COMMENTARY
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Algal genes in aplastidic eukaryotes are not necessarily derived from historical plastids

Jipei Yue and Jinling Huang*
Department of Biology; East Carolina University; Greenville, NC USA; Key Laboratory of Biodiversity and Biogeography; Kunming Institute of Botany; Chinese Academy of Sciences; Kunming, China

In photosynthetic eukaryotes, many genes were transferred from plastids or algal endosymbionts to nuclear genomes of host cells. These transferred genes are often considered genetic footprints of plastids. However, genes of algal origin have also been detected in some plastid-lacking eukaryotes, and these genes are often cited as evidence of historical plastids. In this paper, we discuss two recent publications about algal genes in plastid-lacking eukaryotes. Both studies highlight the point that algal genes are not exclusively derived from historical plastids. Instead, the findings show that gene acquisition through feeding activities is a plausible explanation.

Keywords: endosymbiosis, photosynthetic eukaryotes, gene transfer, phagotroph

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Correspondence to: Jinling Huang;
Email: huangj@ecu.edu

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Endosymbiosis and Eukaryotic Evolution

Endosymbiosis refers to an ecological process in which one partner organism lives within another and both can exhibit various degrees of integration. The origin and diversification of eukaryotes have been tremendously shaped by a series of endosymbioses, the most significant of which involved the origin of mitochondria from α-proteobacteria and the origin of plastids from cyanobacteria.[1] During the course of evolution, mitochondria and plastids gradually transferred their genes to host cells, a process that is often referred to as endosymbiotic gene transfer (EGT). For many of these transferred genes, their protein products are re-imported to mitochondria or plastids for functions. Such genetic integration is often considered the hallmark of organelles.[2]

All contemporary mitochondria evolved from a single endosymbiotic event.[3] By contrast, the history of plastid evolution is far more complex. Plastids of green algae, red algae and glaucophytes evolved from a cyanobacterial endosymbiont, and they were later spread to multiple other eukaryotic lineages such as heterokonts, apicomplexans, dinoflagellates and euglenids through secondary or tertiary (eukaryotic/eukaryotic) endosymbioses.[4,5] Both mitochondria and plastids could be secondarily lost, leading to puzzling relationships among eukaryotes with or without such organelles. For example, several anaerobic parasites, including Entamoeba, Giardia, Trichomonas and Trachipleistophora were thought to lack mitochondria. These taxa and their close relatives were once placed in a group Archezoa, which presumably diverged before the origin of mitochondria.[6,7] The Archezoa hypothesis was abandoned later after findings of vestigial mitochondria or mitochondrial genes in amitochondrial eukaryotes.[8-12] Likewise in plastid-lacking eukaryotes, traces of plastids- or algae-derived genes have been frequently cited as evidence for the past existence of plastids in their evolutionary histories.[13-17] The interpretation of these algal genes has significant implications in at least two intertwined aspects: (1) because plastids span multiple major eukaryotic lineages, they are often considered shared derived characters for some super-groups in large-scale phylogenetic reconstruction; (2) mechanisms of gene acquisition in eukaryotes, i.e., how did these algal genes end up in a distantly related species? Recent analyses

