

# NOTES

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## **MEMORIES**

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Chestnutting, an original illustration by John Exley
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# SCIENCE AND NATURAL HISTORY

Using DNA markers to distinguish among chestnut species and hybrids,
by Tom L. Kubisiak



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# notes



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# FROM THE EDITOR

In this issue of the **Journal** we celebrate the recent opening of our satellite office in Asheville, North Carolina with a focus on memories of the Appalachians.

We start our story of chestnut in the South before the region was even considered as such. Naturalist William Bartram's three short recollections of his trips through Georgia and South Carolina date to 1773 and 1775, and are among the earliest chestnut records we have.

The next installment in the Appalachian chestnut story takes place a little more than a century later. In an original illustration by TACF member John Exley, we see a family chestnutting somewhere in the southern mountains shortly before the turn of the century. The members of the family "pose for our imagination," says John, not knowing that within a few short years the blight would bring an end to their annual outing.

An article in what is now **American Forests** magazine takes us forward to 1915 when, the author P.L. Buttrick writes, "it is thought that [the blight] will all but exterminate the chestnut in the northern states... and may invade the South with like disastrous results." Buttrick's comprehensive essay on the commercial uses of chestnut shortly before the First World War makes clear just how much that mountain family - and the nation - will soon lose. (The article was pulled from the growing collection of chestnut material - much of it donated by Board member Dr. Hill Craddock - now housed at our Asheville satellite office.) The National Archives photographs that accompany the article, collected for TACF by our good friend Carl Mayfield, portray that decline. They begin with a chestnut in bloom along a dirt road in June and an open-grown tree standing, full leaf, in a field at the height of summer. They end with an autumnal portrait of a huge victim of the blight.

Grace Blanchard was born in a hollow in eastern Tennessee just before the blight arrived. Her memories of chestnut, collected by her daughter and TACF member Jo Neuswanger, coincide with the period described by Donald Davis in his upcoming book **Where There Are Mountains: An Environmental History of the Southern Appalachians**. We excerpt portions of his chapter "A Whole World Dying" here.

The photographs scattered throughout the book selection record the death of the American chestnut in Shenandoah National Park in Virginia,



and the uses to which it was put by Depression-era Civilian Conservation Corps workers. The photographs were gathered and beautifully duplicated for TACF by Park volunteer John Amberson.

There's something of the present day in this issue also. There's our annual report on activities and holdings at our research farms in southwestern Virginia. These yearly updates by staff pathologist Fred Hebard and staff geneticist Paul Sisco summarize the results of a huge number of hours of planning, research, and labor. They're a quick reference for members who want to track the growth of our trees. Finally, an article by Dr. Tom Kubisiak of the U.S. Forest Service shows how DNA markers can be used to distinguish American chestnuts from all the rest. Tom's case study grew out of a larger investigation into American chestnut genetic diversity he and University of Massachusetts researcher Dr. Robert Bernatzky began in 1998.

A **Journal** largely devoted to a lost glory could be a sad one. But here's the silver lining: More than a century after the introduction of the disease that so altered eastern landscapes, every year more and more people join the effort the save the American chestnut. Dozens of volunteers arrive each June at our farms in the Virginia mountains to help produce the trees that Fred Hebard and Paul Sisco report on; researchers like Tom Kubisiak whittle away at all we don't know about chestnut and the blight; supporters like John Exley, Carl Mayfield, Jo Neuswanger, Donald Davis, John Amberson, and many others contribute what they're best able to offer; and our organization's growth is large enough to justify opening a new office, providing us with presences both north and south, and with a reach both back to the past and ahead to the future.

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# MEADOWVIEW NOTES 1998-1999

by Fred V. Hebard and Paul Sisco

I n 1998, Meadowview experienced a very warm, wet spring. *Forsythia* started blooming the first week in March and we had no hard freezes after that date. By mid-May, most planted chestnuts were up, and chestnut trees started blooming by June 3. This was about two weeks ahead of last year, which was a late year. The rains stopped in early July, and it became quite dry, although we had two good rains in early and mid-August. Then it didn't rain appreciably until October.

Due to the lack of summer rain, we needed to irrigate orchards where trees were being screened for blight resistance for the first time since 1995. The lack of summer rain also may have increased mortality in seedlings earlier weakened by a "damping off" disease. The disease, apparently caused by a species of *Pythium*, attacks the roots of newly germinated seedlings. The damping off disease was confined to portions of two orchards that had been planted to tobacco in 1994 and 1995. It may have been associated with very high levels of phosphorous in those soils.

Symptoms of the damping off disease were noticeable by early June as a mild chlorosis and lack of enlargement of new leaves, although mortality was not evident until July. Treatment with the fungicide Ridomil reversed symptom development in less severely affected plants.

The disease possibly was promoted by the warm, wet spring. In the two previous and cooler seasons, chestnut seedlings in the same fields were not similarly affected. This is typical of damping off diseases.

We are taking steps to reduce high phosphorous concentrations in other areas of the Price Research Farm. Soil mapping indicated that the high phosphorous levels were confined to the top 6 inches of the soil, so we plowed new fields in 1999 to a depth of 12 inches, trying to turn the soil over, to decrease the phosphorous concentration. We also applied nitrogen as urea (rather than ammonium nitrate, which can increase phosphorous availability), and, of course, did not apply any phosphorous fertilizer. We will treat with Ridomil if symptoms reappear this spring.

Our current holdings are indicated in Table 1, and Table 2 indicates the changes from last year. Despite the difficulties we experienced, there was a net increase last year of over 1,100 new trees growing at the farms.

#### **1998 HARVEST**

We had a good harvest in 1998. In general, the rates of pollen contamination were acceptable, with only a few crosses needing to be remade again this year. The total harvest was down from the previous two years, primarily because we did not make a large number of first hybrids between Chinese and American chestnut. We also lost some trees with numerous bags to high winds, and lost some bags to the winds. Finally, the number of American chestnut trees available for bagging is declining due to blight. Table 3 summarizes the harvest results.

Among the noteworthy crosses, first and foremost are the BC<sub>3</sub>s. In two more years we will have completed advancing most of the lines of 'Clapper' and 'Graves' BC<sub>2</sub>s to third backcross. In 2001, we will begin intercrossing selected BC<sub>3</sub>s to produce the BC<sub>3</sub>-F<sub>2</sub> mother trees that will produce nuts for reforestation, beginning in 2005 or 2006. Then the long wait will begin to see whether they grow like the American chestnut of old!

Other noteworthy crosses include some  $BC_2s$  from which we hope to select trees with only one gene of the two or more which confer resistance. Intercrosses of those  $BC_2s$  should yield nuts that will help us map the genes for blight resistance with high precision. We hope to accomplish that by 2006 or 2007. The mapping may facilitate selection of  $BC_3$ - $F_2$  progeny homozygous for blight resistance, and may help us find additional sources of blight resistance that might help us increase the durability of blight resistance.

#### **BLIGHT RESISTANCE**

Like last year, most  $F_2$  and  $BC_1$ - $F_2$  plants which showed high levels of blight resistance in 1993, when we began inoculating with the blight fungus, continue to fare well; the resistance is holding up. Some are beginning to grow into impressive-looking trees. In 1999, we will perform our first resistance test of  $BC_2$ - $F_2$  trees. These are progeny from open pollination of second backcross trees derived from the 'Clapper' first backcross. The second backcross parents were screened for blight resistance in 1994 and susceptible ones removed from the orchard. The  $BC_1$ - $F_2$  nuts were harvested in 1995 and planted in 1996. We hope to recover some highly blight-resistant trees from among those progenies.

