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notes

Preserving Chestnut Memories

Are you 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.

FROM THE EDITOR

It is said that time flies when you are having fun, and I find that to be quite true as I write the notes for what will be my third publication of *The Journal of The American Chestnut Foundation*. Here in Bennington, the leaves are beginning to turn and a busy pollination season has come to an end. One can imagine, as the reds and yellows begin to creep across the landscape, what a splendid sight a stand of towering American chestnut must have been in autumn.

In his poem entitled *The Chestnut Tree*, Brandon Esten laments the loss of these autumn colors, and the last days of a blight-infected American chestnut. In his letter to me, Brandon wrote:

> (This) poem is dedicated to my aunt, Angela Hayes, of Vallejo, CA, who has been fighting cancer. The metaphor is fairly plain to understand. I learned about the American chestnut tree on your (website), and immediately recognized an opportunity to draw the parallel between the trees and my family. It is an emotional situation, but we are all given challenges in life to meet and find solutions to.

I hope that *The Chestnut Tree* touches your heart, as it did mine.

In the Spring 2006 issue of *The Journal of TACF*, I printed an "advertisement" for stories that featured the American chestnut. Since then, I have received numerous letters, phone calls, and e-mails from members who want to share their memories. These stories are heartwarming, often funny, always nostalgic, and those who share them are full of hope that TACF will ultimately restore the tree that featured so prominently in their childhoods.

In this issue, you will read about the boyhood adventures of Virginia member, Dan Stiles. Dan writes about a hunting expedition with his Uncle Alfred, whose home in the Berkshires of Massachusetts lay in the shadow of a mountain crowded with the skeletons of thousands of American chestnut trees. The skeletons of those trees have long since been reclaimed by the forest's floor, but Dan continues to relive the day when he first experienced "buck fever" among the fallen chestnut.

In the final article of our *Then to Now* section, Maryland member Robert Strasser reflects upon our relationship with nature, and the impact our surroundings can have on the formation of our characters. In his mountain wanderings, Robert comes upon the artifacts of a culture that has succumbed to modern times, and he recognizes the need to preserve what nature has given to us even as we embrace the realities of our ever-changing world.

Our *Science and Natural History* section contains the annual *Notes From Meadowview.* In addition to the inventory listings and the results of blight resistance screening, two new staff members are introduced, including Research Geneticist Dr. Bob Paris and Research Technician William White.

Also included is the second half of Dr. Mahn-Jo Kim's manuscript summarizing decades of chestnut breeding in Korea, as well as part two of Dr. Paul Sisco's *Update on Chestnut DNA Projects.*

Finally, Joe Schibig, Mark Vance, Sandra Cumming, Lloyd Fly, Clint Neel, and Jack Torkelson present their findings about the *Ecology of the American Chestnut and Allegheny Chinquapin on the Cumberland Plateau of Kentucky and Tennessee.*

Please keep those memories coming, and enjoy the gifts of autumn!

Jeanne

from then to now

This poem is dedicated to my aunt, Angela Hayes.

XII. THE CHESTNUT TREE

Brandon Esten, January 2006 (copyrighted)
Dedicated to Angela Hayes
Petrarchian sonnet

I rest my back against the chestnut tree
Reclining there, and idly count each leaf
So greet the tanager's respite so brief
While green withdraws to gold by slow degree.
This early autumn bodes my soul to flee
For all her leaves there's only disbelief
And perches, my heart suffers splendid grief
Because the blight has taken hold of thee!

May this be her last time to yield and change
The colors of her coat to yellows bright
And clumping on the ground her nuts arrange?
The forest's now aglow in rage and fright
Their deep and ruddy colors fall downrange
As all the other trees bemoan your plight!

CHESTNUT MEMORIES

Submitted by Dan Stiles of Fairfax, VA

I must have been 13 or 14 at the time, so the year would have been about 1947. My Uncle Alfred Nichols had given me a brand new lemonwood bow. At least my understanding was that it was lemonwood. I know for sure I loved that bow. It was the kind of longbow that required you to step between the bowstring and bow in order to nock the string on the upper end. It was tricky business because unequal pressure on either the upper or lower limbs would shatter the lemonwood. Re-curved bows were unknown then and compound bows with their laser sights, wheels, cables, mechanical releases, and all the rest of it were still many years away.

I shot reasonably straight wooden arrows. Archery was then a truly primitive sport; one bow, a bowstring, and a couple of arrows. It was called instinctive shooting as there were no sights for aiming. But, with practice, one could become quite proficient. I really could drive an arrow through an apple at 60 feet with surprising regularity. My favorite and only reference book was entitled, *Hunting the Hard Way* by Howard Hill.

My Uncle Alfred was truly a gentleman of the old school. He was an avid hunter, fisherman, and trapper. He always kept several hound dogs, and he had a closet full of shotguns and rifles. He "sighted in" his rifle and patterned his shotguns from his kitchen, resting the gun on the windowsill, aiming at a target outside (Aunt Betty did not approve of this one bit, you can be sure!). He was irascible and cantankerous, in the opinion of some, but he was a hero to me.

"Uncle" Alfred Nichols in 1914

We hunted, camped, and fished together for many of my formative years with never a disagreeable moment. I would later name my son after him.

Uncle Alfred's home was in the town of Williamsburg, Massachusetts, in the Berkshire Mountains and near the town of Goshen. Thus, local residents called this area the "Land of Goshen," meaning a land flowing with milk and honey (Genesis 45:10).

My uncle knew about American chestnut trees and the deadly chestnut blight. He had witnessed trees dying in his backyard and on the mountain above his home, where there were deer, cottontail rabbits, raccoons, beavers, snowshoe hares, squirrels, ruffed grouse, a pond full of trout, migrating mallards, and wood ducks. And there were thousands of American chestnut trees that had succumbed to the blight. Many were still standing but most had fallen into a crisscross network that a person could walk on for long distances without ever touching the ground. The bark had fallen off these dead trees and the wood was smooth and hard as a rock. The standing trees were pretty much without branches, but their main stems still stood straight and tall. What an awesome mountain this must have been with all these huge, amazingly straight trees a few decades prior to my visits!

We were deer hunting, and Uncle Alfred had a plan. I was to stand high on a network of fallen trees and watch on the downhill side. He would make a "drive" through several acres of the whitetails' traditional feeding and loafing areas to "spook" the deer up toward me where they would be seeking heavy cover and concealment in the fallen chestnuts. No more than 10 minutes after he left, a four-point buck approached. I recall it all as clearly as if it had happened yesterday. He was as alert as a whitetail buck can be. Vibrant is the word that says it best. He was also a tremendously beautiful animal, and he seemed to be drawn exactly to where my Uncle had said he would be--and destined to pass beneath me along a trail some 30 feet away. It was a bow hunter's dream come true, and I had the worst case of buck fever ever! It was difficult to breathe. My heart was pounding and my arms were shaking. I could not control my excitement. The deer stopped in the trail thirty feet away. I had never been this close to a deer before. My Uncle would be pleased to know his plan had worked almost beyond belief. It was enough. But the deer stood there. I could tell Uncle Alfred I shot an arrow and that would be even a better story. The deer stood like a statue. I nocked an arrow rather hoping the deer would move on, but he did not. I drew the arrow back, and the next thing I remember is that the arrow struck the ground just in front of his front feet. The deer casually nuzzled the arrow's feather fletching by bending his head down a trifle. He was not alarmed in the slightest. I nocked my last arrow, aimed and released in a kind of frustrated effort, but the arrow glanced off the trunk of a fallen tree. It ricocheted and thumped into a standing chestnut some 30 feet above and a few feet beyond the deer, at a downward 45 degree angle. Thankfully the deer bounded off when my Uncle approached. We talked and laughed about what had just happened and my terrible case of "buck fever." It was truly a great and memorable day.

I returned to that spot and relived the experience often. The arrow remained overhead for many years. I became a forester and wildlife biologist and was employed by the U.S. Fish and Wildlife Service for 35 years. Through these years I have maintained a fascination and admiration for American chestnut trees and have wholeheartedly supported those who are working to bring them back, straight and strong as they were in the Land of Goshen a century ago.

OF PLACE AND TIME

By Robert Strasser

Relationships with our surroundings can teach us many things. It is fascinating to reflect upon the ways in which the place where we live influences who we are as people. I live on Catoctin Mountain, and in the town of Thurmont a few miles east at the base of the mountain, people have a separate designation for "mountain folk." Those who make their lives here on the rocky, wooded elevations which rise above the rolling farmlands of the piedmont below are recognized as having a separate character which has specifically to do with their native terrain. While I am a relative newcomer compared to some of my neighbors whose family histories extend back in time for generations and even centuries, 12 years of residence on Catoctin Mountain have shaped my awareness of this place's unique history.

Little Catoctin Creek

The rugged terrain gives rise to a high degree of self reliance in the people who live here. Weather on the mountain is also influenced by altitude. Summers are cooler and in winter we often get snow and ice when it is only raining 1,000 feet below. A big ice storm in March 1993 brought down several large ash and locust trees behind my cabin, where I have gradually claimed a small garden from what a few years ago was a wooded hillside. As stumps and briars gave way to terraced beds, I realized that I was not the first one to have worked this patch of ground. A rough row of piled greenstone, covered in lichen and gone unnoticed under thickets of multiflora rose, indicated someone's past efforts to clear the rocky earth and make it useful: a plowable field, perhaps, or a backyard garden. I discovered old rusted fence wires and the iron pieces of a bridle. The newly tilled soil yielded a variety of other small artifacts: porcelain dishes, broken redware and salt glazed crockery; glass of jade green canning jars and medicine bottles; a rusty, cast iron coin bank in the form of an elephant; even a remnant of dry-rotted leather with the fine stitchery of an old-fashioned women's dress shoe. Who lived here and left these cast off reminders of their lives behind? What other clues were to be found about how they had lived?

