What makes a flower? What makes these organs, and what makes them appear in the same sepal-petal-stamen-carpel order, time after time, species after species?

Late Bloomer: *Arabidopsis* Arrives

by Rebecca Rothenberg

Professor of Biology Elliot Meyerowitz is surrounded in his meat locker cum nursery by his charges—hundreds of *Arabidopsis* mutants in various stages of development, some just beginning to germinate, some gone to seed.

What makes a flower? A seed, dirt, sun, water, Miracle-Gro, and a little luck, right?

Okay, let's put it another way: what makes *up* a flower? Ah. Well, petals, of course. Those little green leaves that enclose the bud and remain outside the petals—the sepals. The long filaments in the middle of the petals—the stamens, or male, pollen-carrying organs. The other, female, structure at the center—the carpel, which contains the single or compound ovary that becomes a seed.

In fact, as it turns out, almost every flower in the world, from rose to camellia to carnation to wild mustard, has exactly the same parts, or organs, in exactly the same pattern: concentric whorls of—from the outside in—sepals, petals, stamens, and carpels. Check it out in your garden. (The aster, or composite, family, such as daisies, dandelions, and sunflowers, in which each "petal" is actually a complete flower, has different terminology, but the rule holds nonetheless.)

So let's return to our first question: what makes a flower? What makes these organs, and what makes them appear in the same sepal-petal-stamen-carpel order, time after time, species after species? And what tells each plant of a given species to make the right *number* of organs and with the right spacing in between (for example, in the wild, *Arabidopsis* almost always has four petals and six stamens, whereas members of other plant families have different numbers of organs).

Professor of Biology Elliot Meyerowitz thinks he knows the answers to some of these questions. He's identified the sequence of master regulatory genes that turns on the instructions to make flower organs appear in the appropriate whorl. In fact, Meyerowitz can make a flower that's all sepals. Or a lush (but sterile) bloom comprising four whorls of petals. Or what he jokingly calls "a manly thing," consisting only of stamens.

Meyerowitz's work has unfolded in his lab in the northwest wing of Church, which at first glance looks like every other biology laboratory: the Ikea kitchen section run amok. Endlessly replicated shiny counters are covered by glassware and machinery of unknown purpose. But make a wrong turn and suddenly you're in a closet-turned-potting shed, garden spade leaning up against 20-pound sacks of planting mix, green garden hose coiled at your feet. Cross the hall and you're in another familiar milieu, a garden nursery. Actually, Meyerowitz explains, it's a fluorescent-lit meat locker modified into a nursery: refrigerator shelves designed for shrink-wrapped pork chops instead hold hundreds of flats of weedy little plants. Some are barely germinating, some are flowering, some are very strange-looking indeed, with strap-like structures instead of stems, and flowers growing higgledy-piggledy up the sides instead of in an orderly pattern. Some have gone to seed, sending up scaffolds of dry seedpods that give the room the forlorn look of a vacant lot.

The protean *Arabidopsis thaliana* can be classy (right) or funky (below).

Yet these homely weeds—mouse-ear cress, or, more properly, *Arabidopsis thaliana*—are the heart of the lab, and one of Meyerowitz's most important contributions to contemporary genetics.

Arabidopsis thaliana is a diminutive member of the mustard family. It stands about five inches high, has a rosette of leaves at the base of its stem and a stalk of tiny, four-petalled white flowers. In the wild it looks like, and is, a scruffy cousin to sweet alyssum; it was named for Johannes Thal, a 16th-century herbalist who first described it. "Not," says the soft-spoken Meyerowitz in his office behind the closet/potting shed, "for the Greek muse of comedy," Thalia. Though there *is* something comic and endearing about the little plant. It inspires metaphors—"the people's plant" and "the Hyundai of plants"—and pranks: Meyerowitz has a slide of a chia pet furry with sprouting *Arabidopsis*, the gift of Mike Nasrallah, a Cornell colleague.

When Meyerowitz set out to determine which genes tell a particular cell in a plant's growing shoot, or apical meristem, to become part of a sepal, as opposed to, say, a petal or carpel, he knew he would begin in classic Mendelian fashion, by looking at mutations in the plant's phenotype and inferring information about its genotype. Unlike Mendel, however, who had to wait for nature to produce those interesting mutations, 20th-century geneticists can induce mutations by soaking seeds in a mutagenic agent like ethyl methanesulfonate.

