

# Floral Evolution: Dramatic Size Change Was Recent and Rapid in the World's Largest Flowers

Recent studies clarifying the closest relatives of the world's largest flowers, Rafflesiaceae, whose floral diameters range from ~11 to ~100 cm, indicated that they evolved from tiny-flowered ancestors in a burst of floral gigantism. New data now suggest that floral size evolution within Rafflesiaceae may be more dynamic than expected, with both recent and rapid changes in flower size.

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Over 180 years ago, a remarkable plant species was discovered in the Sumatran rain forest. It was a leafless, stemless, non-photosynthetic parasite embedded in its host plant (i.e., an endophytic holoparasite) with flowers that measured nearly a meter in diameter. The plant was described as *Rafflesia arnoldii* R. Br. in honor of its codiscoverers [1] and crowned with the title of world's largest flower (Figure 1). Almost two centuries after this discovery, surprisingly little is known about the evolution of the extraordinary reproductive morphology in this species and its closest large-flowered relatives.

A major factor contributing to our lack of understanding of Rafflesiaceae floral evolution has been the difficulty of pinpointing the closest relatives of holoparasitic angiosperms. Despite a remarkable transformation in our knowledge of plant phylogenetic relationships during the past thirty years, especially in light of the wealth of new DNA sequence data [2], our understanding of holoparasitic plant relationships has remained relatively static. This is largely due to their reduced vegetative morphology, highly modified reproductive structures [3], and anomalous molecular evolution [4–6], which has made them difficult to compare to other free-living relatives. Fortunately, the use of mitochondrial (mt) and low-copy nuclear (nu) genes, combined with model-based phylogenetic approaches using maximum likelihood (ML) and Bayesian inference, is shedding light on the evolutionary relationships of holoparasitic plants [6–9].

Traditional classifications based on morphology have tended to include the majority of holoparasitic genera as members of Rafflesiaceae in the

broadest sense (sometimes termed Rafflesiales [10]), implying a single evolutionary origin of these species. This grouping was based principally on their shared reduced, parasitic vegetative bodies and included both enormous- and tiny-flowered species. Molecular phylogenetic studies, supported by fresh morphological reassessments, however, have overturned this argument by showing that these genera belong to several distantly related groups and that this lifestyle arose at least four times [6–9]. Rafflesiaceae today are restricted to some of the largest-flowered members of the plant kingdom and exhibit remarkable floral size diameter variation spanning nearly one order of magnitude (~11–100 cm).

Several recent studies [6–9] placed Rafflesiaceae as a member of Malpighiales, which is one of the most

diverse flowering-plant lineages. The group encompasses more than 16,000 species spanning tremendous morphological and ecological diversity and includes numerous economically important species, such as cassava, flax, poinsettia, poplar, and the rubber tree [11]. The problem of determining the relationships of Rafflesiaceae within Malpighiales was solved recently by sequencing numerous gene regions from dozens of their closest Malpighialeans relatives. These efforts support the surprising finding that Rafflesiaceae are embedded within the spurge family (Euphorbiaceae), whose flowers measure only a few millimeters in diameter (Figure 2) [11,12].

Until now, only one previous study [12] (see also [13]) has combined detailed phylogenetic information and data on flower size with sophisticated analyses of size evolution to determine the tempo of size evolution in these species. This study estimated that Rafflesiaceae evolved from their tiny-flowered ancestors at a rate that was 91 times faster, on the stem lineage of Rafflesiaceae, compared with their closest relatives. The change in flower size along this stem lineage increased from ~2.4 to ~189 mm at a healthy rate of ~10% per million years, under the most conservative estimates. Furthermore, since it is likely that the period of accelerated size evolution was restricted to a smaller