From my walks through the hardwood forests I know that the slopes and hollows of Catoctin are dotted with hundreds of small terraces of black stained earth, the abandoned open-air hearths where for generations logs of oak, chestnut and other native trees were stacked and reduced to charcoal for the Catoctin iron furnace. Charcoal and timber production must have been an important source of income for many mountain people. Local lore also includes stories of moonshiners who utilized the secluded hollows for distilleries during the early 20th century. Nearby there is an old stone foundation of a one-room schoolhouse, and people who attended classes there as children still live in the area. In a modern world of globalization and human mobility it is an increasingly rare thing to find those who have lived and worked their entire lives in one place, but they exist locally and their knowledge and experience connect us to a past era and way of life.

My fascination with the past also has me wondering about Catoctin Mountain before the arrival of the first European colonists who cleared the primal forests. How large were the ancient trees, and what kinds of wildlife did they support that are now gone or uncommon? I have read of the abundant wildlife that attracted both Native and European alike to hunt on Catoctin. One rare reminder I have of past human habitation on the mountain is an inch-long, white quartz arrowhead. How many centuries ago did some Native American hunter craft this tool, and how long did it lay on the forest floor until a 20th century hand plucked it from among the pebbles exposed on a dirt foot path? How did the flora and fauna differ then, before the ecological changes caused by more than a century of regular clear cutting? In the early 20th century a once dominant tree species, American chestnut, succumbed almost completely to the devastation caused by a fungal blight accidentally introduced from overseas. During the ensuing years oak trees increased in abundance, filling the forest habitat left vacant by the lost chestnuts. The oaks in turn suffered severe mortality in the 1980s due to the destructive infestations of gypsy moths, another introduced alien species. Now I observe black

During the ensuing years oak trees increased in abundance, filling the forest habitat left vacant by the lost chestnuts.

Second growth forest reclaiming an old charcoal hearth.

birch, red maple and sassafras growing in thick patches underneath weathered skeletons of standing dead oaks and wonder what the future holds for these stressed but resilient forests. Despite all of the disturbances, the mountain forests continue to renew themselves with an ever changing complement of living things.

Spring is my favorite season for nature watching, when winter's austerity gives birth to an accelerating progression of returning life. The changes are measurable in a predictable sequence of returning migratory songbirds and in the pattern of emerging ephemeral wildflowers. An ecologist once explained to me that Catoctin Mountain has a much greater diversity of plant species than other ridges in the region, and therefore should be protected as a biological corridor linking with the forests to the north in Pennsylvania. This floral diversity results in part from a greater variety of soil types on the mountain, which in turn derive from the more varied rock formations which give rise to soil minerals. The Appalachians have been formed and weathered away four successive times over hundreds of millions of years. They are among the oldest mountains on Earth. The unique diversity of plant life on Catoctin is an important part of the legacy of these ancient mountains' complex geologic history.

Learning to recognize details in the world around me and relate them to the past has enriched the years of my life spent on Catoctin Mountain. Written on the landscape is a chronicle of ancient natural history. More recently it is the story of the people who have shaped and been shaped by the mountain. We cannot separate ourselves from nature, nor from the consequences of our actions upon the places in which we live. During our relatively short tenure we humans who have called the mountain home and utilized its natural resources for our own purposes may not have been fully mindful of the impacts of our actions. Change can come too fast. Some may call it progress, but as the disappearing gravel roads in my area give way to pavement and the inevitable development it ushers into these forests, I can't help but feel a sense of irreversible loss. The rustic Appalachian character of this distinct part of Frederick County's history is succumbing to the suburban sprawl which has already claimed so much nearby farmland. It is inevitable that this quiet corner of Frederick County will continue to come under development pressures. While we can never return to the more pristine natural conditions of centuries past, many opportunities do remain to preserve and restore what is left.

The Languages of Chestnut

栗子	Chinese
kastanje	Dutch
κάστανο	Greek
Kastanie	German
castagna	Italian
クリ	Japanese
밤	Korean
castanha	Portugese
Kawtah	Russian
castaña	Spanish
ᏘᎵ	Cherokee

science and natural history

MEADOWVIEW NOTES 2005–2006

Frederick V. Hebard
Staff Pathologist

Meadowview experienced low rainfall in the summer of 2005, especially during June. As usual during dry summers, temperatures were fairly hot. This weather pattern is associated with fast rates of canker expansion. Thanks to the irrigation system at our Glenn C. Price Research Farm, the rates of canker expansion there were not so great that we lost promising trees, nor were they so great that it was overly difficult to distinguish intermediate from low levels of blight resistance. However, irrigation could not decrease the hot temperatures that favor the blight fungus, so canker expansion rates were faster than in wetter, cooler years.

Like 2005, it was quite wet in the winter of 2006, delaying plowing until March, as in 2004. But, once again we were able to finish planting by early April, as we now have sufficient equipment to prepare orchards quickly once the weather breaks. We have sufficient equipment because of the generous support of TACF members, and we thank you for this for the third year in a row!

Inventory.

Our current holdings are presented in Table 1, and changes from 2005 to 2006 are indicated in Table 2. We now have more than 26,000 trees and planted nuts, an increase of approximately 4,000 over last year (Table 2). The addition of B_3-F_2 trees has been offset by the removal of straight backcross trees as we have made selections and rogued the rejects. Note also that we have planted our first B_3-F_3 nuts!

Starting with the Spring 2007 issue of this journal, results from our state chapter breeding efforts will be presented by our Regional Science Coordinators, Dr. Paul Sisco in the South, Sara Fitzsimmons in the Mid-Atlantic, and Leila Pinchot in New England. Therefore, chapter results are no longer being presented in Meadowview Notes. Suffice it to say for now that we have vigorous breeding programs fully underway at numerous chapters.

Harvest.

Meadowview Notes for last year were printed late, in time to include the harvest results for 2005. See *The Journal of The American Chestnut*

Foundation 19(2), 27. Next year's notes will include our harvest results for 2006.

Personnel.

Thanks once again to your generosity, TACF was able to hire three new science staff in the last year. Leila Pinchot was mentioned previously. At Meadowview, Dr. Bob Paris was hired as a Research Geneticist and William White as a Research Technician. William came on board just as we were starting to collect pollen this June, during our busiest season, and one of his charges is to supervise pollen collection and distribution to state chapters. It was a real baptism under fire for William. He immediately made some improvements to our germination testing procedures; we hope this will help ensure that we ship highly viable pollen to our state chapters.

Dr. Paris came on board in November of 2005, as things were slowing down for the year. This gave him a bit of time to evaluate various approaches to increasing the number of sources of blight resistance being used in the breeding program, with a view to ensuring long-term stability of blight resistance. With that in mind, Bob initiated a number of crosses this June, as well as exploring rapid methods for evaluating blight resistance in chestnut and aggressiveness in the blight fungus.

William and Bob currently are hard at work initiating various studies to evaluate strains of the blight fungus for ability to break down the blight resistance we are backcrossing into American chestnut.

It has been a real pleasure for me to work with Bob and William and I anticipate that you will enjoy reading *Journal* articles they will be writing about their work for many years to come, including next year's edition of Meadowview Notes.

Blight resistance screening in B_3-F_2 seedlings.

The year 2005 was the second in which we screened 'Clapper' B_3-F_2 seedlings for blight resistance and the first for 'Graves' B_3-F_2 seedlings. The results of the 'Clapper' tests for the past two years are presented in Table 3 and those for the 'Graves' test in Table 4.

I combined the 2004 and 2005 results for blight resistance screening of 'Clapper' B_3-F_2 seedlings (Table 3) because the same genotypes were represented in both years. This increases the sizes of the populations up to levels where we would expect strongly to observe some trees with high levels of blight resistance. There were some individuals classified as highly resistant, but, overall, the populations had mean resistance ratings higher than 3.0, the level expected, roughly, for B_3-F_2 populations. This may be because the 'Clapper' B_3-F_2 seedlings were planted at a farm which is not irrigated.

In contrast, 'Graves' B_3-F_2 seedlings (Table 4) grown under irrigation at the Price Research Farm had mean resistance ratings closer to 3.0, not significantly different from the F_1 controls planted in that orchard. However, no B_3-F_2 seedlings were classified as highly resistant. This could have occurred for several reasons. First, the low number of planted seedlings gave only a small chance that one would be highly resistant. Second, the fact that the B_3-F_2 seedlings' average resistance rating was slightly higher than the F_1 controls—a difference too small to be statistically significant in this study—may be hinting that the B_3 parents of the B_3-F_2 seedlings had inadequate blight resistance. Lesser apparent resistance of the B_3-F_2 seedlings than the F_1 controls alternatively may have occurred because the B_3-F_2 seedlings were smaller than this particular set of F_1 controls, which showed strong hybrid vigor. Nevertheless, I was impressed enough with five progeny from the B3119 x B3176 cross to transplant them to the 'Graves' B_3-F_2 seedling seed orchard being developed at our Wagner Research Farm. Some of those transplanted seedlings are still showing good resistance as of writing this in July 2006.

Overall, I have cautious optimism that we will be able to recover highly blight-resistant progeny among the B_3-F_2 seedlings from the 'Clapper' and 'Graves' sources of resistance, but we certainly do not have firm evidence for this yet. This year we inoculated many more 'Clapper' and 'Graves' B_3-F_2 seedlings than in 2004 and 2005. However, as I discussed last year, we are moving to staged screening for blight resistance in B_3-F_2 seedling seed orchards, which means this year we only inoculated with a relatively non-aggressive, but virulent, strain of the blight fungus. This will separate out the weakest trees, those in resistance classes 4 and 5. It will be one or more years before we inoculate the stronger trees with an aggressive strain of the blight fungus to separate out the strongest trees. Stay tuned!