But at the outset Meyerowitz was faced with a fundamental decision. What would he use as the experimental organism? Peas, like Mendel? Maize, like Barbara McClintock? Some cash crop, like wheat, tomatoes, or tobacco? And here Meyerowitz made a very canny choice, grounded in his instincts and training as a molecular biologist. He chose *Arabidopsis.*

Before Meyerowitz, *Arabidopsis* was not unknown in the lab. It had obvious research advantages: small size, short generation time (four to six weeks), prolific seed production, and the sheer tenacity to flourish in fluorescent-lit labs. As early as 1907, in fact—just about the time zoologist Thomas Hunt Morgan was introduced to an obscure little "fruit" (technically vinegar) fly, *Drosophila melanogaster,* by a colleague at Cold Spring Harbor—a German graduate student named Friedrich Laibach determined the chromosomal content of *Arabidopsis thaliana.* But while *Drosophila* rapidly climbed the biological charts, producing fascinating mutations, many PhDs, one Crafoord and two Nobel Prizes for Caltech professors—most recently in 1995, for Morgan Professor of Biology, Emeritus, Ed Lewis—and, finally, its own on-line arcade game and Web site (http://flybrain.uni-freiburg.de/), *Arabidopsis* remained a wallflower, pretty much sitting out the 20th century.

It was heard from briefly in 1943, when the loyal Laibach returned to his early research and once again extolled the virtues of *Arabidopsis* as a research organism. But plant geneticists continued to work with the useful or the beautiful: familiar species like petunias, tobacco, tomatoes, and maize.

But advances in molecular biology were beginning to promote a whole new approach to genetics: rather than simply inferring a gene's function from its expression in the organism's phenotype, researchers were beginning to understand, and to be able to manipulate, the chemistry of the gene itself. The new techniques were tested and developed using simple animals with few genes—the bacterium *E.coli*; the roundworm *Caenorhabditis*; and of course, *Drosophila*—but by the early 1970s a few forward-looking, or long-memoried, plant geneticists began to take a second look at *Arabi-*

dopsis. Those five chromosomes Laibach had counted in 1907 were the smallest of any known flowering plant, pointing to a modest, manipulable genome.

So when University of Missouri agronomist George Redei in a 1976 review article once again took up the banner of *Arabidopsis*, which he referred to, charmingly, as "our beloved organism," the scientific world was almost persuaded. Chris Somerville, now director of the Carnegie Institution of Washington Department of Plant Biology at Stanford, began to use the plant to investigate the genetics of photorespiration. But his work did not, he has remarked, trigger the groundswell of *Arabidopsis* research he expected. In fact, when Maarten Koornneef and colleagues at the Agricultural University of Wagenigen in the Netherlands put together a linkage map of *Arabidopsis*, the paper had difficulty finding a publisher due to lack of interest.

Then, in the early 1980s, mouse-ear cress got lucky. It caught the attention of Elliot Meyerowitz.

Meyerowitz was an unlikely champion. Though he had attended plant genetics seminars in graduate school at Yale, he had never taken a botany course and to this day claims to be fuzzy on the particulars of plant physiology. He was a fly man: his postdoctoral work at Stanford dealt with *Drosophila*, and at Caltech, which he joined in 1980 as an assistant professor, he investigated the regulatory effects of steroids on a gene that produces a glue-like protein in *Drosophila.*

But sometime in the early '80s he became interested in the developmental genetics of plants. It was a relatively unstudied field and it "seemed like fun," he says. "People were beginning to look at individual genes in animals—at their genetic and genomic structures. I got really curious to know how different plants were." To investigate this he would need a plant that was small, easy to grow—and, in order to take advantage of those new techniques in molecular biology, possessed of a lean-and-mean genome. He would need. . . *Arabidopsis*.