# TABLE 1

Type and Number of Chestnut Trees and Planted Nuts at TACF's Meadowview Research Farms in April 1999, with the Number of Sources of Blight Resistance and the Number of American Chestnut Lines in the Breeding Stock

	Number of		
	Nuts or Trees	Sources of Resistance	American Lines*
Type of Tree			
American	1388		82
Chinese	493	33	
Chinese x American: F <sub>1</sub>	457	18	55
American x (Chinese x American): BC <sub>1</sub>	1010	11	41
American x [American x (Chinese x American)]: BC <sub>2</sub>	2753	8	60
American x {American x [American x (Chinese x American)]}: BC <sub>3</sub>	3273	2	73
(Chinese x American) x (Chinese x American): F <sub>2</sub>	310	3	4
[Ch x Am) x (Ch x Am)] x [Ch x Am) x (Ch x Am)]:F <sub>3</sub>	9	1	2
[Amer x (Chin x Amer)] x [Amer x (Chin x Amer)]: BC <sub>1</sub> -F <sub>2</sub>	464	2	2
{Am x [Am x (Ch x Am)]} x {Am x [Am x (Ch x Am)]}:BC <sub>2</sub> -F <sub>2</sub>	628	2	4
Chinese x (Chinese x American): Chinese BC <sub>1</sub>	142		
Chinese x [American x (Chinese x American)]	41		
Japanese	3	2	
American x Japanese: F <sub>1</sub>	1	1	1
(American x Japanese) x American: BC <sub>1</sub>	38	2	2
Castanea seguinii	48	1	
Chinese x Castanea pumila: F <sub>1</sub>	9		
Large, Surviving American x American: F <sub>1</sub>	342	10	11
(Large, Surviving American x American) x American: BC <sub>1</sub>	198	2	7
Large, Surviving American x Large, Surviving American: $I_1$	93	4	4
Large, Surviving x American: $F_2 = F_1 x F_1$ , same LS parent	198	2	2
Other	34		

#### Total

11,932

\* The number of lines varied depending on the source of resistance. We will have to make additional crosses in some lines to achieve the desired number of 75 progeny per generation within a line. In keeping with past practice, the number of lines for each source of resistance are added separately; thus, progeny from two sources of resistance with the same American parents would be counted as two lines rather than one line (this only occurs rarely).

# TABLE 2

Changes between 1998 and 1999 in the Number of Chestnut Trees and Planted Nuts of Different Types at TACF's Meadowview Research Farms, Including Changes in the Number of Sources of Blight Resistance and the Number of American Chestnut Lines in the Breeding Stock

	Increase or	Decrease* in	Number of
	Nuts or Trees	Sources of Resistance	American Lines
Type of Tree			
American	263		29
Chinese	99	3	
Chinese x American: F <sub>1</sub>	-51	-2	-2
American x (Chinese x American): BC <sub>1</sub>	214	0	4
American x [American x (Chinese x American)]: BC <sub>2</sub>	195	-3	5
American x {American x [American x (Chinese x American)]}: BC <sub>3</sub>	272	0	12
(Chinese x American) x (Chinese x American): F <sub>2</sub>	-1	0	0
[Ch x Am) x (Ch x Am)] x [Ch x Am) x (Ch x Am)]:F <sub>3</sub>	0	0	1
[Amer x (Chin x Amer)] x [Amer x (Chin x Amer)]: BC1-F2	0	0	0
{Am x [Am x (Ch x Am)]} x {Am x [Am x (Ch x Am)]}:BC <sub>2</sub> -F <sub>2</sub>	152	1	3
Chinese x (Chinese x American): Chinese BC <sub>1</sub>	-3		
Chinese x [American x (Chinese x American)]	-3		
Japanese	0	0	
American x Japanese: F <sub>1</sub>	-5	-3	-3
(American x Japanese) x American: BC <sub>1</sub>	33	1	1
Castanea seguinii	0	0	
Chinese x Castanea pumila: F <sub>1</sub>	1		
Large, Surviving American x American: F <sub>1</sub>	68	1	1
(Large, Surviving American x American) x American: $BC_1$	-73	0	0
Large, Surviving American x Large, Surviving American: ${\rm I}_{\rm 1}$	-204	-2	-2
Large, Surviving x American: $F_2 = F_1 x F_1$ , same LS parent	198	2	2
Irradiated American	-48	-3	-3
Other	7		

#### Total

1114

\* The decrease in F1 trees reflects lack of emergence of nuts planted in 1997. The increases in BC1, BC2 and BC3 trees are due to further breeding of those, minus lack of emergence and roguing of trees with inadequate levels of blight resistance. The decrease in Large, Surviving American chestnut BC1 trees reflects lack of emergence of planted nuts, while the decrease in Large, Surviving American chestnut I1 trees reflects reclassification of some of those as F2s. The Large, Surviving American F2 trees have the same tree as their source of blight resistance while the I1 trees have two separate Large, Surviving American chestnut trees as their sources of blight resistance. The decrease in irradiated American chestnut trees reflects roguing of those due to inadequate blight resistance.

# BLIGHT RESISTANCE — FACTORS FROM AMERICAN CHESTNUT

In 1995, we obtained convincing evidence that some American chestnut parents yield backcross trees with better blight resistance than occurs on average. As is illustrated in Figure 1, this is evident for the cross CC1 x 'Clapper', which had significantly smaller cankers than the two other 'Clapper' "families" (or offspring of two specific parents) in that test. There had been hints in previous years that some American parents yield progeny with improved resistance, but we generally lacked sufficiently large families to confirm it statistically or pollen contamination was a potential confounding factor. We don't know what it means but thought it interesting that the improved blight resistance of the CC1 x 'Clapper' family was much more evident when its members were screened for blight resistance at four years of age than at one or two years of age (Table 4).

We obtained additional evidence that some American parents yield backcross progeny with better than average blight resistance from molecular mapping of two first backcross families. The families' source of blight resistance was an  $F_1$  from the 'Nanking' variety of Chinese chestnut. In one family derived from the 'Musick' American chestnut, the marker most significantly associated with resistance came from the 'Musick' tree rather than the  $F_1$  parent. The marker was located on linkage group C. In both the 'Musick' family and the second family, from the 'Mill Creek H' tree, a second marker from American chestnut on linkage group A was significantly associated with blight resistance. The average blight resistance of members of the 'Musick' family was higher than that from members of the 'Mill Creek H' family.

Unlike progeny from the 'Mill Creek H' first backcross family and many progeny from crosses of the 'Clapper' tree with American chestnut, both the 'Musick' first backcross family and the CC1 x 'Clapper' family were noticeably lacking in progeny with premature formation of dead outer bark. It is unclear at present whether this phenomenon was related to the improved blight resistance of the 'Musick' and CC1 x 'Clapper' families.