I would like to thank Lou Silveri, Dave Lazor, Chandis Klinger, Bart Chezar, and Marshal Case(!) for helping out with pollination and inoc-

ulation in 2005. They came down on their own. We also had a group come down under an Elder Hostel program. Sam Fisher, Director of the Southwest Virginia 4-H Center has been very helpful managing the Elder Hostel program and running the crew, which would not occur without her initiative. Thanks to all—this wouldn't get done without their help. If you are interested in helping to pollinate next year, plan on any time in June (call 276-944-4631). If you are interested in learning more about the Elder Hostel program, call 617-426-8055 or write 75 Federal St., Boston, MA 02110.

We would like to remind all TACF members that you are welcome to visit the farms at any time. We are located in a white house on the northeast side of Virginia Route 80, one-third of a mile southeast of Exit 24 on Interstate 81, the Meadowview Exit. We generally are there during normal work hours, but it might be good to call ahead (276-944-4631).

TABLE 1

Type and number of chestnut trees and planted nuts at TACF Meadowview Research Farms in May 2006, with the number of sources of blight resistance and the number of American chestnut lines in the breeding stock.

Type of Tree	Nuts or Trees	Number of Sources of Resistance	Number of American Lines*
American	2162		235
Chinese	692	51	
Chinese x American: F_1	523	22	90
American x (Chinese x American): B_1	425	15	33
American x [American x (Chinese x American)]: B_2	1559	10	91
American x {American x [American x (Chinese x American)]}: B_3	3818	9	77
Am x (Am x {Am x [Am x (Chin x Am)]}):B_4	9	1	1
(Chinese x American) x (Chinese x American): F_2	710	6	6
[Ch x Am) x (Ch x Am)] x [Ch x Am) x (Ch x Am)]:F_3	6	1	1
[Amer x (Chin x Amer)] x [Amer x (Chin x Amer)]: B_1-F_2	769	4	8
{Am x [Am x (Ch x Am)]} x {Am x [Am x (Ch x Am)]}:B_2-F_2	341	5	5
(A x {A x [A x (C x A)]}) x (A x {A x [A x (C x A)]}):B_3-F_2	12376	2	29
B_3-F_3	121	1	2
Chinese x (Chinese x American): Chinese B_1	191	3	4
Chinese x [American x (Chinese x American)]	41	1	1
European x American: F_1	3	1	1
Japanese	3	2	
American x Japanese: F_1	11	2	2
(American x Japanese) x American: B_1	10	2	2
(American x Japanese) x American] x American: B_2	23	1	1
Castanea seguinii	48	1	
Chinese x Castanea pumila: F_1	9		
Large, Surviving American x American: F_1	328	13	29
(Large, Surviving American x American) x American: B_1	403	7	11
[(Large, Surviving American x American) x American] x American: B_2	94	2	3
Large, Surviving American x Large, Surviving American: I_1	689	13	12
Large, Surviving American: $F_2 = F_1 x F_1$, same LS parent	448	6	8
Large, Surviving American Other	175	9	10
Irradiated American x American: F_1	1	1	1
Other	25		
Total	**26,013**		

* 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 that share an American parent would be counted as two lines rather than one line (this only occurs rarely).

TABLE 2

Changes between 2005 and 2006 in the number of chestnut trees and planted nuts of different types at TACF 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	80		29
Chinese	-122	-4	
Chinese x American: F_1	-335	0	-5
American x (Chinese x American): B_1	39	2	5
American x [American x (Chinese x American)]: B_2	47	0	10
American x {American x [American x (Chinese x American)]}: B_3	-1100	1	2
Am x (Am x {Am x [Am x (Chin x Am)]}):B4	-77	0	0
(Chinese x American) x (Chinese x American): F_2	0	0	0
[Ch x Am) x (Ch x Am)] x [Ch x Am) x (Ch x Am)]:F_3	0	0	0
[Amer x (Chin x Amer)] x [Amer x (Chin x Amer)]: B_1-F_2	81	1	5
{Am x [Am x (Ch x Am)]} x {Am x [Am x (Ch x Am)]}:B_2-F_2	-24	0	0
(A x {A x [A x (C x A)]}) x (A x {A x [A x (C x A)]}):B_3-F_2	5081	0	6
B_3-F_3	121	1	2
Chinese x (Chinese x American): Chinese B_1	0	0	0
Chinese x [American x (Chinese x American)]	0	0	0
European x American: F_1	3	1	1
Japanese	0	0	
American x Japanese: F_1	0	0	0
(American x Japanese) x American: B_1	0	0	0
(American x Japanese) x American] x American: B_2	23	1	1
Castanea seguinii	0	0	0
Chinese x Castanea pumila: F_1	0		
Large, Surviving American x American: F_1	56	2	0
(Large, Surviving American x American) x American: B_1	-179	1	2
[(Large, Surviving American x American) x American] x American: B_2	-32	1	1
Large, Surviving American x Large, Surviving American: I_1	215	-1	-2
Large, Surviving American: $F_2 = F_1 x F_1$, same LS parent	-19	1	3
Large, Surviving American: Other	116	7	3
Irradiated American x American: F_1	0	0	0
Other	1		
Total	**3975**		

* The decreases in B_1, B_2, B_3, and large, surviving American B_1 & F_2 trees reflect roguing of trees with inadequate levels of blight resistance. The increases reflect further breeding and collecting.

TABLE 3

Combined data for 2004 and 2005 on number of ‘Clapper’ B_3-F_2 seedlings ranked in various blight resistance classes.

Code of Mother Tree	Code of Resistant Grandparent	Number Tested	Blight Resistance Class* 1	2	3	4	5
CH271	CL285	120	2	6	29	50	33
CH199	CL112	35	0	6	14	10	5
CH34	CL198	84	0	7	11	27	39
CH726	CL130	91	0	3	17	40	31
CH283	CL98	247	5	28	82	69	63
CH526	CL287	145	1	7	30	42	65

* 1 is the most resistant class and 5 the least. A rating of 1 indicates that cankers caused by both strongly and weakly virulent strains of the blight fungus were small (2-3 cm long) after one season of canker expansion. A rating of 2 indicates that cankers incited by the strong strain were intermediate in size (3-6 cm long) while the weakly virulent strain yielded small cankers. A rating of 3 indicates that the strong strain yielded large cankers (>6 cm long) and the weak strain small cankers. A rating of 4 indicates that the strong strain yielded large cankers and the weak strain intermediate cankers, and a rating of 5 indicates that both strains yielded large cankers. Typically, Chinese chestnut trees achieve a rating of 1 or 2 and American chestnut trees a rating of 4 or 5.

TABLE 4

Number of 'Graves' B3-F2 seedlings and check trees ranked in various blight resistance classes in 2005.

Type of Tree	Pedigree	Number of Progeny Tested	Blight Resistance Class* 1	2	3	4	5
B_3-F_2	B343 x B3176	13	0	0	2	5	6
B_3-F_2	B3176 x B343	11	0	2	4	4	1
B_3-F_2	B3119 x B3176	35	0	9	12	10	4
American	seedlings	1	0	0	0	1	0
Chinese	seedlings	3	3	0	0	0	0
F_1	'Nanking' Chinese x American	4	0	1	3	0	0

*See footnote to Table 3.

A Quick Guide to Chestnut Breeding Terminology

PARENT		OFFSPRING
American x Chinese	=	F_1, F-one
F_1 x F_1	=	F_2, F-two
F_2 x F_2	=	F_3, F-three
F_1 x American	=	B_1, first backcross, or B-one
B_1 x American	=	B_2, second backcross, or B-two
B_2 x American	=	B_3, third backcross, or B-three
B_3 x American	=	B4, fourth backcross, or B-four
B_1 x B_1	=	B_1-F_2, B-one F-two
B_1-F_2 x B_1-F_2	=	B_1-F_3, B-one F-three
B_2 x B_2	=	B_2-F_2, B-two F-two
B_2-F_2 x B_2-F_2	=	B_2-F_3, B-two F-three
B_3 x B_3	=	B_3-F_2, B-three F-two
B_3-F_2 x B_3-F_2	=	B_3-F_3, B-three F-three

In the Spring 2006 Issue of The Journal of TACF, *we published the first part of Dr. Mahn-Jo Kim's manuscript summarizing decades of chestnut breeding in Korea. Following is the second half of that manuscript.*
~ JMC

CHESTNUT CULTIVATION AND BREEDING IN KOREA: PART II

Mahn-Jo Kim, PhD, Korea Forest Research Institute (KFRI)

Orchard establishment and management

In Korea, most chestnut orchards are established in the foothill areas. Grafted trees are usually planted at 5 x 5 m spacing (400 trees/ha). When crowding occurs, smaller trees are thinned out in order to maintain high yield and uniform bearing. Chestnut trees require cross pollination from a different compatible cultivar to ensure good nut production. This means that an orchard planting must contain at least two different pollen-producing cultivars in a low-ratio mix, such as 3:1 or 4:1. Chestnut orchards begin to bear the first crop after two to three years of growth, and can reach good production in 10 years.

Intensive management of chestnut orchards is very important to ensure high nut quality and yield. The problem of tree size control has become more and more economically important. Leading Korean chestnut growers have intensively controlled the crown architecture of chestnut trees through pruning and thinning regimes like those used for apple and pear. They have also managed orchards using disease and pest controls, and fertilization and soil management for large nut and high yield.

