# CONTENTS

## Notes



## From Then to Now



## Science and Natural History

notes

## FROM THE EDITOR

Spring is upon us and many of TACF's members and volunteers are busy with plans for planting, pruning, and other seasonal yard work. When you are through with your work (or when your back is—whichever comes first!) please relax with a cold beverage and the Spring 2006 *Journal of The American Chestnut Foundation*! This issue is a delightful mix of whimsy, history, science, research, and culture—truly something for everyone.

In addition to the spring issue, you will soon be receiving a short history of TACF, derived from the Keynote Speech from TACF's President Marshal Case at our Annual Meeting in October 2005. The supplement describes the fascinating 22-year history of TACF, and our ongoing efforts to restore the magnificent American chestnut.

Past issues of the Journal have included a section called Memories, and we believe that capturing these chestnut-related memories is an important part of our work. Perhaps you are one of the fortunate few who was around to witness the majesty of forests full of towering chestnut? Or perhaps you have a parent or grandparent who regaled you with stories that featured this mighty giant? Whatever your story, we want to hear it! Please send articles you would like to be considered for publication to:

Jeanne Coleman, Publications Director
The American Chestnut Foundation
469 Main St., P.O. Box 4044
Bennington, VT 05201
Or e-mail publications@acf.org.

Are you more the talkative type? Please let us call you to record your story. You can leave your name and telephone number with our main office at 802-447-0110.

We look forward to sharing your memories.

# Preserving Chestnut Memories

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from then to now

# THE GREAT (ALAS, FICTIONAL) CHESTNUT OF NASHOBA

By Jane Langton


After 16 mystery novels set in the present, with my characters using cell phones and computers and trash compacters, I found myself being dragged backward to the summer of 1863 and the Civil War. The result was a historical mystery, "The Deserter, Murder at Gettysburg."

After finishing this first historical novel I couldn't bear to abandon the 19th century. Therefore, I wrote a sequel, "Steeplechase" (church steeples, that is, not horses). Published by St. Martin's Press in November 2005, it focuses on an ancient tree rather than on warfare.

From *Meetings with Remarkable Trees*. Courtesy of Jane Langton

## EVELYN'S 'GREAT CHESTNUT'

The story was inspired by a book, Thomas Pakenham's "Meetings with Remarkable Trees." Every one of Pakenham's massive survivors is remarkable—the Bowthorpe Oak, the Goodwood Cedar of Lebanon, the Much Miracle Yew. But it was "John Evelyn's Great Chestnut" that caught my fancy, because the photograph of the gigantic tree in ruin is accompanied by a 19th century engraving of the same tree in its glory.

Meetings With Remarkable Trees, *by Thomas Pakenham, is available through Random House Press*

## "NO! IMPOSSIBLE!"

I was charmed by Evelyn's tree. I began to wonder if an ancient *Castanea sativa* might turn up in a fictional New England village in the year 1868.

"No!" said everyone. "Impossible!"

Well, all right, but why couldn't I put some other kind of tree smack in the middle of my developing story? Any old tree would do, so long as it fulfilled three requirements. Not only must it be very old and very large, its lowest branches must be close to the ground. Why? So that a five-year-old boy can climb it.

*Castanea dentata*, Oak Dale, Dedham, Mass. Circumference 32 ft; 21½ ft. at 3 ft. from ground. Courtesy of Jane Langton

## THE SHORT LEGS OF HORACE

Knowledgeable people made suggestions. Professor Will Weeks in Amherst thought my tree might be a black cherry. Dennis Collins of Mount Auburn Cemetery suggested black oak or European beech. Sheila Connor of the Arnold Arboretum wondered if an American chestnut might fill the bill.

The American chestnut! I asked around, hoping for the endorsement of other knowledgeable people. But Norman Levey produced an old photograph of a forest of American chestnuts with such tall straight trunks that the short legs of five-year-old Horace couldn't possibly climb them.

Still, I yearned for a chestnut as grand as Evelyn's. I went back to that great fountainhead of 19th-century natural history, Henry Thoreau's Journal, and found three entries under the word "chestnut."

## "MEASURED THE GREAT CHESTNUT"

"June 2, 1852: Measured a chestnut stump on Asa White's land, 23 and 9½ feet in circumference, 8½ feet one way, 7 feet the other, at 1 foot from ground."

"August 15, 1854: Crossed from the top of Annursnack to the top of Strawberry Hill, measured the great chestnut. At about 7' from the ground, it is 14¾ feet in circumference, 22 feet at 1 foot from the ground."

"Inches Wood, Boxboro, November 16, 1860: The chestnut is remarkable for branching low, occasionally so low that you cannot pass under the lower limb."

## BETTER YET

Then Sheila Connor dug further into the files of the Arnold Arboretum and came upon a photograph of the perfect tree, a massive American chestnut.

If you look carefully, you'll see a well-dressed young man leaning on the rugged trunk, pretending to read a book —

The problem was solved. Young Horace would have no trouble scrambling up into a tree like this. So from then on the American chestnut was the protagonist of my story - the tragic hero, as it turned out.

## FORTUNATELY, UNFORTUNATELY

In the first chapters of Steeplechase, the Great Chestnut of Nashoba spreads its gigantic crown high and wide above the local burying ground. But then, unfortunately—horrible to relate—the glorious old *Castanea dentata* is brutally cut down.

But then—fortunately!—a champion emerges with a plan for resurrecting the fallen giant by building a church, using lumber milled from its lopped branches.

All Saints Episcopal Church, Linville, North Carolina
Courtesy of Jane Langton

## BOUNCED INTO BEING

But was chestnut good for building? Unfortunately, a few friends shook their heads, and Thoreau spoke of its twisted grain. But Sheila Connor, in her book "New England Natives," explains that many Connecticut tobacco barns were made of American chestnut. "While not quite as hard as white oak, the wood of the American chestnut was durable, stood up well to moisture, and was slightly more elastic and lighter in weight." (187)

Then—fortunately!—Anne Myers of The American Chestnut Foundation sent this miraculous photograph of an actual church built in 1913 of American chestnut, All Saints Episcopal in Linville, North Carolina.

Sometimes I see people on the street who are like my own characters come to life. In the same way the Linville church seemed to have bounced into being to prove that my fictional structure was plausible.

## AN AMAZING COINCIDENCE

So now it was perfectly all right to build my own little chestnut church. Fortunately, by an AMAZING coincidence, there just happens to be a fictional steam-driven sawmill right across the way from my fictional fallen tree. Therefore it is just a matter of rolling the immense trunk and branches across the street to be milled into boards by the great round saw.

My fellow townsman Kim Johnson demonstrated the process with his own sawmill, powered by the engine of a Ford pickup truck and, at Old Sturbridge Village, Tom Kelleher sliced logs with a mighty water-powered saw.

But now my eager characters have to be patient, because their milled boards cannot be hammered together until they have dried for two months in the back yard of my impassioned hero, Josiah Gideon. At last, with the help of defectors from the church of the vandal who destroyed the tree, Josiah is able to dig a foundation and raise his chestnut church, complete with its own small steeple.

