Besting Johnny Appleseed

With a few tricks, and a lot of patience, fruit geneticists are undoing the work of an American legend

KEARNEYSVILLE, WEST VIRGINIA—Ask how many fruit trees he's responsible for, and Michael Glenn just laughs. "I have no idea," he says.

Glenn oversees some 120 hectares as director of the Appalachian Fruit Research Station here, and seemingly every road leads to a new orchard. Glenn tramps through one rolling 6-hectare plot on a bright day in March, pruning season. Half the branches of some trees lie discarded. After thinking for a moment, Glenn guesses that 315,000 trees live on the station's acreage in the eastern handlebar of West Virginia. To fit them in, the station plants row after row of ash-brown trees, two meters tall and about as far apart, in military formations. The regularity is deceptive.

Even though all these trees are the same species, *Malus x domestica*, the apple, there's no end to the variety of shapes and postures they assume. Glenn, trim, white-haired, points out that some trees grow vertically like elms, while some droop like willows. Some have branches with elbows and right angles, still others lack a central trunk and sprout stalks like bamboo. And that's only part of the variety he'll see when their fruit arrives in late April. Cross two adult fruit trees—a wild variety resistant to disease, say, and a domesticated one with sweet fruit—and there's almost no telling what you'll get.

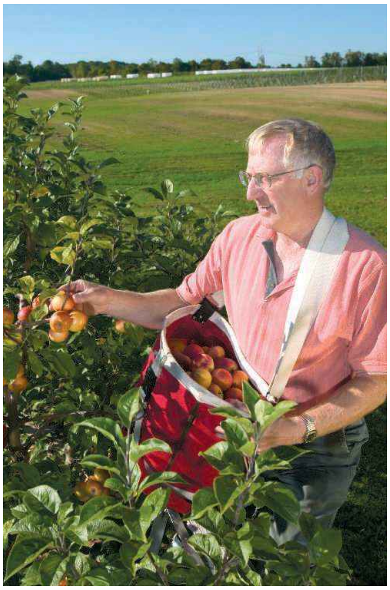
As slower-breeding plants, apples are not far removed from their wild ancestors, so they have had fewer chances to shed unwanted genes. And apple trees cannot reproduce with close relatives because special proteins recognize their own or similar pollen and choke off reproduction. Growers get consistent varieties only through clonal propagation, and today 11 cloned varieties make up 90% of the apples sold in the United States. This leaves apples vulnerable to diseases and environmental stress. To "update" a popular variety to withstand those traumas, a breeder must cross it with apples with quite different genes, which can dilute or scatter its good qualities.

This genetic roulette makes for interesting walks through orchards but frustrates scientists who want to develop consistent but hearty apples, plums, and pears. "Stringent, ugly, bitter, tiny fruit, squishy, doesn't store well—anything you can think of that will be bad in a fruit—it happens," says Cameron Peace, an apple and cherry geneticist at Washington State University, Pullman.

Traditional breeding, part of what Glenn calls "cultural practices," requires crossing two lines of apples, each with one or a few good traits. The result: anonymous brown pips. Once, the only way to sort the duds from sweet and hearty varieties was to plant them all, an expensive and laborious job. And because first crosses rarely meet supermarket standards, breeders improve them by recrossing the best fruit of each generation with other varieties, up to five times.

A faster approach would be tweaking apple DNA directly with the tools of molecular genetics. But until recently, geneticists, their skills honed on *Arabidopsis* and other quick-breeding flora, avoided fruit-tree research like a blight. Of the 11,000 U.S. field tests on plants with transgenic genes between 1987 and 2004, just 1% focused on fruit trees. That's partly because of the slow pace. Whereas vegetables like corn might produce two harvests each summer, apple trees need eons—around 5 years—to produce their first fruit, most of which will be disregarded as ugly, bitter, or squishy. Given the odds, 315,000 trees can look tiny.

