Evolution of the Chestnut Tree and Its Blight

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Recent research into the genetics of the fungal blight provides hope that chestnut trees may someday return to the forests, parks, and orchards of New England.

American chestnuts (*Castanea dentata*) have been in this country for a long time. When biologists started looking at the leaves preserved in the Clarkia fossil beds of northern Idaho, some of the 17- to 20-million-year-old leaves were chestnut. Pollen records prove that they were on Long Island at least as long ago as between the last two glaciers, about 30,000 to 50,000 years ago. They expanded their range northward as the last ice receded, and became a major component of New England forests. The studies of David Foster and his colleagues at the Harvard Forest in Petersham, Massachusetts, revealed abundant chestnut pollen in 2000-year-old soil layers.

When George Emerson reported *On the Trees and Shrubs Growing Naturally in the Forests of Massachusetts* in 1846, he mentioned several very large American chestnut trees in the state. One *Castanea dentata* on Monument Mountain near Sheffield had a trunk that was almost 3 meters (9.5 feet) in diameter at the base. A reliable, yearly crop of tasty and nutritious nuts fed people and their domestic animals, as well as many of the wild creatures of the forest (Figure 1). The hard, durable wood had many uses, and the split-rail fences built in the last century still wend their way through forests where large American chestnut trees are no longer found.

When American chestnut trees suddenly started dying in the Bronx Zoo in New York City in 1904, they were found to have girdling cankers caused by a fungus. At first pathologists thought that a resident fungus had mutated and become lethal to our chestnut trees, but nothing exactly like the fungus could be found in the United States. Murrill reported in 1908 that Japanese chestnut trees (*Castanea crenata*) in the New York Botanical Garden had the disease, and that the Botanical Garden's American chinquapins (*Castanea pumila*) were also attacked. When F. N. Meyer discovered the same fungus in Asia on Chinese and Japanese trees, he reported that they were rarely killed by the disease. Since Japanese trees had been imported and planted here since 1876, the fungus probably hitched a ride on some of them. Pathologists named it *Endothia parasitica*, but the name has now been changed to *Cryphonectria parasitica*.

The Search for Resistance

As chestnut blight disease proceeded unchecked through the whole native range of the American chestnut tree (essentially the eastern half of the United States), a desperate effort was made to find a substitute for these valuable trees (Figure 2). The European chestnut trees (*Castanea sativa*), which had been widely planted since early importation by Eleuthère Irénée Du Pont de Nemours (1799), were also very susceptible. Large orchards of