## "THE VANDAL VILE"

The freedom to mess around with history is one of the perks of a fiction writer. It was fun to exhume Boston poet Oliver Wendell Holmes, who happened in real life to be a lover of very large trees. His fictional shock at the destruction of the great "Nashoba Chestnut" called for a fictional poem by his fictional hand (composing a Holmesian pastiche turns out to be easy as pie)—

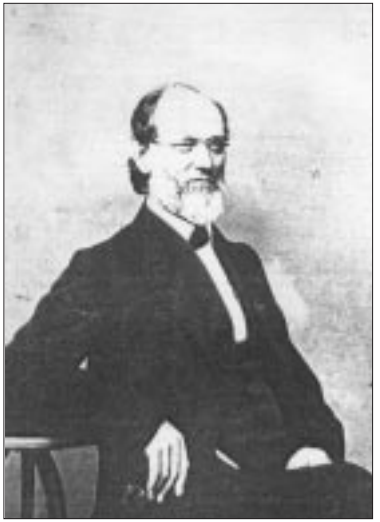
*Let good men curse the vandal vile*
*Who killed our ancient tree.*
*May this foul deed afflict his soul*
*Till he shall cease to be.*

In a fast-forward to right now, some of my modern characters find the vast stump of the Nashoba chestnut, and then at last they recognize the edifice made from its wood. Like the original tree, the chestnut church of Nashoba has undergone a surprising transformation of its own.

## FACES, FACES

All the long-suffering editors of my mysteries have allowed me to add illustrations to the text, my own drawings of the real places where my fictional events happen. But in my two historical novels the drawings are accompanied by 19th-century photographs. These real once-living faces were found in a collection of anonymous cartes de visite in the antiquarian bookshop of Henry Deeks in Maynard, Massachusetts. Here are some of them...

Isabelle and James Shaw



Professor Jedediah Eaton

Reverend Horatio Biddle

They came to seem very real to me as they played their parts around the tree, the great chestnut of Nashoba.

"The Great Chestnut of Nashoba"

# INTERIOR WOODWORK OF APPALACHIAN CHESTNUT

By David G. White
Trade Extension Manager, The Appalachian Hardwood Club
***Southern Lumberman, Dec. 15, 1930***



Along with the rapid strides which have been made in culture, art and science in America during the past few years, there has been an awakened and ever-increasing interest in the esthetic factors influencing human relationships, such as the effect of beauty and attractiveness of surrounding objects in determining the comfort of people and the harmony in and the joy of living.

One of the most important esthetic developments in the trend of modern progress has been the increasing desire and demand for beauti-

*Arcade, De Witt Clinton Hotel, Albany, New York. Furnished in antique Appalachian chestnut.*

ful woodwork interiors in the home, the office, the club house, hotels and other buildings in which both leisure and busy moments are spent.

Architects, interior decorators, and the public at large, in seeking attractive as well as serviceable woods with which to beautify places in which to live and work, are manifesting an increasing demand for the beautiful lace-figured wood of the Appalachian chestnut.

Appalachian chestnut, scientifically known as Castanea dentata, was highly prized and sought after by the Colonists and by their descendants during succeeding decades; not only because of the beauty of the wood, but also because of its serviceability and versatile uses. The renown of chestnut has been echoed through the world in prose and poetry, especially in that immortal poem by Longfellow, "The Village Blacksmith."

Unfortunately, shortly after the beginning of the twentieth century, a fungus scourge, known as the "chestnut blight," was introduced into the North Atlantic and New England states where it destroyed the chestnut forests in those regions. During the early part of the twentieth century, therefore, considerations concerning the charm and the use of chestnut lumber were temporarily semi-obscured by considerations relating to the control of the blight.

It has been scientifically determined that the chestnut blight has no effect upon the properties of the wood of the chestnut tree nor upon its highly prized attractive texture and grain.

The period of semi-obscurity through which chestnut lumber passed, as a result of the publicity and consideration given to the chestnut blight, has made possible a new and fuller appreciation of the charm, beauty and serviceability of this wood to an extent which may cause it to exceed its former popularity, even during the present period of keen competition with numerous other woods and highly advertised materials competitive with wood. It is fitting, therefore, that we refreshen (sic) our memories with a summary of the merits of this splendid and intrinsically valuable wood.

## Availability of Chestnut

The destruction of the chestnut forests in the New England and North Atlantic states by the chestnut blight, and the enormous publicity resulting therefrom (sic), gave some people the impression, especially inhabitants in the states referred to, that good chestnut was no longer available.

The results of a recent survey by the Appalachian Hardwood Club demonstrated the fallacy of this conception. In the Southern Appalachian Mountains, extending from southern Pennsylvania southwesterly to northern Georgia, are large stands of some of the finest chestnut timber ever grown. According to the United States Forest Service, "The finest stands of chestnut are in the southern Appalachians1." The lumber cut from chestnut timber grown in the Southern Appalachian Mountains, because of the splendid texture and grain of the wood resulting from forest site conditions peculiar to the mountains, is commercially known as "Appalachian Chestnut" and should be so specified. The supply of Appalachian chestnut will be ample for many years. Furthermore, stocks of Appalachian chestnut lumber, timbers, ties, poles, posts and other products at the sawmills are always not less than 100,000,000 board feet and go as high as 250,000,000 board feet. Furthermore, the Appalachian chestnut lumber of today is more accurately manufactured, refined and graded to meet the requirements of use than was true in the past.

The chestnut blight has been spreading from the northeastern forests into the chestnut forests of the Appalachian producing territory and it is probable, should blight control measures fail, that at some future date the blight will destroy the Appalachian chestnut forests. This, however, does not affect the availability of Appalachian chestnut lumber and other Appalachian forest products to present day consumers. It will, however, be important to future generations. In the meantime, interior woodwork and other products made from Appalachian chestnut will undoubtedly have rapidly increasing values assigned to them, such as is the case of antiques, when the Appalachian chestnut forests are destroyed.

### Texture and Grain

The beautiful appearance of Appalachian chestnut woodwork derives its charm from the intricate lace-like figures appearing on the surfaces of the rough and finished lumber. The open pores of the wood formed in the spring alternate with the closer-textured wood formed in the summer in each annual ring of growth. When the saw cuts through these alternating layers of springwood and summerwood growth there are produced the beautiful lace-like figures on the surfaces of the lumber, the designs of which are never exactly the same on any two pieces of lumber. The

distinct individuality of the wood designs made by the texture and grain on Appalachian chestnut lumber insures its superior attractiveness over the standardized patterns shown on competitive products on which the finish is an imitation of wood grain. Furthermore, in the case of Appalachian chestnut, the designs are a part of the wood and can not be chipped or scratched off.

## Color of Appalachian Chestnut

The heartwood of Appalachian chestnut is tinged with an exceptionally uniformly very light shade of nut brown. The sapwood, as is true of practically all woods, is lighter in color than the heartwood. Fortunately in the case of Appalachian chestnut trees there is little sapwood and a considerable portion of Appalachian chestnut lumber is free of sapwood.