But everything in apple breeding is about to change. An Italian team plans to publish the decoded apple genome this summer, and scientists at places like Kearneysville, which is run by the U.S. Department of Agriculture (USDA), are starting to single out complex genetic markers for taste and heartiness. In some cases the scientists even plan, by inserting genes from other species, to eliminate the barren juvenile



Mr. Appleseed. In New York, USDA's Philip Forsline studies *M. sieversii* (*top*) from wild fruit collected in Kazakhstan.

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Published by AAAS

stage and push fruit trees to mature rapidly, greatly reducing generation times. Glenn came of age when someone might spend decades breeding the Johnagold or Fuji. No more. Expectations are changing, and as Glenn says, "The orchards of the future will be driven more by genetics than cultural practices."

Legends in their times

In the early 1800s, a nomad named John Chapman began wandering through Pennsylvania, Ohio, and beyond, a burlap gunnysack of seeds (as tradition has it) over his shoulder, to spread the apple to the American frontier. That most apple trees he planted gave squishy or crabby fruit didn't make Johnny Appleseed's gifts any less welcome for farmers and hunters eager to make liquors like cider and applejack.

But not all were stringent, and many "dessert" apples with colorful names like Irish Peach, Maiden Blush, and Sops of Wine thrived in America. Unfortunately, Chapman, no geneticist, had drawn on a narrow stock of European apples—itself a narrow selection of wild apples, which are native to Kazakhstan. Other U.S. stocks, spread by pioneers and missionaries, had the same shortcomings, and grafting clones further tapered the line. (Every single Red Delicious traces back to one farm, Jesse Hiatt's, near Peru, Iowa, for example.) Meanwhile, USDA's efforts to breed heartier varieties have proved inefficient and slow.

The foundations for modern apple research were laid in the 1990s. Fittingly, it all started with another trek to the frontier, by another legend among apple breeders. Philip Forsline had already made a name for himself in the 1980s when Cornell University decided to bulldoze its renowned groves of apple trees. Forsline, an apple curator for USDA's Agricultural Research Service, oversaw the transfer of every variety from Cornell in Ithaca, New York, to his USDA research station 70 kilometers away in Geneva, New York, where buds were meticulously grafted onto rootstock.

Even those holdings, however, were genetically narrow, so USDA drew up ambitious collection trips to China and Kazakhstan. Forsline made seven trips to Central Asia in the 1990s, collecting apples from coastlands and inland steppes, from veritable deserts and dense forests where 90% of trees sprouted apples. He saw groves where bears smashed branches to knock apples loose, and among the thousands of shapes he saw a few 8-centimeter beauties "that looked like they could be at the supermarket," he marvels. In all Forsline collected 949 varieties of *Malus sieversii* trees, forerunner to the domesticated apple.

Wide variety. Although one species, apples have genes that can change their fruit wildly.

Back in New York state, Forsline recreated the Kazakh and Chinese forests (with humans pruning instead of bears) by planting 1600 seedlings. And as he loves to joke, the work is finally bearing fruit. Over the past dozen years, he and his successor at Geneva, Gennaro Fazio, have monitored which apples could weather cold snaps or dry spells, and which handled "The Gauntlet"—a pestilential greenhouse crawling with pathogens that kills three-quarters or more of all prisoners. After these tests, Forsline and Fazio have identified apples that can withstand nearly any natural evil. The next trick will be identifying the key genes and getting them into the 11 apple varieties Americans covet.

