The American Chestnut as a Service Learning Project at Juniata College

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Today I will be talking about my work on the American chestnut (*Castanea dentata*). Until 2007, my knowledge of the American chestnut was limited to theoretical information I got from textbooks. It is a classic case study in conservation biology - a dominant species that was all but wiped out as a result of an introduced disease. In the late 1800s, the estimated population size of the American chestnut was over four billion; it was reduced to a few hundred over a fifty-year period. I had been following ongoing restoration and outreach efforts, but as a wildlife biologist I had not considered working on the species.

In 2007, Ashley Musgrove, a (then) senior in Environmental Studies, expressed an interest in doing research on the American chestnut. She had always been fascinated with the species and its history, and she thought that it would be a good idea to combine her interest in the American chestnut with her career goals in landscape architecture. I agreed to help her find researchers she could work with. I contacted the Pennsylvania Chapter of The American Chestnut Foundation (PA-TACF) at Penn State. The regional coordinator, Sara Fitzsimmons, invited us to a regional conference of The American Chestnut Foundation (TACF) for a quick overview of the work and projects being conducted in the region. Ashley and I attended the meeting at Penn State University on November 10, 2007. It was a very productive meeting for us – we met a lot of professionals in the field and got a summary of the ongoing research. After talking with several researchers, I decided that I would like to be involved in the research and recovery of the American chestnut.

HISTORY OF THE AMERICAN CHESTNUT

The American chestnut is a deciduous tree native to the Eastern United States, with a distribution following the Appalachian Mountains from Maine to Georgia (Fig. 1).[1] Historically, the tree provided a reliable crop of chestnuts each year for many wildlife species, more reliable than oak acorn masts. Chestnuts were also important to Native Americans and pioneers. In the early 1900s, the American chestnut trees began to get infected by the chestnut blight, *Cryphonectria parasitica*. This fungus was accidently introduced into the U.S., coming in from Asia via nursery stock or lumber.[2] The fungus establishes in openings in the bark of the tree and invades the cambium layer, resulting in a canker (swelling) of the infected tree. The infected trees can die within a few months. The disease quickly spread

throughout the trees' native range and decimated almost all standing trees. By 1920, the only remaining populations of *C. dentata* were an introduced population in Michigan and Wisconsin and stump sprouts from the remaining infected trees.[3] Because infected trees regenerated from stump sprouts, the American chestnut is not protected under the Endangered Species Act. The USDA Threatened and Endangered Plants database lists the species as *Endangered* in Kentucky and Michigan; in Tennessee and Maine it is listed as a species of *Special Concern*.

Restoration Efforts

Several organizations such as the U.S. Forest Service, the Connecticut Agricultural Experiment Station, Pennsylvania State University, and the University of Tennessee have been involved in research to restore the American chestnut to the wild. Another organization that has been involved extensively in the restoration effort is TACF, founded in 1983. The stated mission of TACF is "to restore the American chestnut tree to its native range within the woodlands of the eastern United States, using a scientific research and breeding program developed by its founders." The technique being used by the foundation is to develop a blight-resistant tree through a process of hybridization and backcrossing. Most Chinese chestnuts (*Castanea mollissima)* are resistant to chestnut blight; mortality among infected trees is low. American chestnuts were initially hybridized with the naturally blight-resistant Chinese chestnut (Fig. 2, F1). The resulting hybrids were then tested for blight resistance, and resistant hybrids were backcrossed with pure American chestnuts for three generations (Fig. 2 - BC1, BC2 and BC3). At each step, individuals were tested for blight resistance. Selection for the next stage of backcrossing was also based on the physical characteristics of the trees. In the final stages, the backcrosses were crossbred, to increase resistance (Fig. 2 – BC3F2 and BC3F3). This process focused on creating trees that maintain the morphology and genetics of *C. dentata* and the blight resistance of *C. mollissima*. Since 2009, these blight-resistant backcross seeds have been offered to state and federal agencies, as well as private orchards. The purpose of the initial distribution of the seeds is to test blight resistance and growth form in natural settings.

THE AMERICAN CHESTNUT AS A SERVICE LEARNING PROJECT

In 2008, I was one of the recipients of the Southern Alleghenies Learn and Serve Alliance (SALSA) grant to incorporate service learning into the curriculum. SALSA represents a regional service-learning network, founded by Juniata College, Mount Aloysius College, and St. Francis University. The goal of the grant was to create service learning course by identifying community partners and have students work with community partners on goal-specific projects. The difference between service learning and community service is that in service learning programs, students are expected to use skills they developed in their course work and incorporate it in a real-world setting. In a successful service learning partnership, both the community partner and the students benefit from the interaction. While the primary

community partner in this project was PA-TACF, I also collaborated with the biologists at the Raystown branch of The Army Corps of Engineers and the Juniata College Community Service Office.