So Meyerowitz and his colleagues set out to realize the research potential of Laibach's organism. In a 1985 paper in *Science* he and Caltech graduate student Robert Pruitt laid out a campaign. First, they determined just how small *Arabidopsis's* genome was. They reported about 70,000 kilobase pairs—that is, 70 million letters of DNA; current reports are somewhat higher, but the essential estimate of about 20,000 genes remains. If this sounds daunting, maize, another staple of plant genetics, has about 2,500,000 kb pairs and 30,000 genes. The ratio of these two genomes to number of estimated genes hints at another fact about *Arabidopsis's* genome: very little of it is "junk DNA"—DNA that does not translate into proteins, much of it mysterious repetitive sequences, like stutters in a genetic statement, that serve no known purpose other than to bedevil molecular biologists. Meyerowitz calls the elaborate work he had to do to establish these basic facts "a piece of history"; in a field that progresses as rapidly as genetics—when today's graduate students can buy kits from mail-order catalogs to clone genes, isolate DNA, or radioactively label probes—15-year-old methodology seems as archaic and cumbersome as grinding your own flour to make a cake.

In the same 1985 paper Meyerowitz proposed to construct an RFLP (restriction fragment length polymorphism) map, in order to be able to isolate and clone specific genes. Building on Koornneef's work, he published his map in 1988. Now Meyerowitz (and others—the map and the DNA library were made generally available) could begin to exploit *Arabidopsis* to investigate some research questions.

And the question Meyerowitz eventually came

Plant breeders have known for centuries how to produce such beautiful floral mutants as the camellias above, in which petals have turned into stamens (center) and stamens into petals (bottom). They have not been particularly interested in the scraggly mouse-ear cress (posing at right with the far lovelier poppy, which also happens to have four petals), but the humble little weed has a beauty of its own as a research organism.

By charting the patterns of inheritance over several generations, geneticists can puzzle out the order in which the genes lie on the chromosome.

Of Mouse-Ear Cress and Maps

Alfred Sturtevant invented linkage mapping in about 1913. Later he became one of the original faculty members of Caltech's Division of Biology, founded in 1928. Sturtevant was Ed Lewis's adviser in the '30s, and, like Lewis, worked with Drosophila. But, after his retirement, he also did genetic tests with irises, descendants of which are planted in a memorial garden behind Parsons-Gates.

Genes are strung in a fixed order on the chromosome, so the idea of mapping is to determine where exactly a gene lies. In theory, this could go down to exact numbers—a gene lies at letters 36,504 to 37,391, say, in chromosome 5. Creating such maps is one goal of genome sequencing projects. In the meantime, researchers are trying to figure out which genes are close to each other—drawing what are called linkage maps, of which restriction fragment length polymorphism (RFLP) maps are one kind.

Like so many other things, linkage maps start with sex. Ordinary cells have two complete sets of chromosomes—one from each parent. During the early stages of meiosis, the process by which sperm and egg cells are generated, the chromosomes pair off and trade genetic material back and forth. The chromosomes, which look like capital Xes, high-five each other, and wherever the arms (or legs) of the two Xes touch, they swap. It's as if two people bumped elbows and each came away from the encounter wearing the other person's forearm instead of their own. This genetic shuffling determines whether you get your mother's hair and your father's eyes (or your father's petals, if you're a plant), and in the longer term drives variations within a species and, ultimately, evolution. Each egg or sperm gets one set each of the new, mixed'n'matched chromosomes, so when they combine, the fertilized egg has the normal complement of two sets of chromosomes.

The DNA that crosses over is generally hundreds or even thousands of genes long, so if two genes are close to each other on the chromosome, the odds are they'll stay together during the trading session—either both of them will move, or neither will. This is the linkage in linkage mapping. But as they become separated by longer and longer stretches of DNA, they begin to behave more independently. So the frequency with which genes migrate together is a proxy for how close they are. By charting the patterns of inheritance over several generations, geneticists can puzzle out the order in which the genes lie on the chromosome.

In RFLP mapping, the chromosome is treated with a restriction enzyme, which recognizes a four- to eight-letter stretch of DNA code and cuts the DNA wherever that code appears. This gives an assortment of fragments of various lengths. A process called gel electrophoresis sorts them by length—longer fragments are heavier and don't move as far from the point of origin. A series of other treatments eventually makes the fragmentation pattern visible as a set of dark blobs.

