On the Origin of Flowering Plants

IN 1879, CHARLES DARWIN PENNED A LETTER to British botanist Joseph Dalton Hooker, lamenting an "abominable mystery" that threw a wrench into his theory of evolution: How did flowering plants diversify and spread so rapidly across the globe? From rice paddies to orange groves, alpine meadows to formal gardens, prairies to oak-hickory forests, the 300,000 species of angiosperms alive today shape most terrestrial landscapes and much of human life and culture. Their blooms color and scent our world; their fruits, roots, and seeds feed us; and their biomass provides clothing, building materials, and fuel. And yet this takeover, which took place about 100 million years ago, apparently happened in a blink of geological time, just a few tens of millions of years.

The father of evolution couldn't quite fathom it. Darwin had an "abhorrence that evolution could be both rapid and potentially even saltational," writes William Friedman in the January *American Journal of Botany*, which is devoted to this "abominable mystery." Throughout his life, Darwin pestered botanists for their thoughts on the matter, but they couldn't give him much help.

Now, 130 years later, evolutionary biologists are still pestering botanists for clues about what has made this plant group so successful, as well as when, where, and how flowers got started—and from which ancestor. Today, researchers have analytical tools, fossils, genomic data, and insights that Darwin could never have imagined, all of which make these mysteries less abominable. Over the past 40 years, techniques for assessing the relationships between organisms have greatly improved, and gene sequences, as well as morphology, now help researchers sort out which angiosperms arose early and which arose late. New fossil finds and new ways to study them—with synchrotron radiation, for example—provide a clearer view of the detailed anatomy of ancient plants. And researchers from various fields are figuring out genomic changes that might explain the amazing success of this fast-evolving group.

These approaches have given researchers a much better sense of what early flowers were like and the relationships among them. But one of Darwin's mysteries remains: the nature and identity of the angiosperm ancestor itself. When flowering plants show up in the fossil record, they appear with a bang, with no obvious series of intermediates, as Darwin noted. Researchers still don't know which seed- and pollen-bearing organs eventually evolved into the comparable flower parts. "We're a bit mystified," says botanist Michael Donoghue of Yale University. "It doesn't appear that we can locate a close relative of the flowering plants."

Seeking the first flower

One of two major living groups of seed plants, angiosperms have "covered" seeds that develop encased in a protective tissue called a carpel (picture a bean pod). That's in contrast to the nonflowering gymnosperms, such as conifers, which bear naked seeds on scales. An angiosperm's carpel sits at the center of the flower, typically surrounded by pollen-laden stamens. In most flowers, the carpel and stamens are surrounded by petals and an outer row of leaflike sepals. Seeds have a double coating as well as endosperm, tissue surrounding the embryo that serves as its food supply.

Darwin was perplexed by the diversity of flowering plants; they were too numerous and too varied, and there were too few fossils to sort out which were more primitive. Throughout much of the 20th century, magnolia relatives with relatively large flowers were leading candidates for the most primitive living flowers, although a few researchers looked to small herbs instead.

In the late 1990s, molecular systematics came to the rescue, with several reports presenting a fairly consistent picture of the lower branches of the angiosperm tree. An obscure shrub found only in New Caledonia emerged as a crucial window to the past. *Amborella trichopoda*, with its 6-millimeter greenish-yellow flowers, lives deep in the cloud forests there. In multiple gene-based assessments, including an analysis in 2007 of 81 genes from chloroplast genomes belonging to 64 species, *Amborella* sits at the base of the angiosperm family tree, the sister group of all the rest of the angiosperms.

Given that placement, *Amborella*'s tiny flowers may hint at what early blossoms were like. It's one of "the most similar living flower[s]" to the world's first flower, says James Doyle of the University of California, Davis. The petals and sepals of its single-sex flowers are indistinguishable and vary in number; so too do the numbers of seed-producing carpels on female flowers and pollen-generating stamens on male flowers. The organs are spirally arranged, and carpels, rather than being closed by fused tissue as in roses and almost all familiar flowers, are sealed by a secretion.

Most genetic analyses showed that water lilies were the next branch up the angiosperm tree, followed by a group represented by star anise, which also has a primitive look about it, says Doyle, "though each of these has deviations from the ancestral type."

Fossil records

Although some fossil pollen dates back 135 million years, no credible earlier fossil evidence exists. In Darwin's day, and for many decades afterward, paleobotanists primarily found leaves or pollen but almost no fossil flowers. They had the wrong search image, says Else Marie Friis of the Swedish Museum of Natural History in

THE YEAR OF DARWIN

This essay is the fourth in a monthly series. For more on evolutionary topics online, see the Origins blog at **blogs.sciencemag.org/origins.** For more on flower origins, listen to a podcast by author Elizabeth Pennisi at **www.sciencemag.org/multimedia/podcast.**

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Published by AAAS

Out of the past. Tiny *Amborella* sits at the bottom of the angiosperm family tree.