The richness of the neutral and uniform color of Appalachian chestnut lumber makes it highly desirable for interior woodwork for the reason that, in approved interior decorating as applied to many periods of architecture, the woodwork must be neutral in color. The primary colors of red, yellow and blue, and various hues, or secondary colors derived from mixing the primary hues, are usually more correctly shown in furnishings, such as colored rugs, furniture, draperies, lamps and the like, and this is exceptionally important for the reason that the woodwork is permanent and color changes may best be obtained through changes in movable objects from time to time as desired.

## Figured Designs Dependent on Growth and Method of Sawing

The method of sawing Appalachian chestnut logs in the sawmill determines to a large extent the wood grain figures shown on the surfaces of the lumber.

When the lumber is plain-sawed, that is, when a log is sawed so that the broad surfaces of the lumber are approximately at right angles to the radius of the log, comparatively wide patterns of alternating open springwood and closer-textured summerwood growth are visible in beautiful lace-like figures….

When the lumber is rift-sawed, that is, when a log is sawed so that the broad surfaces of the lumber are approximately parallel to the radius of the log, attractive straight-grained figures made by the comparatively nar-

row straight lines of alternating open springwood and closer-textured summerwood growth are visible….

The exact angle of the saw cut with reference to the radius of the log, the thickness and diameter of the various annual rings of growth at that part of the log being sawed, the taper and straightness of the log, deflections of wood fibers due to knots, and the like, influence the width, size, and designs of the figures visible on the surfaces of the lumber.

"Antique" Appalachian chestnut boards are those which have genuine small pin worm holes or special types of knots or a combination of such pin worm holes and knots…. This type of lumber is customarily selected stock produced in the manufacture of plain-sawed lumber. The worm holes, that occur in the sound wormy lumber used in the manufacture of "antique" interior woodwork, are caused by worms that work in the trees and, according to the United States Bureau of Entomology2, the living worms do not occur in the lumber. Even if there were any worms in the green lumber, they would be killed and practically disintegrated when the lumber was kiln-dried.

## Grades and Sizes

Appalachian chestnut is manufactured into practically all standard sizes and grades ordinarily used by the public. When clear, or practically clear, interior woodwork is desired, it is usually manufactured from the grade of firsts and seconds, No.1 common and better or No.1 common and selects. When a natural antique finish is desired, the interior woodwork is usually manufactured from the grade of No.1 common and better, worm holes no defect; or from No.1 common and better sound wormy.

Interior trim and mouldings (sic) of Appalachian chestnut manufactured in accordance with standard patterns are obtainable from some sawmills and from millwork concerns. Trim, paneling and mouldings (sic) manufactured to special patterns, however, are usually produced on order by millwork concerns.


science and natural history

# CORRECTION

Table 1, by Michael A. Steele, Brian C. McCartha, and Carolyn H. Keiffer, from Seed Dispersal, Seed Predation, and the American Chestnut, was incorrectly printed in our Fall 2005 issue (Vol. XIX, No. 2). The corrected table appears below. We apologize to the authors, and for any confusion this caused our readers.

**TABLE 1** Comparison of nutritional value of *Castanea dentata* in relation to the nuts of other selected species common within the historic range of *C. dentata*. Values are taken from Vander Wall (2001); original sources of data are indicated below.

| Species | Dry mass of edible nut (g) | Caloric value (kJ/g)) | Protein (% dry mass) | Lipids (% dry mass) | Carbohydrates (% dry mass) | Crude fiber (% dry mass) | Ash |
|---|---|---|---|---|---|---|---|
| 1. American chestnut *Castanea dentata* | | | 8.6 | 2.3 | 82.9 | 3.4 | 2.8 |
| 2. Northern red oak *Quercus rubra* | 2.12 | 20.4 | 5.3-7.0 | 18.9-20.8 | 67.1-69.1 | 2.8-4.2 | 2.4-3.1 |
| 3. Black oak *Quercus velutina* | | | 6.9-7.0 | 23.0-24.1 | 64.6-65.1 | 3.0-3.1 | 1.7-2.0 |
| 4. White oak *Quercus alba* | 0.4-0.83 | 17.4-17.8 | 6.3-7.8 | 4.8-6.3 | 82.3-83.3 | 2.5-2.7 | 2.6-2.7 |
| 5. Chestnut oak *Quercus prinus* | 1.21 | 18.1 | 5.8-6.9 | 5.1-10.1 | 78.9-83.2 | 2.5-2.6 | 2.2 |
| 6. Hickory | 1.01 | 27.5 | 13.3 | 74.4 | 8.8 | 1.5 | 2 |
| 7. Black walnut *Juglans nigra* | 2.04 | 26.1 | 29.3-32.6 | 36.9-60.2 | 6.7-25 | 1.0-2.1 | 2.8-3.4 |

Original sources
1. McCarthy and Meredith, 1988
2. Wainio and Forbes, 1941; Baumgrass, 1944; Gysel, 1957; Lewis, 1982
3. Baumgrass, 1944; Gysel,. 1957
4. Wainio and Forbes, 1941; Baumgrass, 1944; Gysel, 1957; Smith and Follmer, 1972; Lewis, 1982
5. Wainio and Forbes, 1941; Lewis, 1982; Smallwood and Peters, 1996
6. Wainio and Forbes, 1941; Smith and Follmer, 1972
7. Wainio and Forbes, 1941; Baumgrass, 1944; Smith and Follmer, 1972

# AN UPDATE ON CHESTNUT DNA PROJECTS: PART I

## The Genes for Blight Resistance from Chinese Chestnut

By Paul H. Sisco
Southern Regional Science Coordinator

Staff Scientists of The American Chestnut Foundation (TACF) often get asked the following questions:

**1.** Why doesn't TACF just clone the genes for blight resistance from Chinese chestnut and insert them into American chestnut using the techniques of genetic engineering?

**2.** Could we use molecular markers to screen for blight resistance at the seedling stage, saving years of time, thousands of dollars, and acres of land?

This article is the first of two that will review progress in applying molecular technology to TACF's breeding program. The focus here is the genes for blight resistance that are present in Chinese chestnut. The second article, to appear in a subsequent issue of the Journal, will report on other uses of molecular technologies that could aid TACF's breeding program.

### CLONING THE GENES FOR BLIGHT RESISTANCE

I have personal experience in gene cloning. In the early 1990s, while I was a professor at North Carolina State University, my graduate student Steve Moose and I spent three alternately frustrating and exhilarating years cloning a gene from corn called *glossy15*. (Moose and Sisco, 1994; Moose and Sisco, 1996). *Glossy15* is a **regulatory** gene—**a genetic "switch"** that turns on a host of leaf surface genes, including ones that control wax composition, the number and type of leaf hairs, and even cell wall chemistry. A single regulatory gene can have a major effect on other genes, such as turning them all "on" or "off" at the same time.

How would TACF go about cloning the Chinese genes for resistance? When Steve Moose and I cloned *glossy15*, we had three advantages that

A regulatory gene acts as a genetic switch to turn other genes "on" or "off."

