Floral Gigantism in Rafflesiaceae

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The Rafflesiaceae are leafless, stemless, and rootless nonphotosynthetic parasites that live embedded in host plants (1). With flowers measuring up to a meter in diameter and weighing up to 7 kg, Rafflesiaceae *sensu stricto* [Supporting Online Material (SOM) text] possess the largest flowers of all angiosperms. Like other holoparasitic angiosperms, the phylogenetic affinities of Rafflesiaceae have proved difficult to resolve because of their reduced vegetative morphology, highly modified reproductive structures (1), and anomalous and often accelerated molecular evolution, particularly in plastid (cp) DNA (2–6).
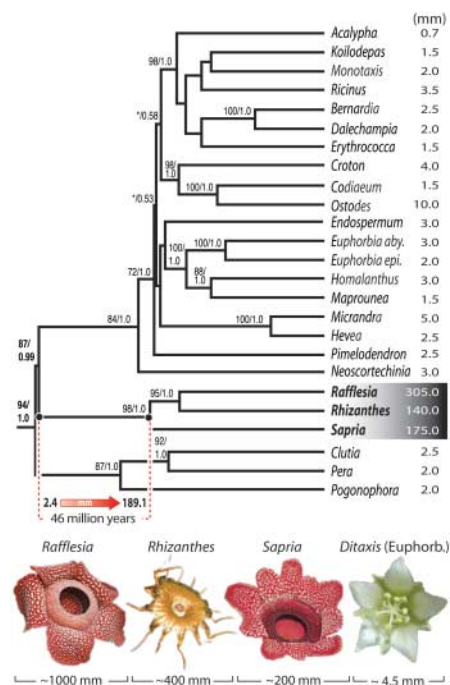

Fig. 1. Phylogeny of Euphorbiaceae [including Rafflesiaceae (bold)] based on a temporally calibrated ML tree (see SOM text for details and fig. S1 for full tree). ML BS and BPP, respectively, are provided. Support values $\leq$ 50%/0.50 are designated with asterisks. Flower size diameters (in mm) are provided (right), and ancestral flower size estimates are indicated at the stem and crown nodes of Rafflesiaceae. Reconstructions indicate a 79-fold increase in floral diameter for stem lineage Rafflesiaceae (with a 95% confidence interval of 74- to 83-fold). For additional ancestral size estimates see SOM text. Color images with scale bars illustrate the approximate sizes of flowers representative of the three genera of Rafflesiaceae (*Rafflesia arnoldii*, *Rhizanthes lowii*, and *Sapria himalayana*), plus a representative of Euphorbiaceae (*Ditaxis neomexicana*), the latter being similar in size to the inferred ancestral flowers at the stem node of Rafflesiaceae.

By analysis of slowly evolving genes, especially from mitochondrial (mt) DNA, Rafflesiaceae were shown to be members of the Malpighiales (4–6), a diverse group of circa (ca.) 16,000 species, with 29 major subclades [mostly recognized as families (7)]. However, the position of Rafflesiaceae within the order was unclear because of either insufficient taxon sampling (4, 6) or a lack of phylogenetic signal (5). We used maximum likelihood (ML) and Bayesian inference (BI) to estimate the phylogeny of Malpighiales from ca. 11,500 base pairs of sequence data (see SOM text for detailed information). Five mt genes (*ccmB*, *cob*, *matR*, *nad6* and *rps3*) and one cp gene [*matK* (8)] were sampled from 111 accessions representing all families of Malpighiales (7) and 22 outgroup species, including Rafflesiaceae's obligate host, *Tetrastigma* (Vitaceae). Nuclear (nr) small- and large-subunit ribosomal DNA regions were also included for a subset of 40 taxa. Examination of ML bootstrap scores (BS) and Bayesian posterior probabilities (BPP) in the individual analyses of the eight gene regions revealed no significant topological discord, and thus the data were concatenated and analyzed in combination.