#### LEAF EMERGENCE

We have now extended molecular mapping to first backcross progeny from the 'Mahogany' Chinese chestnut, to 'Mahogany' x American  $F_2s$ , and to first backcross 'Nanking' families. That mapping has revealed

# TABLE 3

# The American Chestnut Foundation 1998 Nut Harvest from Controlled Pollinations and Selected Open Pollinations.

Nut Female		Pollen	Pollinated		Unpollinated Checks			Number of American	
Туре		Parent	nuts	bags	burs	nuts	bags	burs	Chestnut Lines*
BC <sub>1</sub>	American	Nanking F <sub>1</sub>	103	197	316	3	16	33	4
BC <sub>1</sub>	Mahogany $F_1$	American	44	23	29	0	3	3	2
BC <sub>1</sub>	Nanking F <sub>1</sub>	American	105	55	109	1	3	8	3
BC <sub>2</sub>	American	Mahogany BC <sub>1</sub>	97	230	527	2	24	64	6
BC <sub>2</sub>	American	Nanking BC <sub>1</sub>	267	551	863	24	48	86	25
BC <sub>2</sub>	Mahogany BC <sub>1</sub>	American	24	26	42	0	5	8	2
BC <sub>2</sub>	Nanking BC <sub>1</sub>	American	499	260	723	17	18	47	7
BC <sub>2</sub>	OTR1T7 BC1	American	28	19	49	6	2	5	1
BC <sub>2</sub>	S.LotR1T10 BC <sub>1</sub>	American	13	50	101	0	4	4	1
BC <sub>2</sub>	Sp.LotR4T23 BC <sub>1</sub>	American	1	8	19	1	5	7	1
BC <sub>2</sub> -F <sub>2</sub>	Mahogany BC <sub>2</sub>	Mahogany BC <sub>2</sub>	155	85	451	0	9	64	3
BC <sub>2</sub> -F <sub>2</sub>	Clapper BC <sub>2</sub>	open pollinated	[3260]	ope	en pollina	ated		5	
BC <sub>3</sub>	American	Clapper BC <sub>2</sub>	470	517	1247	12	49	135	19
BC <sub>3</sub>	American	Mahogany BC <sub>2</sub>	204	225	433	0	21	29	14
BC <sub>3</sub>	Clapper BC <sub>2</sub>	American	245	180	431	2	17	45	8
BC <sub>3</sub>	Mahogany BC <sub>2</sub>	American	127	65	220	4	8	28	4
F <sub>1</sub>	Kuling	American	19	40	98	0	4	9	1
Irrad $F_1$	American	NCF 179	120	48	145	2	5	11	1
LSA $F_1$	American	Corrigan	19	63	52	0	8	14	3
$LSAF_1$	Desormeaux	American	11						1
LSA I <sub>1</sub>	Gault F <sub>1</sub>	Scientists' Cliff F <sub>1</sub>	77	37	110	0	2	8	1
$LSAI_1$	Scientists' Cliff $F_1$	Gault F <sub>1</sub>	42	22	39	0	2	5	1
Total Co	ontrolled Pollination	าร	2670	2701	6004	74	253	613	

\*The number of American lines for this table is restricted to the number of American chestnut trees that were direct parents, not grand parents, of progeny.

another trait, the time of leaf emergence in the spring. This trait mapped to linkage group L in both 'Mahogany' and 'Nanking' first backcross progenies.

The time of leaf emergence can be a critical trait with regard to the adaptation of a tree to a particular region of the county. If trees leaf out too early in the spring, the leaves can be killed by a late spring frost. This leads to excessive branching in the trees; they lose a columnar form. It also can kill flower primordia, greatly reducing nut set. And it taxes the trees' energy reserves. On the other hand, trees which leaf out too late in the spring can be shaded out by trees which emerge earlier, and they lose a portion of the growing season. We have been selecting for trees which do not leaf out too early in the spring. It may be helpful to investigate further the molecular basis of this trait.

#### MOLECULAR MAPPING OF BLIGHT RESISTANCE

In general there was very little correspondence between 'Nanking' and 'Mahogany' in the linkage groups associated with blight resistance. The American contribution to blight resistance in 'Nanking' may have obscured the location of Chinese-derived loci contributing to blight resistance, or, as we had hoped to find, 'Nanking' may have a different set of resistance genes than 'Mahogany.'

The mapping of blight resistance in the 'Mahogany'  $F_2$  and  $B_1$  families corresponded quite closely except there was no recombination in linkage group G in the  $B_{1,}$ , so any resistance that may have come from that linkage group was impossible to map. Furthermore, linkage groups B and E from the  $F_2$  were merged in the  $B_1$ . They may have been separated in the  $F_2$  because of segregation distortion where they joined. The markers at that point did not show the expected frequencies of American and Chinese types, so they were dropped from the analysis.

The list of volunteers who helped with bagging and pollination this year is long. We would like to thank Dave Armstrong, Bob BaRoss, Steve and Sue Baum, Jessica Hawk, Danny Honnaker, Carolyn Keiffer, Bill Lord, John Lund, Bernie Monahan, Harry Norford, Larry Patchell, Dan Schilling, Lou Silveri, Ron and Mary Stanley, Bob Summersgill, Welles Thurber, Gene Witmeyer, and Phyllis Yoder. Sam Fisher from the 4-H Center again supplied a corps of Elder Hostelers, who proved once more that even people our



age and older can make a difference! We would not have brought in this harvest without the help of these volunteers. Thank you.

If you would be interested in helping pollinate this year, you could apply for the Elder Hostel program, which will be held the week of June 14 (call 617 426-8055 or write 75 Federal St., Boston MA 02110), or, if you prefer coming down on your own, plan on the weeks of June 7 or June 21. (Call to confirm the dates around June 1 at 540 944-4631).

There also apparently is some confusion as to just when people are welcome at our Research Farms, and the answer is, any time! You don't have to come down during pollination season, and you don't have to work. Come on down and we'll be glad to show your around. It is not necessary to call first, but doing so can be helpful.

### TABLE 4

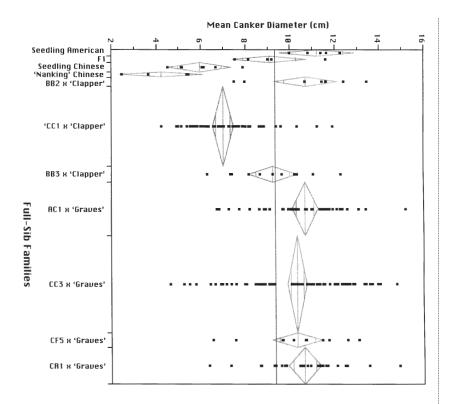
Mean Canker Sizes for Three BC<sub>2</sub> Families, and Control Trees, For Inoculations Made When the Trees Were 1, 2 and 4 years old<sup>1</sup>

Cross	Mean Canker Size <sup>2</sup>				
	1 year old	2 year old	3 year old		
'Mahogany' (1 & 2-yr-old) or 'Nanking' (4-yr-old) Chinese	0.95 (1)	5.0 (2)	4.3 (5)		
Seedling Chinese	1.9 (6)	4.6 (7)	6.0 (7)		
CC1 American x 'Clapper' <sup>3</sup>	4.6 (39)	9.1 (90)	7.0 (60)		
'Meiling' Chinese x American	2.8 (3)	6.7 (6)	9.1 (5)		
CC3 American x 'Graves' <sup>3</sup>	5.1 (46)	10.0 (4)	10.3 (73)		
AC1 American x 'Graves' <sup>3</sup>	5.0 (22)	10.7 (23)	10.7 (40)		
Seedling American	7.2 (6)	11.3 (7)	11.2 (5)		

<sup>1</sup>The nuts from the three BC<sub>2</sub> families were planted in 1994 in separate orchards. All inoculations were made in June of various years. The inoculations in the orchard with 1-year-old trees were made in 1995 and cankers measured in September. The inoculations in the orchard with 2-year-old trees were made in 1996 and cankers measured in January of 1997, while the inoculations in the orchard with 4-year-old trees were made in 1998 and cankers measured in December.