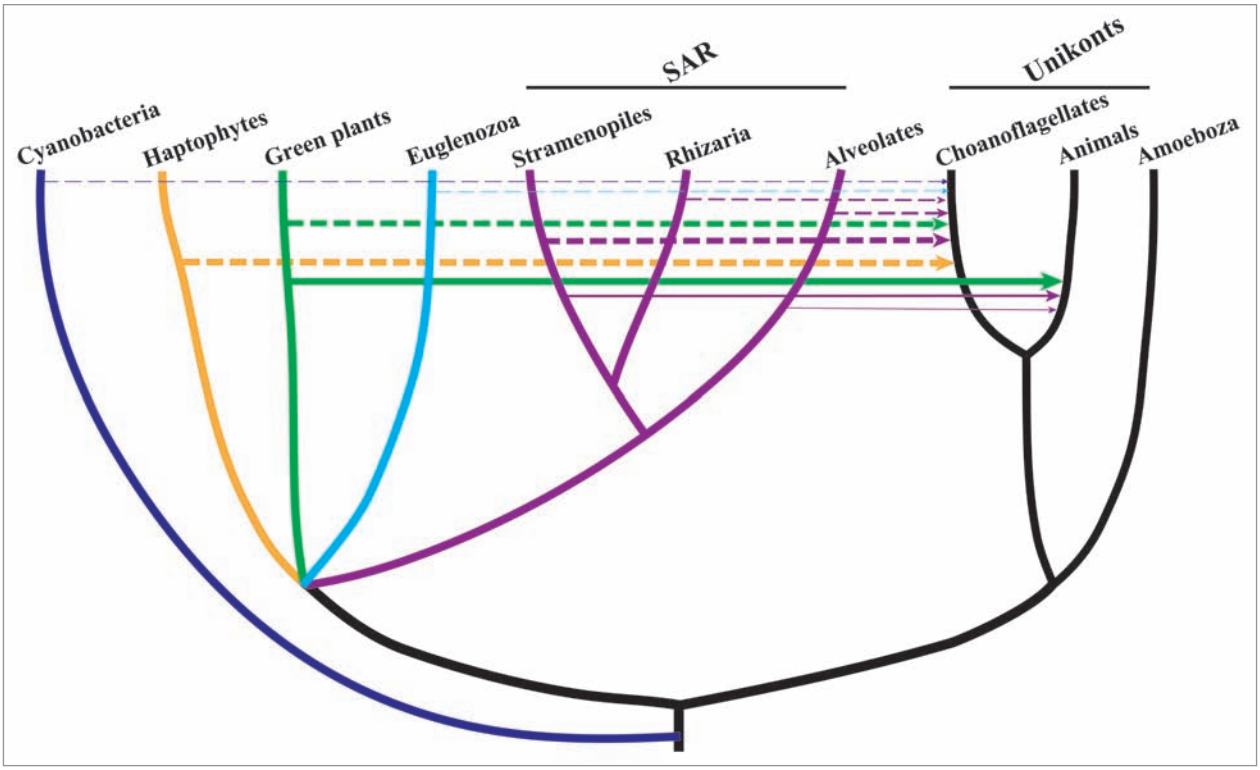

Figure 1. An illustrative diagram demonstrating that putative HGT led to algal genes in choanoflagellates and the ancestral animal.[18,19] Horizontal lines and arrows show HGT donors and recipients. Relationships of different eukaryotes are largely based on.[27] Only groups discussed in the text are shown. Branch lengths are roughly drawn for displaying purpose and not correspondent to accurate evolutionary distances.

show that, although physical association in the form of cyanobacterial or algal endosymbioses often left genetic footprints in nuclear genomes of host cells, phylogenetic signal of algal genes in plastid-lacking eukaryotes might not exclusively constitute evidence for historical plastids.

Algal Genes in Choanoflagellates and Animals

Choanoflagellates and animals are closely related. No anatomical evidence of plastids has ever been reported and, importantly, no hypothesis based on any credible data ever posits the existence of plastids in these two lineages. Because most choanoflagellates are free-living unicellular organisms (and animals had a free-living unicellular ancestor), which tend to acquire genes from other species (horizontal gene transfer or HGT hereafter), the availability of genome sequence data for these two lineages offers an excellent opportunity to test whether algal genes are mostly derived from plastids or algal endosymbionts. In two recent phylogenomic studies, over 100 and 90 genes of possible algal origin were identified in the genomes of choanoflagellate *Monosiga brevicollis* and tunicate *Ciona intestinalis* respectively.[18,19] The numbers of algal genes detected in these two species are comparable to those reported in the oomycete Phytophthora[20] and higher than those in the apicomplexan Cryptosporidium[14] and the ciliates,[16] all of which have been suggested to contain historical plastids. Therefore, if we strictly consider the number of acquired algal genes, a suggestion would have to be made that both Monosiga and Ciona had plastids.