As part of the service learning project, five students worked with PA-TACF to identify areas of need that they could work on – Ashley Musgrove in 2008; and Courtney Goss, Kelly McErlean, Brittany Moyer and Rebecca Goodman in 2009. Together, the students and PA-TACF staff identified three distinct areas of need that could be developed: chestnut growers were interested in techniques that increase the overwintering success of chestnut seeds; TACF wanted more chestnut orchards that could be used for research; and PA-TACF was interested in developing an educational outreach module on the American chestnut.

Overwintering Study

Overwintering refers to the process of getting through or surviving the winter. The seeds of most temperate hardwood species have evolved to adapt to cold winters, so seeds collected in the fall need to go through a cold storage process to germinate. In our study, we examined different temperature and growth medium storage conditions. Students used 100 seeds each from three different species - *C. dentata, C. mollissima* and the blight resistant backcross. The Chinese chestnut seeds were collected by Mr. Rick Entriken, a member of PA-TACF, from trees located around Huntingdon. The pure American chestnut seeds were purchased from F.W. Schumacher Inc., and the backcross seeds were obtained from Dr. Sandra Anagnostakis of the Connecticut Agricultural Experiment Station. The seeds were stored at three different temperatures; 28° F, 34° F, and 40° F, in three different growth mediums - potting soil, sphagnum moss, or sand. The sprouting time and growth rates of each of the chestnut species were measured. Sprouting time and growth rates were highest at 40° F in all three species. Growth medium did not affect the growth rates and sprouting times of the Chinese chestnuts and the backcrosses; American chestnuts grew faster in sphagnum moss. Ashley Musgrove published the results of this experiment in the PA-TACF's *The Chestnut Tree Newsletter*.

Development of the Juniata College Chestnut Orchard

To help us set up the chestnut orchard, the PA-TACF sent a team of experts to help with site selection and design. We selected a plot of 2.5 acres, located behind Brumbaugh Academic Center. The criteria for site selection was based on the guidelines listed in "The Chestnut Grower's Primer."[4] Thirty seedlings each of six different species were planted in the orchard – pure American chestnut; blight-resistant backcrosses; Chinese chestnut; European chestnut (*C. sativa*); Japanese chestnut (*C. crenata*); and Chinquapin (*C. pumila*) (Fig. 3). Chinquapin is a dwarf chestnut species native to the eastern United States. This species is also affected by the chestnut blight. The seedlings were planted in grids, ten feet apart. The plan is to thin the trees in later years to allow more space for trees to develop. With funding from the SALSA grant, we put up an eight-foot wire fence around the orchard to prevent browsing by

white-tailed deer (Fig. 4). In summer 2008, Kelly Crosset (an Environmental Studies Senior) and I received the D. C. Goodman Summer Research Award. The grant was used to develop the orchard and initiate long-term research on the species.

Educational and Outreach Module

As part of the Service Learning agreement, students developed an educational module geared towards the general public that could also be used for school students. The module contained an indoor and an outdoor component. The indoor section included a PowerPoint lecture that introduced the audience to the history of the American chestnut, the different species of chestnuts, conservation concerns, and current efforts to restore the American chestnut. They also developed a website, posters, and brochures for distribution. The outdoor module was designed around the Juniata College orchard, highlighting variations between chestnut species in leaf morphology, nut morphology and tree structure. A kiosk was constructed near the orchard, where information on the orchard is posted. To advertise the event, announcements were put in the *Juniatian*, Huntingdon's *Daily News*, and the Juniata College Announcements. Attendees also participated in planting chestnut seedlings in the orchard.

ONGOING RESEARCH

The SALSA grant ended in spring 2010, but the collaborative partnership with PA-TACF continues. Currently, I am doing some research with PA-TACF and the Army Corps of Engineers on regional variations in the leaf morphology of the American chestnut. In this study, we are examining regional American chestnut populations to look for evidence of hybridization, regional distinctness, and evidence of environmental and genetic stresses. The findings of this study are relevant to PA-TACF's effort to re-establish the American chestnut in Eastern forests.