<sup>2</sup>Canker size in centimeters. Numbers are the mean diameter ([length + width]/2) of either two cankers per tree, for 1 and 2-year-old trees, or four cankers per tree, for 4-year-old trees. The number of trees in a cross are in parentheses. The cankers on the cross CC1 American x 'Clapper' were significantly (p<.05) smaller than those on CC3 American x 'Graves' or AC1 American x 'Graves' in 4-year-old trees, but not in younger trees.

<sup>3</sup> 'Graves' and 'Clapper' are BC<sub>1</sub> trees, ([Chinese x American] x American).



**Figure 1.** Mean canker diameter for various second backcross families, with pure American and Chinese chestnut controls, and  $F_1$  controls. Mean canker diameter is an index of blight resistance: the smaller the canker, the more blight resistant the tree. This figure illustrates the higher level of blight resistance found in the CC1 x 'Clapper' family than in other second backcross families derived from the 'Clapper' and 'Graves' tree. However, there were some trees with acceptable levels of blight resistance in those families. These trees were 4 years old when inoculated in June, 1998. The cankers were measured in November, 1998. Each dot is the mean diameter (length plus width divided by 2) of four cankers on one tree. The tops and bottoms of the diamonds are 95% confidence ranges for the mean value for a family, which is a horizontal line running through the center of the diamond. For second backcrosses, each group of dots with diamond is from a separate full-sib family. Blank Page 16





# EXCERPTS FROM THE TRAVELS OF WILLIAM BARTRAM

The Bartram name is nearly synonymous with New World botany. John Bartram (1699-1777) founded the first botanical garden in North America, on the banks of the Schuylkill River in Philadelphia. His son William (1739-1823), a gifted naturalist, traveled widely throughout the colonies, collecting specimens for patrons in England. William Bartram's record of his four years in the South, later published as **Travels through North and South Carolina, Georgia, East and West Florida**, is the earliest and most detailed natural history of what thereafter became the southeastern United States. It is also one of the earliest and most detailed records of the occurrence of American chestnut (known as <u>Fagus castania</u> to Bartram) in the southern Appalachians. The following excerpts from **The Travels of William Bartram**, edited by Francis Harper and published in 1958 by Yale University Press, are reprinted with permission and in their original form. (Copyright 1958, Yale University Press)

Sometime in late June or early July of 1773, Bartram and his party camped on one of the headwaters of the Broad River, probably in Madison County northeast of today's Athens, Georgia.

This branch of the Broad River is about twelve yards wide, and has two, three, and four feet depth of water, and winds through a fertile vale, almost overshadowed on one side by a ridge of high hills, well timbered with Oak, Hiccory, Liriondendron [tulip tree], Magnolia acuminata [cucumber magnolia], Pavia sylvatica [buckeye], and on their rocky summits, Fagus castania, Rhododendron ferruginium, Kalmia latifolia [mountain laurel], Cornus florida [dogwood], &c. ...After leaving Broad River, the land rises very sensibly, and the country being mountainous, our progress became daily more difficult and slow; yet the varied scenes of pyramidal hills, high forests, rich vales, serpentine rivers, and cataracts, fully compensated for our difficulties and delays.

In May 1775, Bartram passed through Abbeville County, South Carolina, heading northwesterly.

The road this day led me over an uneven country, its surface undulated by ridges or chains of hills, sometimes rough with rocks and stones,

yet generally productive of forests, with a variety of vegetables of inferior growth, i.e. Quercus, various species, Juglans hickory, varieties, Liriodendron, Fraxinus [ash], Fagus sylvatica [beech], Fagus castania, Fagus pumila, s. Chinkapin, Nyssa sylvatica [black gum], Acer rubrum [red maple], Aesculus sylvatica [Carolina buckeye], Magnolia acuminata, Magnolia tripetala [umbrella magnolia], Andromeda arborea [sourwood], Hopea tinctoria [horse sugar], Aesculus pavia [a red form of Carolina buckeye], Vibernum, Azalea flammea and other species; Hydrangea, Calycanthus [sweet shrub], &c.

In late May 1775, Bartram arrived at a Cherokee settlement in what is now Pickens County, Georgia.

The town of Cowe consists of about one hundred dwellings, near the banks of the Tanase, on both sides of the river.

The Cherokees construct their habitations on a different plan from the Creeks, that is but one oblong four square building, of one story high; the materials con-

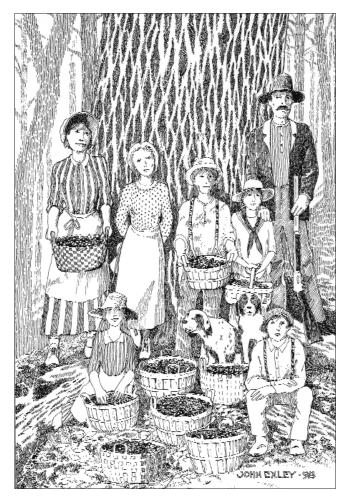
sisting of logs or trunks of trees, stripped of their bark, notched at their ends, fixed one upon another, and afterwards plaistered well, both inside and out, with clay well tempered with dry grass, and the whole covered or roofed with the bark of the Chesnut tree or long broad shingles... each house or habitation has besides a little conical house, covered with dirt, which is called the winter or hot-house; this stands a few yards distance from the mansion-house, opposite the front door.

Mander Millin's Book. Riche Dec. g. 1816. TRAVELS	
THROUGH	
NORTH & SOUTH CAROLINA,	
GEORGIA,	
EAST & WEST FLORIDA,	
THE CHEROKEE COUNTRY, THE EXTENSIVE	
THE MUSCOCULOR	
OR CREEK CONFEDERACY AND THE	
COUNTRY OF THE CHACTAWS	
CONTAINING	
AN ACCOUNT OF THE SOIL AND NATURAL	
TRODUCTIONS OF THOSE RECTORE MORE	
THER WITH OBSERVATIONS ON THE	
MANNERS OF THE INDIANS.	
EMBELLISHED WITH COPPER-PLATES.	
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# CHESTNUTTING

A rtist John Exley, who has produced several original illustrations for The American Chestnut Foundation, was imagining the following when he drew his illustration of an Appalachian family of a hundred years ago.

"Having driven their wagon up the hollow as far as the mountain slopes will let them, they climb with their baskets to gather up this year's harvest of chestnuts to sell to buyers along the railroads for shipment to the



cities. School has started: winter is coming; new shoes, a coat, a supply of coal oil will be needed, so every spare hour until the 'can't see' light of dusk is spent gathering the bounty of the forest floors before the other foragers of the hills have swept them clean. The father carries his rifle, never knowing who else might be in the groves with them. The family has a garden, a good stand of field corn, a couple of cows, but they also have hundreds of acres of chestnuts to gather. And now, for a moment before they start home again, they strike a period pose for our imagination, not knowing that within a few short years their family adventures into the hills will cease, their groves will become logs on a mountain railway, and their world an entire culture for a thousand miles — will disappear."

The October 1915 issue of American Forestry, since renamed American Forests, focused on the American chestnut - on the important role it played in the economy of that time, and on the unhappy fate observers were predicting for it. The following excerpt is reprinted with permission and, except as noted, as it was originally published.

# COMMERCIAL USES OF CHESTNUT By P.L. Buttrick

... it is certainly fair to consider chestnut as one of the leading hard-woods of America.