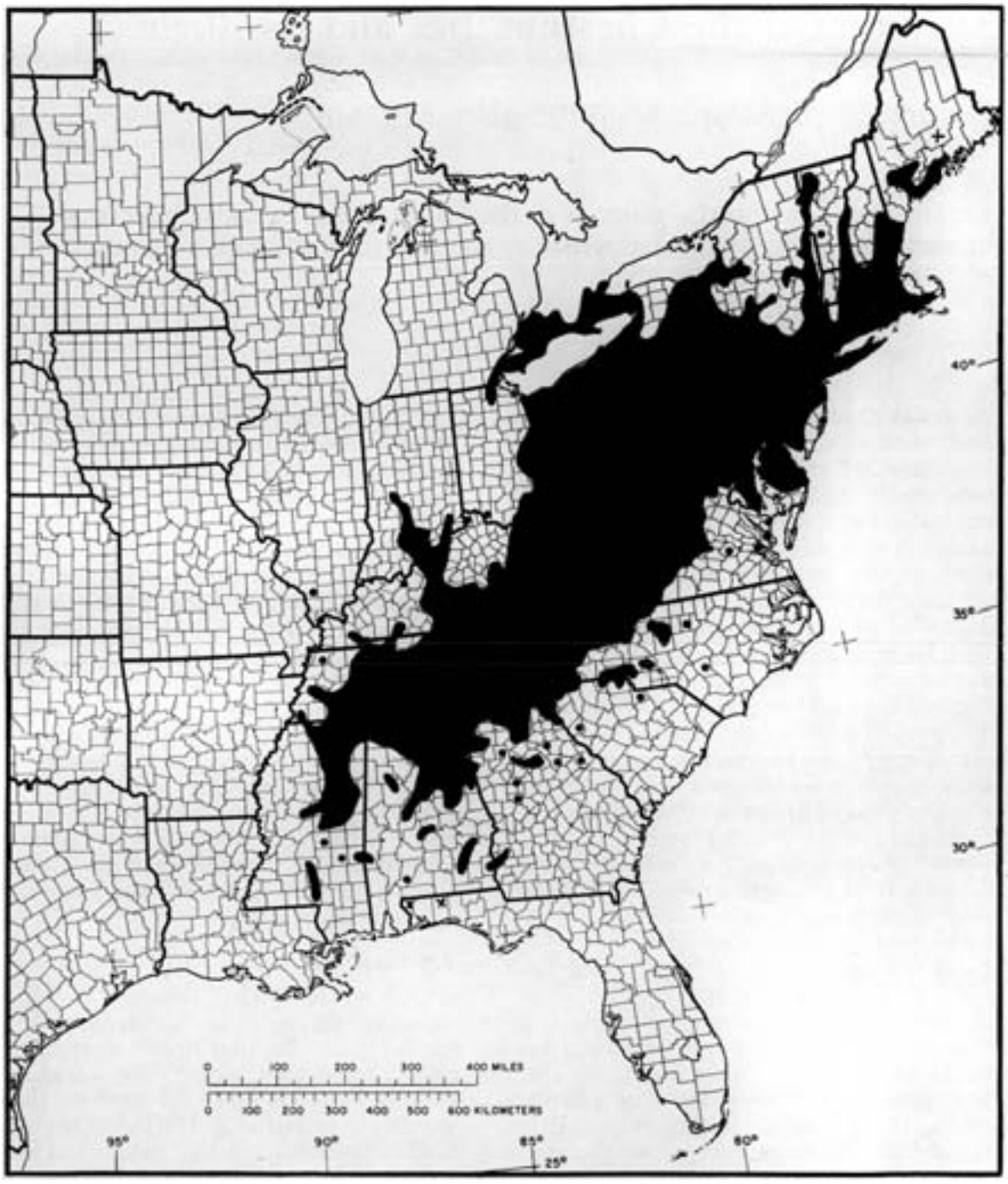

Figure 2. The natural range of the American chestnut. From Atlas of United States Trees. *Vol. 4:* Minor Eastern Hardwoods *by E. L. Little, Jr. USDA Forest Service Misc. Publ. 1342, June 1977.*

C. sativa established in New Jersey and Pennsylvania were decimated by the chestnut blight disease.

Japanese chestnuts that had been imported by S. B. Parsons of Flushing, New York, in 1876 proved both disease-resistant and able to survive our winters. Two of these are still growing in southern Connecticut. Ellwanger and Barry's Mt. Hope Nursery near Rochester, New York, had been selling trees of the Japanese chestnut cultivar 'Japan Giant' by mail order for several years, and Luther Burbank, in Santa Rosa, California, sold 'Miracle'. Three of Burbank's other selections of Japanese chestnut became 'Hale', 'Coe', and 'McFarland' and were sold by the Connecticut nursery of J. H. Hale. All of these Japanese chestnuts were shorter in stature than American trees, with a branching habit that made them unsuitable for timber. Although their nuts were larger than American chestnuts, they were often bitter.

The U.S. Foreign Seed and Plant Introduction Section had imported Chinese chestnuts (*Castanea mollissima*) as early as 1901, and when they proved to be resistant to chestnut blight, imports were increased. C. S. Sargent sent Chinese chestnut seed to the Arnold Arboretum in 1903, but the seedlings raised from these have not survived. The oldest living Asian chestnut in the Arboretum is a Chinese, planted in 1918 (AA #7892A). This tree was sent by the Rochester (N.Y.) Parks Department, with whom the Arboretum had close ties. J. W. Kelly in Rochester is fairly certain that this was one of the seedlings from Plant Introduction #36666, a shipment of 250 pounds of chestnuts collected in the Pang Shan region, northeast of Beijing, by the famous plant explorer F. N. Meyer (Figure 3).