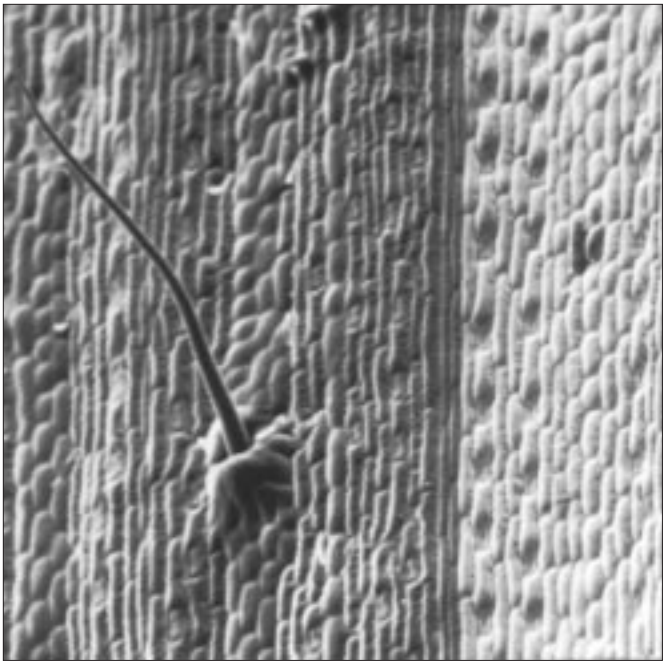


**Figure 1.** This is a B/W scanning electron microscope image of a leaf from a corn plant that has a mutable glossy15 gene caused by the insertion of a transposable element. The gene is "on" in the lighter-colored sector and "off" in the darker-colored sector. When the gene is "on", the surface of the leaf is covered with wax—thus the light color. When the gene is "off", the wax is not present and specialized structures such as leaf hairs appear.

These two linear sectors are descended from two cells—one in which the gene was "off" because of the presence of the transposable element in the gene, and one in which the gene was turned back "on" because the transposable element had moved, restoring gene function. We could isolate DNA from each of the two sectors. This helped us to clone the DNA sequence of the gene that was "on" or "off."

TACF scientists do not have in trying to clone the blight resistance genes: (1) we were dealing with a single gene; (2) the effect of the gene when "on" or "off" was clearly visible on the leaf surface of seedlings (Fig. 1); (3) we had a mutant in the gene caused by the insertion of a transposon, a piece of DNA that we could use as a "fishing hook" to first identify and then clone the gene. Even with these advantages, it took us three years and hundreds of thousands of dollars to clone it.

These two linear sectors are descended from two cells—one in which the gene was "off" because of the presence of the transposable element in the gene, and one in which the gene was turned back "on" because the transposable element had moved, restoring gene function. We could isolate DNA from each of the two sectors. This helped us to clone the DNA sequence of the gene that was "on" or "off."

TACF scientists do not have these advantages in trying to clone the genes for blight resistance. (1) The genetic control of blight resistance is more complex—more than one gene needs to be cloned to get a better understanding of how the genes for resistance work together. Also, since there is more than one gene affecting resistance, it is more difficult to determine when a single one of the genes is "on" or "off." (2) The effect of the genes for blight resistance is less easy to measure, and is more affected by environmental conditions than is the effect of *glossy15*. (3) We do not have a mutant in any of the blight resistance genes that we could use as a "fishing hook."

Nevertheless, more molecular technologies are available in 2006 than were available in the early 1990s when Steve Moose and I cloned *glossy15*.

Machines developed in the course of the human genome project can now get 90% of an organism completely sequenced in less than a year. Other machines can determine which genes are turned on or off when a plant is infected with the blight fungus. These technologies are expensive. Sequencing machines can cost a half million dollars, and the supplies and technicians to run them cost hundreds of thousands more per year. A complete sequence of a chestnut tree's DNA could cost $20 million. But, after spending $20 million, we still would not know what part of that sequence contains the blight resistance genes. What do we really need to clone? To answer this question, genetic markers may be a big help in locating the gene. The final proof that a certain DNA sequence is a gene for resistance makes use of the techniques of genetic engineering. If the DNA sequence confers resistance to a susceptible tree, this is considered proof that the sequence is a gene for resistance.

## MOLECULAR MARKERS AND INDIRECT SELECTION

Genetic markers are segments of DNA used for **indirect** selection.


In the diagram, Marker A and Marker B are segments of DNA located close to a gene that we wish to monitor, such as one of the genes for blight resistance. During meiosis, when sperm and egg cells are being formed, **crossing-over** occurs along a chromosome. During crossing-over, the association between the markers and the gene can be lost, depending on how close the marker is to the gene being selected. This "genetic distance" between marker and gene can be measured in experimental populations. In the diagram above, the genetic distance between Marker A and the gene is 5 centiMorgans (cM), which roughly means that 5% of the time Marker A will be separated from the gene during crossing-over. Marker B is closer to the gene, so that by selecting for Marker B we will lose the gene only 2% of the time. If we should select for **both** Marker A and

Marker B, then we would lose the gene 5% x 2% = 0.1% of the time, only 1 time in 1000, on average.

Molecular markers have been available to the plant breeding community for over 20 years. Like other molecular technologies, they have proved to be very useful in some situations and not so useful in others. Some of the things that must be considered are:

**1. Expense:** The first big expense is in developing a set of markers that will be useful in a particular breeding population. I do not have space here to discuss all the types of markers that have been developed. They are a veritable alphabet soup of acronyms: RAPDs, RFLPs, AFLPs, SSRs, SCARs, and SNPs, just to name a few of the most widely-used types. TACF scientists have one advantage in marker development; it is easier to find markers that distinguish different species, such as Chinese and American chestnut, than it is to find markers that distinguish individuals in highly related populations, such as cultivated tomatoes. Nevertheless, even after a good set of markers has been developed, their use requires DNA extraction and laboratory analysis, costs that can quickly add up if large populations are being screened. If the trait to be analyzed can be scored quickly and cheaply by phenotype (appearance) alone, then it is not practical to use marker-assisted selection. This was the conclusion of a recent study comparing conventional backcrossing versus marker-assisted selection for resistance to southwestern corn borer (Willcox et al., 2002).

**2. Time to do the Analysis:** Another factor that has limited the usefulness of markers is the time it takes to analyze them. Plant breeders for annual crops will sometimes produce as many as three generations in a year. Results from one generation are needed to select in the next generation, and testing may not be rapid enough to accommodate that cycle. Again, TACF scientists have an advantage, since it normally takes two to four years after planting for the trees to get large enough to screen for blight resistance. If we had reliable, closely-linked markers for the resistance genes and enough money to pay for the DNA analysis, we could get test results back in six months after germination. This would allow us to eliminate susceptible trees at the seedling stage, saving years of time and acres of space.

**3. Reliable Correlation between the Markers and the Trait:** To be truly useful, markers must be very reliable. The most reliable markers would be the genes themselves. In almost all cases, however, we do not have the sequence of the gene for the trait. We must be satisfied with markers that are closely linked to the genes and that are highly correlated with the trait we wish to follow. This is where TACF scientists have a disadvantage. The best correlations are obtained when the trait to be studied is controlled by a single gene and when correlations are measured in large mapping populations. Because chestnut blight resistance is controlled by at least two to three genes (Hebard, 2005), it is much more difficult to get a good correlation of markers with any one of the genes. It would take analysis of mapping populations as large as 1,000 trees from a single cross, and that would only give reliable information for the parents of that cross (Beavis, 1995). We would need to repeat such experiments for each source of resistance in the TACF program such as 'Clapper', 'Graves', and 'Nanking', until we were sure we had mapped all the resistance genes available in Chinese chestnut and that our markers were reliable and consistent indicators of resistance.