It is most numerous and important in the Southern Appalachian Mountains, where in the State of North Carolina it forms 27 percent of the total stand, and is the most numerous tree in the forest, occasionally forming almost pure stands, although generally growing in mixture with other hardwoods such as oaks and tulip poplars. Conditions are much the same in eastern Tennessee and southwestern Virginia. In these States most of the stand is composed of virgin timber, but outside of the Appalachians, most of the chestnut is second growth and is apt to be composed of sprouts from old stumps, often several generations having grown up and been cut from the original seedling's stump.

#### CHARACTER AND USES OF THE WOOD

Chestnut is neither a very strong nor very hard wood, not nearly so strong or hard as oak, but it is very even grained and durable. It will outlast almost all the oaks and most other hardwoods, its durability being due to the high percentage of tannin which it contains. It is light in weight and easily worked and does not warp readily...

This lightness, freedom from warping, durability and reasonable strength, and the high percentage of valuable chemical substances which it contains, together with its great abundance have given chestnut a greater variety of uses than almost any other American hardwood. It touches almost every phase of our existence. It serves as a shade and ornamental tree on our parks and estates. Its wood is used in the building and decoration of our houses and the manufacture of our furniture. We sit down in chairs made of chestnut and transact our business at desks, ostensibly



of oak, but generally of chestnut veneered with oak, we receive messages from the distance over wires strung on chestnut poles. We sit on a railroad train and read newspapers into whose composition chestnut pulp has gone, while our train travels over rails supported on chestnut ties and over trestles built of chestnut piles, along a track whose right-of-way is fenced by wire supported on chestnut posts. On the same train travel goods shipped in boxes and barrels made of chestnut boards and staves. Even the leather for our shoes is tanned in an extract made from chestnut wood. In the Fall we munch hot roasted chestnuts and many housewives feel that they are a necessary part of dressings of various kinds. At last when the tree can serve us no longer in any other way it forms the basic wood onto which oak and other woods are veneered to make our coffins.

### **ITS EARLY HISTORY**

The early settlers encountered chestnut pretty well up and down the eastern coast of the United States, and when food was scarce, if we are to believe our school histories, they were glad to make use of its succulent nuts as a serious part of their diet, even as did the Indians... [W]hen the local pine was exhausted... oak and chestnut then began to be used and many Revolutionary and early nineteenth century houses were built of hewn oak and chestnut frames, oak floors, and chestnut sidings and shingles... Country houses and barns are even yet frequently framed of local hardwood timbers, and one does not have to go back many years to find barns built of heavy hand-hewn chestnut beams put together with wooden pins...In the Appalachian Mountains, even as far north as Pennsylvania, to this day log cabins are built of chestnut logs, sometimes in the round, sometimes hewn square.

The earliest use of chestnut still remains one of its important ones, for chestnut has been a fencing wood since Colonial times. Few woods split lengthwise easier and straighter than chestnut, or are lighter or more durable. Fence rails made of it will last a lifetime. The early settlers built their fences of chestnut rails, piling them in the familiar snake or zigzag fashion... Later in the North a form of fence came into use in which the rails were mortised into the posts set in the ground... Posts of larger size are frequently used for the foundations of shore cottages and other buildings which are built without cellars...

The advent of the telegraph and telephone created a demand for large poles. At first, apparently, many woods were used indiscriminately, but for a long time the value of chestnut for this use as well as for trolley and electric light poles has been fully realized. Taking the country as a whole, cedar is the chief pole wood, but east of the Mississippi, where chestnut is available in large quantities, it outranks all other woods used for the purpose, and even taking the United States as a whole, 20 percent of the poles used are chestnut...

The early settlers soon learned that chestnut did not make very desirable firewood, and their descendants have not forgotten the fact. Dry chestnut burns easily and quickly, but it snaps and crackles, throwing out sparks profusely. This makes it undesirable for the fireplace. Yet it is one of the best of hardwoods for kindling, since it splits easily and ignites quickly... Sometimes chestnut is used in rural districts, where the gas range is not, as "summer wood." The fact that it ignites and burns quickly renders it desirable when a light, quick fire is wanted.



### CHESTNUT WOOD AS A SOURCE OF TANNIC ACID

... by far the largest use is as a source of tannic acid; for chestnut wood is rich in that substance. Its tannin content averages more than 8 per cent, occasionally running as high as 12 per cent... Over two-thirds of all the tannic acid produced in the United States is now extracted from chestnut wood and bark.

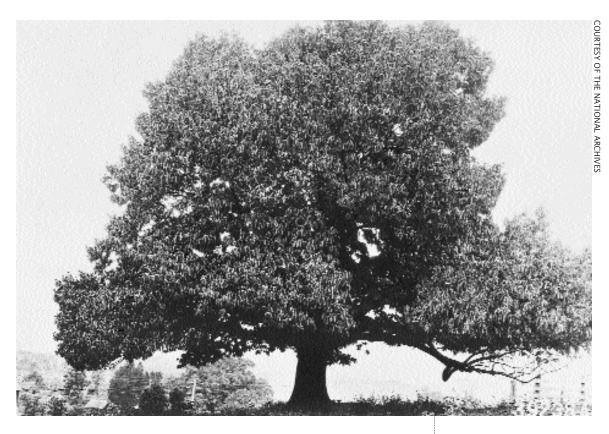
It is used in the manufacture of leather and the dyeing of silk...

The industry of extracting tannic acid from chestnut wood is largely confined to the South... The industry centers in southwestern Virginia, western North Carolina and eastern Tennessee, where some twenty plants, with a combined product of perhaps 1,000 barrels of extract per day, are at work. The process is very simple. The cordwood is ground up into small chips, placed in tanks and leached out by hot water. The product is then evaporated to dryness or the required degree of concentration. Although cordwood is generally used, sawmill waste is sometimes employed... About 100,000 long cords of chestnut wood per annum are used in this industry in North Carolina alone, while Tennessee uses about half that amount, and the total production is reported to be about 250,000 standard cords per annum.

A few paper pulp plants in the South use chestnut in the manufacture of their product. The plant which uses probably the largest amount of this wood for the purpose has or had a contract to supply the Government with the paper for its postal cards...

#### **CHESTNUT LUMBER**

Chestnut lumber is used for house construction, both interior and exterior... Yet in spite of its many uses for building and construction it is much more in demand for house furnishing than house building. It is, in fact, one of our leading furniture woods, quite probably surpassing any one of the oaks in volume used, yet, with the exception of panels in wooden bedsteads, kitchen furniture and the less expensive chairs, bureaus, and tables, we see little furniture finished in chestnut. Its great use comes as core stock for veneers... Tables, desks, bureaus, cabinets, and the like are often made of chestnut and covered with thin veneers of oak, maple, cherry, walnut or expensive tropical woods such as mahogany or rosewood. The essential wood of pianos is frequently chestnut.