And here's the nub: many genes have subtle variations within their DNA sequences—just a letter or two here or there—that don't affect their functions, but alter one or more sites where the restriction enzyme should cut them. Thus, two individuals with different variants of the gene will have different fragmentation patterns—the site that should have been cut but wasn't will now be part of a longer fragment that won't move as far. (Hence the name restriction fragment length polymorphism—polymorphism is a five-dollar word meaning "many forms.") These patterns, again, are inherited with the DNA, and since RFLPs are very common, the odds are good that there'll be one reasonably near the gene you're trying to map. Furthermore, there are hundreds of known restriction enzymes, each of which recognizes a different sequence of letters, and new ones are being discovered all the time. □

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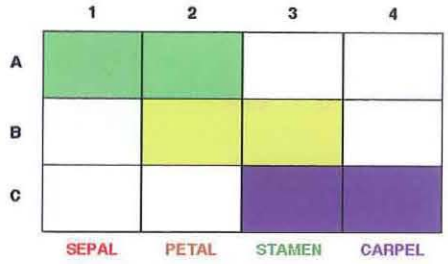
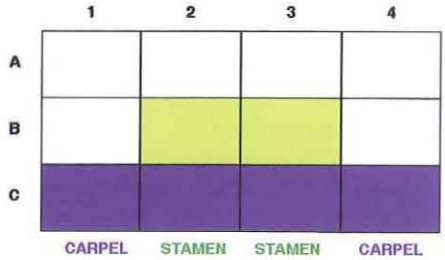
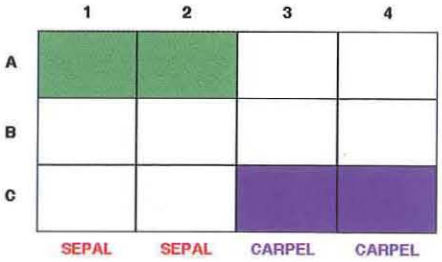
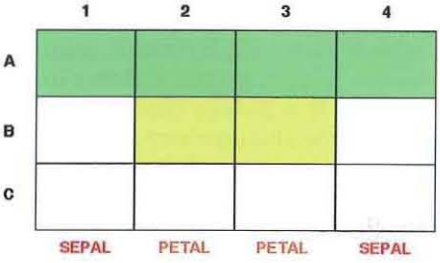
The normal, or wild-type, *Arabidopsis* contains (from outside whorl to inside) four sepals, four petals, six stamens, and two carpels.









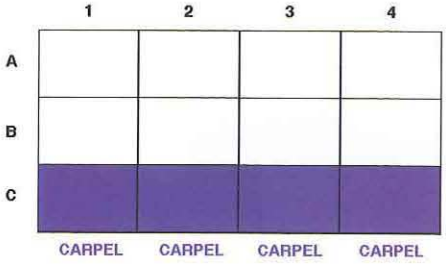
In the graphs above, the numbers along the top indicate the whorl (position from outside to inside), and the organ types run along the bottom axis. Along the vertical axis are the groups of master regulatory genes that specify organ identity; "A" controls the organs in whorls 1 and 2, "B" in 2 and 3, and "C" in 3 and 4. In the wild-type flower at top, the A genes produce sepal and petal; B, petal and stamen; and C, stamen and carpel. Knocking out the A genes (second from top) gives rise to a bizarre flower with carpels and stamens where the sepals and petals should be. When the B genes are disabled, a flower of sepals and carpels emerges. And C-class mutants (bottom) consist of only sepals and petals. In each case, the number of organs in each whorl can remain the same as in the wild-type flower.

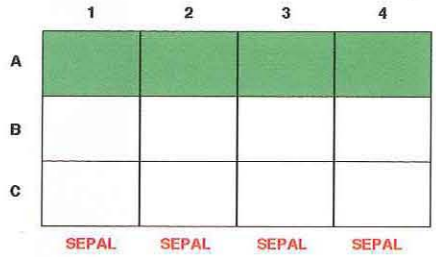
to ask of *Arabidopsis* was the one we began with: How do you make a flower? In other words, what Ed Lewis had done with *Drosophila*—looking at such mutations as an extra thoracic segment or misplaced set of legs to determine which genes were homeotic, that is, regulators of organ identity and position—Meyerowitz proposed to do with *Arabidopsis*.