Crowded trees tend to create excessive shading on lower branches and promote bearing only on one plane in the tops of the trees, resulting in limited production. They eventually grow to a height of 9 to 11 m. Large, timber-form trees are not desirable for nut production because they are more difficult to manage and much of the tree's energy goes into wood production.

A good crop of well-trained chestnut trees with low tree-form

A low tree-form training system has been introduced into the commercial chestnut industry for its efficiency in both fruit quality and cultural care. This training system was developed in Japan in the mid-1980s (Araki and Fujiwara, 1993). Under this system, chestnut trees are maintained at 4-5 meters in height through pruning, including thinning-out cuts and heading-back cuts. The pruning of central leaders and erect branches is preferred for lowering tree height and expanding the crown width. Growers control annually the canopy of chestnut trees by this training system in late winter or very early spring. Pruning allows sunlight into the canopy and stimulates the formation of nut-bearing flower buds.

Chestnut orchard established on a hillside

The most notorious chestnut diseases are the chestnut blight (*Cryphonectria parasitica*) and ink disease (*Phytophthora* spp.). These fungal diseases are very threatening to the American and European chestnut trees. Fortunately, none are currently a significant problem in Korea. The chestnut blight is present in Korea, and is sometimes observed on some moderately susceptible cultivars with weak cold hardiness. However, it is not spreading because most cultivars in orchards have resistance to chestnut blight. Ink disease has recently been reported in the central region, however, it rarely occurs and is not presently a major constraint for chestnut production.

The worst insect problems in Korea are caused by the chestnut gall wasp (*Dryocosmus kuriphilus*), the peach pyramid moth (*Dichocrocis punctiferalis*), and chestnut weevils (*Curculio* spp.). The chestnut gall wasp is currently one of the leading insect pests in the southern region. The wasp was found first in 1958, and then spread rapidly all over the country. Because of its prevalence, most Korean indigenous chestnut trees were heavily damaged. Thereafter, some cultivars with resistance to the gall wasp were introduced from Japan and were planted widely in new orchards. However, infestation of resistant cultivars by the gall wasp was reported first in 1978 at Gyeongju. It is considered to be a new biotype of the insect that can successfully attack cultivars that were formerly resistant. The resistant cultivars, such as Tanzawa, Arima, and Tsukuba, planted widely in the southern region, have been heavily damaged by a new gall wasp. Studies on biological control by *Torymus sinensis* Kamijo, a parasitoid of the chestnut gall wasp, are in progress. Breeding efforts to overcome the breakdown of resistance, as well as physio-ecological and genetic studies about new gall wasp, are underway.

The peach pyramid moth can sometimes cause significant economic loss to chestnut orchards. Nut damage by the larvae occurs at harvesting time from mid-August to mid- September. Like with all orchard crops, diligent pest monitoring is important during the entire chestnut growing season. Sex pheromone traps are recommended for biological control of the peach pyramid moth. Chestnut weevil is yet another insect that can damage nuts at harvesting time. It arrives following the peach pyramid moth, from mid-September to early October. Three species of chestnut weevils have been identified in Korea: *C. sikkimensis*, *C. robustus*, and *C. camellia*. Insecticide is applied with air-blast sprayers like those used in fruit orchards.

Chestnuts are harvested by hand, using methods that have changed little over the past 2,000 years. Mechanical harvesting is impossible because most chestnut orchards are located on hillsides. Hand harvesting means high labor cost, but it assures the highest nut quality because the nutshell is not scratched. Fresh chestnuts contain about 40% water, making them highly perishable. The nuts must be picked at least every two days to prevent drying out. Hand selection for healthy nuts is required. Harvested chestnuts should also be fumigated with carbon disulfide (CS_2) to prevent chestnut weevils from damaging nuts, and then graded according to size.

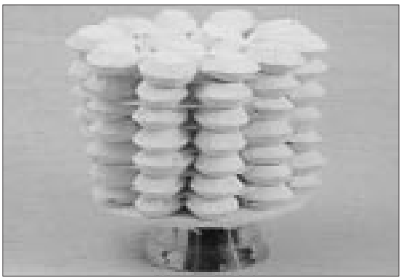
Most growers with small acreage sell their products as soon as the harvest is gathered, since they lack storage equipment. However, large growers are able to store nuts to avoid the low prices that occur at harvest time. Chestnuts should be washed thoroughly and cooled as soon as possible to 3-5°C (37-40°F) for storage. Chestnuts can be stored in breathable polyethylene bags (thickness: 0.03-0.06mm) at 2-4°C (35-39°F) for two months, with enough moisture to keep the kernels from shriveling. For longer storage, chestnuts should be placed in closed polyethylene bags at -2 to -1 °C (28-30°F) under the controlled-atmosphere storage condition.

Chestnut Breeding

Chestnut breeding programs around the world have deliberately hybridized the various species to create superior cultivars for nut and/or timber production. There are no barriers to hybridization among chestnut species, and cultivars derived from their inter- and intra-specific crossings are cultivated in many parts of the world. Japanese/Chinese hybrid cultivars are now found in South Korea and Japan. European/Japanese hybrids are now the common commercial fruiting cultivars in France, Australia, New Zealand, and the western USA. American/Chinese hybrid cultivars are now found in the eastern USA along with even more complex hybrids.

Chestnut breeding programs have a wide range of specific objectives such as improved nut quality, high productivity, and increased disease and insect resistance. In Korea, KFRI began its chestnut breeding program in 1961, and the genetic improvement efforts focused mainly on resistance to chestnut gall wasp, as well as large-sized nut, and high nut productivity. At that time, the fatal chestnut gall wasp spread all over the country, and most Korean indigenous chestnut trees were heavily damaged. As a result, breeding activity tended to concentrate on mass selection and crossing. Some cultivars with resistance to chestnut gall wasp were introduced from Japan and tested for cultivation in Korea (Kim *et al.*, 2003). In the late 1960s, Okkwang, Kwangjujoyul, Sandae, Sunseong, and Daab were developed by mass selection from indigenous chestnut trees widely distributed in Korea. Japanese cultivars such as Tanzawa, Ibuki, Arima, Riheiguri, Tsukuba, Ginyose, and Hyogo 57 were also selected as suitable for cultivation through the local adaptability test.
The cultivars developed by mass selection and introduced from Japan were

used as parents for breeding by crossing. A total of 380 crossings were performed from 1967 to 2002. In order to develop cultivars with a good taste and flavor, a total of 146 crossings were done from 1967 to 1974 and, finally, five cultivars, Kwangeun, Pyeonggi, Juok, Eunsan, and Idae were released in 1988. Three cultivars, Daebo, Parkmi 1, Parkmi 2, with facility of peeling suitable for roasted chestnut were released in 1998 from 54 crossings.



Hand-peeled chestnuts

Nut quality is obviously becoming more important to contribute to the increase of growers' income and to meet the diverse demands of consumers. Breeders need to provide alternatives to meet their needs. Callahan (2003) insisted that fruit breeders should predict consumer desire in 20 years, and have an open mind about what might be considered for high fruit quality on the basis of our current and future lifestyles.

Fruit quality in fruit trees is a complex trait. There are many definitions and standards set by each industry. However, the simple definition of fruit quality is whatever the consumer desires (Barritt, 2001; Kupferman, 2002; Callahan, 2003). Because people are different, and their desires and ideas of quality are different, breeders need to provide alternatives to meet these market needs. The appearance and the taste of fruit are very important. The characteristics that affect the appearance are primarily size and color. Taste generally depends on a combination of sweetness, texture, flavor, etc.

With chestnut, nut size, kernel sweetness, kernel hardness, ratio of polyembryonic nuts, ratio of pericarp split, pellicle intrusions, and facility of peeling are all very important nut quality characteristics. These characteristics directly affect marketability and storage properties. Korean consumers prefer bigger nuts, and are willing to pay more. Over 40% of chestnuts produced in Korea are consumed fresh (Table 1).

TABLE 1

Consumption of domestic chestnut as a percentage of the total crop in Korea

Fresh	Flour	Soup	Roasted	Boiled	Rotting
44.1%	17.6%	11.8%	4.5%	4.4%	17.6%

SOURCE: Korea Forest Administration Handbook 1999.

This consumption pattern is closely related to a tradition wherein fresh chestnuts, hand-peeled by knife, have been used in memorial services as an ancestor worship. Due to this custom of eating raw chestnuts, Korean people tend to prefer large-sized, monoembryonic nuts with high kernel sweetness.

TABLE 2

Selection criteria of major nut traits according to uses of chestnut

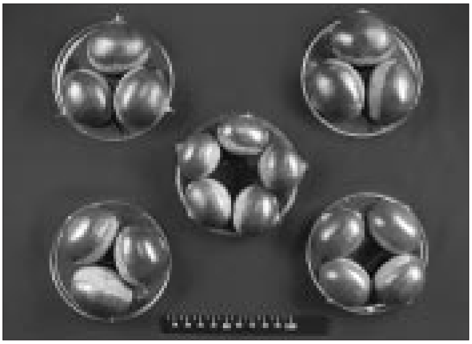
Uses	Nut weight (g)	% of poly-embryonic nuts (%)	Soluble solid content (%)	Kernel hardness (kg/cm²)	% with pericarp split (%)	Facility of peeling* (%)
For general quality	20g	<5%	11%	9kg	<5%	60%
For fresh hand-peeled	22g	<2%	12%	10kg	<5%	60%
For roasted	18g	<5%	14%	11kg	<5%	85%
For processing	20g	<5%	10%	8kg	<10%	40%

*Tanaka's method (1992)

KFRI has been conducting a nut breeding program with breeding goals that correspond to the diverse demands of consumers. Hybridization among the best parents selected from the characterized data is conducted, and then superior offspring are selected by independent culling with the selection criteria of major nut traits, and then by index selection (Table 2).

