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Letters

Chromalveolate plastids: direct descent or multiple endosymbioses?

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In their recent Opinion article on endosymbiosis and the tree of life, Lane and Archibald [1] focused on the chromalveolate hypothesis [2] that all eukaryotes with plastids derived secondarily from a red alga are descended directly from a single, common ancestor. They pointed out that no data actually support a monophyletic grouping of all these taxa, and highlighted growing phylogenetic conflicts with the traditional Chromalveolata. To resolve these contradictions, they offered an expanded version of the 'chromalveolates,' with additional independent plastid losses from the added heterotrophic lineages. More recent phylogenetic analyses suggest that, if a model of direct vertical descent of these plastids is to be accommodated, further expansions of the original Chromalveolata are needed [3–5]. In fact, several investigations, including perhaps the broadest combined sampling of genes and taxa to date [3], indicate that Archaeplastida (=Plantae) is descended from the same ancestor as chromalveolate taxa [3–5]. Interpreted within the confines of the chromalveolate model of plastid descent, this would require a secondary red algal endosymbiont to have existed before red algae ever originated, clearly an evolutionary impossibility (Figure 1).

The failure to find support for a monophyletic Chromalveolata is particularly significant given that predicted phylogenetic artifacts, described by Lane and Archibald [1], should favor that association. Therefore, in light of growing difficulties with the chromalveolate hypothesis, we propose an alternative model of plastid evolution, a single secondary red algal endosymbiosis followed by tertiary endosymbioses that moved these plastids between unrelated, previously heterotrophic taxa (Figure 1). We argue that serial endosymbioses are more consistent with available data than is the vertical descent required by the chromalveolate model.

Plastid-related characters do not favor the chromalveolate model

Much of the published support for chromalveolates comes from plastid-derived characters, particularly shared products of endosymbiotic gene replacement such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [1]. Phylogenies of *gapdh* and similar genes are complicated by duplications and horizontal gene transfers [6], however, and are inconsistent with the pattern of vertical plastid inheritance indicated by the chromalveolate model [7]. In fact, it is not yet clear that plastid characters as a whole support a single secondary origin of chromalveolate plastids [8], or even that all 'chromalveolate' plastids are derived originally from a red alga [9]. More importantly, any plastid-related character that links two or more chromalveolate taxa is, by itself, equally consistent with serial plastid transfer as it is with direct descent. In several cases, these data are more consistent with tertiary transfers [7,10]. Thus, as Lane and Archibald reiterate, plastid characters themselves do not support the chromalveolate model directly; rather, it is the enormous *a priori* weight placed on plastid loss over plastid gain when interpreting those characters [1].

Complete loss of plastids is a rare evolutionary event

From the beginning, the Chromalveolata was based largely on the proposition that converting endosymbionts into organelles must be so difficult that plastid losses should be favored enormously over gains [1,2]. This argument has not received empirical support. Although there are numerous cases of loss of photosynthesis from plants and algae, evidence for complete plastid loss from a verified photosynthetic lineage is exceedingly rare. Plastids fulfill numerous vital, non-photosynthetic functions (e.g. fatty acid and isoprenoid biosynthesis), and nearly all known plant and algal parasites retain reduced forms of the organelle [11]. The numerous independent losses from free-living species required by the chromalveolate model are even more unlikely [12]. In fact, upon closer inspection, reduced plastids have been found in several derived lineages once thought to have lost them completely [13,14].

Citing several recent studies, Lane and Archibald [1] suggest that the presence of algal-like genes in heterotrophic or parasitic chromalveolates represents direct evidence for plastid loss. We strongly disagree with this interpretation. Recovery of small numbers of such sequences in aplastidic protists could be explained by stochastic variation and unidentified phylogenetic biases within large, complicated genomes [15], or by horizontal gene transfer from countless prey species (the 'you are what you eat' hypothesis) [16]. For example, phagotrophic ciliates harbor long-term algal endosymbionts and contain various genes from prey species in their nuclei [17]. There appears to be no clear justification for an *a priori* assumption that putatively 'algal' genes favor plastid losses over reasonable alternative hypotheses.