In the next few years, the U.S. Plant Introduction Section shipped, to Massachusetts alone, 3,441 Chinese chestnut trees and about 30 pounds of seed, 553 Japanese chestnut trees and 60 pounds of seed, 15 seguine (*Castanea seguinii*) from China, and 11 trees and 1 pound of seed of *Castanea henryi*, the Chinese chinquapin. Since other Asian chestnut trees were also available from nurseries, we have no way of knowing with certainty how many non-American chestnuts have been planted in Massachusetts (Table 1).

What happened to all of these trees? Many were planted in forested areas by people hoping to restore some kind—any kind—of chestnut to the Massachusetts woods. Plant breeders, who were trying to cross Asian and American chestnut trees to produce American-like trees with blight resistance, soon discovered that all of the species of chestnut were cross-fertile. Thus, all of the Asian chestnut trees in Massachusetts were able to cross with each other, and with any American sprouts that survived long enough to flower.

Chestnuts planted by diligent squirrels may survive the winter, unfound or unneeded for food. As these germinate and grow, many will be killed by deer browsing on the foliage and tender stems. Those that survive to grow are the beginning of a "natural" breeding experiment. In time, chestnut trees with the cold-hardiness of the American chestnut, the tall stature that would allow them to compete in the forest canopy, and the blight resistance genes of the Asian chestnuts would probably evolve in our New England forests. Many people who have found chestnut trees surviving with blight in Massachusetts have sent leaf samples to the Connecticut Agricultural Experiment Station for identification. All of these have been hybrids.

Since all of the sprouts formed from the bases of killed chestnut trees come from a few dormant cells, mutations could lead to some blight resistance. Trees have been located in Connecticut and New York that are clearly American but are resisting the blight better than expected. If this is really genetic resistance, it is another potential starting point for the evolution of trees able to survive in the presence of chestnut blight.

However, plant breeders are impatient to see results sooner than the slow progress likely to occur without our intervention. A backcross breeding program was outlined for the

*Figure 3. The trunk of a large specimen of the Chinese chestnut (*Castanea mollissima*) described by Frank Meyer as "showing some big wounds caused by bark fungus. My assistant, Mr. J. J. C. de Leuu, is standing beneath. Near San tun ying, Chili Province, China, June 1, 1913." Photo by F. N. Meyer. From the Archives of the Arnold Arboretum.*

American chestnut by Charles Burnham. His experience in corn genetics convinced him that a few generations of crossing resistant Asians, and then their hybrids, to susceptible American trees and of selecting resistant progeny would provide us with the kinds of trees we want much more quickly. Chestnut breeding was started in the 1930s at the Connecticut Agricultural Experiment Station, and its collection of trees of all of the species of *Castanea* is probably the finest in the world. Hybrids of all kinds were made by A. H. Graves, W. Van Fleet, J. D. Diller, H. Nienstaedt, R. A. Jaynes, and many others. The fact that some of their best trees still survive in the Connecticut orchards makes it possible to select the fittest for new breeding experiments.

Evolution of the Blight Fungus

The blight fungus has maintained its destructive vigor in New England for at least eighty years (Figure 4). The original chestnut trees, "killed" by *C. parasitica* in the early 1900s, sprouted from the base only to have the new stems reinfected by the blight and "killed" again. This seemingly endless cycle of sprouting and reinfection has continued unabated

