *Zoochlorella* revisited employing an integrative approach. *Environ Microbiol* 13:350–364

- Rodríguez-Ezpeleta N, Brinkmann H, Burey SC et al (2005) Monophyly of primary photosynthetic eukaryotes: green plants, red algae, and glaucophytes. *Curr Biol* 15:1325–1330
- Rueckert S, Betts EL, Tsaousis AD (2019) The symbiotic spectrum: where do the gregarines fit? *Trends Parasitol* 35:687–694
- Small DP, Bennett RS, Bishop CD (2014) The roles of oxygen and ammonia in the symbiotic relationship between the spotted salamander *Ambystoma maculatum* and the green alga *Oophila amblystomatis* during embryonic development. *Symbiosis* 64:1–10
- Sproles AE, Oakley CA, Matthews JL et al (2019) Proteomics quantifies protein expression changes in a model cnidarian colonised by a thermally tolerant but suboptimal symbiont. *ISME J* 13:2334–2345
- Strassert JFH, Jamy M, Mylnikov AP et al (2019) New phylogenomic analysis of the enigmatic phylum Telonemia further resolves the eukaryote tree of life. *Mol Biol Evol* 36:757–765
- Stroud JT, Losos JB (2020) Bridging the process-pattern divide to understand the origins and early stages of adaptive radiation: a review of approaches with insights from studies of *Anolis* lizards. *J Hered* 111:33–42
- Summerer M, Sonntag B, Sommaruga R (2007) An experimental test of the symbiosis specificity between the ciliate *Paramecium bursaria* and strains of the unicellular green alga *Chlorella*. *Environ Microbiol* 9:2117–2122
- Tonooka Y, Watanabe T (2002) A natural strain of *Paramecium bursaria* lacking symbiotic algae. *Eur J Protistol* 38:55–58