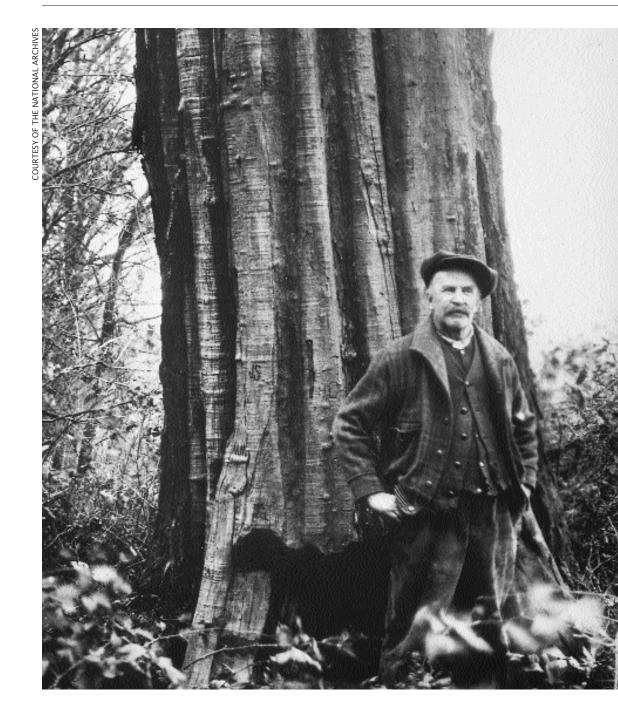
There are two reasons for the popularity of chestnut in the furniture industry. First, it is abundant, light, holds its shape well, does not warp, is not affected by moisture, and can be obtained in wide widths... Second, its open, porous structure, combined with freedom from knots, pitch or blemishes, and the frequent presence of numerous small holes... caused by a boring insect, known as the chestnut timber worm, enable the glue which binds the core to the veneer to take a good grip...

Coffins are hardly to be classified as furniture, yet they are made of much the same woods, and the process of manufacture is quite similar to that of many more cheerful articles. The coffin manufacturers probably use more chestnut than any other wood. It is used solid in coffins... of the less expensive grades, and as a backing for veneered coffins of higher price.

... we have failed to mention chestnut as a shingle wood. Most of our shingles are of soft wood... Chestnut is seventh on the list, but it is the lead-



# memories



ing hardwood. In 1909, 91,760,000 chestnut shingles were manufactured... The above figure presumably does not include the large number of hand-made chestnut shingles made in the Southern Appalachians... Chestnut shingles are very durable and weather to an attractive shade... It is... hard to get much chestnut shingle stock free from the worm holes previously mentioned, and these cause them to leak. It seems as though it ought to be possible to work up a market for chestnut shingles to be used as side shingles on cottages and suburban residences. Their attractive gray color when weathered is as pleasing as white cedar, and the supply is unlimited.

### CHESTNUT AS A NUT TREE

Chestnuts whether roasted or in turkey dressing require no introduction. There are no statistics to show how many bushels of chestnuts are marketed every fall, but one has only to visit the produce houses in our large cities at the proper season - or, better still, the country stores and express offices in the small towns in the Appalachian Mountains - to realize that it is large indeed, for the bulk of the nuts on the market come from these mountains. With the first frost, the women and children seek the woods to collect the freshly fallen nuts, taking them to the country stores, where they are sold or exchanged for other commodities... Realizing the value of the nuts, some of the mountaineer farmers have selected suitable tracts of chestnut growth... and thinned out the trees so as to develop specimens with large crowns, which results in increased nut production. These places are locally called chestnut orchards...

#### CHESTNUT IN THE FUTURE

Aside from its value for all sorts of uses, chestnut was long regarded as a valuable woodlot tree... But its popularity was short lived, for today, notwithstanding all its good points, it is no longer upon the forester's list of desirable trees, and far from encouraging it, he is advocating its removal from the woodlot as speedily as possible. Enemies now attack this tree on every side, and it is very poor forestry to favor a tree against which nature has so definitely set her hand...

One of these enemies has risen with almost drastic suddenness. Less than fifteen years ago the chestnut blight was unknown to the scientist or the woodsman... At present it is found from Maine to North Carolina, and it is thought that it will all but exterminate the chestnut in the



Northern States... and may invade the South with like disastrous results... So the forester is recommending the removal of all chestnut of commercial value in the region of blight infestation in order that it may be marketed before it is destroyed... Thirty years or less at the present rate of cutting will exhaust the supply of virgin chestnut timber in the Southern Appalachians... If the blight and the other agents of destruction continue their devastation, it looks as though within our lifetime the chestnut will have to be added to that melancholy list of American plants and animals, like the buffalo... of which we say "formerly common, now rare."





# A COOKE COUNTY, TENNESSEE CHESTNUT RECOLLECTION

ember Jo Neuswanger's mother Grace Blanchard has lived in the same house her father built in an eastern Tennessee hollow all her 81 years. When she was a child, her family (there were seven girls) grew their own food, made their own clothes, butchered their own animals, and took the corn to the mill over the next ridge to have it ground into meal. Running water, electricity, and phone service didn't arrive until the 1950s.

Jo says the tiny mountain community has changed enormously since her mother's childhood. No one in the hollow makes a living farming anymore. Some people have jobs in factories; others drive to Gatlinburg or Pigeon Forge to work in the restaurants and motels that serve visitors to Great Smoky Mountain National Park. Kids no longer walk to a one-room school house but are bused to a consolidated school several miles away. And there are no large chestnuts in the surrounding woods. Grace Blanchard told Jo her own chestnut story:

"Do I remember the chestnut trees? My goodness, yes. We had two big chestnuts, one on the west side of the house, one straight across on the east side. It was a sure sign of fall when we gathered the chestnuts. Besides just that the nuts were ready, it meant it was time to put on shoes again. All of us young 'uns went barefoot in the summer, of course. Have you ever seen a chestnut hull? If you have, I guess you know why you wouldn't go barefoot around a chestnut tree. Those hulls are about the spiniest thing I've seen. Anyway, those trees really produced. We gathered chestnuts by the gallon. The burs would open when they were ripe, and then we would step on them with our heels to open them up more and get the nuts out.

I hear some folks talk about roasting chestnuts, but we never roasted them; we boiled them. Some of them had worms - a tiny white worm; you could spot the little hole in the nut and tell which ones had worms. You could get the worms out, though. Some people said the ones that had the worms were actually better to eat - sweeter. I don't know about that. The flowers sure were pretty too. Sort of silvery white, long fronds that hung down. One thing the chestnut tree wasn't good for was burning in the fireplace. It would pop a lot and send burning sparks out into the room.

The chestnuts were the biggest trees we had on this place, so that made them good to put swings in. We always had our swings in the chestnut trees."

Dr. Donald Davis is a professor of sociology at Dalton State College in Dalton, Georgia with a special interest in social and historical ecology. His Ecophilosophy: A Field Guide to the Literature came out in 1989. His most recent book, Where There Are Mountains: An Environmental History of the Southern Appalachians, published by the University of Georgia Press, will be released in late summer 1999. The following excerpt is reprinted with permission. (We've omitted the author's footnotes.)

# FROM "A WHOLE WORLD DYING" by Donald Davis

A simportant as commercial logging and dam construction was to environmental and cultural change in the southern Appalachian region, few single events in North American environmental history compare with the loss of this unique tree species. Chestnut trees once comprised rough-

ly twenty-five percent of the entire forest in the mountains although in specific areas they accounted for as much as one-third of all trees. William Ashe reported seeing locales where the trees "occur pure or nearly pure over areas as large as 100 acres." In 1901, he and Horace Ayers estimated that their southern Appalachian study area contained more than 884,000 acres of chestnut timber. The tree was largely confined to the Blue Ridge Mountains and Cumberland Plateau where they commonly grew at altitudes between 1,000 and 4,000 feet. The Ridge and Valley province had a few important stands of chestnuts as well, but these were found only on the



slopes of the highest ridges where richer soils and heavier rainfall predominated. A reconstruction of nineteenth century forests in northwest Georgia found chestnut trees comprising no more than six percent of the area, with hickories, the most dominant tree species, making up 10 percent of the total forest. William MacDonald, professor of plant pathology at West Virginia University and a leading expert on the tree, estimates that chestnut-dominated forests once covered more than 200 million acres of land from Maine to Georgia.

