ECOLOGY

The American Chestnut's Genetic Rebirth

A foreign fungus nearly wiped out North America's once vast chestnut forests. Genetic engineering can revive them

By William Powell

ABOUT 70 FEET TALL and six feet in diameter, one of the largest remaining American chestnut trees grows in Oregon (*left*). At the right is a leaf from the species.

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IN 1876 SAMUEL B. PARSONS RECEIVED A SHIPMENT OF CHESTNUT SEEDS FROM JAPAN AND DECIDED TO grow and sell the trees to orchards. Unbeknownst to him, his shipment likely harbored a stowaway that caused one of the greatest ecological disasters ever to befall eastern North America. The trees probably concealed spores of a pathogenic fungus, *Cryphonectria parasitica,* to which Asian chestnut trees—but not their American cousins—had evolved resistance. *C. parasitica* effectively strangles a susceptible tree to death by forming cankers—sunken areas of dead plant tissue—in its bark that encircle the trunk and cut off the flow of water and nutrients between the roots and leaves. Within 50 years this one fungus killed more than three billion American chestnut trees.

Before the early 1900s the American chestnut constituted about 25 percent of hardwood trees within its range in the eastern deciduous forests of the U.S. and a sliver of Canada—deciduous forests being those composed mostly of trees that shed their leaves in the autumn. Today only a handful of fully grown chestnuts remain, along with millions of root stumps. Now and then these "living stumps" manage to send up a few nubile shoots that may survive for 10 years or longer. But the trees rarely live long enough to produce seeds because the fungus almost always beats them back down again.

In its prime, the American chestnut was a keystone species, crucial to the health of a multitude of organisms in its ecosystem. Many different birds, insects and small mammals nested in its branches and burrowed into its bark. Bears, deer, turkeys, blue jays, squirrels and other animals ate the large, nutritious chestnuts. After losing so many mature chestnut trees, wildlife populations declined and became less diverse. The oaks that have since replaced the chestnut cannot support as many animals; the acorns they produce are only half as nutritious. And chestnuts once generated larger quantities of nuts than oaks do today, in part because they flowered after frosts that might have destroyed delicate buds.

The American chestnut also had great economic value. Its nuts can be used for food or ethanol fuel. Because the American chestnut grows quickly, has sturdy, straight-grained wood and is very rot-resistant, it provides excellent timber. In fact, if the chestnut were still abundant, most decks would likely be made from its wood instead of from pressure-treated lumber, which often contains heavy metals and other preservatives that endanger the environment and people's health when they find their way into soil and food. Last, the American chestnut has been an especially beloved tree, immortalized in poetry, songs, books, street signs, and the names of many schools, hotels and parks across the country.

We do not have to stand by as the American chestnut becomes a distant memory for most people. The culmination of decades of research suggests that science can restore the tree and all the resources it once offered people and wildlife. After a century of ineffective efforts to combat chestnut blight, two approaches are now meeting with some success. One strategy attempts to create blight-resistant American chestnuts with an ancient horticultural technique: hybridization. By mating American chestnuts with far smaller, fungus-resistant Chinese chestnuts, researchers "backcross" the resulting hybrids with other Ameri-

IN BRIEF

In its prime, before the early 1900s, the American chestnut flourished in the eastern forests of North America, providing shelter and food for many other creatures. Within 50 years, however, a foreign fungus introduced by humans eradicated more than three billion trees.

To revive the American chestnut, some scientists have hybridized it with its more resilient Chinese cousin. A more precise and successful approach inserts genes from wheat and other plants into American chestnuts to yield fungus-resistant trees.

If researchers receive federal approval to plant these transgenic trees in the wild, which could happen in the next five years, the American chestnut will be the first genetically engineered plant used to restore a threatened species to its native range.

PRECEDING PAGES: GARY BRAASH *Getty Images (tree);* JOEL SARTORE *(leaf)*

can chestnuts to Americanize the trees as much as possible while, it is hoped, keeping all the genes responsible for blight resistance. In addition to being rather imprecise, however, backcross breeding requires many generations and thousand of trees to produce individuals suitable for restoration.

For those reasons, my many collaborators and I are focusing on a second approach, which relies on altering the chestnut tree's DNA in a much more exact way than traditional breeding and which has the potential to produce more fungus-resistant trees more quickly. By borrowing genes from wheat and the Chinese chestnut, among other plants, and inserting them into the American chestnut's genome, we have created hundreds of transgenic trees, some of which defend themselves against *C. parasitica* as well as, if not better than, their Asian counterparts. If the U.S. Department of Agriculture, the Environmental Protection Agency, and the Food and Drug Administration approve our trees—which could happen as soon as five years from now—they will be the very first transgenic organisms used to restore a keystone species to its native environment.

