

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 aplastic 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.

References

- Lane, C.E. and Archibald, J.M. (2008) The eukaryotic tree of life: endosymbiosis takes its TOL. *Trends Ecol. Evol.* 23, 268–275
- Cavalier-Smith, T. (1999) Principles of protein and lipid targeting in secondary symbiogenesis: euglenoid, dinoflagellate, and sporozoan plastid origins and the eukaryote family tree. *J. Eukaryot. Microbiol.* 46, 347–366
- Burki, F. *et al.* (2008) Phylogenomics reveals a new ‘megagroup’ including most photosynthetic eukaryotes. *Biol. Lett.* 4, 366–369
- Patron, N.J. *et al.* (2007) Multiple gene phylogenies support the monophyly of cryptomonad and haptophyte host lineages. *Curr. Biol.* 17, 887–891
- Kim, E. and Graham, L.E. (2008) EEF2 analysis challenges the monophyly of Archaeplastida and Chromalveolata. *PLoS ONE* 3, e2621
- Takishita, K. *et al.* (2008) Origins of plastids and glyceraldehyde-3-phosphate dehydrogenase genes in the green-colored dinoflagellate *Lepidodinium chlorophorum*. *Gene* 410, 26–36
- Bodyl, A. and Moszczyński, K. (2006) Did the peridinin plastid evolve through tertiary endosymbiosis? A hypothesis. *Eur. J. Phycol.* 41, 435–448
- Iida, K. *et al.* (2007) Assessing the monophyly of chlorophyll-*c* containing plastids by multi-gene phylogenies under the unlinked model conditions. *Mol. Phylogenet. Evol.* 45, 227–238
- Auch, A.F. *et al.* (2006) Genome BLAST distance phylogenies inferred from whole plastid and whole mitochondrion genome sequences. *BMC Bioinformatics* 7, 350
- Bodyl, A. (2005) Do plastid-related characters support the chromalveolate hypothesis? *J. Phycol.* 41, 712–719
- de Koning, A.P. and Keeling, P.J. (2004) Nucleus-encoded genes for plastid-targeted proteins in *Helicosporidium*: functional diversity of a cryptic plastid in a parasitic alga. *Eukaryot. Cell* 3, 1198–1205
- Borza, T. *et al.* (2005) Multiple metabolic roles for the nonphotosynthetic plastid of the green alga *Prototheca wickerhamii*. *Eukaryot. Cell* 4, 253–261
- Sekiguchi, H. *et al.* (2002) Vestigial chloroplasts in heterotrophic stramenopiles *Pteridomonas danica* and *Ciliophrys infusionum* (Dictyochophyceae). *Protist* 153, 157–167
- Matsuzaki, M. *et al.* (2008) A cryptic algal group unveiled: a plastid biosynthesis pathway in the oyster parasite *Perkinsus marinus*. *Mol. Biol. Evol.* 25, 1167–1179
- Leigh, J.W. *et al.* (2008) Testing congruence in phylogenomic analysis. *Syst. Biol.* 57, 104–115
- Doolittle, W.F. (1998) You are what you eat: a gene transfer ratchet could account for bacterial genes in eukaryotic nuclear genomes. *Trends Genet.* 14, 307–311
- Archibald, J.M. (2008) Plastid evolution: remnant algal genes in ciliates. *Curr. Biol.* 18, R663–R665
- Rice, D.W. and Palmer, J.D. (2006) An exceptional horizontal gene transfer in plastids: gene replacement by a distant bacterial paralog and evidence that haptophyte and cryptophyte plastids are sisters. *BMC Biol.* 4, 31
- Cai, X. *et al.* (2003) Apicoplast genome of the coccidian *Eimeria tenella*. *Gene* 321, 39–46

0169-5347/\$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tree.2008.11.003 Available online 4 February 2009

Letters Response

Reply to Bodyl, Stiller and Mackiewicz: “Chromalveolate plastids: direct descent or multiple endosymbioses?”

Christopher E. Lane^{1,2} and John M. Archibald¹

¹The Canadian Institute for Advanced Research, Integrated Microbial Biodiversity Program, Department of Biochemistry and Molecular Biology, Dalhousie University, 5850 College Street, Halifax, NS B3H 1X5, Canada

²Current address: Department of Biological Sciences, University of Rhode Island, 100 Flagg Road, Kingston, RI 02881, USA

Bodyl, 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. Bodyl, Stiller and Mackiewicz [1] take issue with the chromalveolate hypothesis [3] and our views on the tempo and mode of

Corresponding author: Lane, C.E. (clane@mail.uri.edu).

plastid gain and loss among chromalveolates which aim to minimize secondary endosymbiotic events. They favor a model of 'serial plastid transfer,' whereby red algal-derived secondary plastids have been passed between 'chromalveolate' taxa on multiple occasions by tertiary endosymbiosis.

