PLANT GENETICS

Something to Sniff At: Unbottling Floral Scent

Plant biologists are blending modern genomics and classic biochemistry to restore scents lost when flowers are bred for looks and long vase life

David Weiss knows where to look for fragrant roses—and it's not the corner florist. Instead, Weiss turns to a growing rose DNA database, hunting for scent genes that could enhance future flowers. His search might mean more romantic bouquets for us all—and a bonanza for the flower industry.

Many modern roses, bred for stunning blossoms and long vase life, have all but lost their scent. In fact, although cut roses bring in some $10 billion worldwide every year, the flowers often smell mostly of wax-paper wrapping. And these floral favorites are not alone. Carnations, chrysanthemums, and some lilacs, among other modern cultivars, lack fragrance.

But Weiss, a plant biologist at the Hebrew University of Jerusalem, and other researchers might bring back petal perfume. Using both genomics and classic biochemistry, these scientists have begun unraveling the mechanics of scent in roses, snapdragons, and Brewer's clarkia, among other bloomers. "We now have the opportunity to go deep into this field," Weiss says, "in a way that wasn't possible just a few years ago."

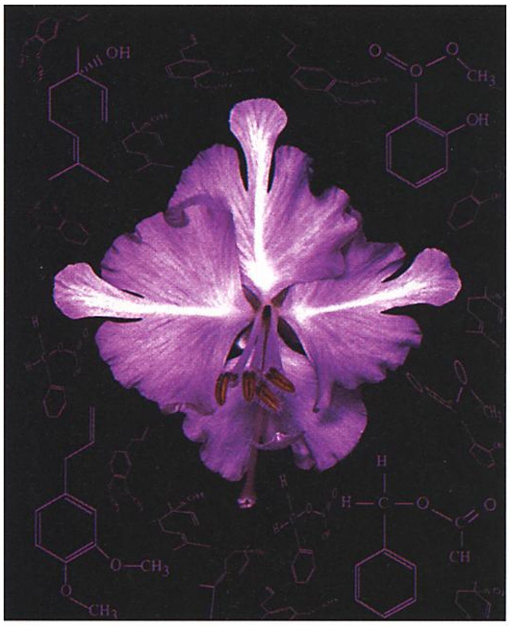
Unbottling the basics

In the garden, fragrance is fickle. Blossoms can be sweet or spicy, faintly citrus, or starkly intoxicating. A plant's signature scent is a unique combination of volatile compounds: small organic molecules that evaporate off petals, filling the air with perfume to attract bee and moth pollinators. Although all flowering plants likely share some genes for floral scent, each species expresses a particular gene mix.

For decades, floral scent was a sensual delight, difficult to measure or even describe. Perfumers have been the primary consumers of floral scent chemistry, bottling the sweetness of freesia, gardenias, and the like. But they merely require the chemical structure of a scent. Until recently, no one had tried to take a biochemical step backward—to see precisely how flowers produce scents in the first place.

That began to change in 1994, with a study led by Eran Pichersky of the University of Michigan, Ann Arbor. Pichersky had originally been interested in evolution: How have some plant species evolved an ability to make scent when others, even close relatives, have not? Casting about the literature, however, he quickly realized that researchers didn't understand even the basics of floral scent. "We had no genes, no enzymes," Pichersky says. "In order to see how scent evolves, we first had to learn how it's made."

Pichersky and his colleagues decided to study Brewer's clarkia (*Clarkia breweri*), an annual wildflower native to California that wears a strong, sweet scent and lavender blossoms. In the lab, the team trapped volatile compounds emitted by Brewer's clarkia in a plastic chamber. They injected them into a gas chromatograph, which separated the compounds, and a mass spectrometer, which identified and quantified them. In all, the team found eight to 12 major volatile compounds.



Wildly fragrant. The wildflower Brewer's clarkia offered researchers the first biochemical details of floral scent enzymes.