Several different cultivars (genotypes) could be used as parents for diverse breeding goals. The Korean germplasm collection of chestnut is housed at KFRI National Chestnut Gene Bank in Hwasung. It contains many cultivars (genotypes) with good nut quality, blight resistance, gall wasp resistance, good growth performance, etc. The collection includes some 320 clonal accessions (or cultivars) of Korean native chestnut, 97 cultivars introduced from Japan, and 17 cultivars from five other countries. From a total of 434 germplasm collections, morphological traits of 98 commercially or academically important cultivars have been investigated for the construction of data base during the last four years.

Korean native chestnut accessions have desirable nut characteristics such



New cultivar candidates for processing

as high sweetness, easy peeling, and hard kernel (Table 3). Therefore, they can be used as good breeding materials to develop new cultivars suitable for fresh and/or roasted chestnut.

Until the 1950s, most chestnut orchards were established from seedlings of Korean native chestnut and were used to produce both nuts and timber. Since the 1960s, due to the severe damage by chestnut gall wasps, new chestnut orchards have been established for commercial nut production by planting grafts of profitable cultivars with resistance to gall wasps. Grafts usually grow as bushes with multiple trunks and are not suitable for timber production, whereas seedlings grow upright and live longer.

TABLE 3

The comparison of nut characteristics between 5 selected Korean native chestnut accessions and prevailing cultivars

	Accessions/ Cultivars	Nut drop mode*	Nut weight (g)	Soluble solid content (%)	Kernal hardness (kg/cm²)	% of poly-embryonic nuts (%)	% with pericap split (%)	Facility of peeling (%)
Korean Native Chestnut	Gwacheon 11	A+B	17.6	15.2	12.5	0.0	0.0	57.1
	Uljin 3	A+B	18.9	18.6	12.1	0.0	0.0	71.9
	Hyunri 7	A	15.3	14.1	9.9	4.4	2.0	100.0
	Hongcheon 8	A	15.3	19.8	11.6	0.0	0.7	73.3
	Hongcheon 22	A	17.6	18.1	12.0	3.3	3.9	100.0
Prevailing Cultivar	Tanzawa	A+B	20.4	11.4	7.8	4.8	5.4	31.4
	Okkwang	A	17.2	11.2	8.3	0.3	6.3	17.6
	Ginyose	A+B	22.5	11.6	9.4	2.3	3.6	17.1
	Riheiguri	A+B	20.2	12.0	8.5	0.2	5.8	83.3
	Tsukuba	B	18.4	12.6	9.4	0.5	0.7	0.0

* A=nuts fall separated from bur, B=nuts fall in the bur (protected by the involucre)

Today, as a result of depopulation, urbanization, and the availability of agricultural products, there are increasing areas of abandoned farmland that are suitable for reforestation in the hilly and mountainous areas. The chestnut tree could contribute financially to the improvement of these areas, since they produce nuts and timber.

KFRI is planning another chestnut breeding program for both nut and timber production that involves planting seedlings. It is very important to select cultivars and/or genotypes usable as seed parents suitable for this goal. These cultivars should have desirable traits, such as high nut quality, good growth performance and high resistance to the chestnut blight and gall wasps. They should also have high general combining ability of these traits. Unfortunately, there is little genetic information about the inheritance of major nut traits so far. Therefore, we are planning to establish the clonal seed orchard using carefully selected cultivars, and then to evaluate the performance of open-pollinated progeny.

In addition, molecular marker research using ISSR and micro satellite makers is in progress to develop a simple PCR-based procedure for cultivar identification and use in marker-assisted selection. Breeding work should be greatly assisted by molecular markers, as selection will be made much easier.

Acknowledgements

I would like to thank Dr. Kim C. Steiner and Dr. John E. Carlson of The Pennsylvania State University for giving me an opportunity to study their American chestnut breeding and restoration program during my stay at Pennsylvania State University. I am also grateful to Dr. Kim C. Steiner and to Sara Fern Fitzsimmons for their useful comments and proofreading of the manuscript.

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AN UPDATE ON CHESTNUT DNA PROJECTS: PART II

Other Uses of Molecular Markers in the TACF Breeding Program

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In the last issue of *The Journal of TACF*, I described how molecular markers might be used to screen for the presence of genes for blight resistance in chestnut seedlings. Although molecular markers are currently being used to select for disease resistance in hazelnut and loblolly pine, as of this date we do not have reliable markers for blight resistance in chestnut. It would take years of work and a great deal of money to develop such markers (Sisco, 2006). We are hopeful that a grant application now being considered by the National Science Foundation will provide financial support for a four-year effort to find better markers for resistance to chestnut blight. The grant would fund work at several universities and government research labs and would utilize the genetic resources of TACF's Meadowview Research Farms and the Pennsylvania Chapter's Graves BC_3 orchard at the Pennsylvania State University.

Screening for blight resistance is not the only way molecular markers can be used. In this article I will describe several other uses, some of which have very practical and immediate application to TACF's breeding program.

TABLE 1

Percent pollen contamination in progeny of controlled crosses. Data from analysis with Simple-sequence repeat (SSR) markers by Dr. Tom Kubisiak, USDA Forest Service, Southern Institute of Forest Genetics, in collaboration with Dr. Fred Hebard of TACF's Meadowview Research Farms. These unpublished data are presented with Dr. Kubisiak's permission.

Family	Year Pollinated	Total Trees	# Contaminants	% Contamination
'Mahogany' F_2	1990	102	18	17.6
CC1 x 'Clapper' BC_2	1993 & 1994	95	17	17.9

Accurate Identification of Parents

Most of us are familiar with the use of DNA markers to prove paternity in humans. A baby's father can now be identified with almost 100% certainty with the use of DNA samples, except in the rare cases when either of two identical twins could be the father.

In the same way, DNA markers can prove parentage of chestnut trees, if the parents are still alive to be sampled. Why is this important to the breeding program of The American Chestnut Foundation?

Accidents happen!

Even though the BC_2 and BC_3 trees in TACF's breeding program were produced by controlled pollination using bagged flowers, contaminating pollen can occasionally land on a female flower while it is uncovered. Table 1 shows that in the families that we have analyzed with highly-discriminating DNA markers, about 18% of the trees are the products of contaminating pollen.

The contamination rate in more recent years has undoubtedly been lower, because the workers at Meadowview bag the female flowers at an earlier stage than they did in the early 1990s (Dr. Fred Hebard, personal communication). The Meadowview crew also leaves one out of every 10 bags unpollinated as a control. If too high a percentage of nuts are found in the control bags, the family is discarded. Nevertheless, pollen contamination is a common occurrence in plant breeding, and accurate knowledge of the pollen parent is important.

Molecular markers are not always needed to eliminate contaminants. In two cases, contaminants are obvious from appearance (phenotype) of the seedlings in a family:

Case 1: Contaminating pollen from a pure Chinese chestnut tree.
In the BC_3F_2 orchards at Meadowview, a few of the seedlings obviously had a Chinese chestnut as their male parent instead of a BC_3 tree. These contaminants were easily eliminated, because their appearance is strongly influenced by the Chinese pollen parent, whereas the true BC_3F_2 seedlings are very "American" in their appearance.

Case 2: Contaminating pollen from a pure American chestnut tree.
Some of the early BC_2 families at Meadowview were created by bringing 'Clapper' and 'Graves' pollen down from the Connecticut Agricultural

Experiment Station to pollinate female flowers on American chestnut trees in the Mount Rogers National Recreation Area. In this case, all the contaminating pollen would be from other American chestnut trees in the opening, so all contaminants would be pure American chestnut seedlings that would be eliminated at inoculation time. Only the true BC seedlings that had either 'Graves' or 'Clapper' as their parent were likely to survive screening for blight resistance.

The Meadowview BC_3F_2 trees and BC_3F_3 seeds are the products of open-pollination.

In order to increase seed production, the 'Clapper' and 'Graves' BC_3 trees at Meadowview have been allowed to open-pollinate to create BC_3F_2 families, where it is hoped that highly-resistant trees can be selected as parents for testing and eventual reforestation. In order to prevent high rates of pollen contamination from surrounding orchards, the Meadowview crew and Elder Hostel volunteers have painstakingly removed all male catkins from trees in nearby orchards. Nevertheless, even a few stray catkins from nearby orchards or more numerous catkins from trees farther away might result in some contaminants. And 'Graves' x 'Clapper' BC_3F_2s would be almost impossible to distinguish by appearance alone from BC_3F_2 seedlings that came only from 'Clapper' or 'Graves' sources. In this case, molecular markers could be extremely helpful in identifying contaminants.

The markers could also identify which BC_3 trees were the pollen parents of the BC_3F_2 selections. This would be useful for at least two reasons:

1. It may be that some BC_3 selections will contribute more to blight resistance than others, and analysis of both the pollen and seed parents of the BC_3F_2 trees could help figure this out.

2. To maintain as much genetic diversity as possible, it is important to have a diversity of pollen parents of trees in the BC_3F_2 orchard. There are numerous reasons why a particular BC_3 tree might be represented as pollen parent more often than others. It could produce more pollen, it could be closer to the seed parent trees, the timing of its pollen production could be closer to the time of receptivity of the female flowers, or genes for pollen incompatibility could prevent certain trees from crossing, as discussed in Sisco (2004).