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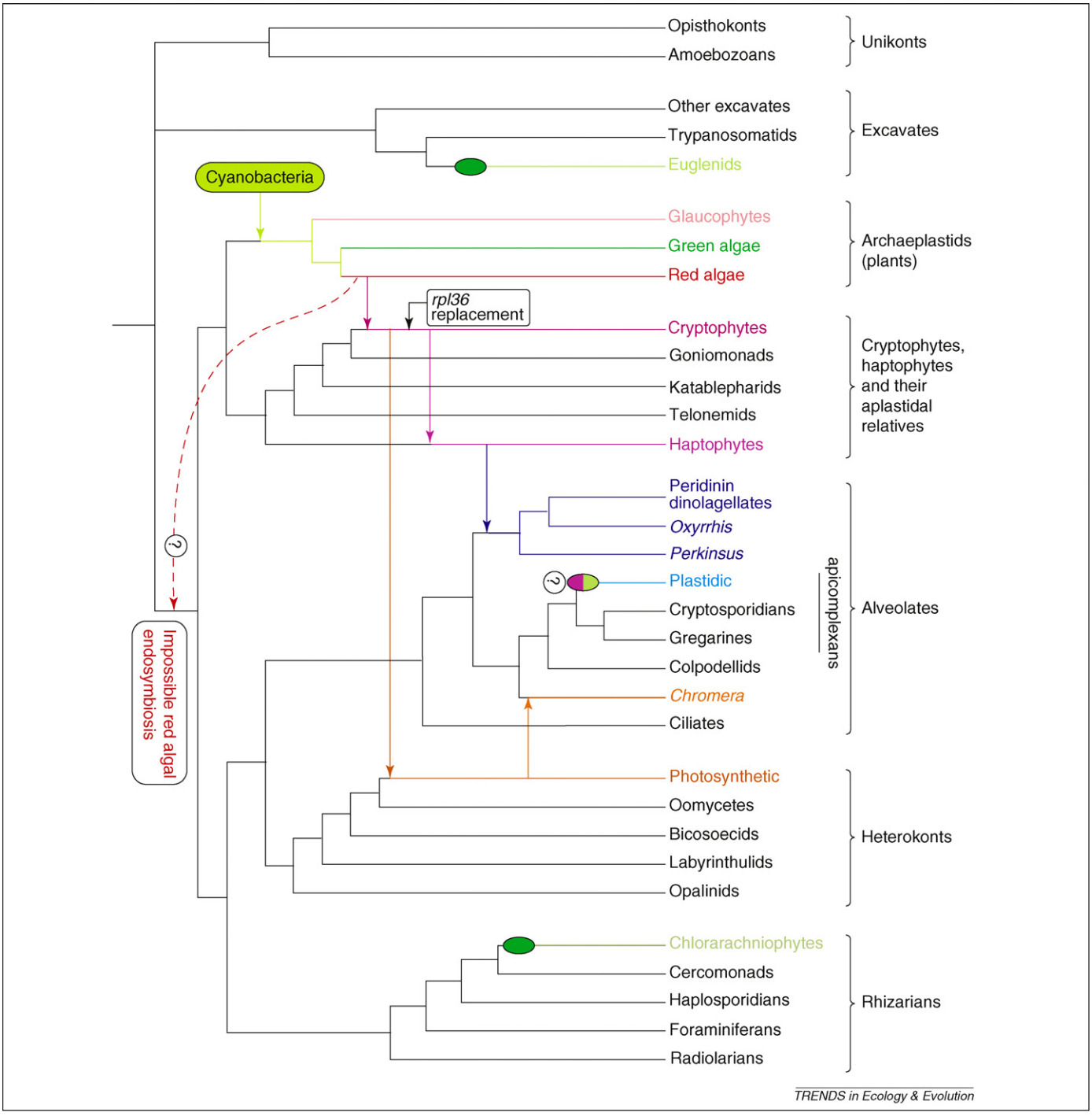