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Soon, the team accomplished a floral first: They isolated and characterized an enzyme that confers scent—linalool synthase, which helps form linalool, a common scent volatile in flowers—and its corresponding gene. Since then, the scientists have discovered three more floral scent enzymes and their relevant genes in Brewer's clarkia. "We found that almost all scent biochemistry happens in the epidermal surface of the flower—especially in the petals, where scents escape easily," says Pichersky, who calls the system beautifully simple.

Now, the snapdragon (*Antirrhinum majus*), a perennial garden favorite, is getting its day in the sun. Using a similar biochemical approach, plant biologist Natalia Dudareva of Purdue University in West Lafayette, Indiana, and her colleagues have identified and characterized the enzyme benzoic acid carboxyl methyltransferase (BAMT), which helps form methyl benzoate, a major scent volatile in snapdragons. BAMT shows up solely in snapdragon petals. "What's interesting," Dudareva says, "is that floral scent is restricted to the areas of snapdragon petals that come in contact with pollinators, like close to the mouth of the corolla."

Dudareva aims to sort out the snapdragon's perfume patterns. In a study published last year in *The Plant Cell*, for instance, she and her colleagues reported that the flowers follow a circadian rhythm, releasing four times as much methyl benzoate during the day—prime pollinating time for bees—than at night. They also found a similar circadian pattern in petunias and flowering tobacco, although these plants release maximum methyl benzoate at night, when their moth pollinators are most likely to visit. "We don't know much about this clock yet," Dudareva says, "but it could work in many plants."

Like many flowers, snapdragons also turn down their scent shortly after pollination—a strategy that might steer insects toward unpollinated blossoms. To catch this scent loss in action, Dudareva's team recently hand-pollinated snapdragon and petunia flowers over several days. Within 72 hours of being fertilized, snapdragons lost 90% of their scent production; petunias lost that much in just 48 hours. The researchers zeroed in on a candidate gene that might trigger this slump in scent, Dudareva says, in work to be published later this year.

Growing genomics

These early scent experiments on Brewer's clarkia and snapdragons brought classic biochemistry into the garden: Researchers worked backward to characterize each scent enzyme and its gene, one at a time. The science might have stopped there. Lacking a simple way to spot subtle fragrance mutants, researchers cannot use Mendelian genetics to easily breed and test plants for scent.

Now, however, there's a promising alternative: genomics, which enables speedy screening of plant tissue DNA for candidate

A rose to remember. Unlike some modern cultivars, the Fragrant Cloud rose is rich in scent—and notably volatile.

genes. In Israel, Weiss heads the Petal Genomics project—a 3-year-old effort to build a database of DNA expressed in rose petals; from there, the researchers hope to identify scent genes.

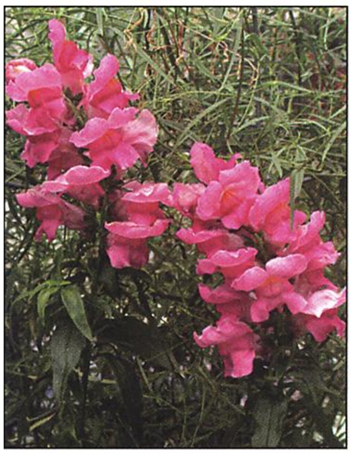
To do so, Weiss and his colleagues, including Pichersky, are cloning and sequencing active DNA from rose petals. They pinpoint candidate scent genes by searching GenBank and other databases for similar genes that encode enzymes known to help build volatiles. Finally, the team expresses those candidate genes in bacteria, analyzing the proteins' activity. "The idea is to find the volatiles produced by flowers, as well as the genes expressed by petals during different stages of scent production," Weiss explains.

To launch the Petal Genomics project, funded by Israel's Ministry of Science, Weiss's team chose two cultivars: Fragrant Cloud, a strongly scented red rose, and Golden Gate, an almost odorless yellow rose. When analyzed, petals from the two roses revealed strikingly different volatile compounds—and yielded more than 3000 potential genes expressed in petals, including at least 15 candidate fragrance genes, Weiss says. Already, the team has characterized four novel enzymes—including orcinol O-methyltransferases dubbed OOMT1 and OOMT2, which catalyze a common scent component in roses known as 3,5-dimethoxytoluene. The first description of a scent enzyme in rose flowers, that work will be published in *Plant Physiology* in August.