## Two Case Studies of the Use of Molecular Markers in Breeding for Disease Resistance in Trees

**Breeding for Resistance to Eastern Filbert Blight in European hazelnut.** Dr. Shawn Mehlenbacher and his colleagues at Oregon State University are routinely using molecular markers in breeding for resistance to Eastern filbert blight, *Anisogramma anomala*, in European hazelnut cultivars, *Corylus avellana* (Mehlenbacher et al., 2004; Mehlenbacher et al, 2006). Interestingly, this is a native American disease to which American hazelnuts are resistant. The blight is potentially devastating to the Oregon hazelnut growers, who use European cultivars selected for large nut size and quality traits. While searching for resistance, Dr. Ron Cameron and Dr. Maxine Thompson at Oregon State found one rarely-used European cultivar named 'Gasaway' that has a single, dominant gene for resistance to Eastern filbert blight (Mehlenbacher et al, 1991). Because this was a single, completely dominant gene, it was relatively easy to find molecular markers that were highly correlated with the resistance gene. And, because it normally takes 16 months after inoculation to get good resis-

tance data, it was cost-effective to use markers flanking the 'Gasaway' gene to eliminate susceptible trees at the seedling stage. To be successful, hazelnut cultivars must have many other qualities, such as good nut size, ease of blanching, etc., so being able to eliminate the susceptible seedlings quickly made the effort and expense of molecular marker analysis worthwhile. Dr. Mehlenbacher and his colleagues are also investigating other sources of resistance, some that may be single genes and others that are polygenic in nature, in case a strain of the blight fungus can overcome the 'Gasaway' gene. Nevertheless, the 'Gasaway' gene has held up for over 25 years.

**Breeding for Resistance to Fusiform Rust Disease in Loblolly Pine.** Dr. Henry Amerson of North Carolina State University and colleagues have used molecular markers to identify at least eight different genes in loblolly pine, *Pinus taeda*, for resistance to fusiform rust disease, caused by *Cronartium quercuum* f sp. *fusiforme*. Information about only one of these eight genes has been published (Wilcox et al., 1996; Kuhlman et al., 1997), but the NCSU Fusiform Rust Program and the Tree Improvement Cooperative at North Carolina State University have been using markers for several of the genes in pine breeding research (Henry Amerson, personal communication). One of Amerson's colleagues, Dr. Tom Kubisiak of the USDA Forest Service's Southern Institute of Forest Genetics, noted that for fusiform rust disease there is a clear gene-for-gene interaction between host and pathogen (Kubisiak et al. 2005). In other words, one particular strain of the fungus might be avirulent against one of the known resistance genes, resulting in no disease, whereas others might be virulent, resulting in disease. Thus, Amerson and co-workers were able to use different pine families and different single-genotype strains of the fungus to uniquely identify the different resistance genes. The markers so far have been very reliable. For the first resistance gene tested, an associated marker was 95% predictive of phenotype in a disease screening test (Kuhlman et al., 1997). The 5% "misses" could be due to several factors, including genetic map distance between marker and gene; low levels of pollen contamination in the full-sib pine family used; low levels of spore contamination in the single-genotype inoculum; simple escapism; or an inability to accurately rate disease phenotype in all cases (Henry Amerson, personal communication). The tracking of resistance alleles with molecular markers should prove extremely useful in future efforts to "pyramid" fusiform rust resistance genes in pine. "Pyramiding" means putting

several different disease resistance genes into a single tree, thus making it resistant to multiple strains of the pathogen.

## Molecular Markers and Breeding for Chestnut Blight Resistance: The Future

Mapping studies in populations of about 100 trees have indicated the rough location of up to three resistance genes coming from the Chinese cultivar 'Mahogany' (Kubisiak et al., 1997; Sisco, 2005; Hebard, 2005). Dr. John Carlson of Pennsylvania State University and Dr. Laura Georgi of Clemson University are in the process of developing a more complete set of molecular markers to target the known location of these resistance genes. Dr. Georgi has also cloned almost the entire genome of the 'Mahogany' Chinese chestnut cultivar into bacteria, in what is known as a "Bacterial Artificial Chromosome (BAC) Library." Proposals for sequencing and more extensive mapping have been submitted to the Community Sequencing Program of the Department of Energy's Joint Genome Institute and to the Grants Program of the National Science Foundation. These proposals, if funded, will greatly aid us in the development of useful and reliable genetic markers for blight resistance, and may help in eventually cloning one or more of the resistance genes.

Meanwhile, molecular markers have other, more immediate uses in our breeding program, as will be discussed in Part II, to appear in a future issue of *The Journal of The American Chestnut Foundation.*

Dr. Paul Sisco is TACF's Regional Science Coordinator at the Southern Appalachian Regional Office (SARO). Paul develops, coordinates, and oversees cooperative breeding projects with our regional partners.

*Note:* Thus far in chestnut, no strains of the blight fungus have been discovered that can attack our resistant trees. This eliminates one means of furthering our understanding of the genetics of blight resistance, as outlined by Dr. Sisco in his discussion of resistance in pines to fusarium rust. However, it is not unlikely that strains of the blight fungus will arise in the future that are capable of breaking our resistance. Efforts are underway to find such strains, so we can prepare ourselves.

—Dr. Fred Hebard, Pathologist, Meadowview Research Farms

Dr. Hebard has headed up TACF's research in Meadowview, VA since the farm was established in 1989.

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*In 2005, Drs. Kim Steiner and John Carlson of Penn State University sponsored a visiting scholar to Pennsylvania State University, Dr. Mahn-Jo Kim, from the Korean Forest Research Institute (KFRI). Over five months, Dr. Kim studied the breeding program of TACF, traveled to various breeding orchards, assisted in planting a Graves backcross orchard in eastern Pennsylvania, and attended several TACF meetings, including a chapter meeting in Ohio and the board and cabinet meetings in Abingdon, VA. In addition, Dr. Hill Craddock and the Tennessee Chapter sponsored a visit and lecture from Dr. Kim at the University of Tennessee at Chattanooga. From that visit, arrangements for the exchange of germplasm occurred and, over the spring of 2006, Dr. Craddock will be grafting scion sent to him from Dr. Kim. Overall, Dr. Kim's visit was a delight to those who met him, especially toward the purposes of learning new techniques in both grafting and orchard management. The following article, which is to be published over two issues of The Journal, is the culmination of Dr. Kim's work in the United States, and is geared toward summarizing decades of chestnut breeding in Korea. We hope you enjoy this first look into the biogeography, culture, and tradition of the Korean chestnut.*