Stockholm. "When we started, the search profile was bigger, a magnolia [flower]," she recalls. But 30 years ago, she and others discovered tiny ancient flowers by sieving through sand and clay sediments. With this technique, they have now collected hundreds of millimeter-size flowers, some preserved in three dimensions, from Portugal and other locations with Cretaceous deposits 70 million to 120 million years old.

This fossil diversity shows that angiosperms were thriving, with several groups well-established, by 100 million years ago. In some, the flower parts are whorled like those of modern flowers; in others they are spiraled, considered by some researchers as the more primitive arrangement. Some flower fossils have prescribed numbers of petals, another modern feature, whereas in others the petal count varies.

In 1998, Chinese geologist Ge Sun of Jilin University in Changchun, China, came across what seemed to be a much older flower. The fossil, called *Archaefructus*, was an aquatic plant that looked to be 144 million years old. By 2002, Sun and David Dilcher of the Florida Museum of Natural History (FLMNH) in Gainesville had described an entire plant, from roots to flowers, entombed on a slab of rock unearthed in Liaoning in northeastern China.

In one sense, *Archaefructus* wasn't much to look at. "It's a flowering plant before there were flowers," Dilcher notes. It lacked petals and sepals, but it did have an enclosed carpel. When Kevin Nixon and colleagues at Cornell University compared its traits with those same traits in 173 living plants, *Archaefructus* came out as a sister to living angiosperms and closer to the common ancestor than even *Amborella*.

Archaefructus's distinction was short-lived, however. Within months, better dating of the sediments in which it was found yielded younger dates, putting this first flower squarely with other early fossil flower parts, about 125 million years old. Also, a 2009 phylogenetic analysis of 67 taxa by Doyle and Peter Endress of the University of Zurich, Switzerland, placed the fossil in with water lilies rather than at the base of the angiosperms, although this conclusion is contested.

> "We are realizing that this huge diversity is probably the result of one innovation piled on top of another innovation."
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> —Peter Crane, University of Chicago

These fossils often spark debate because specimens tend to be imperfectly preserved and leave room for interpretation. To help remedy that, Friis and her colleagues have begun to examine flowers using synchrotron radiation to generate a 3D image of their inner structures, allowing the fossil to remain intact while Friis peers inside it from many angles (*Science*, 7 December 2007, p. 1546). "We can get fantastic resolution," says Friis. "It's really exciting." But so far, the flowers Friis finds are too diverse to trace back to a particular ancestor. "From these fossils, we cannot say what is the basic form," she says.

Before flowers

Although they have yet to find the oldest fossil flowers, researchers assume that the ancestral angiosperm evolved from one of the nonflowering seed plants or gymnosperms, whose heyday was 200 million years ago. Modern gymnosperms include conifers, ginkgoes, and the cycads, with their stout trunks and large fronds. Before angiosperms came along, these plants were much more diverse and included cycadlike species, such as the extinct Bennettitales, and many woody plants called Gnetales, of which a few representatives, including the joint firs, survive today (see family tree, p. 31). Also common in the Jurassic were seed ferns, a group now long gone; their most famous member is *Caytonia*, which seems to have precarpel-like structures. These groups' perceived relevance to flower evolution and their relationships to angiosperms have ping-ponged between camps, depending on how the evolutionary trees were constructed.

In the mid-1980s, Peter Crane, now at the University of Chicago in Illinois, proposed a solution, the anthophyte hypothesis. Using several lines of evidence and noting that both Bennettitales and Gnetales organize their male and female organs together in what could be construed as a preflower, he considered them, along with angiosperms, as comprising a single angiosperm entity called anthophytes. For the next decade, most family trees based on morphology sup-

Larger than life. Although merely 2.2 millimeters in diameter, this 3D fossil flower shows that grasses date back to 94 million years ago.

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Flowers, food, fuel. Darwin marveled at the diversity of angiosperms. Given that they represent nine in 10 land plants, it's no surprise that they serve as mainstays of both our welfare and sense of beauty. Clockwise from left: aspens, orchids, grasses, sunflowers, tulips, apples, walnuts.

ported this idea. Crane and others carefully dissected and described fossils of these groups, looking for the precursors to carpels, the seed's double coat, and other distinctive angiosperm traits.