Compared with other efforts to revive endangered or extinct species with genetic engineering and related biotechnologies—such as the proposed restoration of the passenger pigeon, thylacine and mammoth—the efforts to reinstate the American chestnut face far fewer hurdles and offer much clearer benefits. Unlike cloned mammoths and pigeons, trees do not require surrogate mothers, parenting or socialization. And as a massive organism that is home to many others, the American chestnut can improve the health of the forest more than any one animal.

PLANT PATHOLOGIST Gary J. Griffin of Virginia Tech uses a hand lens to examine a swollen canker on a chestnut tree infected with a harmful fungus.

GARY BRAASH

SEEDS OF SALVATION

LIKE MANY ADULTS in the U.S. today, all I knew about chestnuts while I was growing up was what I learned from a certain iconic Christmas song. Yet in 1983, when I became a graduate student working with plant pathologist Neal Van Alfen, then at Utah State University, I began to develop a deep appreciation and sympathy for the magnificent chestnut tree and its demise at the hands—or rather the fungal fingers—of an exotic pathogen.

In 1989, when I had moved to the S.U.N.Y. College of Environmental Science and Forestry, Stan Wirsig of the American Chestnut Foundation approached my colleague Charles Maynard and me with a proposition. He wanted to complement the foundation's ongoing chestnut tree hybridization program with a new restoration project focused on genetic engineering, which was a cutting-edge technology at the time and promised a speedier and more precise way to create resistant American chestnuts. One of my tasks was to find a gene that could endow the trees with resistance to *C. parasitica* while Maynard and Scott Merkle of the University of Georgia developed the techniques that would allow us to introduce that gene to chestnut tree embryos—tiny bundles of swiftly multiplying cells that would eventually grow into adult trees. If everything worked as planned, the young trees would grow into sturdy adults with the ability to battle the fungus.

At that time, no one had ever tried to genetically engineer a tree to fight a virulent fungus, but we had a few clues about how to get started. Over the years researchers had learned some important details about how *C. parasitica* damages chestnut trees. The pathogen grows feathery lattices of fungal tissue called mycelial fans that produce oxalic acid, which eats through the tree's bark to make room for the fungal invasion. As the fungus wedges its way into the tree, a canker girdles the trunk.

Initially we focused on finding a way to weaken the mycelial fans. We knew that the immune systems of many plants and animals contain small chains of amino acids known as antimicrobial peptides (AMPs) that can disrupt fungal cells. Using AMP genes in the African clawed frog as a model, we assembled genes from scratch to produce AMP peptides that could fight *C. parasitica*. We hoped that if we could engineer the chestnut trees to produce even small amounts of these AMPs, they would make mycelial fans go slack and thereby render them benign. Such peptides are notoriously unstable molecules, though, so we needed a backup plan.

Around the same time, a then graduate student named Kim Cameron stopped by my office and dropped off a book summarizing many of the studies presented at the recent annual meeting of the American Society of Plant Biologists. When I read about a study conducted by Ousama Zaghmout and Randy Allen, both then at Texas Tech University, I had a eureka moment. The study described a wheat gene for an enzyme called oxalate oxidase (OxO), which breaks down oxalic acid—the very same caustic substance produced by the chestnut blight fungus. Even better, the researchers had worked out a way to introduce this gene into other plants. They put the gene into *Agrobacterium,* a microbe that can inject DNA into the command center of plant cells, and exposed plants to clones of that microbe. The resulting transgenic plants became resistant to an acid-spewing fungus known as *Sclerotinia sclerotorium*. Maybe we could do something similar with the American chestnut.


ULMUS AMERICANA

What Happened to the American Elm?

Throughout the country, the American elm once sheltered many city streets in cathedrals of green. In addition to its beauty, it was a hardy tree, tolerant of the compacted, salty soil and periodic droughts characteristic of urban life. Like the American chestnut, however, this native species fell victim to a virulent fungus from Asia. Although the American elm is not extinct, it is now very rare to see these trees in urban settings.

The American elm succumbed to a fungus known as Dutch elm disease (DED), which is spread by bark beetles. Once in the tree, the fungus grows through tubes of xylem, conduits for water and minerals. The tree attempts to contain the fungus behind walls of tissue, thereby inadvertently clogging its own passageways and depriving itself of sustenance. Through many decades of selective breeding, however, researchers have produced 23 DED-tolerant varieties of American elm, such as the New Harmony, Valley Forge and Liberty elms.

