

Water lilies, loss of woodiness, and model systems

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The delicate necklace of threaded petals from the tomb of Rameses II, midnineteenth century glass houses built for the newly discovered *Victoria amazonica*, and Monet's giant canvases in the Musée de l'Orangerie all testify to a deep human attraction to water lilies: beguiling plants with showy flowers that seem to arise nymph-like out of the mud. Like orchids, cacti, succulents, and carnivorous plants, water lilies have a dedicated band of horticulturalists devoted to growing and exploring their endless variety. The late nineteenth century craze for water lilies that attracted Monet was fueled by one such enthusiast, Joseph Bory Latour-Marliac (1), who developed hardy water lily cultivars with dazzling new flower colors ranging from "delicate yellow to fuchsia and deep red." *Nymphaea thermarum*, the focus of the recent paper by Povilus et al. (2), is another unusual water lily variant. The smallest water lily known, *N. thermarum* was discovered and described in the late 1980s (3). Endemic to hot spring lakes in the Albertine Rift Valley of Rwanda, now, just a few decades after its discovery, it appears to be extinct in the wild (4, 5).

Crucially, as recognized by Povilus et al. (2), it has a small genome, only about three times larger than that of the botanical model of choice, *Arabidopsis*. Povilus et al. (2) provide initial documentation of that genome and use it to explore the genomic correlates and evolutionary significance of an unusual water lily trait, the complete loss of the vascular cambium that is responsible for the formation of woody tissues in almost all seed plants.

Part of the interest in water lilies for Povilus et al. (2), and for contemporary plant science, flows from the phylogenetic position of water lilies in flowering plant (angiosperm) evolution. It has long been suspected that water lilies were among those plants of special interest for studies of early angiosperm diversification (6), and this is now confirmed by phylogenetic analyses based on extensive molecular data that place water lilies as sister group to all other flowering plants but



Fig. 1. Colored print illustrating flower bud, flowers, and leaves of *Victoria amazonica*, the giant Amazonian water lily, from the monograph by John Fisk Allen published in 1854 (21) based on plants grown at Salem, MA. In contrast to the minute size of *N. thermarum*, the leaves and flowers of *V. amazonica* are gigantic.

one (7, 8). Only the lineage that today comprises *Amborella*, a monotypic shrub endemic to New Caledonia, appears to have diverged before the common ancestor of water lilies and all other flowering plants (7, 8). Since all nonangiosperm seed plants are woody, as also is *Amborella* and other closely related lineages (such as Austrobaileyales), the obvious conclusion is that the loss of woodiness in Nymphaeales is a modification that probably occurred early in the diversification of angiosperms, most likely, in association with the transition from a terrestrial to aquatic existence.

Angiosperms are hugely diverse, with perhaps 400,000 living species. Many are herbaceous, but complete loss of the vascular cambium, which is responsible for the production of woody tissues, has

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Author contributions: P.R.C. and E.M.F. wrote the paper.

The authors declare no competing interest.

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See companion article, "Water lily (*Nymphaea thermarum*) genome reveals variable genomic signatures of ancient vascular cambium losses," [10.1073/pnas.1922873117](https://doi.org/10.1073/pnas.1922873117).

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been documented in only five lineages. In Nymphaeales, the “hornwort” (*Ceratophyllum*), the lotus (*Nelumbo*), and the tropical riverweed family Podostemaceae, the convergent loss of the vascular cambium appears to be associated with the transition to an aquatic habit, which obviated the need for mechanical support or additional water-conducting tissues. A semiaquatic habitat has also been invoked for the last common ancestor of all monocots, the fifth clade of angiosperms to have independently lost a vascular cambium (9).

Based on their sequencing and assembly of the *N. thermarum* draft genome, Povilus et al. (2) are able to compare the genomes of monocots and *Nelumbo* with the genomes of plants with a vascular cambium, such as *Arabidopsis thaliana*, *Populus trichocarpa*, and *Zinnia elegans*. Consistent with currently accepted phylogenetic patterns, they detect different genomic signatures correlated with the loss of cambial activity in *N. thermarum*, *Nelumbo*, and monocots. In *N. thermarum*, especially notable was the loss of *REVOLUTA*, an HD-ZIP III transcription factor that is known to regulate the differentiation of vascular tissues by the cambium in other angiosperms, including *Arabidopsis*. Interestingly, *Nelumbo*, once thought to be closely related to water lilies (10), shows no evidence of gene loss comparable to that seen in *N. thermarum*. Understanding more about cambial loss in *Nelumbo* could be illuminating for understanding the origin of the herbaceous habit, and the reduced cambial activity among buttercups and their allies (Ranunculales), as well as complete cambial loss in *Ceratophyllum*. In current phylogenetic hypotheses, these three lineages are not widely separated (7, 8), and how complete cambial loss relates to the evolution of the herbaceous habit more generally is an important question. Presumably, there were herbaceous intermediates between woody shrubs like *Amborella* and fully herbaceous aquatics.