In retrospect, flowers, with their simple, familiar, nearly ubiquitous pattern, seem ideal for this kind of research. Meyerowitz displays a German text from the 1930s that scrupulously documents and catalogues mutations in snapdragons. "It's hard to understand why they didn't go on to the next step, to try to look at flower development," he says with a kind of bemusement. "They had all the mutants but they never made the theories."

But Meyerowitz did. Patiently knocking out genes in the *Arabidopsis* seeds and observing the results in the nursery, he and his team eventually demonstrated that three groups of genes govern the four whorls of flower organs in an overlapping fashion: group "A" specifies organ identity in whorls one and two (normally sepal and petal); "B" in two and three (petal and stamen); and "C" in three and four (stamen and carpel). A and C are also mutual antagonists: the action of one suppresses the other. So in A-class mutants, that is, mutants in which the "A" genes have been disabled, carpels replace sepals and stamens replace petals, and carpel-stamen-stamen-carpel flowers develop; B-class mutants create sepal-sepal-carpel-carpel flowers; C-class, sepal-petal-petal-sepal. If all three groups are missing, a flower consisting entirely of leaves is produced.

Meyerowitz is at a loss to explain why no one did this work earlier, since this part of his work, which resulted in the construction of the A-B-C model, is simple in concept—"not 'deceptively simple,'" he insists, "just simple"—and was accomplished with the techniques of classic genetics. Certainly there were no technical limitations;





These examples of *Arabidopsis* are both double mutants. Knocking out the B and C genes produces a "flower" that is all sepals (above, right), and when the A and B genes are disabled, you get all carpels (right).

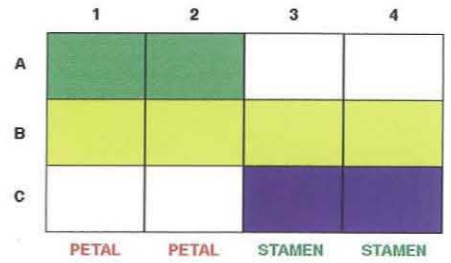
about this Meyerowitz is adamant. His model was constructed with 50-year-old techniques and classic, zap-it-and-see-what-happens methodology; that is, induce mutations, observe phenotypic changes, infer genetic changes, cross and back-cross to identify mutated gene. He sometimes wonders if somebody *did* do the work before him; "Maybe I'll come across it in the library one day, somebody's PhD thesis done decades before I did it," he says. In fact, at about the same time, similar, and complementary, work was being done with snapdragons by Enrico Coen of the John Innes Institute in Norwich, UK.

But the definitive tests of the Meyerowitz model, the tests that moved his work beyond classical genetics, involved actually isolating the genes of interest, cloning them, and reinserting them into plants in which they'd been knocked out, to confirm that they did in fact perform the predicted function. This capability didn't exist before the early 1980s, and it didn't exist for all organisms. But because Meyerowitz had done the initial legwork with *Arabidopsis,* he was able to perform the final tests—with the labor and collaboration of many, many graduate students and postdocs, he emphasizes.

In fact, the *Arabidopsis* genes performed the same regulatory functions when inserted into other plants—in petunias, for example, and in tobacco. Transgenic tests like these are perhaps the most persuasive arguments in modern genetics, and the most profound. They demonstrate the conservation of genes down through the evolutionary process; or, as Meyerowitz has said more eloquently, "the unity of life—one of the great, satisfying conclusions of modern genetics."

