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

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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.

Brunkard et al. (2) identified novel paralogous LFY sequences in a single moss species and concluded 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 paralogy 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 postgenomeric 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. On the second, on the basis of a sequence alignment alone, Brunkard et al. propose that the novel Polystichum 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
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**


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