

# Reply to 'Molecular clocks provide little information to date methanogenic Archaea'

**To the Editor** — In the accompanying Correspondence, Roger and Susko<sup>1</sup> dispute our evidence for dating the evolution of microbial methanogenesis<sup>2</sup>. Here we contest these claims.

Two major concerns are raised: (1) given uncertainties about the placement of the root within Archaea and the possibility of methanogenesis as an ancestral metabolism, the upper bound on the root is invalid; and (2) that the similarities between posterior and effective prior age distributions indicate that the sequence data do not significantly inform our results.

The placement of the root of Archaea is a challenging phylogenetic problem, investigated by several studies using varied evolutionary models and datasets<sup>3–6</sup>. As Roger and Susko<sup>1</sup> point out, models that include site-dependent substitution heterogeneity recover a root within Euryarchaeota<sup>3,6</sup>. Other substitution models and optimality criteria<sup>3</sup>, and an alternative rooting method reconciling large numbers of gene tree histories<sup>7</sup>, recover a monophyletic Euryarchaeota. This strong model dependence for the rooting of Archaea is likely to reflect the limited phylogenetic information within sequence alignments for resolving the placement of the bacterial outgroup. In fact, other recently published work dating methanogens, in the same journal and issue as our paper, also uses a tree with monophyletic Euryarchaeota (ref. <sup>5</sup>, Supplementary Fig. 3). Therefore, the critique presented in this Correspondence seems better suited to a broader discussion of the state of the field, rather than a claim

that our model choice and subsequent results are invalid in particular.

Roger and Susko<sup>1</sup> further claim that ancestral methanogenesis is evidenced by the presence of methanogen-specific genes within Verstraetearchaeota and Bathyarchaeota, clades that group within TACK based on 16S sequences<sup>8</sup>. Phylogenetic analyses of McrA/B proteins, however, support the acquisition of these proteins by horizontal gene transfer (HGT): homologues from Verstraetearchaeota group with Methanomassiliococcales, and homologues from Bathyarchaeota group with Syntrophoarchaeum (ref. <sup>4</sup>, Fig. 4; ref. <sup>8</sup>, Supplementary Figs. 7 and 8; ref. <sup>9</sup>). Furthermore, Mcr genes in Syntrophoarchaeum have been linked to anaerobic butane oxidation<sup>10</sup>, which may also be their function in Bathyarchaeota<sup>4</sup>. Scenarios wherein microbial methane production evolved in the archaeal ancestor are far less parsimonious<sup>9</sup>, requiring at least five to seven independent losses of this metabolism across many groups<sup>6</sup>, as opposed to only three losses with a monophyletic Euryarchaeota. Therefore, monophyletic Euryarchaeota containing the origin of methanogenesis remains a reasonable hypothesis, and our root prior is reasonable. We acknowledge that a more explicit discussion of these assumptions is valuable for communicating the findings of divergence time analyses.

Roger and Susko<sup>1</sup> correctly reiterate our observation<sup>2</sup> that, for the ancient nodes under discussion, sequence data do not significantly contribute to age estimates, as is expected given the antiquity of the nodes under study, which will be associated

with the greatest uncertainty<sup>11</sup>. Sequence data can only 'overwhelm' the user prior if there is conflict between constraints, causing truncation of the effective prior. In our paper<sup>2</sup>, we demonstrate that ancient HGT provides a calibration that improves our estimates for the effective prior and posterior of methanogens by ~400 Ma (ref. <sup>2</sup>, Fig. 2a, Supplementary Fig. 8). Future studies including more HGT events, providing both relative time constraints and fossil calibrations, will further improve precision in these investigations. □

Joanna M. Wolfe\* and Gregory P. Fournier

Department of Earth, Atmospheric & Planetary Sciences, Massachusetts Institute of Technology, Cambridge, MA, USA.

\*e-mail: [joawolfe@mit.edu](mailto:joawolfe@mit.edu)

Published online: 24 September 2018  
<https://doi.org/10.1038/s41559-018-0685-1>

## References

1. Roger, A. J. & Susko, E. *Nat. Ecol. Evol.* <https://doi.org/10.1038/s41559-018-0687-z> (2018).
2. Wolfe, J. M. & Fournier, G. P. *Nat. Ecol. Evol.* **2**, 897–903 (2018).
3. Lasek-Nesselquist, E. & Gogarten, J. P. *Mol. Phylogenet. Evol.* **69**, 17–38 (2013).
4. Spang, A., Caceres, E. F. & Ettma, T. J. *Science* **357**, eaaf3883 (2017).
5. Davin, A. A. et al. *Nat. Ecol. Evol.* **2**, 904–909 (2018).
6. Raymann, K., Brochier-Armanet, C. & Gribaldo, S. *Proc. Natl Acad. Sci. USA* **112**, 6670–6675 (2015).
7. Williams, T. A. et al. *Proc. Natl Acad. Sci. USA* **114**, E4602–E4611 (2017).
8. Vanwongherghem, I. et al. *Nat. Microbiol.* **1**, 16170 (2016).
9. Boyd, J. A. et al. Preprint at <https://www.biorxiv.org/content/early/2018/08/13/390617> (2018).
10. Laso-Pérez, R. et al. *Nature* **539**, 396–401 (2016).
11. dos Reis, M. et al. *Curr. Biol.* **25**, 2939–2950 (2015).

## Competing interests

The authors declare no competing interests.