We selected four *C. dentate* populations in Pennsylvania. The first site is located in the northwestern part of the state, at Clear Creek State Park in Jefferson County. This population is probably the most isolated of the four study sites. We collected leaf samples from several older chestnut trees, some at least sixty to seventy years old. We also collected samples from a recent clear-cut. For the remaining three sites, all samples were collected from re-sprouts in recent clear-cuts, with the clear-cuts having occurred within the last five years. These sites are Laurel Hill State Park in Somerset County, Michaux State Forest in Franklin County, and private lands in the city of Hazelton in Luzerne County. Control samples were collected from the Juniata College chestnut orchard.

Increased asymmetries of morphological traits are often an indication of environmental and genetic stress.[5] We measured fluctuating asymmetry in chestnut leaves, a random non-directional deviation from perfect symmetry, as an indicator of stress. Our analyses revealed that none of the sampled trees at all four sites showed signs of stress. We used shape variation to identify between-tree and between-site distinctions in leaf morphology. Differences in shape were analyzed using two techniques –

leaf landmarks and leaf outlines. We found that leaves from all four sites are morphologically distinct. Finally, to look for evidence of hybridization, we examined trichome shape, size, and density, and the presence of stellate hairs using a Scanning Electron Microscope. Trichomes or epidermal hair differ distinctly between chestnut species. *C. dentate* epidermal hairs are bulb-like (Fig. 5C); *C. mollissima* are filamentous (Fig. 5B). While the trichome size and density differed between regions, we found no evidence of *C. mollissima* epidermal hair. The three students that have been working with me on this project, Anna Jaworski, Adam Fehn, and Ian Gardner, presented the results of this study at the 2011 annual joint meeting of the Pennsylvania Academy of Science and the Pennsylvania chapter of The Wildlife Society.

The chestnut trees in the orchard have flowered this summer, so we are likely to have our first chestnut crop in the fall. We plan to hand-pollinate the different species when the trees get older. PA-TACF conducts inoculation studies at the Penn State facility – the orchard at Juniata College provides an alternate site.

NOTES

1. E. Lucy Braun, *Deciduous Forests of Eastern North America* (Philadelphia: Blackston,1950).
2. Douglass F. Jacobs and Larry R. Severeid, "Dominance of interplanted American chestnut (Castanea dentata) in southwestern Wisconsin, USA," *Forest Ecology and Management*, 191 (2004):111–120.
3. Susan Freinkel, *American Chestnut: The Life, Death, and Rebirth of a Perfect Tree* (Berkeley, CA: University of California Press, 2007).
4. Tom Wahl, *The Chestnut Grower's Primer* (Wapello, IA: Southeast Iowa Nut Growers, 2002).
5. G.M. Clarke, G.W. Brand, and M.J. Whitten, "Fluctuating Asymmetry: A Technique for Measuring Developmental Stress Caused by Inbreeding," *Australian Journal of Biological Sciences*, 39 (1986): 145–154; A. Richard Palmer and C. Strobeck, "Fluctuating Asymmetry: Measurement, Analysis, Patterns," *Annual Review of Ecology and Systematics*, 17 (1986): 391–421; P.A. Parsons, "Fluctuating Asymmetry: A Biological Monitor of Environmental and Genomic Stress," *Heredity*, 68 (1992): 361–364.

Figure 1: Natural Range of the American chestnut (in dark green). The first signs of infection were reported from New York in 1904; by 1914, most of the chestnut trees in Pennsylvania were infected. By 1950, the entire range all the way down to Mississippi and Alabama were affected. Map courtesy of The American Chestnut Foundation.

