Dedicated to restoring the American

Chestnut Tree

PA-TACF

VOLUME 25, ISSUE II SEPTEMBER 2020

PANJ Chapter of TACF — Fall Member Brownbag Webinar

Saturday, November 14, 2020 / 11:30 a.m. to 2:00 p.m.

To join the webinar online use this link: https://psu.zoom.us/s/96761815405
Or dial in using either of these phone numbers: (646) 876 9923 or (301) 715 8592
Webinar ID: 967 6181 5405

Featured Speakers

Dr. Jenise M. Bauman
Associate Professor Huxley on the Peninsulas, Western Washington University

An 11-year assessment of hybrid chestnut growth, blight incidence (Cryphonectria parasitica), and tree recruitment under various soil restoration treatments

Laurel Fischer Mueller
President, Certified Professional Soil Scientist and Soil Classifier (CPSS/CPSC), Geomorphologist, SEO

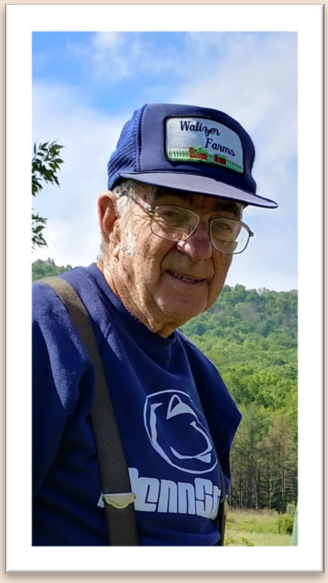
Forest Soil 101 -- a refresher and intro into understanding soils in the woods.

To learn more about the featured speakers and their presentations please visit our website:
www.patacf.org

Meeting Program

Login starting at 11:15 to test your connection.

11:30 —11:45 AM	**Business Meeting Mary Ayres, President**
11:45—12:00 PM	**Restoration Update Sara Fitzsimmons**
12:00–12:50 PM	**Laurel Fischer Mueller Presentation and Q & A**
12:50—12:55 PM	***Break***
12:55—1:45 PM	**Dr. Jenise M. Bauman Presentation and Q & A**



Volunteer Recognition

At our Virtual Fall Meeting this November 14th, the Chapter will recognize the long-term contributions of Board member, Jim Walizer. A member since 2000, Jim has pursued a program to create a timber-type Chinese chestnut, collecting seeds from promising trees, and planting them on his farm outside Bellefonte, PA. He has served on the Chapter Board of Directors since 2004.

President's Corner

This is a time of transitions. In terms of a blight-resistant American chestnut that can thrive as a forest canopy tree, our organization has followed the science and successfully pivoted to a multi-faceted approach to our science plan. If I've learned nothing else over the 10+ years I've been a member of the PA/NJ TACF is that tree biology is very, very complicated. Nonetheless, with the genomics revolution, our understanding of what it will take to get a blight-resistant chestnut has advanced at warp speed. The other great area of rapid advancement has been in the real world understanding of breeding, ecosystems, and phenotypic variations. I continue to be in awe of all the professional scientists, citizen scientists and growers that populate our organization.

Our Chapter is also transitioning. I am concluding my two-year term as Chapter President and Vice President Jim Searing is preparing to take the reins starting in January 2020. Our By-Laws outline the volunteer structure of succession, from Vice President to President to Past President. This structure ensures continuity at the same time it brings new ideas and energy to our volunteer board. This is one reason we are continually on the lookout for new board members. We need volunteers not only to grow trees, work in orchards, and work at outreach events (when we're not dealing with a pandemic) but also to provide guidance and oversight to the organization. If you know someone who might be a good candidate for the board, please call the office and let us know.

As an organization, one of our great assets that contributes mightily to our success is having a part-time Chapter Administrator. As you all know, for several years that position has been most competently held by Jean Najjar. I want to express my deepest thanks to Jean for all that she does to make our Chapter run so successfully. I could not have fulfilled my role as President without her support and guidance. But I need to thank our members as well, as it is your generous donations each year that enable our chapter to pay for a part-time administrator.

As I write this letter, the State University of New York – College of Environmental Science and Forestry (SUNY-ESF) application for approval of the transgenic tree is opening up for public comment.* This portends exciting developments for our organization over the next couple of years, and—we hope—for the eventual restoration of the mighty Chestnut to our eastern forests.