Baring phylogenetic artifacts, an alternative explanation for the presence of algal genes in Monosiga and Ciona is the gene ratchet mechanism, often called "you are what you eat," proposed by W. Ford Doolittle.[21] Under this scenario, phagotrophic protists may acquire foreign genes from food sources. *Monosiga brevicollis* is a unicellular organism feeding on bacteria and marine phytoplankton. *Ciona intestinalis* has a similar lifestyle, but is multicellular. Algal genes detected in Ciona are closely related to homologs from multiple other animal groups, suggestive of ancient HGT events by the ancestral animal. Theoretically, genes derived from a single endosymbiont should be related to a specific donor whereas those from independent feeding activities would involve miscellaneous groups. In practice, such an expectation rarely holds due to various reasons including insufficient taxonomic sampling and gene losses.[22] Nevertheless, most algal genes identified in Monosiga are derived from haptophytes and diatoms. Algal genes in Ciona and other animals were also predominantly from microscopic green plants and chromists (**Fig. 1**). All of the above organisms are widespread and major components in marine ecosystems. Therefore, without any additional evidence for historical plastids in choanoflagellates and animals, it appears at least equally likely that these algal genes were acquired through feeding activities. Further analyses suggest that genes acquired from algae and bacteria account approximately for 4.4% of the nuclear genome of *M. brevicollis* (Huang et al.,

unpublished data). In this case, any suggestion of historical plastids in Monosiga based strictly on the number of acquired genes could lead to suggestions of multiple other bacterial endosymbionts, which appears to be a very unlikely scenario.

When Should Endosymbioses be Invoked as an Explanation for the Observed Data?

Because HGT is widespread in unicellular eukaryotes and exists in multicellular eukaryotes, it is somewhat expected that all eukaryotes would contain genes acquired, either recently or anciently, from other species. However, the intensity of phylogenetic signal from a specific donor group may not always be related to the presence/absence of a symbiont. In terms of mechanisms of algal gene acquisition, not every algal gene carries the same weight when assessing the existence of historical plastids. Only genes that show clear plastid affinity may provide more convincing evidence. For instance, multiple algal genes were identified in Cryptosporidium, but only the gene encoding leucine aminopeptidase is clearly of plastid origin.[14] This gene forms a strongly supported monophyletic group with plastid precursors of other apicomplexans and plants as well as with cyanobacterial homologs. All other identified algal genes in Cryptosporidium are important as additional evidence, but they are not decisive and could be explained by other scenarios.

For many nuclear-encoded plastid-derived genes, their protein products often function in the original plastids. Loss of plastids would undoubtedly affect the retention of algal genes that are related to plastid functionality. It is also possible that acquired genes might have been secondarily lost over time, especially if the recipient lineage was subject to major ecological, physiological or genomic shift. If so, the available methods, either based on phylogenetic analyses or statistical genomics,[23] may not be able to accurately detect traces of historical plastids. The absence of algal genes in a eukaryote should not be interpreted as evidence for absence of historical plastids. On the other hand, unless other circumstantial evidence exists, there are always other alternative, sometimes more plausible, explanations for algal genes identified in a plastid-lacking eukaryote.

When should a historical plastid (or a stable endosymbiont over an extended period of time) be inferred? There probably is no single best criterion to address this question. However, because any major evolutionary event such as plastids or endosymbionts will likely trigger serial changes at molecular, cellular and morphological levels in the recipient organism, a historical endosymbiont may be more reliably inferred based on collective evidence, including ideally a strong and consistent phylogenetic signal from multiple genes. The notion of a historical plastid in Cryptosporidium is less questionable largely because of the existence of plastids in many other extant apicomplexans. Such a plastid was speculated even before the finding of algal genes in Cryptosporidium.[24,25] In this case, the algal genes identified in Cryptosporidium genome only provided additional supporting evidence for a historical plastid. Similarly, chlamydial genes in photosynthetic eukaryotes were attributed to an obsolete endosymbiont in the ancestor of Plantae (red algae, green plants and glaucophytes).[26] Such a suggestion was made not only based on the number of chlamydial genes in photosynthetic eukaryotes, but also largely on the fact that all extant chlamydiae are obligate endosymbionts.[26] In the case of choanoflagellates and animals, no any other evidence exists for historical plastids in these lineages. Because the recipients in both cases are phagotrophic and because acquired genes are mostly from major components of marine food web, independent HGT through feeding activities appears to be a more plausible explanation.

Conclusion

Inference of historical plastids or endosymbionts from genomic data is not only highly important, but also highly complex and controversial. The two studies on choanoflagellates and animals suggest that algal genes in plastid-lacking eukaryotes are not necessarily linked to historical plastids or algal endosymbionts. To better understand the mechanisms of algal gene acquisition and evolutionary histories of the host lineages, independent evidence from other aspects should be considered before historical plastids are invoked as an explanation.

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