CHESTNUT SPECIES

SECTION Castanea [three nuts per bur]	
Castanea dentata (Marshall) Borkhausen	American Chestnut
Castanea sativa Miller	European Chestnut
Castanea mollissima Blume	Chinese Chestnut
Castanea crenata Siebold/Zuccarini	Japanese Chestnut
Castanea seguinii Dode	Chinese Dwarf Chinquapin
SECTION Balanocastanon [one nut per bur]	
Castanea pumila (Linnaeus) Miller	American Chinquapin, Bush Chestnut
variety *pumila*	
variety *ozarkensis* (Ashe) Tucker	Ozark Chinquapin
Castanea X *neglecta* Dode	Possible wild hybrid between *dentata* and *pumila*
SECTION Hypocastanon [one nut per bur]	
Castanea henryi (Skan) Rehder/Wilson	Henry Chinquapin

Table 1. Taxonomy of the genus *Castanea*

to this day. It was only with the discovery of weakened strains of the fungus in Italy that researchers in the United States were spurred on to search for similar "hypovirulent" strains in this country that were not virulent enough to kill healthy chestnut trees.

The European hypovirulent strains lacked the orange pigment of virulent strains, and produced fewer spores (the "seeds" of fungi). As these spread through the chestnut orchards of Italy and France, the trees began to survive longer, and to "heal" over the blight cankers with lumpy bark tissue. Similarly, cankers in Michigan, Tennessee, Virginia, and West Virginia yielded orange strains of the blight fungus that were also less able to kill chestnut trees. Virologists have now confirmed that these American strains, and the European hypovirulent strains of *C. parasitica*, are infected with viruses (see back cover).

When scientists put bits of a hypovirulent blight fungus into holes in the bark around killing cankers, viruses move into the virulent strains that caused the cankers. The cankers then stop expanding, and the tree's natural defenses of walling off invaders succeed in protecting the tree's living cambium. Once hypovirulence has been established in a chestnut blight population, hypovirulent spores are moved around in test orchards and in the forest by every creature that moves up and down the trees.

The European hypovirulent strains have effected a biological control of chestnut blight in the orchards of *C. sativa* in France and Italy (Grente and Sauret, 1978). Tests were begun here in 1978, and for four years in a row, blight cankers in an orchard of American chestnut trees at the Experiment Station Farm in southern Connecticut were stopped by treat-

ing them with mixtures of hypovirulent fungal cultures. No cankers on these trees have been treated for the last ten years, although new infections occur every year. A few branches are killed, but most cankers are swollen and superficial by the time that they are large enough to be noticed. The trees have continued to grow and produce nuts, and are now being used for breeding purposes.

In the Connecticut forest, hypovirulent strains have survived and have spread slowly. They have allowed American chestnut trees on good soil, with plenty of water, to grow large and bear nuts, but on poor sites the trees do not compete well. Many other woody species are striving to capture the sun and nutrients, and the energy used by the chestnut trees to deal with blight infections puts them at a disadvantage. A little more resistance, or more effective viruses, might give them a competitive edge.

What Are These Viruses, and Can They Change?

Don Nuss and his colleagues at the Roche Institute of Molecular Biology in New Jersey have determined that the closest relatives of these viruses are plant viruses that are responsible for many important plant diseases. The viruses from hypovirulent strains discovered in the United States are not closely related to viruses from European hypovirulent strains, based on tests of their nucleic acids, but most appear to have evolved from the same progenitor. In a recent summary of the research on hypovirulence viruses in *C. parasitica*, Hillman suggests they can be divided into at least three distinct families, but most of them belong to one common family (Figure 5). The European types, which belong to this common family, have been studied the most, and all strongly affect the way strains of the fungus look in culture in the laboratory.

When Peter Bedker was searching wooded areas in New Jersey for American chestnut trees to use in his experiments, he found some that were surviving in spite of many blight cankers. Hillman tested the normal-looking

Figure 4. Chestnut blight canker on an American chestnut tree. The fungus has grown in concentric circles in and under the bark from the point of infection, which was probably the broken branch on the left. Photo by R. A. Jaynes.

C. parasitica isolates from these cankers and found that they contained viruses very similar to the European hypovirulence viruses. The genes in the New Jersey viruses were different enough to allow more sporulation by the fungus and to allow the fungus to make its normal orange pigment (the European hypovirulent strains are white in culture and produce few spores). We have no idea whether the pigment change will help the hypovirulent strains survive, but the increased sporulation will certainly help them spread around.