As their phylogenetic position has come into sharper focus, more attention devoted to water lilies has produced unanticipated new perspectives. A dramatic systematic realignment has significantly expanded the range of morphologies known among living Nymphaeales through the addition of the Hydatellaceae, a family with flowers so simple that they were formerly classified with grasses (11). Detailed studies of the process of fertilization have shown that water lilies are an exception to the classic mode of angiosperm embryology (12, 13) whereby the main provisioning of the embryo is by the endosperm, a triploid nutritive tissue formed by two maternal and one paternal genome. In *N. thermarum*, other Nymphaeales, and all nonangiosperm seed plants, the perisperm (or its equivalent), an entirely maternal tissue, is a key regulator and the primary provisioning tissue during embryo development (14), and their endosperm, unusually, is diploid, which is perhaps the ancestral condition for angiosperms (12). And, while it has been long known that the water lilies have an extensive fossil record in the Early Cenozoic, that record is now extensive from the Early Cretaceous. The oldest fossil assemblage known to yield unequivocal angiosperm flowers, fruits, seeds, and stamens, the Late Barremian–Early Aptian Torres Vedras locality in central Portugal, includes several different kinds of seeds with characters indicating a close relationship to extant Nymphaeales and Austrobaileyales (15). There are also structurally preserved flowers and seeds, as well as whole-plant compression/impression fossils, in only slightly younger deposits from the Early Cretaceous (16, 17).

Given the demonstrated antiquity and phylogenetic position of water lilies, the possibility of using *N. thermarum* as an experimental model to investigate potentially general aspects of angiosperm

biology and development is undeniably alluring. However, as with all model organisms, a key issue is the likely generality of the systems being investigated, a judgment that requires comparative context. In particular, in the case of *N. thermarum*, the antiquity of the Nymphaeales is a two-edged sword. The antiquity of a lineage is not the same as the antiquity of a species. *N. thermarum* likely differs substantially from its ancestors that flourished on Early Cretaceous landscapes populated by *Iguanodon* and its cousins.

The initial exploration of the water lily genome provided by Povilus et al. not only sheds light on the loss of the vascular cambium, and the transition to the aquatic habit, but also provides a system for experimental work with a plant in a very interesting phylogenetic position.

The fossil record shows that all living water lily species are separated from their Early Cretaceous ancestors by countless speciation events that occurred over a vast span of time, no doubt along with many morphological and molecular changes. So, if we were able to fully untangle the developmental genetics responsible for the polymerous flowers of *N. thermarum*, whether those insights would apply to the genus *Nymphaea* or the family Nymphaeaceae or have more general relevance to the supposedly polymerous flowers of the first angiosperms (18) would require a comparative perspective. And there is a further complication: the comparative context itself is dependent on sampling, and the ancient plants that were participants in the origin of angiosperms 140 million years ago are long gone. The fossil record shows that their living relatives are a mere shadow of their former diversity. It would therefore be prudent to treat with caution confident extrapolations about the characteristics of the first angiosperms based solely on extant taxa, let alone based solely on *N. thermarum*.

Notwithstanding these complications, further studies of *N. thermarum*, and also *N. colorata* for which the genome has recently been described (19), will surely be instructive. Floral variation in the Nymphaeales ranges from the tiny simple floral structures of *Hydatella* to the massive polymerous flowers of *Victoria* (Fig. 1), and the experience of recent decades is that the water lilies get more interesting, not less, as new techniques allow us to explore their structure and biology, both as fossils and as living plants. The initial exploration of the water lily genome provided by Povilus et al. (2) not only sheds light on the loss of the vascular cambium, and the transition to the aquatic habit, but also provides a system for experimental work with a plant in a very interesting phylogenetic position. Other genomes are available from other early-diverging angiosperm lineages, for example, for *Amborella* (20), but none of these plants have a genome as small as that of *N. thermarum*, nor are they such a tractable experimental subject. *N. thermarum* can be grown by the hundreds on a greenhouse bench, produces abundant seed, and goes from germination to flowering in just 4 mo to 5 mo (4, 5). Once close to extinction, *N. thermarum* now seems to have a secure future, at least in the laboratory. Its study is likely to be illuminating from many perspectives, including for those interested in probing the earliest phases of angiosperm evolution and its profound consequences for all terrestrial life.

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