TECHNICAL RESPONSE

EVOLUTION

Response to Comment on "A promiscuous intermediate underlies the evolution of LEAFY DNA binding specificity"

Samuel F. Brockington,[1*] Edwige Moyroud,[1*] Camille Sayou,[2] Marie Monniaux,[3] Max H. Nanao,[4,5†] Emmanuel Thévenon,[6,7,8,9] Hicham Chahtane,[6,7,8,9] Norman Warthmann,[10] Michael Melkonian,[11] Yong Zhang,[12] Gane Ka-Shu Wong,[12,13] Detlef Weigel,[14] Renaud Dumas,[6,7,8,9] François Parcy[6,7,8,9†]

Brunkard *et al*. propose that the identification of novel *LEAFY* sequences contradicts our model of evolution through promiscuous intermediates. Based on the debate surrounding land plant phylogeny and on our analysis of these interesting novel sequences, we explain why there is no solid evidence to disprove our model.

In Sayou *et al.* (*1*), we explained how conserved LEAFY (LFY) homologs could recognize different DNA motifs (types I, II, and III) by determining the key residues affecting LFY DNA binding specificity. We identified a hypothetical promiscuous LFY variant (key residues His-Cys-His) through phylogenetic reconstruction, but also discovered a promiscuous variant (Gln-Cys-His) in a lineage of early diverging land plants, the hornworts. We proposed that these promiscuous forms acted as intermediates, enabling a gradual transition from type III to types I and II binding specificities.

[1]Department of Plant Sciences, University of Cambridge, Downing Street, Cambridge CB2 3EA, UK. [2]Wellcome Trust Center for Cell Biology, Michael Swann Building 5.1, King's Buildings. Edinburgh, EH9 3JR, UK. [3]Department of Comparative Development and Genetics, Max Planck Institute for Plant Breeding Research, Carl-von-Linne Weg 10, 50829, Köln, Germany. [4]European Molecular Biology Laboratory (EMBL), 6 Rue Jules Horowitz, BP 181, 38042 Grenoble, France. [5]Unit of Virus Host-Cell Interactions, Université Grenoble Alpes (UGA), Centre National de la Recherche Scientifique (CNRS), EMBL, UMI 3265, 6 Rue Jules Horowitz, 38042 Grenoble Cedex 9, France. [6]CNRS, Laboratoire de Physiologie Cellulaire et Végétale (LPCV), UMR 5168, 38054 Grenoble, France. [7]UGA, LPCV, F-38054 Grenoble, France. [8]Commissariat à l'énergie atomique et aux énergies alternatives, Direction des Sciences du Vivant, Institut de Recherches en Technologies et Sciences pour le Vivant, LPCV, F-38054 Grenoble, France. [9]Institut National de la Recherche Agronomique, LPCV, F-38054 Grenoble, France. [10]Research School of Biology, The Australian National University, Acton, ACT 0200, Australia. [11]Botanisches Institut, Lehrstuhl I, Universität zu Köln, Biozentrum Köln, Zülpicher Strasse 47b, 50674 Köln, Germany. [12]Beijing Genomics Institute, Shenzhen, Beishan Industrial Zone, Yantian District, Shenzhen 518083, China [13]Department of Biological Sciences, Department of Medicine, University of Alberta, Edmonton AB, T6G 2E9, Canada. [14]Department of Molecular Biology, Max Planck Institute for Developmental Biology, 72076 Tübingen, Germany.
*These authors contributed equally to this work. **†Corresponding author. E-mail: francois.parcy@cea.fr (F.P.); mnanao@embl.fr (M.H.N.)**

Brunkard *et al.* (*2*) identified novel paralogous LFY sequences in a single moss species and conclude that changes in LFY binding specificity evolved only through duplication. Here, we explain why we do not agree with their conclusions.

First, Brunkard *et al.* constrain the *LFY* phylogeny to a single organismal topology in which liverworts, mosses, and hornworts constitute a paraphyletic grade leading to the vascular plants. This choice does not acknowledge the debate surrounding early land plant phylogeny. The topological constraint they employed is based on the phylogenetic hypothesis provided by Qiu *et al.* (*3*). However, Cox *et al.* reanalyzed the Qiu *et al.* data set and concluded that the paraphyly of bryophytes, and the support for the hornworts as the sister group to the tracheophytes, is a methodological artifact (*4*). Furthermore, other studies support alternative scenarios (*5*–*10*), and recent publications (*4*, *11*) stress that the early land plant phylogeny remains profoundly uncertain, even in the postgenomic era. As emphasized in these publications and discussed in our original manuscript (*1*), there are four alternative topologies still in play [see figure S9 in (*1*)]. In this context, we think that it is inappropriate to constrain the *LFY* phylogeny to only one of several competing hypotheses of organismal relationships. Instead, in Sayou *et al.*, we evaluated the incongruence between the *LFY* phylogeny and these four competing organismal phylogenies, establishing that our model is robust in the context of different organismal hypotheses.