Figure 1. Evolution of chlorophyll *c*-containing plastids through tertiary endosymbioses. The tree topology reflects the results of several recent phylogenomic investigations [3–5]. We presume that all modern plastids are derived from a single cyanobacterial endosymbiosis in the common ancestor of the kingdom Archaeplastida (=Plantae). These primary plastids then were spread to distinct eukaryotic lineages by multiple endosymbioses involving green algae (green ellipses) as well as red algae and algae with red algal-derived plastids (arrows). Cryptophytes acquired their plastid directly from a red alga via secondary endosymbiosis. Our tertiary model postulates that, before acquisition of a eubacterial *rpl36* gene [18], a photosynthetic cryptophyte was transferred to one group within the Heterokonta, and a heterokont was adopted subsequently by the *Chromera* lineage. The predecessor of haptophytes engulfed a different cryptophyte, containing the new *rpl36* gene, and a haptophyte was further enslaved by the common ancestor of peridinin dinoflagellates, including *Oxyrrhis* and *Perkinsus*. The evolutionary origin of the apicoplast remains controversial, with data suggesting an origin from either the red [7] or green [9,19] lineages. The chromalveolate model requires the very early acquisition of a red alga shown in the common ancestor of cryptophytes, haptophytes, alveolates, heterokonts and rhizarians. To accommodate the terminal branching positions of most plastid-bearing taxa, it must presume multiple independent plastid losses from all aplastidic descendent lineages, shown on this tree with gray branches. If the Archaeplastida also descended from this same common ancestor [3–5], the chromalveolate model becomes impossible. A tertiary model pictured minimizes the number of plastid losses, provides an explanation for modifications of chlorophyll *c* plastids (e.g. distinct number of envelope membranes in different lineages) and reconciles plastid phylogenies with those of host cells [7,10]. Well-established tertiary plastid origins in non-peridinin dinoflagellates (*Lepidodinium*, *Karenia* and *Kryptopteridinium*) [7] are omitted for clarity, but do not differ between the two models.

The phylogenetic distribution of chromalveolate plastids favors independent tertiary origins

Red algal descended plastids are found in clearly polyphyletic taxa, each nested within lineages containing aplastidic relatives (Figure 1). Moreover, photosynthetic taxa frequently occur at derived positions, with multiple heterotrophic groups branching ancestrally. The number of independent losses required to account for this pattern is unreasonable (see above); it is far more parsimonious to presume several tertiary transfers (Figure 1).

It is clear that plastid evolution in eukaryotes has resulted in a complex phylogenetic pattern with many conflicts among molecular data. Although the chromalveolate model cannot be rejected formally, neither should it serve as the *a priori* framework for interpreting plastid character evolution, endosymbiotic gene transfers or other intragenomic phylogenetic conflicts. We argue that an alternative model of serial tertiary endosymbioses is more consistent with available data, and should be taken into account in phylogenomic investigations of eukaryotic diversity.

Acknowledgements

We are grateful to Katja Bargum for valuable comments on the manuscript. A.B. is supported by funds from Wrocław University grant BS/1018/2008.

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0169-5347/$ – see front matter
doi:10.1016/j.tree.2008.11.003 Available online 4 February 2009

Letters Response

Reply to Bodył, Stiller and Mackiewicz: "Chromalveolate plastids: direct descent or multiple endosymbioses?"

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Bodył, Stiller and Mackiewicz [1] have written in response to our recent Opinion piece [2] in which we discussed progress and problems in resolving the deepest branches of the eukaryotic tree using phylogenomics. The focus of our paper was the challenge of inferring accurate phylogenies from multigene data sets that include sequences from organisms that have acquired plastids by secondary (i.e. eukaryote-eukaryote) endosymbiosis and whose nuclear genomes are a composite of genes from two (or more) distinct nucleocytoplasmic lineages. We highlighted the controversial eukaryotic supergroup 'chromalveolates' as a case in point and discussed changing views on the evolution of photosynthesis in this diverse lineage in light of recent large-scale phylogenomic studies. Bodył, Stiller and Mackiewicz [1] take issue with the chromalveolate hypothesis [3] and our views on the tempo and mode of

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