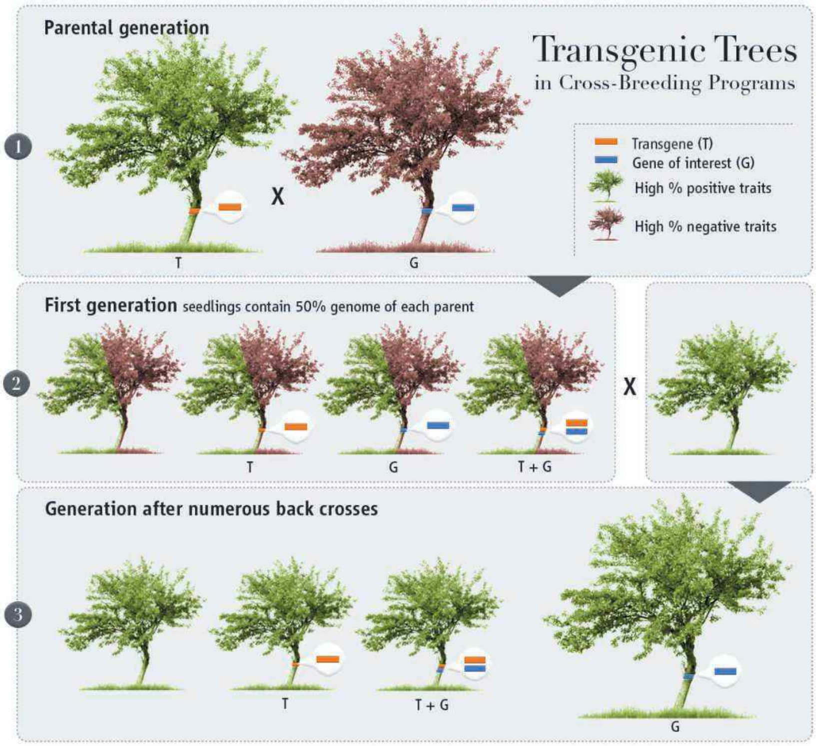
Double trouble

Traditional breeders face two barriers to making better apples, plums, and pears: the staggering inefficiencies and the tedious wait between generations. Genetic work has the potential to eliminate both problems.

As for the inefficiencies, "you can use molecular tools … to screen a large population of seedlings" for genetic markers associated with a desirable trait, explains Gayle Volk, a geneticist at USDA's National Center for Genetic Resources Preservation in Fort Collins, Colorado. If scientists screen for multiple traits, only 10 or so of 1000 seeds may have the right combination, she says, but scientists can focus on those few.

Peace discovered a great example of the potential savings through work on ethylene, a natural hormone that causes fruit to ripen. Breeders want low ethylene levels so that fruit doesn't turn mushy in trucks on the way to stores. Peace has discovered two gene markers for fruit that produce 90% less ethylene, and with his tests, breeders can discard three of every four seeds and possibly save 60% on maintaining groves.

Ultimately, Peace wants to find markers for taste, the most desirable and elusive trait that fruit breeders pursue. Texture and crispness are largely fixed by genes, while all-important sweetness depends heavily on environmental factors, and



The transgene vanishes. A fast-flowering poplar gene (pink) speeds new fruit varieties. In the middle generations, scientists select for trees with both the poplar gene and a gene of interest (blue). In the last generation, scientists select trees without the poplar gene to avoid the stigma of genetically modified (GM) food.

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scientists don't know the details. But thanks to collaborations like USDA's $14.4 million RosBREED project, Peace thinks geneticists can start finding robust correlations for taste within about 2 years.

Still, even the best screening won't solve the second problem, that fruit trees mature so slowly. But scientists at Kearneysville, in collaboration with scientists at a few universities, may have a shortcut. In the next 6 months or so, Kearneysville geneticist Jay Norelli will begin a project to introduce two alleles for resistance to fire blight, a common apple plague, into dessert apples. Using traditional methods, it would take 20-some years to fix this trait into a supermarket-quality apple. But by introducing a "fast-flowering" gene from a poplar tree, Norelli believes he can cut the generation time down to roughly a year.

The process works like this. First, scientists introduce the fast-flowering gene into the chromosomes of a dessert apple, with traditional genetic engineering tools (such as a bacterium loaded with the fast gene). Scientists then cross this altered apple with a complementary variety that might not taste good but is resistant to disease. After this cross, scientists will, as usual, get many dud seeds. But they can screen their DNA and find varieties likely to taste good, mature quickly, and withstand diseases. They discard the rest. Scientists then cross the best of the new generation with another tasty (but not fast-flowering) apple. And once again, they select the best offspring. With each subsequent cross, they become more like dessert apples, except disease-resistant.