Second, on the basis of a sequence alignment alone, Brunkard *et al.* propose that the novel *Polytrichum commune LFY* sequences they isolated are the product of duplications at the base of mosses, rather than derived events. However, they did not perform the phylogenetic analysis necessary to establish whether these duplication events occurred before or after the changes in DNA binding specificity. In addition to duplications within mosses, their model also requires a likely duplication within hornworts and a deep duplication at the base of the land plants (mediating type III to type I specificity change). In the absence of this deep duplication, their model requires an abrupt switch between different binding specificities, which would likely be deleterious. To date, there is no support for any of these additional duplications, and Brunkard *et al.* do not provide a nondeleterious mechanism to account for an abrupt switch.

Finally, Brunkard *et al.* rely on the parsimony criterion to substantiate their model. However, their parsimony reconstruction analysis [figure 2B in (*2*)] does not take into account that the three critical amino acid residues occupy well-separated positions and were reconstructed as such in Sayou *et al.* [figures 4 and S6 of (*1*)]; instead, Brunkard *et al.* appear to have reconstructed them as a single linked trait. Reanalyzing the Brunkard *et al.* data with the three amino acids sites individually reconstructed, but using their tree topology, we obtained an equal probability of the promiscuous intermediate (His-Cys-His) preceding key transitions in binding specificity (Fig. 1). Furthermore, the topology supplied by Brunkard *et al.* is the more challenging scenario for our model. In the other three competing organismal hypotheses (i.e., liverworts-plus-mosses as monophyletic, all bryophytes as monophyletic, or hornworts as sister to all land plants), a Gln-Cys-His ancestral promiscuous intermediate is always recovered. Therefore, we reject the idea that promiscuity is merely a derived state and maintain that the promiscuous model holds.

We agree that gene duplication plays a role in *LFY* evolution because *LFY* duplicates may occasionally acquire different functions, likely through divergence in expression patterns (*12*, *13*). In Sayou *et al.*, we carefully noted all examples of known LFY duplications, even if not associated with a change in DNA binding specificity. We also agree that limited taxon sampling and genomic data make the presence of gene duplication difficult to disprove and concluded, "we cannot completely rule out the occurrence of transient ancient duplications" (*1*). In their Comment, Brunkard *et al.* treat the duplication and promiscuity scenarios as mutually exclusive. In contrast, we maintain that "it is plausible that the mechanisms we describe could also contribute to the evolution of TFs encoded by multigene families" (*1*). Promiscuous intermediates may be easier to detect in a predominantly single-copy gene lineage, but promiscuous forms could themselves be duplicated and obtain novel function in derived paralogous lineages, as recently invoked in the evolution of HOX genes (*14*).

In summary, we feel that Brunkard *et al.* misjudge the extent of the phylogenetic support for their model, and we suggest that their proposed scenario does not adequately explain the existence of the promiscuous form. Brunkard *et al.* highlight the need to densely sample *LFY* sequences from emerging genomic resources to