DETERMINING THE PERCENTAGE OF AMERICAN CHESTNUT IN OUR ADVANCED TREES

Another way molecular markers can be used is to determine the percentage of Chinese and American chestnut in our advanced generation selections. One would need to score many markers spread over all 12 chromosome pairs to do this accurately, because the blocks of Chinese genes in our advanced generation trees are likely to be small and difficult to detect. But finding these small blocks of Chinese origin could also give us a clue as to what regions of the Chinese genome are most important for blight resistance. If all our blight-resistant selections have one or more Chinese blocks of genes in common, then these segments of the Chinese genome probably contain the resistance genes. Figure 1 illustrates a situation where one Chinese segment is common among three BC_3F_2 trees that have been selected for a high level of blight resistance.

DISCOVERING CHROMOSOMAL ABNORMALITIES AMONG OUR TREES

One interesting discovery that came about from our molecular markers studies so far is that a few of the trees in one backcross family we analyzed had three alleles* of certain markers—one allele of Chinese origin and two alle-

*any of the alternative forms of a gene that may occur at a given locus.

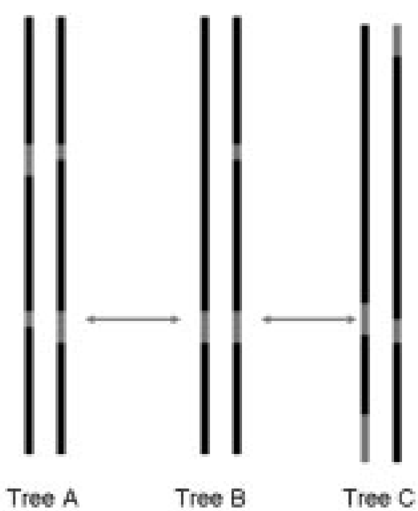

Fig. 1. This hypothetical diagram, called a "graphical genotype", compares one of the 12 chromosome pairs from three BC_3F_2 trees that have been selected for high levels of blight resistance. The **gray** segments of each chromosome represent blocks of genes remaining from the Chinese ancestor, whereas the **black** segments represent blocks of genes from one of several American chestnut trees in its ancestry. The Chinese blocks are small because of repeated backcrossing to American chestnut. The blocks of Chinese genes indicated by the arrows are common to all three trees and may contain genes for blight resistance. The other Chinese blocks of genes likely remain only because of random chance. If TACF scientists had such diagrams for each of the trees in the orchard, they would choose the type shown by Tree B, because it has the highest percentage of American genes. A careful observer will note that Trees A and B may have a parent in common, because they share an identical pattern for one of their sister chromosomes.

Another less detailed method for determining the percentage of American chestnut in TACF's advanced generation trees uses total DNA, rather than markers on individual chromosomes (Liu and Carlson, 2006).

les of American origin (Dr. Tom Kubisiak, unpublished data). This indicated that they had an extra copy of the chromosome on which that marker resides. A tree that has three copies of every chromosome would be called a "triploid." A tree that has three complete copies or parts of some chromosomes but not others would be called an "aneuploid." Normal trees with two copies of every chromosome are called "diploids." The unusual trees—WV5, WV156, and WV280—were the only ones among 78 BC_2 trees that had extra alleles for the markers we tested. WV5 and WV156 had extra alleles for at least four linkage groups, while WV280 had extra alleles for at least two linkage groups. In other words, these trees appear to be aneuploids. Thus they can have three doses of a single gene instead of the usual two. If they happened to have three doses of a single resistance gene, these trees could be more resistant to the blight than a normal diploid tree. However, they would not pass along this resistance to their offspring in a regular fashion. Offspring of aneuploids can be aneuploids themselves or normal diploids, depending on how many chromosome segments they get from their aneuploid parent. Aneuploid pollen from the male parent often aborts, but egg cells in the female parent can tolerate extra chromosome segments and pass them on to the next generation.

ASSESSING THE GENETIC DIVERSITY IN TACF'S ADVANCED FAMILIES

One of the goals of TACF's breeding program is to create a genetically diverse population of blight-resistant American chestnut trees. The state chapter breeding efforts are especially important in this regard. Because of a baseline study by Dr. Tom Kubisiak and James Roberds at the Southern Institute of Forest Genetics, DNA markers can help in assessing the amount of genetic diversity in TACF's breeding program compared to the remnant population of American chestnut in eastern North America. Kubisiak and Roberds (2003 and 2006) analyzed DNA markers from 993 surviving American chestnut trees from 22 sites across the natural range. For some markers, many different forms (alleles) of the markers were discovered among the 993 trees analyzed.

For example, Table 2 shows data for marker *CsCAT01*, which had 31 different alleles among a total of 834 trees throughout the range. These alleles varied in size from one repeat to 23 repeats of a certain DNA sequence. Note that *CsCAT01* marks a highly variable region that is use-

TABLE 2

Allele numbers and frequencies for marker *CsCAT01* over the entire range of American chestnut and in four of the 22 sites analyzed by Kubisiak and Roberds (2003, 2006).

Allele Designation	Allele Size	Entire Range		Greenville Co SC		Qtr Br Smyth Co VA		Middlesex Co CT		Litchfield Co CT	
		Number	%	Number	%	Number	%	Number	%	Number	%
1	15.5	362	21.7%	13	22.4%	22	18.6%	8	8.0%	27	24.1%
2	12.5	215	12.9%	6	10.3%	7	5.9%	10	10.0%	24	21.4%
3	14.5	194	11.6%	4	6.9%	9	7.6%	7	7.0%	30	26.8%
4	11	121	7.3%	0	0.0%	4	3.4%	9	9.0%	6	5.4%
5	13.5	96	5.8%	5	8.6%	7	5.9%	0	0.0%	1	0.9%
6	16.5	95	5.7%	3	5.2%	11	9.3%	4	4.0%	1	0.9%
7	7.5	86	5.2%	2	3.4%	12	10.2%	17	17.0%	1	0.9%
8	9.5	80	4.8%	6	10.3%	1	0.8%	28	28.0%	0	0.0%
9	13	79	4.7%	3	5.2%	0	0.0%	5	5.0%	16	14.3%
10	11.5	76	4.6%	3	5.2%	11	9.3%	10	10.0%	0	0.0%
11	17.5	58	3.5%	2	3.4%	13	11.0%	0	0.0%	1	0.9%
12	15	57	3.4%	0	0.0%	2	1.7%	0	0.0%	0	0.0%
13	10.5	38	2.3%	1	1.7%	6	5.1%	0	0.0%	0	0.0%
14	1	23	1.4%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
15	8.5	18	1.1%	3	5.2%	5	4.2%	2	2.0%	4	3.6%
16	19	13	0.8%	1	1.7%	1	0.8%	0	0.0%	0	0.0%
17	19.5	8	0.5%	1	1.7%	0	0.0%	0	0.0%	0	0.0%
18	21.5	8	0.5%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
19	16	7	0.4%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
20	18.5	7	0.4%	4	6.9%	0	0.0%	0	0.0%	0	0.0%
21	10	6	0.4%	0	0.0%	4	3.4%	0	0.0%	0	0.0%
22	18	6	0.4%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
23	20.5	3	0.2%	0	0.0%	2	1.7%	0	0.0%	0	0.0%
24	8	2	0.1%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
25	12	2	0.1%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
26	14	2	0.1%	0	0.0%	1	0.8%	0	0.0%	0	0.0%
27	22.5	2	0.1%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
28	9	1	0.1%	0	0.0%	0	0.0%	0	0.0%	1	0.9%
29	17	1	0.1%	1	1.7%	0	0.0%	0	0.0%	0	0.0%
30	22	1	0.1%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
31	23	1	0.1%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
Total Alleles		1668	100.0%	58	100.0%	118	100.0%	100	100.0%	112	100.0%
Total Trees		834		29		59		50		56	
Different Alleles		31	100.0%	16	51.6%	17	54.8%	10	32.3%	11	35.5%

ful simply because of its variability. It does not code for a gene of any consequence in chestnut. Rather, it is what is often called "junk DNA;" DNA that occupies filler regions of the genome. Markers for "junk DNA" are called "neutral genetic markers" because they have no effect on the plants and animals in which they reside. This also means that the plant or animal can tolerate a lot of DNA diversity in the regions of these markers, simply because the differences have no effect on the organism.

Nevertheless, a careful study of Table 2 can provide some worthwhile lessons for preserving genetic diversity in American chestnut. I have numbered the alleles from 1 to 31 on the basis of their frequency in the entire range, with allele 1 being the most frequently found. Alleles 28 through 31 were found only once among the 834 trees analyzed. "Allele size" refers to the number of DNA repeats in that particular form of the marker. The number of repeats was the only difference among all these alleles.

Question 1: How many of these alleles are worth preserving?

The quick answer here is "none," because *CsCAT01* is a marker in "junk DNA." But the frequency distribution of these alleles is probably typical of many genes in chestnut that are important. So the question "How many of these alleles should we try to preserve?" is a very relevant one. Forest geneticists Gösta Eriksson, Gene Namkoong, and James Roberds discussed the preservation of alleles in their paper, "Dynamic conservation of forest tree gene resources" (1995). They noted that most genes are additive in nature. In other words, several genes act together to affect tree height, wood quality, and other important factors. So the loss of only one form of one gene is usually not of much consequence. The authors suggested not trying to preserve any alleles that are at frequencies of 1% or less. Looking back at Table 2, we can see that half the alleles were found at frequencies of 1% or less in the entire range. So, if these markers represented genes that actually had an effect on the tree, we only need concern ourselves with alleles 1 - 15. I have drawn a heavy line between alleles 15 and 16 to show where the break point is.

Question 2: How many locations should we sample?