—Sara Fern Fitzsimmons

Sara is TACF's Tree Breeding Coordinator at Penn State

# CHESTNUT CULTIVATION AND BREEDING IN KOREA

By Mahn-Jo Kim, PhD, Korea Forest Research Institute

The chestnut has long been cultivated and consumed throughout Asia, Europe, and America, and is one of the most important nut crops found throughout the temperate zone. There are four commercially important species of chestnut: Japanese (*C. crenata* Sieb. and Zucc.), Chinese (*C. mollissima* Bl.), American (*C. dentata* [Marsh.] Borkh.) and European (*C. sativa* Mill.). Chestnut trees produce a marketable crop and can be used in many ways as a basic carbohydrate source like grains and vegetables. Chestnut has quite a remarkable nutritional composition that sets it apart from all other nuts and makes it an outstanding food source. Most nuts are high in fat and low in carbohydrates and best reserved for treats. However, chestnuts are made up of primarily complex carbohydrate, have a high-quality protein (< 5%), and are very low in fat (< 1%). They also have reasonable quantities of vitamin C and potassium and are very low in sodium. The flavor, texture, and sweetness of the nuts varies widely according to chestnut species and cultivars, from tasteless and bland, to very sweet and flavorful.

## Chestnut production

Chestnut trees have been cultivated for more than 2,000 years in Korea. Koreans consider chestnuts a wholesome food for their nutritional value and chestnuts were especially important as a food source during times of famine. Chestnuts are consumed raw or roasted during winter in Korea.

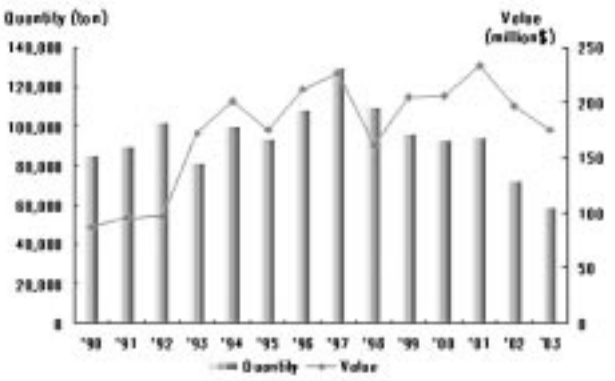


Figure 1. Chestnut production in Korea

Low res


Figure 2. Main production regions (black circles)

Korea is currently the second-largest producer of chestnuts worldwide, following China. Since 1997, when the peak production was 130,000 tons, however, Korea's yield has been slowly declining (Figure 1). Today, total chestnut orchard areas are estimated at about 60,000 hectares, and annual production is about 70,000 tons. One-third of the annual harvest is exported to Japan.

Chestnut trees are economically the most important nut tree species cultivated in rural and mountainous areas. Most chestnut-producing areas had been built up from late 1960s to mid-1970s by 15,000-35,000 ha per year. The main producing area has been the southern part of the Korean peninsula. Most orchards established in the last 10 years are located in the central area, and the production of the central area is increasing. More than 60% of total production is at Chungju, of Chungbuk Province; Gongju and Buyeo, of Chungnam Province; Kwangyang, of Cheonnam Province; and Sanchung and Hadong, of Gyeongnam Province (Fig. 2). Harvesting time is from late August to early October.

Proper management of chestnut orchards is a challenge at the present time. Some 53% of chestnut trees in orchards all over the country are over 25-years-old, resulting in smaller nut size and lower nut productivity than is possible on younger trees. The difficulty of finding labor for harvesting nuts, aging of growers, and the decline of chestnut trees through aging are increasingly important constraints for chestnut production. Recently, the recovery of old chestnut trees by heavy pruning has been recommended as a means of improving of nut production. Some growers have successfully converted their old orchards to renewed orchards with high productivity.

Although the average yield per hectare is still low (Table 1), leading growers of the central region (Chungbuk and Chungnam Province) produce over four tons of chestnuts per hectare every year, with yields as high as six tons per hectare having been reported.

## TABLE 1

*Average chestnut production by province.*

| Region of Production | Cultivation Area (ha) | Tree Age (year) | Number of Trees (trees/ha) | Production (kg/ha) |
|---|---|---|---|---|
| Chungbuk | 11.4 | 19 | 383 | 1,385 |
| Chungnam | 17.9 | 19 | 289 | 1,539 |
| Cheonbuk | 12.3 | 20 | 277 | 683 |
| Cheonnam | 11.2 | 27 | 254 | 1,015 |
| Gyeongnam | 11.2 | 27 | 168 | 1,149 |
| Total average | 12.7 | 22 | 269 | 1,184 |

### Chestnut species and cultivars

The Genus *Castanea* comprises seven species (Rutter et al., 1990). Four species are native to Asia: Japanese chestnut on the Japanese islands and the Korean peninsula, Chinese chestnut, Sequin chestnut (*C. seguinii* Dode.), and Henry chestnut (*C. henryi* Rehd. & Wils.) on the mainland China. Two species are native to North America: the American chestnut and chinkapin (*C. pumila* Mill.). European chestnut is native to southern Europe and western Asia.

Paleontological studies indicate that chestnut trees were already present in the Korean peninsula during the Miocene Era (24.6 million ~ 5.1 million years ago) (Kong, 2003). Chestnut pollen and wood charcoal have been found in fossil layers correlated to the Pleistocene and Holocene Eras. Chestnut distributions fluctuated during the Quaternary Era, widening towards the North and tightening towards the South as glacial periods alternated with warm periods. The Korean peninsula and the eastern area of the mainland China were not divided by sea when the last glaciations developed about 20,000 years ago. The present shoreline was formed about 6,000 years ago (Park and Kong, 2001). It seems that Korean native chestnut and Chinese chestnut trees have evolved independently since the last ice age.

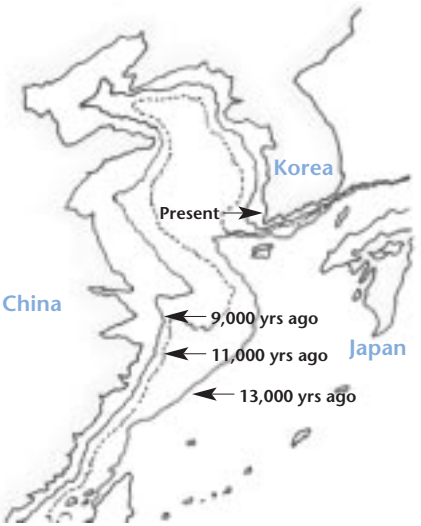


Figure 3. Changes of the shoreline since the last Ice Age.

Figure 4. 90-year-old Korean native chestnut tree

Korean native chestnut is scattered near farmhouses and over the piedmont area (below 1,500 m above sea level) of the Korean peninsula, except in the cold alpine area. It grows spontaneously with other broad-leaf trees in forests. It reaches 15-20m in height, is resistant to the chestnut blight, and its winter hardiness is good down to -25°C (-13°F).