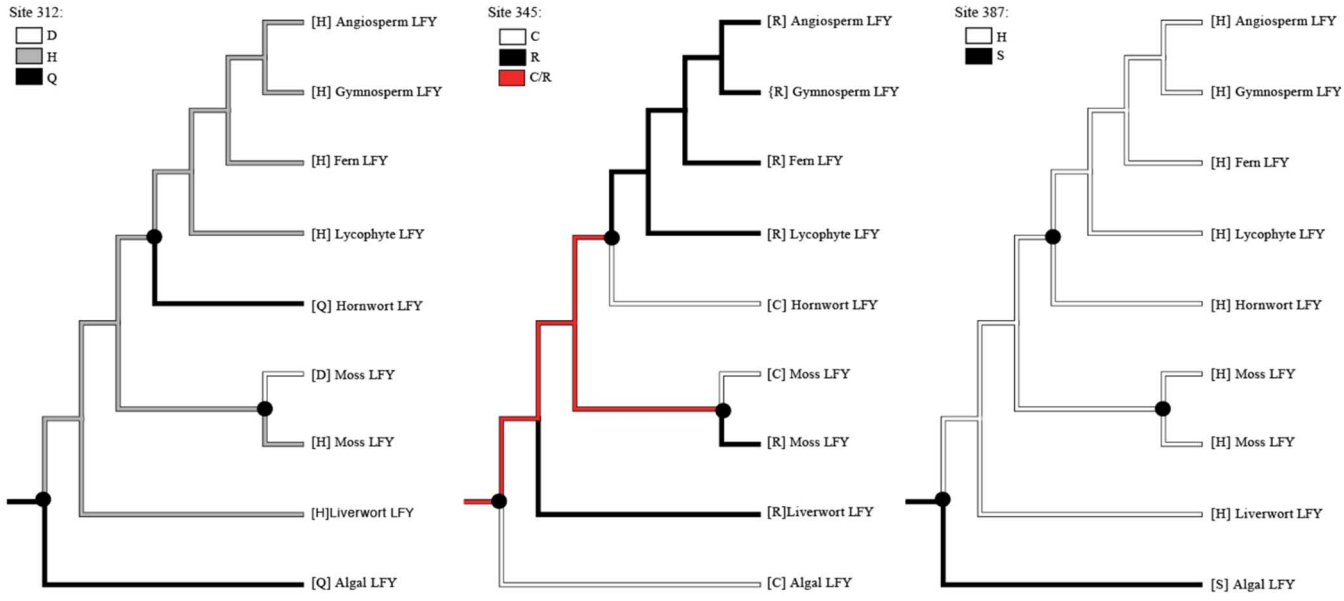

Fig. 1. Phylogenetic reconstruction with the parsimony criterion applied to the same topology and data used by Brunkard *et al*. Reconstruction analyses were implemented in Mesquite Version 2.75, with each amino-acid site (312, 345, and 387) reconstructed individually (2, 8, and 2 most parsimonious reconstructions, respectively). Reconstructions were refigured so that each lineage of *LFY* genes is represented by a single terminal branch, following the methodology of Brunkard *et al*. Black circles indicate sites of binding specificity change inferred in the model of Brunkard *et al*. Taken together, the separate reconstructions indicate that a promiscuous intermediate (His-Cys-His) is as equally likely as the His-Arg-His variant before changes in binding specificity, even under the constrained topology imposed by Brunkard *et al*.

improve estimates of the *LFY* phylogeny and to more accurately infer changes in binding specificity. We concur, but given that the phylogenetic location of gene duplication events is revealed through reconciliation of gene and species trees, uncertain organismal relationships will continue to challenge precise inference on the timing of putative duplication events. As such, parsimony-based phylogenetic reasoning alone cannot be the sole criterion with which to distinguish among competing models of LFY evolution. Rather, we believe that an integrated functional, biochemical, and phylogenetic approach is essential.

REFERENCES AND NOTES

1. C. Sayou *et al*., *Science* **343**, 645–648 (2014).
2. J. O. Brunkard, A. M. Runkel, P. C. Zambryski, *Science* **347**, 621 (2015).
3. Y.-L. Qiu *et al*., *Proc. Natl. Acad. Sci. U.S.A.* **103**, 15511–15516 (2006).
4. C. J. Cox, B. Li, P. G. Foster, T. M. Embley, P. Civán, *Syst. Biol.* **63**, 272–279 (2014).
5. T. Nishiyama *et al*., *Mol. Biol. Evol.* **21**, 1813–1819 (2004).
6. K. G. Karol *et al*., *BMC Evol. Biol.* **10**, 321 (2010).
7. T. Nishiyama, M. Kato, *Mol. Biol. Evol.* **16**, 1027–1036 (1999).
8. C. Finet, R. E. Timme, C. F. Delwiche, F. Marlétaz, *Curr. Biol.* **20**, 2217–2222 (2010).
9. S. Wodniok *et al*., *BMC Evol. Biol.* **11**, 104 (2011).
10. F.-W. Li *et al*., *Proc. Natl. Acad. Sci. U.S.A.* **111**, 6672–6677 (2014).
11. E. D. Cooper, *Trends Plant Sci.* **19**, 576–582 (2014).
12. K. Bomblies *et al*., *Development* **130**, 2385–2395 (2003).
13. A. Vázquez-Lobo *et al*., *Evol. Dev.* **9**, 446–459 (2007).
14. S. Nakagawa, S. S. Gisselbrecht, J. M. Rogers, D. L. Hartl, M. L. Bulyk, *Proc. Natl. Acad. Sci. U.S.A.* **110**, 12349–12354 (2013).

ACKNOWLEDGMENTS

We thank J. Leebens-Mack and E. Carpenter for discussions. This work was supported by funds from the Max Planck Society (D.W.) and the Agence Nationale de la Recherche (grant Charmful SVSE2–2011). The 1000 Plants (1KP) initiative, led by G.K.-S.W., is funded by Alberta Ministry of Enterprise and Advanced Education, Alberta Innovates Technology Futures, Innovates Centre of Research Excellence, Musea Ventures, and BGI-Shenzhen.

27 May 2014; accepted 2 December 2014
10.1126/science.1256011

Science
AAAS

Response to Comment on "A promiscuous intermediate underlies the evolution of LEAFY DNA binding specificity"

Samuel F. Brockington *et al.*

Science **347**, 621 (2015);
DOI: 10.1126/science.1256011