Dead chestnut trees in Shenandoah National Park, Virginia. Courtesy Shenandoah National Park Archives. Gathering and removing dead

chestnut stems and stumps in

Shenandoah National Park, Virginia. Courtesy Shenandoah

National Park Archives.



an exotic blight introduced in the United States from Japanese chestnut nursery stock [around] the turn of the century. A forester at the New York Zoological Park first reported the disease in 1904, after observing an immense number of dead and dying chestnut trees on park lands under his supervision. Five years later, the first scientific bulletin appeared about the disease, a fungus later named *Endothia parasitica* [and now known as *Cryphonectria parasitica*]. Only a year after the bulletin's publication, an editorial in **Southern Lumberman** referred to

The death of the American chestnut was due to

a "mysterious blight" that had recently been observed in Pennsylvania and New York. "Large timbered sections of [Pennsylvania] are already and in an alarming manner affected by the disease," stated the report. By 1912, all the chestnut trees in New York City were dead and the chestnut blight had reached no fewer than 10 states. Scientists in Pennsylvania launched a vigorous control program,



which included burning dead trees, monitoring its advance, and spraying infected trees. This effort, a scientist later commented, was a little like using toy swords to battle an enemy equipped with atomic bombs. Yet foresters told the public that "the control and ultimate extermination of [the chestnut blight]...will sooner or later become a real accomplishment."

The disease spread relentlessly southward, at an astounding rate of some fifty miles per year. Aided by woodsmen who carried it on their shoes and axes, the blight first entered North Carolina near Stokes and Surry counties

Chestnut logs were drilled for drinking fountains in Shenandoah National Park, Virginia. Courtesy Shenandoah National Park Archives. about 1913. Shady Valley, in upper east Tennessee, was hit by 1915. By 1920 the American chestnut in the Great Smokies was ultimately doomed, though there were few visible signs of the blight there before 1925. North Carolina lumbermen even used the imminently encroaching disease as a last-ditch effort to defeat the proposed Great Smoky Mountains National Park. "Certainly nothing could be more unsightly than the gaunt and naked trunks of these dead trees, standing like skeletons in every vista in which the eye turns," they wrote. By the 1930s, the blight had reached much of north Georgia, and by 1940 there was scarce-

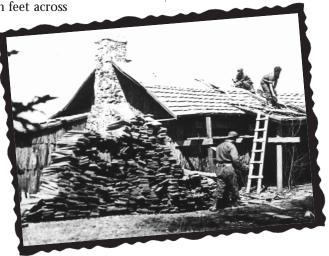


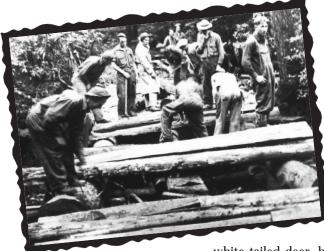
ly a tree in the entire region that was not dead or showed sign of being infected with the disease.

Although few people alive today remember what the southern Appalachian forests looked like before the blight devastated the region, those who did provide indisputable testimony to their significance to the mountain environment. "This is an unbelievable thing: how many chestnuts there were," remembered Paul Woody, who grew up near Cataloochee, North Carolina. Gifford Pinchot himself recalled seeing chestnut stands with individual trees thirteen feet across Removing dead chestnut trees to reduce the threat of fire in Shenandoah National Park, Virginia. Courtesy Shenandoah National Park Archives.

and with crowns spreading more than 120 feet above the forest floor. Charles Grossman, one of the first rangers at the new Great Smoky Mountains National Park, recorded a chestnut tree 9 feet, 8 inches in diameter at a point six feet off the ground. "The hollow portion is so large that [an adult] could stand up in it," wrote Grossman after discovering it. "This hollow

Civilian Conservation Corps workers roof a cabin with chestnut shingles. Courtesy Shenandoah National Park Archives.





Civilian Conservation Corps workers use chestnut logs to construct a bridge in Shenandoah National Park, Virginia. Courtesy Shenandoah National Park Archives. runs more than 50 feet up the trunk and at its narrowest point is not less than three feet. This must be the tree of which I heard. A man lost some stock during a snowstorm and later found them safe in a hollow chestnut tree."

Due to their abundance and enormous size, the American chestnut ranked as the most important wildlife plant of the eastern United States. The largest trees could produce ten bushels or more of nuts. Reports of chestnuts four inches deep upon the forest floor were not uncommon in the southern mountains. Many of the wildlife species that people thought of as game - squirrels, wild turkey,

white-tailed deer, bear, raccoon, and grouse - depended on these chestnuts as a major food source. "The worst thing that ever happened in this country was when the chestnut trees died," said Walter Cole of east Tennessee. "Turkeys disappeared, and the squirrels were not onetenth as many as there were before." Will Effler, who grew up on the West Fork of the Little River in what is today the Great Smoky Mountains, recalled shooting a wild turkey that contained no fewer than ninety-two chestnuts, "still in the hulls and undigested," in its swollen craw. Other non-game animals were equally dependent on the chestnut, including several unique insect species that relied upon chestnut trees as their principal food source...The loss of the chestnut also slowed the recovery of wildlife populations already suffering from loss of habitat by logging operations. Randolph-Macon College biologist James M. Hill ascribes the slow recovery of deer, wild turkey, goshawks, Cooper's hawks, cougar, and bobcat in the mountains to habitat destruction directly caused by the chestnut blight.

Of course, humans seasonally ate chestnuts too, making them an important dietary supplement when the trees dropped their nuts after the first major frost. Each October, children living in the mountains scooped up chestnuts by the sackful, hanging their cloth bags on nails outside the door until December when the nuts would begin to get "wormy." Cherokees made more use of the nut, which they frequently added to cornmeal dough that "was boiled or baked." Some families gathered bushels of chestnuts, taking them by wagon to urban markets. John McCaulley, whose family foraged for chestnuts in the Great Smoky Mountains around 1910, remembered seeing in one mountain cabin a "hundred bushels of chestnuts piled up there, and about four men packing off, every day." McCaulley himself recalls gathering as many as seven bushels of chestnuts in a single day's outing. These, he said, were taken to Knoxville on mules where they were sold for "four dollars a bushel." Chestnuts were

also routinely shipped by rail to major cities on the eastern seaboard. In 1911, West Virginia reported that one railroad station alone shipped 155,000 pounds of chestnuts to destinations along the train's northerly route.



A portable sawmill cuts chestnut logs in Depression-era Shenandoah National Park, Virginia. Courtesy Shenandoah National Park Archives. Blank Page 36

# science and natural history



# SCIENCE AND NATURAL HISTORY

Using DNA markers to distinguish among chestnut species and hybrids by Tom L. Kubisiak

Dr. Tom Kubisiak of the U.S. Forest Service's Southern Institute of Forest Genetics and Dr. Robert Bernatzky of the University of Massachusetts have been using molecular genetic markers to pinpoint hot spots of American chestnut genetic diversity. Using samples collected on a roughly 135-mile grid covering the entire range of the tree, the two researchers are analyzing chestnut DNA to assess overall levels of diversity and to map out the sources of the greatest genetic variation. This paper grew out of an analysis of a sample from a Maine chestnut conducted as part of that study.