Korean native chestnut has been considered a variety of Japanese chestnut due to differences in morphological characteristics such as leaf, branch, nut traits, and so on. (Park *et al.*, 1965; Gu *et al.*, 2001). Korean native chestnut trees have a high variability of nut traits such as shape, size, sweetness, and facility of peeling because they have been propagated by seeds. Korean native chestnut is supposed to be classified into *C. crenata* var. *dulcis* Nakai and *C. bungeana* Blume, although the identification between the two is still obscure because of intermediate traits. *C. crenata* var. dulcis is distributed over central and southern area of the Korean peninsula. It has relatively large nut size, low sweetness, and low ability of peeling. On the contrary, *C. bungeana* is mainly distributed in northern region and has small nut size, high sweetness, and high ability of peeling.

Chinese chestnut contrasts with Japanese chestnut in nut characteristics. Chinese chestnut has smaller nut size, smaller hilum, higher sweetness, harder kernel, and easier peeling ability than Japanese chestnut. There are some historical documents that Chinese chestnut was introduced to the Korean peninsula through several cultural missions about 2,000 years ago, and was subsequently cultivated in the northern area. That suggests the possibility that Chinese chestnut germplasm has moved into adjacent Korean native chestnut by natural crossing and introgression. Among the progenies, chestnut trees with good nut quality such as large nut, high sweetness, and good peeling ability have been preferred for cultivation, and their seeds might be easily spread long distances by humans for the establishment of new orchards. Therefore, Korean native chestnut may be a hybrid swarm which might be a continuous series of morphologically distinct hybrids resulting from hybridization between Japanese chestnut and Chinese chestnut species by natural pollination of subsequent generations (Park et al., 1965; Gu *et al.*, 2001).

In 1958, chestnut gall wasp (*Drycocosmus kuriphylus* Yasumatsu), which causes fatal damage to chestnut trees, appeared for the first time in Korea and then spread rapidly all over the country. As a result, almost all Korean indigenous chestnut trees of the orchards and natural chestnut stands were heavily damaged. Thereafter, gall-wasp-resistant cultivars have been planted for the establishment of new orchards.

The chestnut cultivars grown commercially in Korea are usually Japanese and Japanese-Chinese hybrids with resistance to gall wasp and blight. The currently prevailing cultivars are Okkwang, Daebo, Tanzawa, Arima, Riheiguri, Tsukuba and Ginyose (Figure 5). Okkwang and Daebo were released by Korea Forest Research Institute (KFRI) in 1965 and 1998, respectively. The others were introduced from Japan between the late 1960s and the early 1970s, and are well adapted to the Korean environment. Most cultivars from Korea belong to Japanese chestnut and were derived from individual selection in seedling orchards or intraspecific hybridization. However, Daebo and Riheiguri were derived from interspecific hybridization between Japanese and Chinese chestnut (Kim et al., 2003b). Table 2 lists characteristics of some of the varieties cultivated commercially in Korea. The data was obtained from the chestnut cultivar archives of KFRI during four years (2001-2004). These plantings have been intensively managed with pruning and fertilization, so nut traits are probably high compared with commercial plantations.

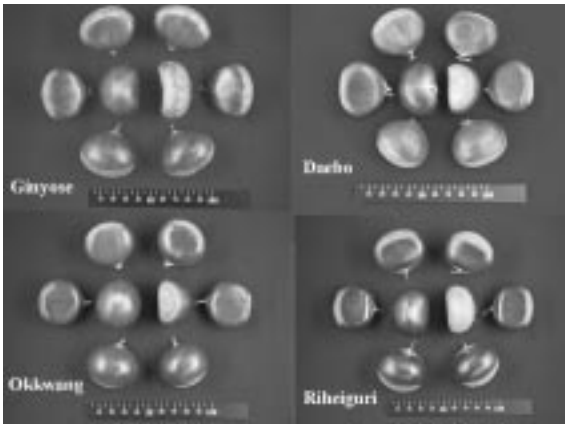


Figure 5. The nuts of common cultivars

Dr. Mahn Jo Kim resides in the Republic of Korea, where he serves as a researcher for the Ministry of Agriculture and Forestry. Dr. Kim attended Seoul National University, where he earned multiple degrees, including a BA, MA, and PhD in Agriculture.

## TABLE 2

*Characteristics of chestnut cultivars.*

| Cultivars | Pedigree* | Harvest Period | Nut weight (g) | Soluble** solids content (%) | Kernel hardness (kg/cm²) | % with pericarp split | % of poly-embryonic nuts |
|---|---|---|---|---|---|---|---|
| Arima | C | Sept.17-Sept.28 | 17.5 | 11.8 | 8.4 | 3.3 | 0.2 |
| Daebo | CxM | Sept.20-Oct.01 | 20.1 | 13 | 10.3 | 3.6 | 0.1 |
| Eunsan | C | Sept.24-Oct.05 | 18.6 | 13.6 | 9.3 | 10 | 3.3 |
| Ginyose | C | Sept.23-Oct.04 | 22.5 | 11.6 | 9.4 | 3.6 | 2.3 |
| Hyogo 57 | CxM | Sept.21-Oct.01 | 22.4 | 12.1 | 8.6 | 1.8 | 0.5 |
| Ibuki | C | Sept.07-Sept.16 | 18.6 | 12.1 | 8.3 | 8.3 | 3.1 |
| Idae | C | Sept.20-Sept.30 1 | 9.8 | 13.6 | 10 | 14.5 | 1 |
| Ishizuchi | C | Sept.22-Oct.04 | 21 | 13.2 | 9.2 | 3.1 | 0.2 |
| Isseumo | C | Aug.28-Sept.06 | 23 | 10.9 | 8 | 2 | 2 |
| Juok | C | Sept.07-Sept.17 | 16.1 | 12.4 | 9.6 | 17.9 | 2.3 |
| Kunimi | C | Sept.06-Sept.15 | 21.2 | 10.7 | 7.2 | 0.3 | 3.3 |
| Kwangeun | C | Sept.13-Sept.23 | 19.5 | 13.3 | 9.2 | 4.8 | 2.4 |
| Okkwang | C | Sept.17-Sept.26 | 17.2 | 11.2 | 8.3 | 6.3 | 0.3 |
| Riheiguri | MxC | Sept.15-Sept.23 | 20.2 | 12 | 8.5 | 5.8 | 0.2 |
| Sandae | C | Sept.25-Oct.05 | 18.2 | 13.5 | 10 | 11.4 | 4.2 |
| Tanzawa | C | Aug.29-Sept.06 | 20.4 | 11.4 | 7.8 | 5.4 | 4.8 |
| Tsukuba | C | Sept.14-Sept.25 | 18.4 | 12.6 | 9.4 | 0.7 | 0.5 |

*C = *C. crenata*; M = *C. mollissima* ** measured soon after harvest

**Editor's Note:** Dr. J. Hill Craddock, a member of the TACF Science Cabinet, and a faculty member for the Department of Biology & Environmental Sciences at the University of Tennessee at Chattanooga, spent a few days in March, 2006 in quarantine at the USDA Animal and Plant Health Inspection Service (APHIS) Plant Germplasm Quarantine Program in Beltsville, MD. Using Chinese and Japanese chestnut rootstocks, Dr. Craddock was there to graft scionwood sent to the facility by Dr. Mahn-Jo Kim of the Korea Forest Research Institute, in Suwon, Korea. The ten cultivars were selected for resistance to both chestnut blight and chestnut gall wasp. Dr. Craddock said, "By importing these clones from Korea, we are taking advantage of 50 years of tree breeding effort by Korean and Japanese scientists. These cultivars may be the best new sources of blight and gall wasp resistance we have available. I am very excited about this opportunity." It is important to note that Castanea scionwood is in the "Forbidden" category for plant material because of the risk of accidentally importing new chestnut diseases and/or insect pests. For this reason, these cultivars can be handled only at the USDA-APHIS Quarantine facility, where they will be grown for two years under very close scrutiny.

