Models with unequal transition rates favor marine origins of Cyanobacteria and photosynthetic eukaryotes

Teofil Nakov^{a,1}, James D. Boyko^a, Andrew J. Alverson^a, and Jeremy M. Beaulieu^a

Using a broadly sampled, time-calibrated phylogeny of Cyanobacteria and photosynthetic eukaryotes, Sánchez-Baracaldo et al. (1) report that the most-recent common ancestor (MRCA) of Archaeplastida likely inhabited a low-salinity (freshwater) habitat, providing an advance on a longstanding debate about the ecological context for the origin of plastids (2).

The freshwater ancestry for Cyanobacteria and Archaeplastida was based on reconstructions that assumed equal transition rates among marine, brackish, and freshwater habitats (1), which likely oversimplified the 2.5-billion-year history of these groups. We relaxed the assumption of a single transition rate by fitting a model with additional parameters for independent transition rates among marine, brackish, and freshwater habitats. This model provided a better fit to the data (relative model probability, w_i , was 99%) and revealed strongly unequal transition rates, with the probability of colonizing freshwaters from the marine habitat more than double the rate of the reverse ($q_{m \rightarrow f} = 0.36$ vs. $q_{f \rightarrow m} = 0.16$ transitions per billion years). Consequently, the marine environment was strongly supported as the likeliest ancestral habitat for Cyanobacteria and Archaeplastida (3).

It is possible that direct transitions between marine and freshwaters are uncommon (4) and proceed through an intermediate brackish habitat (5). We fit models with ordered transitions and again found unequal transition rates, with marine taxa far more likely to colonize brackish waters than their freshwater counterparts (3). Models that assumed equal rates, either unordered or ordered, were highly improbable ($w_i < 0.01\%$), whereas an unequal-rates model with ordered transitions (freshwater $\leftrightarrow$ brackish $\leftrightarrow$ marine) provided the best overall fit to the data ($w_i = 85.6\%$). With this

model, the freshwater habitat was no longer favored for the MRCAs of Cyanobacteria (marginal probability for freshwater, $P_f < 0.01\%$) and Archaeplastida ($P_f = 2.8\%$).

When assuming unequal rates for multistate characters, low frequency states, like the brackish state in this dataset (just 2 of the 119 species are brackish), require extremely high transition rates away from them to account for their low observed frequency and can, as a result, affect ancestral reconstructions (6). The data deposited by Sánchez-Baracaldo et al. (7) contain a second coding scheme, with brackish taxa coded as marine and a different scoring for one *Nostoc* accession (scored as marine instead of freshwater). With this dataset, a model with unequal transition rates was again favored ($w_i = 61\%$ vs. 39%). We accounted for uncertainty in model choice by averaging transition rates across the two models. The results again favored asymmetry in the rates of marine–freshwater transitions ($q_{m \rightarrow f} = 0.56$, $q_{f \rightarrow m} = 0.34$) and strongly supported marine ancestry for both Cyanobacteria ($P_m = 94\%$) and Archaeplastida ($P_m = 82\%$).

The comparative framework employed by Sánchez-Baracaldo et al. (1) represents a promising way forward for reconstructing the history of endosymbiosis. However, as demonstrated here, even modest heterogeneity in the evolutionary process can clearly have profound effects not just on the rates of transition among states, but also—and perhaps most importantly—on inferences about events that have occurred in the very distant past (8).

Acknowledgments

This work was supported by a grant from the Simons Foundation (403249) (to A.J.A.).

- 1 Sánchez-Baracaldo P, Raven JA, Pisani D, Knoll AH (2017) Early photosynthetic eukaryotes inhabited low-salinity habitats. *Proc Natl Acad Sci USA* 114:E7737–E7745.
- 2 Lewis LA (2017) Hold the salt: Freshwater origin of primary plastids. *Proc Natl Acad Sci USA* 114:9759–9760.
- 3 Nakov T, Boyko JD, Alverson AJ, Beaulieu JM (2017) Ecology of Primary Endosymbiosis. GitHub repository. Available at <https://github.com/teofiln/Ecology-of-Primary-Endosymbiosis>. Accessed September 20, 2017.

^aDepartment of Biological Sciences, University of Arkansas, Fayetteville, AR 72701

Author contributions: T.N., J.D.B., A.J.A., and J.M.B. designed research, performed research, analyzed data, and wrote the paper.

The authors declare no conflict of interest.

Published under the [PNAS license](#).

¹To whom correspondence should be addressed. Email: tnakov@uark.edu.

- 4 Logares R, et al. (2009) Infrequent marine-freshwater transitions in the microbial world. *Trends Microbiol* 17:414–422.
- 5 Ruck EC, Nakov T, Alverson AJ, Theriot EC (2016) Phylogeny, ecology, morphological evolution, and reclassification of the diatom orders Surirellales and Rhopalodiales. *Mol Phylogenet Evol* 103:155–171.
- 6 Beaulieu JM, Donoghue MJ (2013) Fruit evolution and diversification in campanulid angiosperms. *Evolution* 67:3132–3144.
- 7 Sánchez-Baracaldo P, Raven JA, Pisani D, Knoll AH (2017) *Data from: Early photosynthetic eukaryotes inhabited low-salinity habitats*. Available at datadryad.org/resource/doi:10.5061/dryad.421p2. Accessed September 20, 2017.
- 8 King B, Lee MS (2015) Ancestral state reconstruction, rate heterogeneity, and the evolution of reptile viviparity. *Syst Biol* 64:532–544.