To answer this question, we should look at the allele distribution in individual sites where samples were taken. I have included data from four of the 22 sites—Greenville County, SC; Quarter Branch in Smyth County, VA; Middlesex County, CT; and Litchfield County, CT. The number of trees sampled varied from only 29 in Greenville County to 59 in Smyth County. The number of alleles is twice the number of trees, because each tree usually has two alleles (forms) of any one marker. The first thing to notice is that the three most common alleles (Alleles 1, 2, and 3) were found in all four locations. But Allele 4** was not found among the sample of 29 trees from Greenville County. If we had sampled only from that county, we would have missed the fourth-most common allele. Now look at the distribution of Allele 8. It is completely missing in the sample from Litchfield County, was only found once among 59 trees from Smyth County, and yet was the most common allele found in Middlesex County, at a frequency of 28%! **The lesson to be learned here is that the chapter breeding programs are very important, because they are sampling many different sites in many different states and counties.** At any one site, a common allele may be missing completely, or a rarer allele overall may be the common one. To maximize the number of alleles we capture in the program, we need to sample as many different sites as possible.

Caution: Neutral genetic markers can lead to false conclusions in genetic diversity studies

Of all the uses of genetically neutral molecular markers, their relevance to genetic diversity studies is one of the most controversial. Neutral markers are excellent for determining parentage, because they are so variable. A tree can tolerate many forms of a non-essential bit of DNA. But neutral markers, by definition, do not affect any trait upon which natural selection acts. The most important genes for assessing genetic diversity are ones that **do** matter to the trees: genes for the timing of bud break in the spring, genes for growth rate, and genes for disease resistance, for example. The best way to determine variation in these genes is to collect chestnut trees from many different locations in one site and measure these differences. Such "common garden studies" could be a big help to TACF in assessing what diversity exists and what needs to be preserved.

**see red outlined areas in Table 2.

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ECOLOGY OF THE AMERICAN CHESTNUT AND ALLEGHENY CHINQUAPIN ON THE CUMBERLAND PLATEAU OF KENTUCKY AND TENNESSEE

Joe Schibig, Mark Vance, Sandra Cumming, Lloyd Fly, Clint Neel, and Jack Torkelson

INTRODUCTION

Ashe (1911) described the pre-blight abundance of the American chestnut *(Castanea dentata)* on the Cumberland Plateau (CP) in Tennessee—"It is common on the slopes of the Cumberland tableland, especially on the sandstone soils which have a sufficient depth and are not too rocky; in such situations it forms a large portion of the forest. Chestnut is almost entirely absent, however, on the thin-soiled and stony portions of the tableland, especially near the southern end. In Claiborne, Campbell, Anderson, Morgan, and Cumberland Counties, it forms possibly 15% of the timber." Rhoades and Park (2001) described the pre-blight abundance of chestnut on the CP of Kentucky as intermediate, and they stated that chestnut density was highest in the Cumberland Mountains of southeastern Kentucky. Information on pre-blight abundance of Allegheny chinquapin *(Castanea pumila)* is scarce, presumably because it was not an important timber tree like the American chestnut. Sargent (1922) stated that it rarely attained a height of 50 ft.

The CP, for the most part, consists of tablelands averaging about 1,800 ft in elevation, and the western and eastern escarpments are highly dissected. The CP is typically capped with Pennsylvanian sandstone, and most soils are sandy, acidic, and well-drained. Braun (1950) classified the CP vegetation as mostly Mixed Mesophytic Forest, dominated by species such as maples (*Acer* spp.), buckeye (*Aesculus* spp.), beech *(Fagus grandifolia)*, tulip-poplar *(Liriodendron tulipifera)*, oaks (*Quercus* spp.), and previously American chestnut *(Castanea dentata)*. Hinkle (1989) characterized the forests of the CP as predominantly mixed-oak with Mixed Mesophytic forests restricted to protected sites on the rich soils of escarpment slopes, coves, and deeper ravines.

OBJECTIVES OF THIS STUDY

Our goals were to (1) inventory American chestnut and Allegheny chinquapin specimens on the CP to determine frequencies of flowering and blight infection, size class distributions, geographic occurrence, site affinities, and associated tree species; and (2) find relatively large American chestnut trees > 4 in. dbh to serve as mother trees in the backcross breeding program of The American Chestnut Foundation (TACF).

METHODS

From 2004 to 2005, we recorded data on American chestnut and Allegheny chinquapin specimens on the CP of Tennessee and southeastern Kentucky. Specimens were usually located by searching areas where they had been reported, and most of the sites were in state parks. Global Positioning Satellite coordinates for each specimen were recorded and mapped with ArcView. At Topozone (2005), the chestnut and chinquapin site coordinates were plotted to determine their elevations from Topozone maps. A hand-held compass was used to determine slope aspect. Notes were taken on signs of blight, flowering/fruiting, soil conditions, and asso-

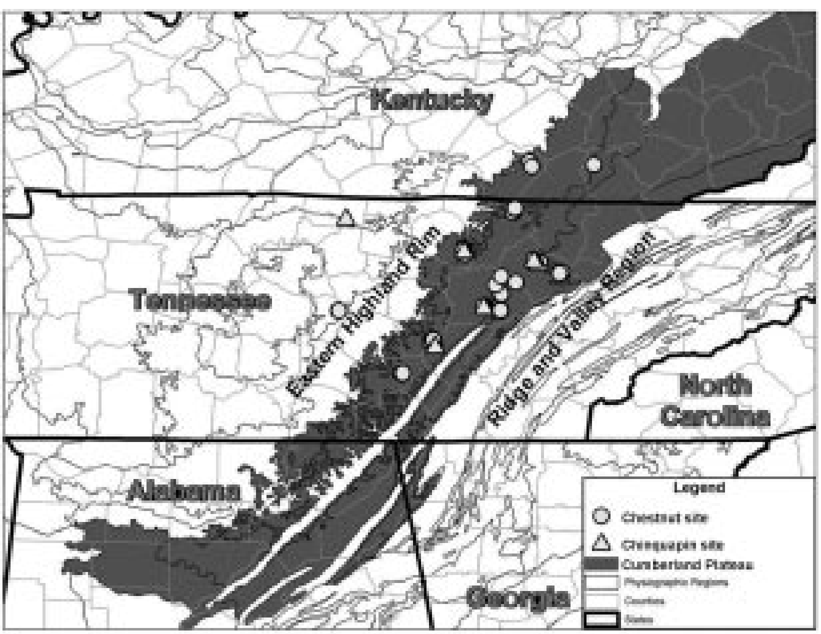

Fig.1. Distribution of American chestnut and Allegheny chinquapin sites on the Cumberland Plateau of Kentucky and Tennessee; specimens were recorded in 2004-2005.

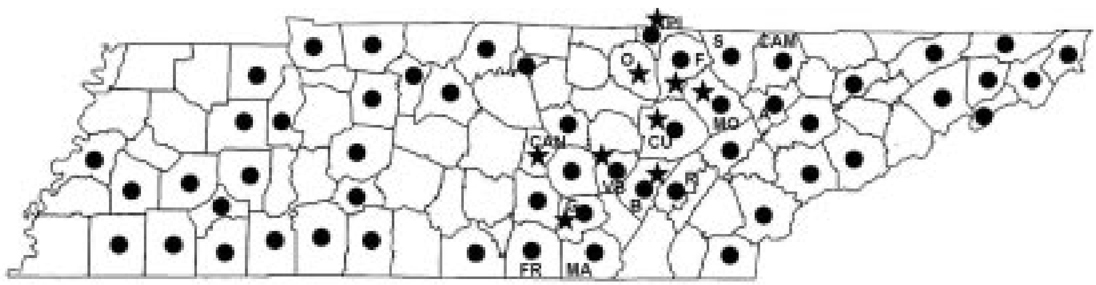

Fig.2. Tennessee counties where American chestnut specimens had been officially recorded by 1997 are represented by a dot (Chester *et al.,* 1997). Counties where we recorded new chestnut specimens in 2004-2005 are indicated with a star. Cumberland Plateau counties include: A = Anderson Co., B = Bledsoe Co., CAM = Campbell Co., CU = Cumberland Co., F = Fentress County, FR = Franklin Co., G = Grundy Co., O = Overton Co., PI = Pickett Co., PU = Putnam Co., MA = Marion Co., MO = Morgan Co., R = Rhea Co., S = Scott Co., and VB = Van Buren Co.; CAN = Cannon Co. (Short Mountain) site which is an outlier of the Cumberland Plateau within the Eastern Highland Rim.

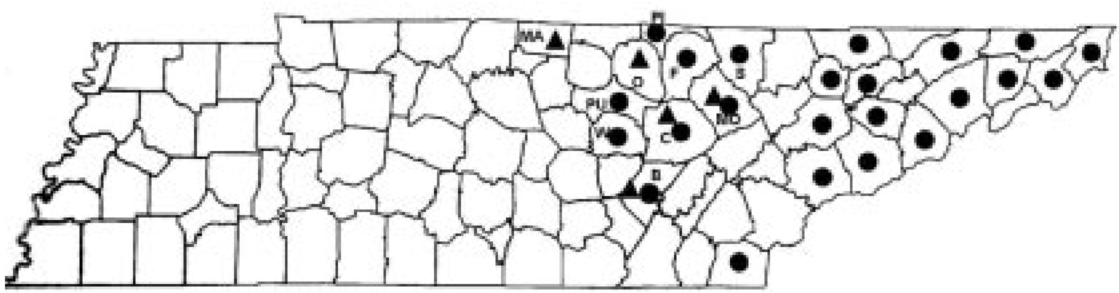

Fig.3. Tennessee counties where Allegheny chinquapin specimens had been officially recorded by 1997 are represented by a dot (Chester *et al.,* 1997). Counties where we recorded new chinquapin specimens in 2004-2005 are represented with a triangle. Cumberland Plateau counties include: B = Bledsoe Co., C = Cumberland Co., F = Fentress Co., O = Overton Co., PI = Pickett Co., PU = Putnam County, MO = Morgan Co., S = Scott Co., and W = White Co.; MA = Macon Co. on the Eastern Highland Rim.

ciated tree and shrub species. Chestnut and chinquapin stem diameters at 4.5 ft above ground (dbh) and estimated height were recorded. If stems were in a cluster arising from the same root system, only the largest stem was measured. For some sites, soil series were determined by referring to county soil maps. All data were entered into an Excel database.