## TABLE 6

*Castanea scionwood sent to the USDA APHIS from Korea*

| Cultivar | Origin | Pedigree | Remark |
|---|---|---|---|
| Arima | Japan | *C. crenata* | |
| Daebo | Korea | *C. mollissima* x *C. crenata* | Patented variety |
| Ginyose | Japan | *C. crenata* | |
| Ibuki | Japan | *C. crenata* | |
| Ishizuchi | Japan | *C. crenata* | |
| Okkwang | Korea | *C. crenata* | |
| Riheiguri | Japan | *C. mollissima* x *C. crenata* | |
| Sandae | Korea | C. crenata | |
| Tanzawa | Japan | *C. crenata* | |
| Tsukuba | Japan | *C. crenata* | |

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*The American Chestnut Scholar program began in the fall of 2004 as a pilot in Maryland to enable middle and secondary school students to participate in student research projects related to restoration of the American chestnut. More than 500 students became Scholars in Carroll County and were instructed using a specialized curriculum in 7th through 12th grades that utilized the American chestnut story to illustrate lessons in genetics, ecology, and biology.*

*In addition, selected science research students in 11th and 12th grades at South Carroll High School conducted independent American chestnut research projects. Dr. Donald L. Nuss of the University of Maryland's Biotechnology Institute (UMBI) and his research assistant, Dr. Chris Root, trained the biology faculty to enable the students to sample blight cankers, grow blight fungus, and determine vegetative compatibility among the samples. After successfully completing these lab projects and associated background research, they were invited to present their work at the American Chestnut Jamboree held on May 14, 2005, at ThorpeWood Environmental Learning Center in Thurmont, MD.*

*The Jamboree presented all aspects of TACF's American chestnut restoration program to the Carroll County middle school American Chestnut Scholars and their families. To round out the presentations to include the techniques used in backcross breeding, students described controlled pollination and illustrated pollen viability testing using microscopes to show the germination of two types of dried pollen provided by Dr. Fred Hebard at Meadowview Research Farms.*

*The illustrative learning stations at the Jamboree included chestnut history, a tour of the TACF backcross breeding orchard established at ThorpeWood in 1999, and the science research projects presented by the students and described by them in the following paragraphs.*

*—Essie Burnworth*

Essie Burnworth is the President of TACF's Maryland Chapter, and has been instrumental in the development of the Scholars Program.

# ACTIVITIES FOR THORPEWOOD ENVIRONMENTAL CENTER

By Robert Foor-Hogue

On May 14th, 2005, students from South Carroll High School, accompanied by their teacher, Robert Foor-Hogue, made a presentation to 300 people at The American Chestnut Foundation Jamboree, located at Thorpewood Environmental Center near Cunningham Falls State Park in Maryland. The presentation included nine display boards and six science research students demonstrating chestnut laboratories. The students from South Carroll High School include Trish Riordan, Aaron Wingert, Sean Considine, Nicole Diven, Greg Poole, and Bryan Wiles.

Labs demonstrated included Chestnut Blight Isolation, Vegetative Compatibility, and Pollen Viability testing.

The purpose of the Chestnut Blight Isolation Lab is to grow the fungus, *Cryphonectria parasitica*, on an agar plate so that various tests and experiments such as the Vegetative Compatibility Lab can be performed. The students used a sterilized bone marrow biopsy instrument to remove samples of the disease from a chestnut canker. The sample, known as a plug, is placed on a microtiter plate where it is cleansed using bleach to remove any outside infection. Next, the plate is placed in distilled water to remove the bleach. Then, using sterilized forceps, the plug is moved to a 2% water agar where it is allowed to grow for up to three days. After the initial growing phase, the plug is moved to a PDA (potato-dextrose agar) where it grows for up to two weeks. It is on the PDA that the usable fungus is grown and can be tested. The fungus radiates outward in a ring-like pattern with characteristic shades of orange.

The Vegetative Compatibility Lab was designed to test if different strains of the virus will grow together and exchange genetic information. The lab begins with two previously grown *Cryphonectria parasitica* PDA plates as well as a scalpel, alcohol, matches, and a BGM agar. After sterilizing the scalpel with alcohol and flame, a 1 mm cube is cut from the PDA and placed in the center of the BGM. Then the scalpel is sterilized again and a 1 mm cube from the second PDA is cut. This cube is placed 3 mm away from the first cube and the BGM is allowed to grow for one week. If the two strains are compatible the filaments will grow together, exchange genetic material and form one large colony. However, if the plugs are not compatible there will be a visible line separating the two different strains because the hyphae cannot fuse. Currently at South Carroll High School, we are testing eight different lesions from two different regions including Sugarloaf Mountain and South Carroll High School to determine the number of different strains we have isolated.

The Pollen Viability Lab is the preparation lab for the Controlled Pollination Lab. In the Controlled Pollination Lab, the objective is to cross trees using pollen from each in order to create a specific type of backcross. The process requires nearly one year, and the first step is to determine if the pollen has the ability to procreate. The materials needed for the lab include a 1% sugar-water solution, a tube of pollen, a microtiter pollen-plate, and a pollen staff. First, pour the solution into the individ-

ual wells in the microtiter pollen plate. Then, using the pollen staff, remove a few grains of pollen from the tube and place it into one of the wells filled with the sugar-water solution. Last, place the microtiter pollen plate into the incubator. Growth can be seen after one hour, however, more noticeable growth can be seen after three to five hours.

Last year, students built a research garden to provide a controlled environment where chestnut studies can take place. A chain-link fence surrounds the garden and a large gate provides access. A gravel pathway surrounds one large handicapp-accesible raised bed. Growing in the garden are five chestnut trees from Sugarloaf Mountain and two trees from Thorpewood. Also included is a bog built by the students of South Carroll High School to study unique bog plants.

A density study has been performed in the South Carroll woodlands. The purpose of which is to determine the percentage of American chestnut trees that are still sprouting from the roots of trees killed by the blight. Over an area of 44,100 sq. ft., students counted and marked the number of chestnut trees present, as well as the total number of trees. A total of 471 trees were present in the area, with 21 chestnut trees dispersed among them; this means that 4.5% of the trees in the area were chestnuts.

In front of the school, students are planting a comparative anatomy tree orchard with Japanese, European, and American chestnut trees. Already in place are five American chestnuts that were planted last year. Three display boards from The American Chestnut Foundation explain the chestnut blight problem, as well as history and other general information about the trees. Ecology, biology, and science research classes will use the orchard for comparative botany studies between the different species of the trees.