Both the ML and BI analyses showed that Rafflesiaceae are nested within Euphorbiaceae (Fig. 1). Strong support was found for both the Rafflesiaceae plus Euphorbiaceae clade (BS = 94% and BPP = 1.0) and the clade that includes Rafflesiaceae and all Euphorbiaceae except *Pera*, *Clutia*, and *Pogonophora* (BS = 87% and BPP = 0.99).

The phylogenetic association of Rafflesiaceae and Euphorbiaceae is robust and not attributable to phylogenetic artifacts (9). Although the morphology of Rafflesiaceae prevents identification of unambiguous synapomorphies, some reproductive traits (10) are consistent with a placement of Rafflesiaceae within Euphorbiaceae.

We conducted a quantitative analysis of floral size evolution in the context of the estimated phylogeny (SOM text). Flower sizes were determined from the literature and herbarium data. A likelihood ratio test (11) rejects the hypothesis that there was a single rate of flower size evolution in the entire Euphorbiaceae-plus-Rafflesiaceae clade. Instead, the optimal model assigns one rate to all Euphorbiaceae lineages and crown-group Rafflesiaceae but a different, higher rate to the stem lineage of Rafflesiaceae. This demonstrates that floral gigantism evolved principally along the stem lineage of Rafflesiaceae, whereas subsequent flower-size evolution within crown group Rafflesiaceae reverted to the original euphorbiaceous

rate. Flower size evolved about 91 times faster along the stem lineage than in the rest of the phylogeny. By using Brownie (12), we estimated flower diameter to have increased from 2.4 [confidence interval (CI) of 1.1 to 5.3 mm] to 189.1 mm (CI of 91.2 to 392.2 mm) along the stem lineage of Rafflesiaceae (Fig. 1 and SOM text): a ca. 79-fold increase in size in a period of ca. 46 million years (Fig. 1). Thus, a placement of giant-flowered Rafflesiaceae within Euphorbiaceae, whose flowers are nearly all tiny, only increases the evolutionary enigma of "the greatest prodigy of the vegetable world" (13).

References and Notes

1. J. Kuijt, *The Biology of Parasitic Flowering Plants* (Univ. of California Press, Berkeley, CA, 1969).
2. D. L. Nickrent, Y. Ouyang, R. J. Duff, C. W. dePamphilis, *Plant Mol. Biol.* **34**, 717 (1997).
3. D. L. Nickrent et al., in *Molecular Systematics of Plants II: DNA Sequencing*, D. E. Soltis, P. S. Soltis, J. J. Doyle, Eds. (Kluwer Academic, Boston, 1998), p. 211.
4. T. J. Barkman, S.-K. Lim, K. Mat Salleh, J. Nais, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 787 (2004).
5. C. C. Davis, K. J. Wurdack, *Science* **305**, 676 (2004).
6. D. L. Nickrent, A. Blarer, Y.-L. Qiu, R. Vidal-Russell, F. E. Anderson, *BMC Evol. Biol.* **4**, 40 (2004).
7. C. C. Davis, C. O. Webb, K. J. Wurdack, C. A. Jaramillo, M. J. Donoghue, *Am. Nat.* **165**, E36 (2005).
8. Plastid *matK* is most likely absent in Rafflesiaceae (SOM text).
9. The phylogenetic placement of Rafflesiaceae is strongly supported by ML and BI methods of both mt and nr data (see SOM text for further evidence).
10. P. F. Stevens, Angiosperm Phylogeny Website, ed. 4 (2001), www.mobot.org/MOBOT/research/APweb.
11. B. C. O'Meara, C. Ane, M. J. Sanderson, P. C. Wainwright, *Evolution* **60**, 922 (2006).
12. Brownie, 2.06b, B. C. O'Meara, University of California, Davis (available by download at www.brianomeara.info/brownie/index.html).
13. R. Brown, *Trans. Linn. Soc. London* **13**, 201 (1822).
14. We thank W. Anderson, H. Cowles, P. Endress, J. Finarellli, J. Hall, A. Knoll, E. Kramer, J. Losos, C. Moreau, B. O'Meara, A. Pringle, B. Ruhfel, and P. Stevens for helpful discussion. C.C.D. was supported by NSF Assembling the Tree of Life (EF 04-31242) and by the Michigan Society of Fellows.

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