Unfortunately, DED is not the only problem. American elms are also highly vulnerable to another disease known as elm yellows, spread by American leafhoppers carrying phytoplasma bacteria. These microbes destroy the tree's roots and phloem tubes, which transport sugars. An infected elm droops at first and eventually dies. In this case, genetic engineering might be useful. Instead of producing American elms that can resist both DED and elm yellows through many decades of breeding, scientists may be able to engineer immunity in only a few generations, using what we have learned from work on the American chestnut. In fact, some of the same Chinese chestnut genes currently under investigation to save the American chestnut may help defend the American elm against elm yellows. Allison Oakes, a graduate student at the S.U.N.Y. College of Environmental Science and Forestry, is currently exploring this possibility. —*W.P.*

We could not test either approach on chestnuts at that point, because we were still figuring out how to grow the finicky chestnut in the laboratory. So we decided to achieve a proof of principle in a different tree—the hybrid poplar, which was well studied and often used in experiments. Haiying Liang, then a graduate student at the College of Environmental Science and Forestry, would deliver both the *OXO* gene and our AMP gene, and when the trees were old enough, we would infect them with *Septoria musiva,* a fungus that produces a good deal of oxalic acid and can cause leaf spot and canker diseases in hybrid poplars. Most of the trees treated in this way remained relatively healthy. We had made one tree fungus-resistant with genetic engineering. Now we needed to do it with the right tree and the right fungus.

While Liang was conducting the poplar experiments, Linda McGuigan, also then a graduate student at the college, set to work figuring out how to raise chestnut trees from embryos in the lab. Some plants, like carrots and petunias, are remarkably easy to grow in the lab. Provided with enough water, nutrients and certain hormones, they will grow new shoots and roots from a tiny piece of leaf, for example. The American chestnut was not one of these cooperative plants. McGuigan, building on the work of previous students, spent two and a half years learning how to successfully introduce the wheat gene into chestnut embryos using *Agrobacterium* and to subsequently shepherd the embryos into young adulthood in the lab. Usually the cluster of rapidly dividing cells that make up a chestnut tree embryo grow within the protective husk of a chestnut seed that has fallen to the ground, eventually pushing roots through the seed and into the soil and pushing green shoots toward the sun. McGuigan learned how to control lighting, humidity and temperature to mimic what would normally happen inside a chestnut seed and fine-tuned the delivery of various hormone cocktails at different stages of the miniature tree's early development to induce growth of roots and shoots.

In 2006 we were able to plant the first transgenic American chestnut trees in experimental fields sectioned off from the forest. It takes at least two to three years for the trees to reach a size at which we can challenge them with the blight fungus. We had attached the *OXO* gene to a promoter—a kind of genetic switch that controls how often a cell reads the instructions in a gene—to limit the production of OxO to certain tissues. We were hoping the resulting low levels of the enzyme would be sufficient to take on the fungus without causing any unwanted side effects. Unfortunately, we were mistaken. This first line of trees was not able to resist the fungus; they died a little slower than is typical but ultimately succumbed to their illness.

By 2012 we had designed a new promoter for the *OXO* gene and engineered a new line of trees that produced much more of the acid-degrading enzyme. Success! These trees evaded disease almost as well as the Chinese chestnut, which had evolved resistance on its own. We have now developed a way of gauging disease resistance by testing the leaves of chestnut trees that are only a few months old, so we no longer have to wait three years to see if our experiments are working. In this test, we make small cuts in leaves, infect them with fungus and wait for a circle of decaying tissue to spread from the wound. The smaller the spot of death, the more resistant the tree. Some of our newest trees, which make OxO in all their tissues and were planted in the field in 2013, appear to be even more resistant than the Chinese chestnut. We need to confirm this finding as

WILLIAM HARLOW Getty Images

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the trees get older, but it appears that the gene we borrowed from wheat has exceeded our expectations.

People often ask us why we do not simply find the genes that make the Chinese chestnut resistant and use them instead of the wheat gene. When we first started our research, no one had thoroughly studied the Chinese chestnut genome, and it would have taken too much time and too many resources to locate the numerous different genes responsible for a complex trait like blight resistance. Each of those genes would contribute only a small portion of the tree's ability to battle the fungus, and any one of them would probably have been ineffective as a defense on its own.