Norelli is confident the poplar transgene shortcut can work because colleagues at the research station, led by horticulturist Ralph Scorza, have used it to create fast-flowering plums, a close relative of the apple.

Control plum trees in the Kearneysville greenhouse, which lack the accelerating gene, have sturdy, woody trunks and stand a good meter tall after 12 months. The controls are also barren—and will be for several years until they reach fruit-bearing age. The experimental, "FasTrack" trees look softer and greener, and they slouch. But that's partly because their branches are bowed down from the weight of plums. Sixty more plum-poplar trees are growing outdoors on a few of Kearneysville's 120 hectares. And like Norelli, Scorza will soon begin introducing new traits into the plums, at speeds that would have seemed miraculous 50 years ago.

To determine how sweet the FasTrack plums are, Scorza's team will chemically test their sugar concentration this summer. But no one will know how they taste for some time. Including a poplar gene makes the trees genetically modified (GM) food, and scientists would need government permission to sample them.

Fast track. Genetically modified in the lab (*inset*) to include a poplar gene, plums grow fruit in less than a year, allowing faster breeding.

What's more, no one knows whether poplar genes in fruit will do something unwanted, like shortening the life of the tree.

But the Kearneysville geneticists have a neat trick to circumvent these concerns. Only a fraction of the offspring in each generation contain the fast-flowering gene. Early in the breeding process, scientists discard the trees that lack it. (They search for the gene by doing PCR on a tissue sample.) But once they have fixed a new trait in the fruit, they select *against* fast-flowering in the final generation. This selection involves no genetic engineering: They simply screen the trees (again, usually with PCR), and toss out the ones with the poplar gene. The leftover trees, which mature normally, are no different than if Scorza's team bred them the traditional way. And as long as any new genes came from wild or semiwild fruit, the trees are, almost magically, no longer GM.

As for potential controversies, Scorza argues that the FasTrack system combines "the latest methods of modern biology with a breeding tradition as old as agriculture." And he thinks it will become valuable with global climate change. "Fifteen, 20 years is no longer good enough" to deliver new fruit varieties to a hungry world, he says.

Still, it's telling that not even FasTrack breeders can eat their fruit—a far cry from the American frontier farmer. And opponents of GM food seem unlikely to accept a "non-GM" label for such varieties. Bill Freese, a science policy analyst at the Center for Food Safety in Washington, D.C., has not studied the new FasTrack program but said, "the genetic engineering process is very disruptive" and often changes surrounding DNA. "Our experience with USDA is that they tend to downplay risks with genetic engineering."

What's next

For the next few years, apple, plum, and peach trees will dominate the grounds of places like Kearneysville. But scientists elsewhere are developing ways to maintain the genetic diversity of fruit with as few trees as possible. In Colorado, Volk has studied cryogenic preservation of dormant buds in liquid nitrogen, and the results show promise for reducing the number of trees that must be kept in dirt. Nitrogen tanks cost $1.50 per year per bud to maintain compared with $50 to $75 per orchard tree. And the germ plasm shows no ill effects.

Even at Kearneysville, breeders spend more and more hours indoors in their labs and fewer in the groves. In fact, Glenn says he's become an anomaly—someone who even prunes on occasion. But most traditional breeders, Glenn included, are eager to eliminate all the tedium and heartache of traditional methods. "Personally, I am nostalgic for the so-called better days, but I think this is the natural progress of science," he says. What's more, in the past 5 years, "the molecular biologists are coming back to the field-oriented scientists to collaborate with them on projects in the field."

So although the informal days of Johnny Appleseed are gone forever, fruit breeders still have a place, and scientists are trembling with excitement at the possibilities. Forsline estimates that his trips to Asia have doubled the known stock of apple genes in the world. Johnny Appleseed may have made the American apple in the 1800s, but as Forsline has written of modern fruit work, "All of this—and forthcoming findings—may one day put the impact of [USDA research] on a par with that of John Chapman's legendary work."

–SAM KEAN

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