Mary Ayres
President 2019-2020

** See page 7 for more on how to participate in public comment.*

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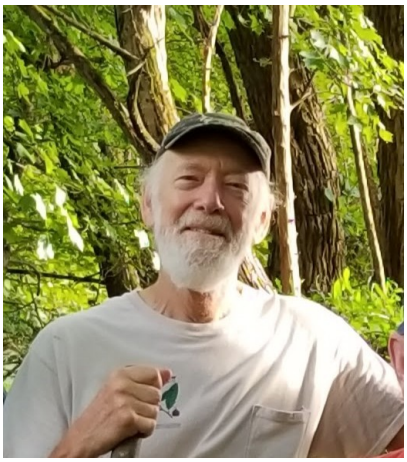
Chapter News

Volunteer Spotlight: Mike Aucott

Mike Aucott grew up in Glenside, PA, a suburb of Philadelphia. After attending Haverford College, he started his own painting and paperhanging business. He eventually, went to work for NJ Department of Environmental Protection (NJDEP) and returned to school to study environmental science at Rutgers University. He got an MS and a Ph.D. in Environmental Science and worked as a research scientist at NJDEP for many years before retiring in 2012. Currently, Mike teaches chemistry part-time at The College of New Jersey.



If you attended the 2019 TACF Annual Meeting you would have seen Mike enjoying one of his many hobbies, music. He plays the banjo and guitar.

In addition to chestnut trees he maintains a big vegetable garden. For a while he used to fix up old cars restoring a 1949 Chrysler and a 1964 Plymouth Valiant. He claims to have pretty much gotten this out of his system, but he still works on small engines. And he still finds time to hike and canoe.

For 7 years in the late 70s to mid-80s along with his wife Louise, Mike operated a small farm in northern PA, growing pick-your-own strawberries, cauliflower, and broccoli. He says, "we were part of what can be called the "back-to-the-land" movement then and tried to be self-sufficient; grew much of our own food; heated with wood. The main lesson for us from this time was how inter-dependent we humans are; that trying to be self-sufficient in today's world isn't practical. We decided to leave our homestead, build on our educations, and develop careers where we could be more effective. Louise (past PATACF Board Member) went back to school and became a midwife, and ran her own practice for many years, doing mostly home births. Mike shared that "I was always amazed at how cheerfully she'd go off to do a birth, often in the wee hours of the morning. Three of our four kids were born at home". Mike and Louise held on to their little parcel of land in northern PA, and today they have an orchard of 600 chestnut trees as well as some hazelnuts.

How did he end up at TACF? Like many of us, he remembers collecting leaves for a school assignment in 5th grade and reading about the American chestnut. He was struck by this story and it set him on a course to hunt for and find an American chestnut. Flash forward many years, Mike's son, who is a woodworker near Asheville, NC, was involved in a project to make furniture from salvaged chestnut. He directed Mike to TACF and our Chapter is stronger for it.

Recently Mike had an article published in Conservation Biology, *Medical biotechnology as a paradigm for forest restoration and introduction of the transgenic American chestnut* . To read an abstract of the article please follow this link: https://conbio.onlinelibrary.wiley.com/doi/abs/10.1111/cobi.13566

Asked for a main take away from the article Mike said this: *"... we have few if any qualms about using the best science when it comes to human health. Why shouldn't we have the same acceptance of the best science when it comes to forest health? Genetic engineering techniques, have led to new medicines and treatments for people. There is reason to be optimistic that itcan also help our forests, specifically the American chestnut ".*

A Walk in Penn's Woods — 2020

Penn State Extension is inviting folks to "Walk in the Penn's Woods" throughout October in 2020. Explore the woodland adventures featured on their website or find some of your own. Mike Aucott who led a walk through Allegheny National Forest as part of last year's event is helping to develop a self guided tour for his chestnut walk. Look for more details on our website later in September.

To learn about other walks visit:
https://sites.psu.edu/walkinpennswoods/

CURRENT CHESTNUT GENOMIC PROJECTS LED BY TACF
By Sara Fitzsimmons

Advances in sequencing technology and analysis have allowed TACF to spearhead recent genotyping projects which will greatly improve the work we all do toward American chestnut restoration. TACFs Director of Science, Jared Westbrook, has been conceptualizing and driving these efforts across the Foundation. Although there are additional projects ongoing, members of the PA/NJ Chapter have directly participated in the four projects detailed below, the results of which will directly impact future work within the Chapter.