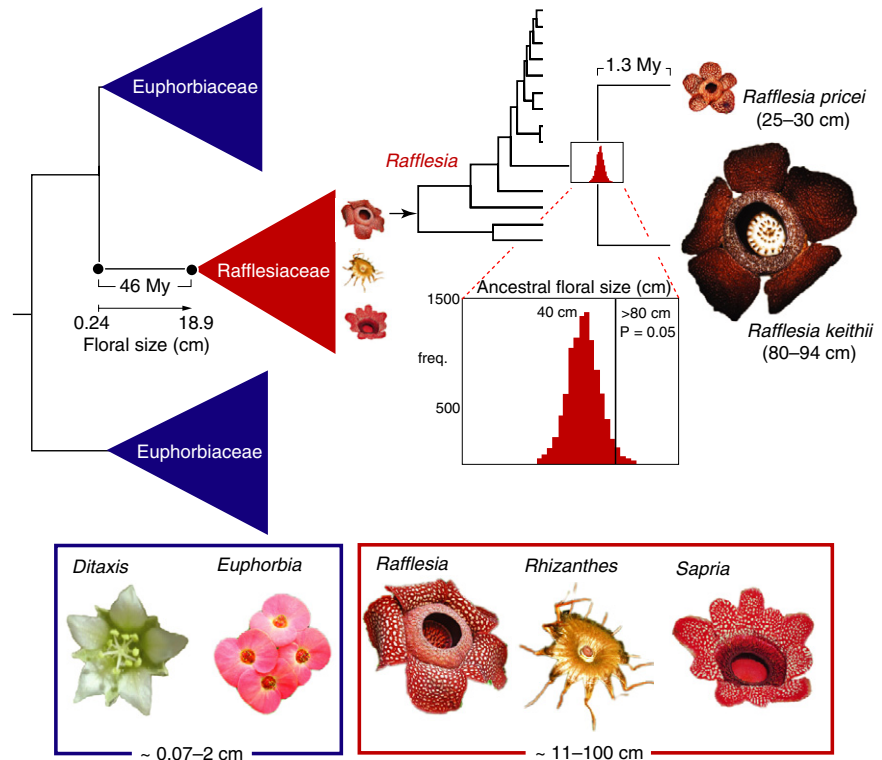


Figure 1. An onlooker gazes deep into the blossom of the world's largest flower, *Rafflesia arnoldii* R. Br. (Photograph courtesy of J. Holden.)

portion of this lineage's ~46 million year duration, the rate of floral size increase may have been dramatically higher [13]. The methods available for ancestral character state reconstruction, however, do not allow us to determine whether size increase along this lineage was gradual or occurred in one or more rapid bursts. Now, Barkman *et al.* [14], in a recent issue of *Current Biology*, have presented new evidence suggesting that rapid change may be the better interpretation.

Barkman *et al.* [14] further explored patterns of floral size evolution within Rafflesiaceae by assembling a nearly complete phylogeny of the family. Their analyses of three mt and one nu gene regions reveal that there is surprisingly little sequence divergence between these species, despite remarkable interspecific variation in floral size, color, scent, and texture [15]. High levels of morphological variation, complemented by very low sequence divergence, are also seen in several adaptive radiations [16], including Hawaiian silverswords and columbines. Molecular divergence time estimates in the largest subgroup of the family, *Rafflesia*, suggest that much of the family's diversity is young and arose within the last 12 million years. Moreover, most speciation events in *Rafflesia* appear to have occurred within the last 1–2 million years, with some divergences as recent as 600,000 years ago.

This rapid and recent burst of speciation has important implications for understanding the evolution of floral size in Rafflesiaceae. Using this improved phylogenetic framework, the authors identified a significant shift in the rate of floral size evolution in *Rafflesia*, which exhibits an average rate of size change that is a hefty 20 times faster than elsewhere in the family (not including the stem lineage Rafflesiaceae). Their size reconstructions indicate that impressive changes in floral diameter size occurred over very short intervals, and perhaps repeatedly. Three especially large-flowered species (>60 cm) are closely related to smaller-flowered ( $\leq 30$  cm) taxa. The smaller-flowered *Rafflesia pricei* (minimally 25 cm), for example, is placed sister to the large-flowered *Rafflesia keithii* (minimally 80 cm) with a high degree of support. This nearly three-and-one-half-fold disparity in



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Figure 2. Phylogenetic placement of Rafflesiaceae, the world's largest flowers.