Identification of American chestnut trees in the wild for inclusion in breeding programs is currently done using morphological traits. Distinguishing traits include leaf shape, stipule size, presence or absence of leaf and stem trichomes, and stem color. Application of these traits is reasonably clear if the trees are pure American chestnut, but identification of hybrids is very difficult. Hybrids that are primarily American chestnut may look like American chestnut. Such individuals can be frequently found in densely forested areas because of extensive plantings of species and hybrids in public and private woodlots. Since the breeding programs are designed to include as much native diversity of American chestnut as possible, positive genetic identification would be very helpful.

Along these lines, I recently received a bag of chestnut leaves and a letter from Mr. Robert P. BaRoss of Cape Elizabeth, Maine. In this letter, Mr. BaRoss explained that the chestnut leaves he sent were taken from a 70-foot chestnut tree on the Ram Island Farm in Cape Elizabeth. Mr. BaRoss stated that Maine members of The American Chestnut Foundation (TACF) were interested in using this tree in their breeding program, but the leaves and bark of the tree did not look typical of American chestnut. Therefore, there was some suspicion as to the tree's ancestry. Maine members hoped that DNA marker techniques would be able to determine whether this tree was a "pure" American chestnut that should be included in their breeding program. I promptly replied to Mr. BaRoss that it should be possible to determine whether the tree is of hybrid ori-

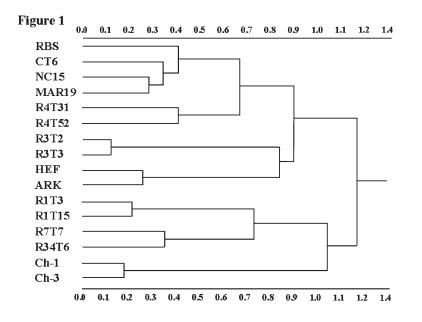


Figure 1. Unweighted pair group mean cluster dendrogram constructed from a distance matrix based on 97 random amplified polymorphic DNA markers collected on 16 chestnut samples. The samples included: one unknown chestnut tree from Cape Elizabeth, Maine (RBS); three American chestnut trees (CT6, NC15, and MAR19); two Chinese chestnut x American chestnut F1 hybrids (R4T31 and R4T52); two European chestnut trees (R3T2 and R3T3); two chinkapin trees (HEF and ARK); two Chinese chestnut trees (R1T3 and R1T15); two Japanese chestnut trees (R7T7 and R34T6); and two Henry chestnut trees (Ch-1 and Ch-3).

gin using DNA techniques, and that I would be happy to genotype the tree in light of its importance to the Maine program. The following is a description of the results of this study.

Leaf or dormant bud tissue from a total of 16 trees was collected and shipped to the USDA Forest Service, Southern Institute of Forest Genetics, in Saucier, Mississippi. The samples included: one unknown chestnut tree from Cape Elizabeth, Maine; three American chestnut trees (one from Connecticut, one from North Carolina, and one from West Virginia); two Chinese chestnut trees (R1T15 'Mahogany' PI#70315 and R7T3 'Nanking' from the South and Spring Lots, respectively, of the Sleeping Giant Chestnut Plantation in Hamden, Connecticut); two Japanese chestnut trees (R7T7 and R34T6 from the South and West Lots, respectively, of the Sleeping Giant Chestnut Plantation in Hamden, Connecticut); two European chestnut trees (R3T2 and R3T3 from the Humphrey Hill Lot of Lockwood Farms in Hamden, Connecticut); two Henry chestnut trees (both from Guang Xi, China); two chinkapin trees (one from Mississippi and one from Arkansas); and two Chinese chestnut x American chestnut  $F_1$  hybrid trees (R4T31 and R4T52 from the Spring Lot of the Sleeping Giant Chestnut Plantation in Hamden, Connecticut).

DNA was isolated from approximately two grams of leaf tissue or 0.25 grams of dormant bud tissue using a modification of the CTAB-based procedure outlined in Wagner et al. (1987). The polymerase chain reaction (PCR) technique was used to generate random amplified polymorphic DNA (RAPD) markers. Oligonucleotide 10-mer primers used in PCR were obtained from either Operon Technologies (Alameda, CA), or J. Hobbs (Univ. of British Columbia, Vancouver, B.C., Canada). RAPD amplification was based on the protocol reported by Williams et al. (1990). The completed reactions were electrophoresed in 2% agarose gels, stained with ethidium bromide, and photographed under UV light using Polaroid 667 film.

To identify informative RAPD markers, 24 primers were screened against DNA extracted from the 16 different chestnut samples. Those RAPD markers showing differences among the 16 samples were scored as potentially informative. Markers were subjectively chosen based on the intensity of amplification (only intensely amplified bands were scored) and the absence of co-migrating DNAs. RAPD fragments were identified by the manufacturer primer code corresponding to the primer responsible for their amplification, followed by a four-digit number indicating the approximate fragment size in base pairs. (A list of these loci is available upon request from the author.)

Chestnut samples were placed into groups or clusters using the unweighted pair-group mean method (UPGMA) available under the CLUSTER procedure in the statistical analysis software SAS (SAS Institute Inc., Cary, NC). A distance or dissimilarity matrix was constructed based on the RAPD fragment data. Chestnut samples were scored for the presence or absence of a band at each of the RAPD markers. The distance matrix was constructed by tallying the total number of marker differences found between pair-wise comparisons of samples. In other words, I simply counted up the number of markers at which two trees differed. For the 16 chestnut samples, a total of 136 pair-wise comparisons were made.

A total of 97 RAPD markers were identified and scored on the chestnut samples. UPGMA analysis suggested two primary groups; one including all the eastern or Asian chestnut species and another including the western or European and North American chestnut species (Figure 1). Within the eastern or Asian chestnut grouping, each species formed its own distinct cluster, with Henry chestnut being further separated from Chinese chestnut and Japanese chestnut. Within the western or European and North American grouping, each species formed its own distinct grouping. European chestnut and chinkapin were slightly more separated from American chestnut than were the Chinese chestnut x American chestnut  $F_1$  hybrids. Although the Chinese chestnut x American chestnut  $F_1$  hybrids clustered closely with the American chestnut trees, they did form their own distinct cluster. The unknown tree from Cape Elizabeth clustered very closely with the three American chestnut trees.

Using the RAPD marker data, it was possible to distinguish among the six chestnut species and Chinese chestnut x American chestnut  $F_1$ hybrids included in this study. The American chestnut trees appear to be quite different than all of the other chestnut species. The American chestnut grouping differed from the European chestnut grouping by an average of 26 markers, the chinkapin grouping by an average of 27 markers, and the Chinese chestnut, Japanese chestnut, and Henry chestnut groupings by an average of 40 markers each. Based on this analysis, the unknown tree from Cape Elizabeth appears to be of American chestnut ancestry as it clusters very closely with the other American chestnuts. It does not appear to be an  $F_1$  hybrid with any of the other chestnut species as it did not form its own distinct cluster, as did the Chinese chestnut x American chestnut  $F_1$  hybrids (Figure 1).

This study provides support to the hypothesis that RAPD markers can be used to quickly determine the genetic identity of putative American chestnut trees in the forest for inclusion in breeding programs. RAPD markers will be useful for quickly identifying "pure" species and first generation hybrids, but may not be useful for identifying more advanced generation hybrids unless large numbers of markers are assayed to provide the desired resolution. ACKNOWLEDGMENTS - The author would like to personally thank Glen Johnson and Kristel Davis for sharing their technical expertise in DNA extraction and the polymerase chain reaction.

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