Ultimately, Weiss hopes to spice up floriculture by engineering more fragrant roses. "The ornamental flower industry in Israel is an important aspect of agriculture, partly because we export cut flowers to Europe during their cold, dark winters," says Weiss, whose family owns a flower business. "I'd like to see if we can bring scent back to these modern cultivars."

But the work will be tricky, cautions Alan Blowers, biotechnology project manager at Ball Horticultural in Chicago. "We have an elementary understanding of floral scent at this point," Blowers notes. "While there is commercial potential, I think we're a ways from that."

Indeed, early attempts to engineer floral scent have had mixed results. In the first reported effort, published last year in *The Plant Journal*, scientists in the Netherlands engineered petunias with the linalool synthase gene found in Brewer's clarkia. The transgenic petunias expressed linalool in many tissues but failed to actually release the scent compound. Weiss's lab recently had more success breeding the same gene into carnations, which did release linalool—although not enough for people to detect a stronger scent.



Snapping in time. Snapdragon scent follows a circadian rhythm, spiking during the day to attract bee pollinators.

Another tack has proved intriguing. In a study to be published next month in *Molecular Breeding*, Weiss and his colleagues boosted scent production in carnations by blocking the flowers' pigment. Because color compounds and scent compounds begin in the same biochemical pathway, Weiss says, blocking color likely redirects metabolic flow toward scent. Researchers might even return to carnations their rightful legacy: At one time, the flowers smelled richly of cloves and spice, thanks to heavy amounts of the volatile eugenol, a minor ingredient in today's cultivars.

Pichersky, for one, is confident that flowers spiked with scent, or transgenic fruits with a special flavor (see sidebar), will eventually hit the marketplace, despite the current lukewarm climate for genetically modified foods. "It can happen and will happen," he says. If so, you just might find yourself stopping to smell the roses.

–KATHRYN BROWN

Plants 'Speak' Using Versatile Volatiles

Inside the garden, methyl jasmonate is the sweetest scent around. Rising off jasmine flowers, the compound's sultry perfume stops pollinators and people alike. But sweetness can be deceiving: When plants are wounded by hungry herbivores, that same scent, shot from the leaves, serves as an SOS.

In fact, the emerging biochemistry of floral scent confirms what researchers have long suspected: Plants are versatile perfumers, flinging similar—and in some cases, identical—chemicals into the air to turn organisms on or off, depending on the situation. "Think of plant volatiles as a language," says Jim Tumlinson of the U.S. Department of Agriculture's Agricultural Research Service in Gainesville, Florida. "If you put them together into one message, you have a scent that attracts pollinators. Put together another way, you have a scent that attracts natural enemies of herbivores threatening a plant."

It's been about 20 years since entomologist Jack Schultz of Pennsylvania State University, University Park, and then-student Ian Baldwin first proposed that "talking trees"—in that case, damaged poplars and maples—could signal distress to their neighbors with airborne chemicals. Scientists scoffed. But last year, Baldwin—now director of molecular ecology at the Max Planck Institute for Chemical Ecology in Jena, Germany—and graduate student André Kessler reported in *Science* the first field evidence for indirect plant volatile defenses against herbivores (16 March 2001, p. 2141).

Working in southwestern Utah's Great Basin desert, Baldwin and Kessler found that tobacco plants under assault from caterpillars, leaf bugs, and flea beetles naturally release three compounds that can cut this herbivore activity by more than 90%. Now, Baldwin is looking for the genes behind these plant defense volatiles. "What is it that controls volatile release?" he asks.

Plant scents also attract human grazers. Researchers have recently identified key volatiles that flavor sweet basil and strawberries, among other edible plants. Last November in *Plant Physiology*, Eran Pichersky of the University of Michigan, Ann Arbor, and his colleagues reported engineering tomatoes with S-linalool, a scent and flavor volatile also found in flowers, with the ultimate goal of improving both scent and taste. In fact, he adds, new plant volatiles—and uses—continue to emerge.

–K.B.

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