The group Rafflesiaceae (red, also pictured bottom right) is embedded within Euphorbiaceae, the tiny-flowered spurges (blue, also pictured bottom left) [12]. Floral diameter size increase along the 46 million year (My) stem lineage of Rafflesiaceae resulted from very high rates of floral evolution. New analyses [14] focused on one subclade of Rafflesiaceae, *Rafflesia*, indicate that dramatic size change may have been rapid and recent within the genus. In one striking example, the nearly three-and-one-half-fold disparity in size between the sister species *Rafflesia keithii* and *Rafflesia pricei* appears to have arisen in ~1.3 My. Floral size estimates of the ancestor of these two species are wide (graph inset), however, and do not allow us to clearly determine the direction of size changes. (Photographs courtesy of A. Kocyan and V. Steinmann.)

size appears to have arisen in a little over one million years. Whether such impressive instances of size change represent a significant departure in the background rate of size evolution within *Rafflesia* will be an exciting area for future investigation.

These analyses imply that dramatic and rapid change in floral size is likely to have occurred in Rafflesiaceae, but determining the directionality of this dynamism is less clear. While Barkman *et al.* [14] favor the origin of larger-flowered species from smaller-flowered ancestors within the family, their confidence intervals of ancestral floral diameter sizes are wide, leaving open the possibility that ancestral species of *Rafflesia* were large. If this were the case, size disparity within the genus may be due to repeated instances of dwarfism from large-flowered ancestors.

Continued evolutionary research is required to better reveal the directionality of floral size evolution and should be complemented with rigorous ecological and functional investigations to evaluate whether the mode and tempo of size change in these record breakers is different from other lineages in the Tree of Life [13]. Although the jury is still out on this question, Barkman *et al.* [14] have demonstrated that this area of research is, indeed, expanding our knowledge of floral evolution.

#### References

1. Brown, R. (1822). An account of a new genus of plants, named *Rafflesia*. *Trans. Linn. Soc. London* 13, 201–234.
2. Stevens, P.F. (2001 onwards). Angiosperm Phylogeny Website. Version 9, June 2008 [and continuously updated since].
3. Kuijt, J. (1969). *The Biology of Parasitic Flowering Plants* (Berkeley: University of California Press).

4. Nickrent, D.L., Duff, R.J., Colwell, A.E., Wolfe, A.D., Young, N.D., Steiner, K.E., and dePamphilis, C.W. (1998). Molecular phylogenetic and evolutionary studies of parasitic plants. In *Molecular Systematics of Plants II: DNA Sequencing*, 2nd edn, D.E. Soltis, P.S. Soltis, and J.J. Doyle, eds. (Boston: Kluwer Academic), pp. 211–241.
5. Nickrent, D.L., Ouyang, Y., Duff, R.J., and dePamphilis, C.W. (1997). Do nonasterid holoparasitic flowering plants have plastid genomes? *Plant Mol. Biol.* **34**, 717–729.
6. Davis, C.C., and Wurdack, K.J. (2004). Host-to-parasite gene transfer in flowering plants: phylogenetic evidence from Malpighiales. *Science* **305**, 676–678.
7. Barkman, T.J., Lim, S.-K., Mat Salleh, K., and Nais, J. (2004). Mitochondrial DNA sequences reveal the photosynthetic relatives of *Rafflesia*, the world's largest flower. *Proc. Natl. Acad. Sci. USA* **101**, 787–792.
8. Nickrent, D.L., Blarer, A., Qiu, Y.-L., Vidal-Russell, R., and Anderson, F.E. (2004). Phylogenetic inference in Rafflesiales: the influence of rate heterogeneity and horizontal gene transfer. *BMC Evol. Biol.* **4**, 40.
9. Barkman, T.J., McNeal, J.R., Lim, S.H., Coat, G., Croom, H.B., Young, N.D., and dePamphilis, C.W. (2007). Mitochondrial DNA suggests at least 11 origins of parasitism in angiosperms and reveals genomic chimerism in parasitic plants. *BMC Evol. Biol.* **7**, 248.
10. Cronquist, A. (1981). *An Integrated System of Classification of Flowering Plants* (New York: Columbia University Press).
11. Wurdack, K.J., and Davis, C.C. (2008). Malpighiales phylogenetics: gaining ground on one of the most recalcitrant clades in the angiosperm tree of life. *Am. J. Bot.*, in press.
12. Davis, C.C., Latvis, M., Nickrent, D.L., Wurdack, K.J., and Baum, D.A. (2007). Floral gigantism in Rafflesiaceae. *Science* **315**, 1812.
13. Davis, C.C., Endress, P.K., and Baum, D.A. (2008). The evolution of floral gigantism. *Curr. Opin. Plant Biol.* **11**, 49–57.
14. Barkman, T.J., Bendiksby, M., Lim, S.-H., Mat Salleh, K., Nais, J., Madulid, D., and Schumacher, T. (2008). Accelerated rates of floral evolution at the upper size limit for flowers. *Curr. Biol.* **18**, 1508–1513.
15. Nais, J. (2001). *Rafflesia of the World* (Kota Kinabalu: Sabah Parks).
16. Givnish, T.J., and Sytsma, K.J., eds. (1997). *Molecular Evolution and Adaptive Radiation* (Cambridge: Cambridge University Press).