CHAPTER GENOTYPING: BACKCROSS SELECTIONS

The original goal of the TACF Chapters' backcross breeding programs was to create a diverse collection of blight-resistant selections representing a minimum of 20 different American chestnut backgrounds (called a "line"). In the PA/NJ Chapter, this was achieved in both the Clapper and Graves sources of resistance, eventually creating over 40 lines between the two sources. Due to recent findings in progeny testing results, we now know that blight-resistance in controlled by many more than 2-3 genes, making finding the best selections in the backcross program more difficult than originally hypothesized.

This genotyping work offer three primary objectives: 1) to quantify the percentage of American genome inheritance; 2) prediction of resistance of a given individual to Phytophthora root rot (PRR); and 3) to quantify genetic diversity (see "Landscape Genomics" section below). By comparing these three metrics within remaining backcross selections, TACF science staff can help Chapter leaders better distinguish the best trees in the backcross breeding program.

After assessing each individual tree for phenotype (i.e. giving them a blight-resistance rating by looking at inoculated cankers and other criteria), trees which were inoculated more than 2 years ago and which have traits indicative of blight resistance will be sent for genotyping. To date, about 700 backcross trees from across all Chapters have been genotyped with another 700 trees in the pipeline. Current results suggest that that at least half of these advanced backcross trees have more than 95% American chestnut ancestry.

In some cases, a given line may not have any selected trees, i.e. all trees within that American chestnut background will exhibit inferior blight resistance. Those trees represent an important aspect of TACFs plans toward species restoration: genetic diversity. Trees from those lines will therefore be crossed with F_1s to create BC_1s to increase blight-resistance in that line (and then used in additional breeding), and/or they may be bred with transgenic trees to further diversity that population.

CHAPTER GENOTYPING: TIMBER PROGRAM

In the early 2000s, the late Dave Armstrong and Dr. Bob Leffel embarked on a breeding program to create a "timber-type chestnut". This led to the creation of an F1 orchard at Codorus State Park in 2005, followed by an F2 orchard started there in 2010. While these trees do not follow a course to restore the American chestnut, they could hold the key to better understanding the inheritance of blight-resistance, as well as tree form/architecture in American, Chinese, and Japanese chestnuts.

Friends of Codorus State Park help take diameter measurements of a 15-year old F1 hybrid. A crew of 12 people spent 8 hours measuring height, diameter, and other metrics on over 300 F1 and F2 trees in mid-August. -Photo by Betsy Murtha

This summer, Chapter volunteers have visited the orchards at Codorus State Park and logged dozens of hours collecting leaves for genotyping, inoculating the F2 trees, and phenotyping both the F1 and F2 trees for tree form and architecture. Further work will take place this fall with additional canker phenotyping, followed by intensive selection and rogueing in 2021.

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LANDSCAPE GENOMICS: GENOME-INFORMED GERMPLASM CONSERVATION

Based on their data analysis in 2016[1], Dr. Harmony Dalgleish and others estimated that some 431 million American chestnut stems persist across the eastern United States, of which 84% are less than 1" diameter at breast height (DBH). Given that TACF estimates we need some 1000 American chestnut backgrounds (or lines) to properly restore a diverse population of trees, how does one prioritize which trees of those 431 million will be used for diversification? In some locations, one can go to a single clear-cut to find 1000 trees, but that single population is unlikely to hold enough genetic variety for proper species restoration.

Most research suggests that a majority of diversity for the American chestnut lies within the most southern portion of the range. During the last ice age, the American chestnut, along with many other native species in the eastern US, sought refuge in the southeastern US. Then, as the glaciers receded, trees spread northward. Most diversity estimates to date have been on relatively small samples of American chestnuts and looked primarily at non-coding regions of DNA. The landscape genomics project aims to give a more holistic analysis of species diversity using a sample of at least 300 trees from across the range.

To date, 192 samples of American chestnut have been sequenced, with 40 million SNPs detected (1 SNP for every 20 bases)[2]. Almost twice as many trees still await sequencing. Volunteers for the PA/NJ Chapter have collected leaves of American chestnuts from the sandy coasts of NJ to the high Allegheny Plateau in PA for this work to be possible. Analysis is estimated to take at least two years. Once those results are available, we can finally know how different a given population is from another and will thus guide future conservation of American chestnut germplasm for diversification of disease-resistant populations.