Chatting with Meyerowitz in his office, where he is self-effacing—describing himself as "father and couch potato"—informal, digressive, and wryly humorous, you might not think him capable of such ringing statements. But in formal talks, such as last year's Watson and Bi 0.1 lectures, he is passionate and lucid, and has a gift for communicating the Byzantine, recursive complexities of current genetic theory in concrete language. It is perhaps this articulateness (not to mention the photogenicity of *Arabidopsis* itself), that has led to his work being recognized not only by his peers—Meyerowitz is a member of the American Academy of Arts and Sciences and the National Academy of Science, and has recently won the Medal of the Genetics Society of America, the Mendel Medal of the UK Genetical Society, Japan's International Prize for Biology, and the "Science pour l'Art" Prize of LVMH Moet Hennessy·Louis Vuitton—but by the popular press as well. He and his work have been profiled in *Newsday, Discovery, Mosaic,* and *The New York Times.*

Meanwhile, both in his own lab and as former chair of the Multinational Arabidopsis Genome Research Project (similar to the Human Genome Project), Meyerowitz continues both to map *Arabidopsis* and to put *Arabidopsis* on the map,



Activating the B genes throughout the flower leads to replacement of sepals with petals and of carpels with stamens, creating a flower with two whorls of petals, for a total of eight, and a set of extra stamens where the ovary would be.

coordinating an effort that is, at least relative to other sequencing projects for cereal crops such as corn and rice, a model of international data sharing.

And Meyerowitz continues to explore the mysteries of the regulation of cell division in developing flowers. "The organ identity stuff was nice," he says; "it came to a pretty simple set of answers"—(and some possible practical applications for agriculture: an all-carpel flower, for example, might produce several times the usual number of seeds)—"but it raised a series of more complex questions." You might call them the "downstream questions": what is happening to the genes that the master regulators regulate—the genes that control organ *number*, for example? Despite the shuffling of organ identity produced by the manipulations of the A-B-C model, *Arabidopsis* produced a normal *number* of organs in each whorl: four in the first, four in the second, six in the third, two in the fourth.

However, Meyerowitz's lab has identified a class of genes—the CLAVATAs, so-called for the "club-shaped" mutations they produce—that regulate not organ identity but organ number. CLAVATA1, for example, seems to set up the apical meristem, the plant's growing tip, which forms the substrate for flower organs. When mutant, this gene produces the strap-like structure with too many flowers that's growing in the nursery. Jenn Fletcher, a postdoc, is on the verge of isolating CLAVATA3, and grad student Mark Running has isolated PERIANTHIA, which actually makes an *Arabidopsis* with *five* petals—a taxonomic disaster for botanists, who rely on characteristics like number of petals and sepals to classify plants.

And the lab has discovered something about a gene involved in the regulation of the number of stamens. A mutant plant missing the gene may produce a dozen or more stamens—not as "manly" as the all-stamen flower Meyerowitz produced by tinkering with the master regulatory genes, but still a "superman." But Meyerowitz and postdoc Steve Jacobsen have discovered a way to merely modify the activity of the gene through DNA methylation, producing less macho "clark kent" mutations. Methylation is of particular interest to biologists, since it appears to play a role in cell memory, and, in mammals, in the inactivation of one of the two X chromosomes in females. This work sheds light on the process, since it shows that overall disruption of methylation to *Arabidopsis* is accompanied by hypermethylation in certain sequences of the plant's genome—a discovery that may have implications for medical research, since certain cancer tumors have been associated with overmethylation of genes.

In a glorious finale to a century of relative obscurity, Astronaut *Arabidopsis* is about to ride a space shuttle in an experiment that will provide insight into the effect of gravity (or no gravity) on root growth. And if a plan to convert the abandoned USDA greenhouse at the corner of Del Mar and Michigan into greenhouses for Meyerowitz is approved, his *Arabidopsis* may be leaving its meat locker for swankier digs.

So Elliot Meyerowitz has been good to mouse-ear cress, and vice-versa. But Meyerowitz dismisses any special fondness for "our beloved organism." "Look around," he says, gesturing at the walls of his office; "do you see any needlepoint of *Arabidopsis*? Any statues?" Well, no. At the time there were a couple of classy botanical posters from the Huntington Library, and a large tapestry of dogs playing poker—a running joke, which has since been replaced by a painting presented to him by Maya Lin, designer of the Vietnam Memorial in Washington, DC, and a fellow winner of *Science pour L'Art*. No monuments to *Arabidopsis*. It's simply a vehicle, he says; if there were another organism that served his research purposes better, he'd use it.

But you have to be careful with *Arabidopsis*. After about 60 or 70 years, it grows on you. □