RESULTS AND DISCUSSION

We recorded data on 718 American chestnut specimens on 17 sites on the CP in eight Tennessee and two Kentucky counties. Data on 104 Allegheny chinquapin specimens on five sites in four counties of the CP of Tennessee were recorded. Figures 1-3 show the distributions of chestnut and chinquapin on the CP.

The maximum elevation for American chestnut on the CP was 2,820 ft in Cumberland County, Tennessee, the minimum was 1,140 ft in Whitley County, Kentucky, and the average was 1,843 ft. The maximum elevation for Allegheny chinquapin was 1,740 ft in Cumberland County, Tennessee, the minimum was 1,480 ft in Morgan County, Tennessee, and the average was 1,670 ft.

On the CP, only 2% of the American chestnut trees were obviously infected with *Cryphonectria parasitica*. This low incidence of blight was likely due to the small size of the chestnut sprouts which made them small targets for the blight spores, and most had smooth bark which impeded infection. On the Highland Rim (HR), the physiographic region just west of the CP, Schibig *et al.* (2005) found the incidence of blight to be 17%; we believe the greater frequency of blight on the HR was related to the larger stem sizes in that region. Allegheny chinquapin stems on the CP were even less affected—only one (1%) was obviously blighted.

Out of 718 chestnut specimens recorded on the CP, most (629) had a dbh of < 1 in.; 62 were 1-2 in.; 17 were 2-3 in.; 6 were 3-4 in. and only 4 (0.6%) were ≥ 4 in. dbh with the largest stem in Wayne County, Kentucky having a dbh of 10.5 in. On the HR, Schibig *et al.* (2005) found that 12.7% of the chestnut trees had a dbh ≥ 4 in. with 19 specimens that were 1- 2 ft in diameter. The height class distribution for chestnut trees on the CP was 625 stems (< 10 ft tall), 52 (10-15 ft), 18 (15-20 ft), 12 (20-25 ft), 4 (25-30 ft) and only 6 (> 30 ft) with the tallest at 55 ft. It is possible that the sandstone-based soils of the CP are generally drier and less fertile than the Mississippian limestone-derived soils of the HR causing an overall slower growth rate of chestnut trees on the CP. Also, most of the chestnut specimens on the HR were on privately owned woodlands where frequent logging had released chestnut sprouts allowing them to grow faster and attain greater size than our specimens on the CP where many were on protected (unlogged) park lands. Most (89.4%) of the Allegheny chinquapin stems were < 1 inch dbh, 9.6% were 1-2 in.

dbh, and only 1% (1 individual) was in the 2-3 in. dbh class. In height, 83.8% of the chinquapin stems were < 10 ft tall, 14.3% were 10-20 ft, and only 2 (1.9%) were 20-30 ft.

On the CP, 61% of the American chestnut specimens occurred on relatively mesic (moist) sites (ravines, north-facing, east-facing, and northeast-facing slopes), 17% were on sites of intermediate moisture levels (southeast and northwest-facing slopes) and 22% were on comparatively dry sites (ridges, south-facing, west-facing, and southwest-facing slopes). In contrast, Schibig *et al.* (2005) reported that on the HR, most chestnut trees (74%) were found on the drier sites (ridges and mostly south to west-facing slopes) while 26% were on the more mesic sites (ravines and mostly north to east-facing slopes). Hinkle (1989), in his study of forest communities on the CP, also found more chestnut trees on relatively mesic sites: they occurred in 4.8% of his 231 ravine plots, but in only 1.8% of his 331 generally drier upland plots. On the CP, most Allegheny chinquapin specimens (73%) were recorded on the relatively dry, sunny sites (ridges and slopes which were south-facing, west-facing and southwest-facing). Many of the chinquapin specimens in Bledsoe County, TN, were on sites where they had been released by the death of overtopping Virginia pine trees.

In Cumberland County, TN, American chestnut trees were on Muskingum fine sandy loam; in Overton County, TN, they were on DeKalb stony loam; in Van Buren County, TN, they were on Ramsey loam and Ramsey rock outcrop soils; in Pickett County, TN, they occurred on Ramsey-alticrest-rock outcrop and Lily loam. Soil series for Allegheny chinquapin sites were obtained only for the 54 Bledsoe County, TN, specimens, which occurred on Lily loam and Ramsey sandy loam.

American chestnut sprouts were found on four of the five Allegheny chinkapin sites on the CP. The tree and shrub species which were most frequently associated with chestnut are listed in Table 1.

Only one of the 718 American chestnut trees we found on the CP was blooming, but it was severely blighted. Three other trees, one blighted and two not blighted, were over four in. dbh and could soon become mother trees if released from competition. There is a scarcity of potential mother trees on the CP of Kentucky and Tennessee compared to the HR (south-central Kentucky and central Tennessee) where we currently have about 14 mother trees that have been pollinated and at least six new ones

TABLE 1

The 20 tree and shrub species most often associated with 718 American chestnut trees recorded on the Cumberland Plateau of Tennessee and southeastern Kentucky. Most (648) of the chestnut trees were recorded in Tennessee and 70 were in Kentucky.

Common Name	Scientific Name	No. of times found within a 50 ft. radius of a chestnut tree	Percent of 718 possible associations
Red maple	*Acer rubrum*	538	74.9
Blackgum	*Nyssa sylvatica*	517	72.0
Sourwood	*Oxydendrum arboreum*	463	64.5
Sassafras	*Sassafras albidum*	472	65.7
Tulip-poplar	*Liriodendron tulipifera*	441	61.4
White oak	*Quercus alba*	394	54.9
Dogwood	*Cornus florida*	333	46.4
Chestnut oak	*Quercus montana*	292	40.7
Serviceberry	*Amelanchier arborea*	212	29.5
Mockernut hickory	*Carya tomentosa*	200	27.9
Eastern white pine	*Pinus strobus*	152	21.2
Black oak	*Quercus velutina*	132	18.4
Virginia pine	*Pinus virginiana*	121	16.9
Red hickory	*Carya ovalis*	116	16.2
Pignut hickory	*Carya glabra*	115	16.0
Mountain-laurel	*Kalmia latifolia*	77	10.7
Shagbark hickory	*Carya ovata*	75	10.4
American holly	*Ilex opaca*	69	9.6
Sugar maple	*Acer saccharum*	67	9.3
Blueberry	*Vaccinium* spp.	61	8.5

to be pollinated in 2006. Our field research has revealed there are presently many more large (>10 in. dbh) chestnut trees on the HR (especially the eastern HR) compared to the CP, but chestnut appears to be more abundant on the CP than on the HR. On the CP, we found no chestnut tree producing viable seed, but 26% of the Allegheny chinquapin specimens were flowering, and many of them produced viable seeds.

ACKNOWLEDGEMENTS

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Castanea Guide: A Quick Comparison of Chestnut Species

	CHINKAPIN	JAPANESE	EUROPEAN	CHINESE	AMERICAN
Leaf Taper to Stem	straight	curved	curved	curved	straight
Taper to tip	straight	curved	curved	curved	straight
Teeth	1-3 mm, small, sharp, no hook	Tiny, often only bristles, no hook	Big, sharp or rounded, no hook	Large or small, no hook	6mm, big, sharp, and often curved (hooked)
Leaf Underside	*Sun leaves noticeably hairy	Sun leaves noticeably hairy	Sun leaves noticeably hairy on some specimens but not others	Sun leaves obviously hairy	Sun leaves not hairy, long sparse hairs only on midrib
Twig (those that have overwintered at least one year)	hairy tips, purple or brownish grey	Pink to light red, large white **lenticels	Stout, dark, brown, small white lenticels	Hairy tips, Tan to pea green Large elliptical yellow lenticels	Slender, smooth, hairless reddish brown, small white lenticels
Bud	Up to 3 mm, downy dark red, pointed, longer than wide, sticks out from stem	Glossy brown, As long as it is wide (rounded)	Dark red, fat and globular	Hairy, tan, dull brown to black, rounded and flat against stem	Up to 6mm, smooth, reddish brown to yellow, pointed, or longer than it is wide, sticks out from stem.
Nut* **	1 nut, ½" tip pointed with a round cross section	2-3 nuts, 1-2" no sunburst pattern at base, moderate brown	2-3 nuts, 1-2 " no sunburst pattern, dark brown, black stripes	2-3 nuts, ¾- 2", rounded hairy tip, no sunburst pattern, often light brown	2-3 nuts, ½ -1", pointed tip, top ⅓ to ⅔ downy, sunburst at base
Taste*****	sweet	not sweet	starchy	sweet	sweet
Resistance to blight:	None	Moderate	Slight	High	None

*Sun leaves arethose leabes that are most exposed to the sunlight on a tree.
** A lenticel is an aerating organ on the surface of a twig or branch. They may appear as bumps on the surface of twigs.
*** Nut size may vary a lot within each species. Sizes provided are maximum possible.
****Taste refers to those commonly found in the U.S. and may not reflect that of all members of a species.
Be aware that all chestnuts can cross-pollinate, so that a tree that seems clearly of one species or another, may actually be a mix of two or more different types of chestnuts, known as hybrids. Please refer to TACF's website www.acf.org for more information on identifying American chestnuts.

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