But they have run into problems. "We do not really know how to compare them because the structures are very different-looking; figuring out what's homologous is quite a difficult thing," says Crane. He and his colleagues argue, for example, that the seeds in the Bennettitales have two coverings, which may be a link to angiosperms. But in the January *American Journal of Botany*, Gar Rothwell of Ohio University, Athens, and two colleagues disagree, saying that what Crane calls the outer layer is the only layer, and find fault with the hypothesis in general.

To make matters worse for anthophyte proponents, gene-based evolutionary trees break up this grouping, pulling the Gnetales off any angiosperm branch and placing them among or next to the other gymnosperms. "The molecular work points in one direction; the paleobotanical work points you in another direction," Crane says.

And if the molecular work is correct, then the field doesn't know in which direction to turn, because in most analyses the genetic data don't place any living plant close to angiosperms. The angiosperms group together, the living gymnosperms group together, and there's nothing in between. "The nonangiosperm ancestor just isn't there," says paleobotanist William Crepet of Cornell. "I'm starting to worry that we will never know, that it transformed without intermediates."

Seeds of success

The angiosperm's ancestor may be missing, but what is very clear—and was quite annoying to Darwin—is that the angiosperm prototype so readily proved a winner. Seed ferns and other gymnosperms arose about 370 million years ago and dominated the planet for 250 million years. Then in a few tens of millions of years, angiosperms edged them out. Today, almost nine in 10 land plants are angiosperms.

Inside and out. Synchrotron radiation helped produce a 3D rendering (gold) of this fossil male flower (*right*) and insights into its internal structure.

The exact timing of the angiosperms' explosion and expansion is under debate, as is the cause. At least one estimate based on the rate at which gene sequences change—that is, the ticking of the molecular clock—pushes angiosperm evolution back to 215 million years ago. "There appears to be a gap in the fossil record," says Donoghue, who also notes that molecular dating methods "are still in their infancy" and, thus, could be misleading. He and others think that flowering plants lingered in obscurity for tens of millions of years before radiating toward their current diversity.

Whatever the timing, there was something special about the angiosperm radiation. During the 1980s and again in 1997, Cornell's Karl Niklas compiled a database showing the first and last occurrences of fossil plants. When he and Crepet used that and more recent information to look at species' appearances and disappearances, they found that new angiosperms appeared in bursts through time, whereas other plants, such as gymnosperms, radiated rapidly only at first. Moreover, angiosperms proved less likely to disappear, somehow resisting extinction, says Crepet.

Once the angiosperms arrived, how did they diversify and spread so quickly? Darwin suspected that coevolution with insect pollinators helped drive diversification, though

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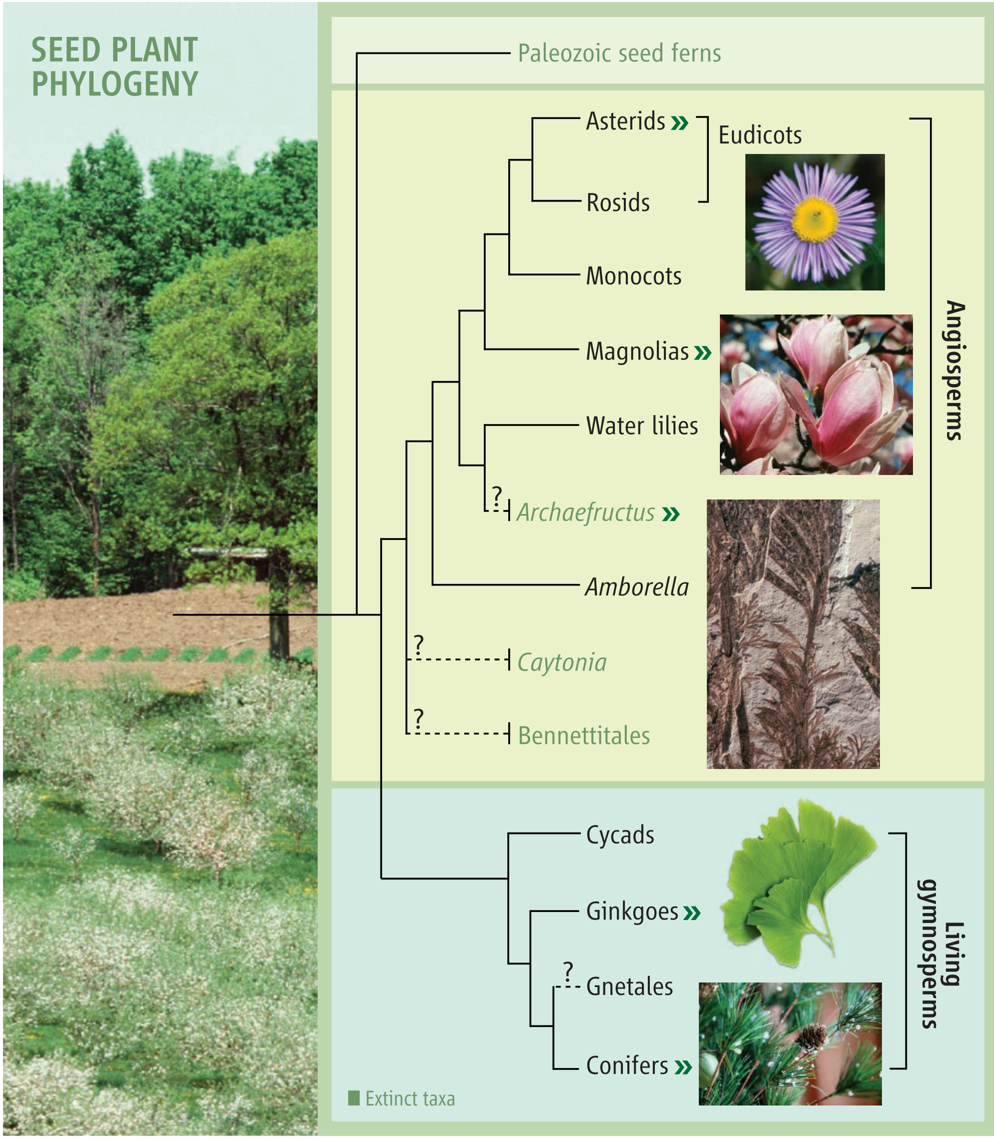
such a causal relationship is not settled. Later, animals that ate fruit and dispersed seeds likely helped evolving species expand quickly into new territory. Some think the answer lies in genes: duplications that gave the angiosperm genome opportunities to try out new floral shapes, new chemical attractants, and so forth. This flexibility enabled angiosperms to exploit new niches and set them up for long-term evolutionary success. "My own view is that in the past, we have looked for one feature," says Crane. Now, "we are realizing that this huge diversity is probably the result of one innovation piled on top of another innovation."