The New Jersey discovery resulted in a phone call to Connecticut to discuss the find. A search of Experiment Station records revealed that European hypovirulent strains had been sent to experimenters in New Jersey five times, beginning in 1978. Thus the strains found by Bedker and Hillman were either the fittest survivors of the early canker treatments, or were strains that contained mutants of those original viruses, which were rapidly

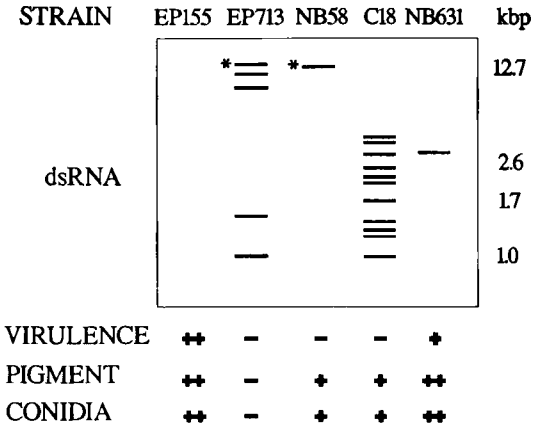

Figure 5. Characteristics of the viruses that cause hypovirulence in the chestnut blight fungus, based on the research of Bradley Hillman. The genes that cause hypovirulence are on nucleic acids called double-stranded Riboses Nucleic Acids (dsRNA). When hypovirulent strains of C. parasitica *are ground up and their nucleic acids purified and separated on gels using an electrical current (electrophoresis), different patterns of dsRNA pieces result. Strain EP155 is a typical virulent strain of the fungus and contains no dsRNA; the others are all different hypovirulent strains, representing three different types of viruses. EP713 and NB58 are related to one another, while C18 and NB631 are distinct.*

selected because they were better adapted to the New Jersey woods. Rapid mutation and selection are qualities typical of viruses adapting to new situations. It is interesting that no white hypovirulent blight strains have been found in New Jersey, even though many were used to treat cankers there. Anagnostakis in Connecticut and MacDonald in West Virginia both have noted that orange isolates of the blight fungus are now common in test plots where European hypovirulence was used to control chestnut blight. No tests have been made of the nucleic acids of these orange strains, but Hillman's results suggest that they should be checked. This may be our first glimpse of the evolution of hypovirulence viruses, adapting to the American chestnut blight and to the American climate.

Connecticut records show that hypovirulent strains were sent to twenty-one other states after the U.S. Plant Quarantine Office lifted the restriction on transfer in 1976. Fourteen such shipments were made to people in Massachusetts, and Anagnostakis has been treating the chestnut trees at the Arnold Arboretum for several years. Terry Tatter and his student Jong-kyu Lee at the University of Massachusetts are now studying chestnut blight in the state and using hypovirulent strains to establish biological control. So far, they have not found any "natural" hypovirulence in Massachusetts.

Reason to Hope

American chestnut trees in Connecticut are surviving better, thanks to hypovirulence, making it easier for us to carry on Burnham's backcross breeding program. In a decade we should have true-breeding resistant hybrids to plant in our forests. The presence of hypovirulence viruses in the blight fungus population should also make it easier for resistant trees to evolve in the forest—whether from rare mutations or because of resistance genes in natural hybrids derived from planted Asian trees. Perhaps the hypovirulence viruses that we have introduced will adapt to provide even better control of chestnut blight disease in the future. We hope to be able to tell our grandchildren that we had a hand in restoring chestnut trees to the forests, parks, and orchards of New England. In the words of Robert Frost:

> Will the blight end the chestnut?
> The farmers rather guess not.
> It keeps smoldering at the roots
> And sending up new shoots
> Till another parasite
> Shall come to end the blight.
>
> —"Evil Tendencies Cancel," 1932

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