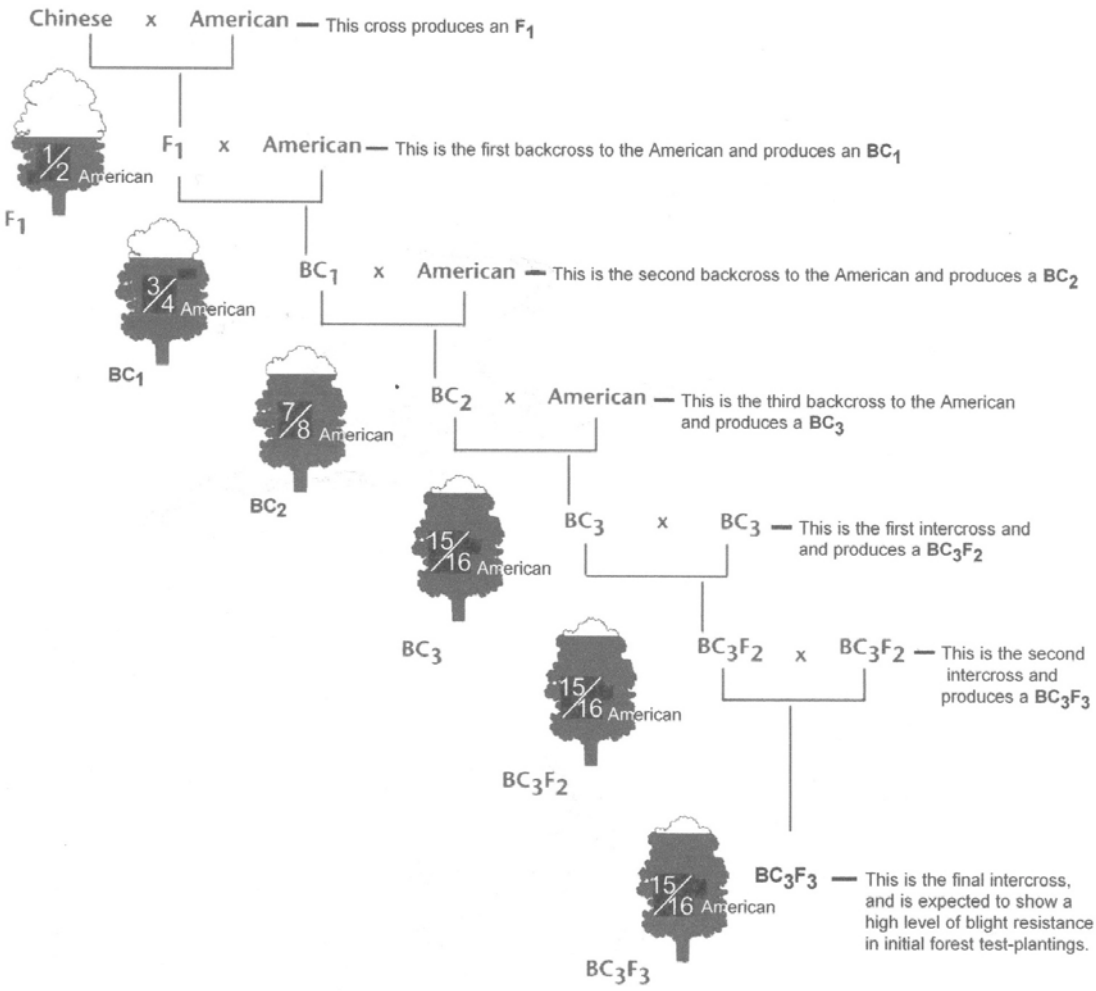

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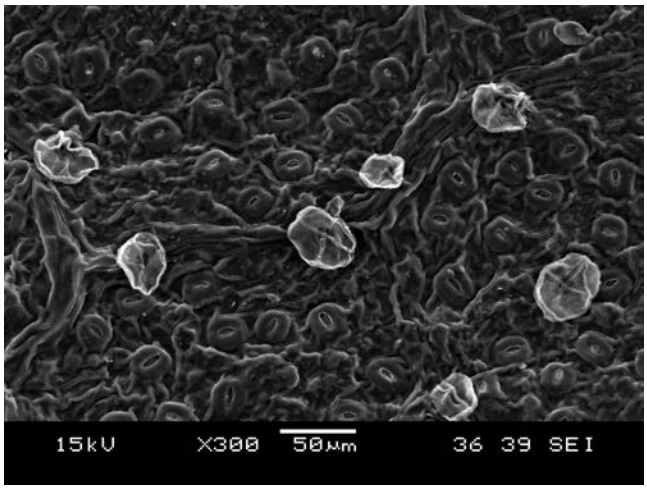
Figure 2: The American Chestnut Foundation breeding program. American chestnuts were hybridized with blight-resistant Chinese chestnut. Resistant hybrids were backcrossed with pure American chestnut, resulting in a cultivar that is ninety-three percent American chestnut, with all of the morphological characteristics of the American, and the resistance of the Chinese chestnut. Figure courtesy of The American Chestnut Foundation.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1												***CHR1***	CHR2	CH16	***J30***	***CHR4***	CE45	
2												***CHR5***	CH46	E30	CHR7	J28	J79	***AM1***
3												CE14	AM84	***CE10***	***AM54***	E29	J27	***CE30***
4					BX77	E24	***CE40***	AM44	CH22	CH25	AM103	CH26	BX20	BX15	CE32	BX83	CE34	BX2
5					CH17	CE35	AM99	E23	J24	BX23	BX86	J25	J26	CH24	AM137	AM118	CH27	***AM2***
6					E18	***E19***	BX93	***CE10***	E21	BX79	BX95	J20	BX72	CE20	J21	BX7	J22	E31
7					AM126	CH20	J18	E15	***CE27***	CH23	BX87	BX101	BX99	CH21	CE38	***E17***	CH30	CE4
8					***CE39***	***CE36***	AM121	***E11***	BX88	BX71	AM117	***CE24***	CH35	J17	E13	AM156	E14	J31
9	E1	BX81	***CE27***	CH39	***E10***	BX90	AM163	AM111	AM155	BX103	CE22	AM122	J16	AM157	BX94	AM152	BX70	E32
10	***J2***	BX96	E2	BX100	AM136	J12	AM162	J13	CH32	J14	***E7***	***CH42***	CE12	CH34	CH19	***J15***	***E9***	CE5
11	AM115	AM161	***J3***	CH45	CH36	AM127	J6	E6	***J7***	J8	AM131	J9	***CH37***	AM125	J10	***J11***	CH33	CH9
12	E40	CE28	BX82	AM128	***CH38***	J4	BX84	***AM140***	AM135	J5	CH40	E4	AM134	E5	BX97	BX74	BX69	E33
13	CE37	E39	J35	CE38	E38	BX1	E37	CE41	E36	CE17	J34	E35	J33	CE31	***E34***	***CE26***	J32	CE38