The latest insights into diversification come from gene studies. From 2001 to 2006, Pamela Soltis of the FLMNH and Claude dePamphilis of Pennsylvania State University, University Park, participated in the Floral Genome Project, which searched for genes in 15 angiosperms. Now as a follow-up, the Ancestral Angiosperm Genome Project looks at gene activity in five early angiosperms and a cycad, a gymnosperm.

DePamphilis and his colleagues matched all the genes in each species against one another to determine the number of duplicates. They then looked at the number of differences in the sequences of each gene pair to get a sense of how long ago the duplication occurred. In most early angiosperms, including water lilies and magnolias, they saw many simultaneous duplications—but not in *Amborella*, they reported in the January 2009 *American Journal of Botany*, confirming earlier reports. The data suggest that a key genome duplication happened after the lineage leading to *Amborella* split off but before water lilies evolved. "We're beginning to get the idea that polyploidization may have been a driving force in creating many new genes that drive floral development," dePamphilis says.



Shifting branches. As this simplified family tree shows, gene studies have helped clarify the relationships of many living angiosperms, but fitting in extinct species is still a challenge, and some nodes are hotly debated.

Others have noted that a duplication occurred in the evolution of grasses, and the Floral Genome Project confirms that yet another duplication paved the way for eudicots, the group that includes apples, roses, beans, tomatoes, and sunflowers. "There are some real 'hot spots' in angiosperm evolutionary history," says dePamphilis, who is working to fully sequence the genome of *Amborella* with his colleagues.

The Floral Genome Project also looked to see whether the genetic programs guiding flower development were consistent throughout the angiosperms. "We found that there are fundamental aspects that are conserved in the earliest lineages," says Soltis. "But there are differences in how the genes are deployed."

Take the avocado, a species on the lower branches of the angiosperm tree. In most angiosperms, the flower parts are arranged in concentric circles, or whorls, around the carpels, with stamens innermost, then petals, and finally sepals. Each tissue has its own distinct pattern of gene expression, but not in the avocado. Genes that in *Arabidopsis* are active only in, say, the developing petals spill over in avocado to the sepals. Thus in the more primitive plants, petals and sepals are not as well-defined as they are in *Arabidopsis*. This sloppiness may have made development flexible enough to undergo many small changes in expression patterns and functions that helped yield the great diversity in floral forms.

In his letter to Hooker, Darwin wrote that he would like "to see this whole problem solved." A decade ago, Crepet thought Darwin would have gotten his wish by now. That hasn't happened, but Crepet is optimistic that he and his colleagues are on the right track, as analyses of various kinds of data become more sophisticated. "We are less likely to go around in circles in the next 10 years," he says. "I believe a solution to the problem is within reach. … The mystery is solvable."

–ELIZABETH PENNISI

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