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## Deep-Sea Ecology: Infectious Impact on Ecosystem Function

Deep seas cover more than half the planet and depend due to their lack of light on inflow of nutrients from above. Viruses play a critical role for the trophic supply of the deep-sea environments.

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Life on earth depends on the primary production of photosynthesising organisms. In the marine realm, food availability is a function of depth, as primary production depends on light and, therefore, only occurs in the upper layer of the ocean, the euphotic zone. The deeper the ocean, the less food (i.e. organic matter) is available for the organisms. Abyssal deep-sea ecosystems are huge and cover 54% of the Earth's surface. Especially for deep-sea sediments, microbes are important in turning over the organic matter on its way to the seafloor. In the so-called 'microbial loop' organic matter of aggregates is constantly changed and can be transformed from particulate organic matter to dissolved organic matter, which is then available for other microbes. Viruses are the most abundant life forms in the ocean and infect presumably every type of organism — from the tiniest bacterium to the giant blue whales. Through their contribution to organismal mortality, viruses are likely to play a major role in the flow of energy and carbon through the trophic levels of the oceanic ecosystem. A recent paper by Danovaro *et al.* [1] shows how viral

infection plays an important role for the functioning of the largest ecosystem of the biosphere — the deep sea — by controlling benthic prokaryotic biomass, by stimulating prokaryotic metabolism and by accelerating biogeochemical processes. Due to viral infection about 80% of the prokaryotic heterotrophic organisms are killed and made available for the foodweb. This process obviously depends on pressure, as with increasing depth and below 1000 m almost 100% of the prokaryotic heterotrophic production is transformed into detritus.

On the basis of a data set of 232 sediment samples, Danovaro *et al.* [1] calculated that on a global scale the death of prokaryotes due to viral infection (viral shunt) releases 0.37–0.63 gigatonnes of carbon per year. Therefore, the 'victims' of viruses are an essential source of labile organic detritus — fine organic matter that is consumed by a lot of small omnivorous invertebrates and that plays an important role in the food web as it is also consumed by organisms on higher trophic levels. The viral shunt thus sustains a high prokaryotic biomass and provides an important contribution to prokaryotic metabolism. The prokaryotes are important in the

oceanic foodweb, as they can be consumed by higher organisms (metazoans) or serve to alter organic matter or aggregates in the water column. This microbial loop allows the deep-sea system to cope with the severe organic resource limitation which is a characteristic of the dark and extreme abyssal environments that lack photosynthetic primary production and instead depend on the carbon export from the surface oceans. Viruses are thus expected to have a direct impact on ecosystem functioning, which refers to processes such as the microbial and also biogeochemical flow of energy and matter (which is enhanced by the viral shunt) within and between ecosystems [2].

In the recent past, experiments in various ecosystems analysed the relationship between biodiversity and ecosystem function. It was shown that the more complex an ecosystem is, the higher the biodiversity tends to be and the 'healthier' the ecosystem [2]. Nowadays, the composition and diversity of biological communities is altered by anthropogenic impact through a variety of activities [3–6]. These activities increase the rate of species extinction and species invasion from local to global scales. Therefore, the effects of biodiversity loss on ecosystem functioning have been intensely investigated over the past decade [7], showing that species diversity generally has a positive but saturating effect on ecosystem processes [7–10]. This pattern is remarkably consistent across trophic