Figure 3: Layout of the multi-species chestnut orchard at Juniata College. This orchard contains six species of chestnut trees: the American chestnut (purple)*;* Chinese chestnut (dark green); Japanese chestnut (yellow); American-Chinese backcross (light green); European chestnut (blue); and Allegheny Chinquapin (pink).

Figure 4: Photograph of the orchard. Tubes were used to protect the seedlings from rodents and birds. An eight-foot fence was constructed around the orchard to keep out white-tailed deer.

A



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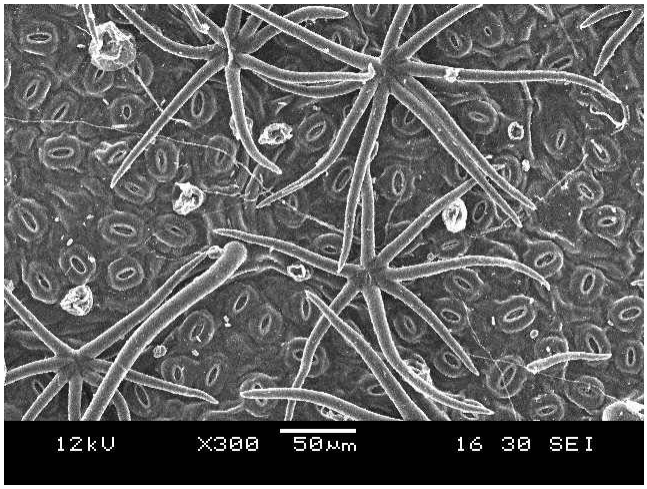

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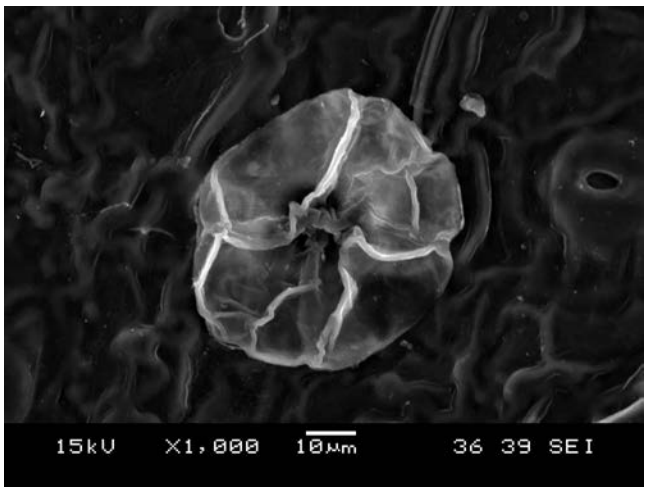

Figure 5: Scanning Electron Microscope image of an American chestnut leaf at 300X magnification (A); a Chinese and American chestnut hybrid at 300X magnification (B); and a trichome at 1000X magnification (C)A. Note the stellate hairs in the hybrid, typical of Chinese chestnut leaves; trichomes are typical of American chestnut leaves.