At this point, however, scientists have identified 27 genes that might be involved in the Chinese chestnut's blight resistance—the fruits of a recent collaborative effort under the Forest Health Initiative between many researchers at the College of Environmental Science and Forestry, the University of Georgia, Clemson University, Pennsylvania State University, the U.S. Forest Service, North Carolina State University, the Connecticut Agricultural Experiment Station and the American Chestnut Foundation. So far two of these genes each appear to endow trees with an intermediate level of resistance. Testing is ongoing with the other candidate genes. Joseph Nairn of the University of Georgia has also given us copies of two other genes to test: one for a grape enzyme that helps to make resveratrol, which is toxic to fungus, and a pepper gene encoding an AMP that directly inhibits the growth of fungal cells.

Eventually we hope to fortify American chestnuts with many different genes that confer resistance in distinct ways. Then, even if the fungus evolves new weapons against one of the engineered defenses, the trees will not be helpless.

GOING OUT ON A LIMB

TODAY MORE THAN 1,000 transgenic chestnut trees are growing in field sites, mostly located in New York State. The next hurdle for American chestnut restoration involves the federal regulatory process. Before we can plant trees in the forest, the FDA, USDA and EPA will want to make sure that genetically engineered chestnut trees are not significantly different from typical trees in some unexpected way. As opposed to hybridized trees, which are genetically quite different from American chestnuts because they have large chunks of Chinese chestnut DNA, our transgenic trees have only a few new genes. Preliminary tests show that the roots of typical chestnut trees and engineered trees form the same kinds of symbiotic relations with helpful fungi and that similar communities of smaller plants grow underneath the canopies of both modified and unmodified trees. Likewise, the same insect species visit both transgenic and typical chestnut trees, and nuts from both types of trees have the same nutritional composition.

Once such tests are complete, we will petition the USDA, EPA and FDA for the same unregulated status that they give to genetically engineered crops. Here is where the American chestnut will introduce a new dilemma in the usual regulatory process. We are not growing a genetically modified organism on cropland for profit; rather we are producing trees for restoration without monetary gain. Like researchers working on golden rice enriched with a precursor of vitamin A, we are motivated by the public good—and the health of the forest. The EPA generally grants seed companies licenses to sell transgenic seeds, but in our case, we have no one to hold the license and nothing to sell. It is not clear what kind of alternative approval they would give us, but we are determined to set a precedent.

A final hurdle is public acceptance. Encouragingly, many people who are typically opposed to genetic modification make an exception for the American chestnut tree. Some people reason that because humans caused the demise of the chestnut in the first place, humans should fix it. Others are accepting because we are not seeking profit and are not patenting the trees.

Many people are also happy to learn that the environmental risks of American chestnut restoration are negligible. The chances of transgenic chestnut tree pollen spreading introduced genes to other plant species are very small. Pollen from one tree species can fertilize only the same species or a closely related one. The American chestnut has no closely related species in the northern part of its natural range. In the southern parts of its range, chinquapins occasionally cross with American chestnuts. But chinquapins are also infected by chestnut blight and would benefit from some genetic resistance. Ideally, some of the transgenic pollen will spread resistance to at least a fraction of the remaining American chestnut stumps that manage to flower, rescuing as much of their total genetic diversity as possible. If the stumps do benefit, they could spawn a blight-resistant population that, over the centuries, could return this once towering keystone species to its former glory in the eastern forests.

Chestnut blight is not the only enemy of biodiversity that genetic engineering can eradicate. We are losing the battle against many other exotic pests such as the hemlock woolly adelgid—a bug that sucks the sap from hemlock trees—and the emerald ash borer—a metallic green beetle whose larvae tunnel under the bark of ash trees—as well as the pathogens responsible for sudden oak death and walnut thousand cankers disease, to name a few. To turn the tables, we have to act quickly, and in most cases, traditional breeding techniques are just too slow to make a difference. Now, more than ever, we need genetic engineering in our toolbox to maintain diverse and healthy forests.

Completely restoring the American chestnut to its previous status as a king of the forest is a centuries-long endeavor. Once the chestnut trees pass regulatory and public approval, a good place to begin restoration is on reclamation lands. With the help of the Forest Health Initiative and Duke Energy, test plots are now being planted on mine reclamation sites. Other areas might include abandoned farmland and historic locations that once had abundant chestnut trees. And perhaps some individuals will want to have these iconic trees in their own yards. An old Chinese proverb says, "One generation plants a tree, the next generation enjoys its shade." In the case of the American chestnut, we are that first generation. SA

MORE TO EXPLORE

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