We agree with several of the points raised by Bodył, Stiller and Mackiewicz [1]: the apparent conflicts between molecular data sets brought to bear on the chromalveolate hypothesis need to be taken seriously, and alternative hypotheses for the spread of plastids among 'chromalveolate' lineages should not be discounted [4,5]. However, it should be pointed out that the recent phylogenomic analysis [6] cited by the authors as indicating 'that Archaeplastida is descended from the same ancestor as chromalveolate taxa' [1] was not published at the time we wrote our article. Indeed, we would caution against unconditional acceptance of even the most strongly supported multigene phylogenies, such as the 135-gene Burki *et al.* analysis [6]. To be sure, such trees provide an important framework for assessing plastid gain/loss scenarios, but they are not immune to phylogenetic artifacts (e.g. [7]) and might strongly support erroneous relationships for the very reasons we discussed [2].

Finally, Bodył, Stiller and Mackiewicz argue that complete plastid loss is 'exceedingly rare' [1]. We agree, but rather than view the recent data suggesting a plastid relict in the alveolate *Oxyrrhis* [8] and the discovery of a remnant plastid in *Perkinsus* [9] as evidence against the chromalveolate model, we consider such findings as providing data where it was previously missing. Cryptic mitochondria [10,11] and plastids [12] have been discovered time and time again in organisms once thought to lack them: demonstrating the *absence* of an organelle is extraordinarily difficult, but it only takes a single discovery to fill in a key gap and push the origin of a particular organelle further back in time. In the case of dinoflagellates, Bodył has argued previously for the recent tertiary endosymbiotic origin of the peridinin plastid [5,13], but the above-mentioned discoveries in *Oxyrrhis* [8] and *Perkinsus* [9] now lead him to conclude that the ancestor these organisms shared with dinofla-

gellates was photosynthetic (Figure 1 in Ref. [1]). We predict that further investigation, particularly of poorly studied non-photosynthetic lineages within the alveolates (e.g. colpodellids, gregarines) and heterokonts (e.g. bicosoecids, labyrinthulids) will reveal additional examples of previously undescribed plastid relics. With each new piece of information, the process that gave rise to plastids in both non-photosynthetic and photosynthetic lineages will need to be reconsidered in the context of the most comprehensive and rigorous multigene phylogenies we have available to us.

References

- 1 Bodył, A. *et al.* (2009) Chromalveolate plastids: direct descent or multiple endosymbioses? *Trends Ecol. Evol.* 24, DOI: 10.1016/j.tree.2008.11.003
- 2 Lane, C.E. and Archibald, J.M. (2008) The eukaryotic tree of life: endosymbiosis takes its TOL. *Trends Ecol. Evol.* 23, 268–275
- 3 Cavalier-Smith, T. (1999) Principles of protein and lipid targeting in secondary symbiogenesis: euglenoid, dinoflagellate, and sporozoan plastid origins and the eukaryote family tree. *J. Eukaryot. Microbiol.* 46, 347–366
- 4 Sanchez-Puerta, M.V. and Delwiche, C.F. (2008) A hypothesis for plastid evolution in chromalveolates. *J. Phycol.* 44, 1097–1107
- 5 Bodył, A. and Moszczynski, K. (2006) Did the peridinin plastid evolve through tertiary endosymbiosis? A hypothesis. *Eur. J. Phycol.* 41, 435–448
- 6 Burki, F. *et al.* (2008) Phylogenomics reveals a new 'megagroup' including most photosynthetic eukaryotes. *Biol. Lett.* 4, 366–369
- 7 Brinkmann, H. *et al.* (2005) An empirical assessment of long-branch attraction artefacts in deep eukaryotic phylogenomics. *Syst. Biol.* 54, 743–757
- 8 Slamovits, C.H. and Keeling, P.J. (2008) Plastid-derived genes in the non-photosynthetic alveolate *Oxyrrhis marina*. *Mol. Biol. Evol.* 25, 1297–1306
- 9 Teles-Griolo, M.L. *et al.* (2007) Is there a plastid in *Perkinsus atlanticus* (Phylum Perkinsozoa)? *Eur. J. Protistol.* 43, 163–167
- 10 Tovar, J. *et al.* (2003) Mitochondrial remnant organelles of *Giardia* function in iron-sulphur protein maturation. *Nature* 426, 172–176
- 11 Williams, B.A.P. *et al.* (2002) A mitochondrial remnant in the microsporidian *Trachipleistophora hominis*. *Nature* 418, 865–869
- 12 McFadden, G.I. *et al.* (1996) Plastid in human parasites. *Nature* 381, 482
- 13 Bodył, A. (2005) Do plastid-related characters support the chromalveolate hypothesis? *J. Phycol.* 41, 712–719

0169-5347/\$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tree.2008.11.002 Available online 3 February 2009