CASTANEA GENOME REFERENCE PANELS

Most recently, TACF has been investigating ways to create genomic signatures for the major species within the *Castanea* genus. Beginning in the spring of 2020, samples from 20 individual trees were collected and shipped for sequencing in order to discover informative markers. Once these reference panels for the wild-type species can be created, they can then be used for two primary purposes: 1) to better detect ancestry make-up in complex hybrids; and 2) to find disease-resistance genes which may be present in resistant trees but absent from susceptible ones (a way to detect candidate genes for resistance).

To date, almost all of TACFs identification work for wild-type American chestnuts has been performed by morphological analysis on leaf and stem characteristics. While this is usually sufficient, complex hybrids, —

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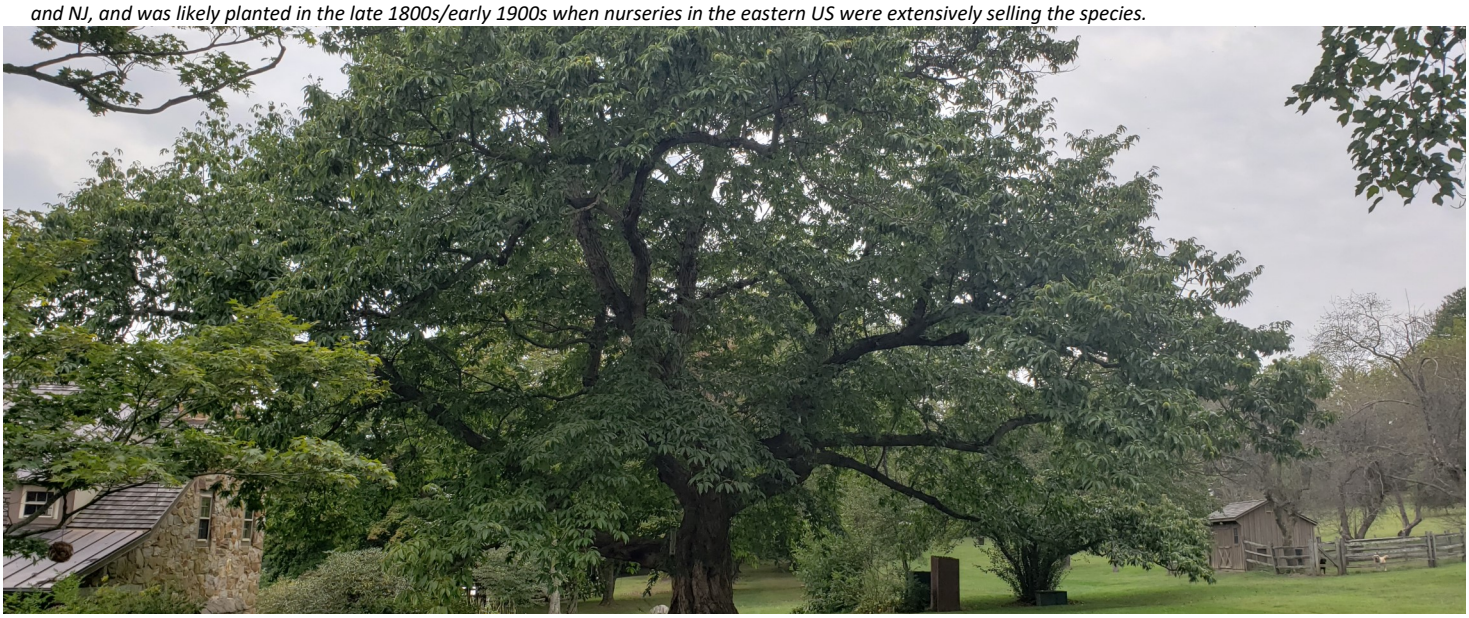
[1] Dalgleish, H.J.; Nelson, C.D.; Scrivani, J.A.; Jacobs, D.F. Consequences of Shifts in Abundance and Distribution of American Chestnut for Restoration of a Foundation Forest Tree. *Forests* **2016**, *7*, 4.

[2] Pronounced "snip", SNP stands for "single nucleotide polymorphism". While much of the genetic code in individuals of a given species are the same, SNPs represent small differences (a single nucleotide) that are present in a small proportion of a given population but not others, and can allow analysts to pinpoint important differences both within and between given populations within a species.

Continued on page 7

This Japanese chestnut is on the grounds of a longtime TACF member and supporter. The spreading form is similar to many other Japanese chestnut found across PA and NJ, and was likely planted in the late 1800s/early 1900s when nurseries in the eastern US were extensively selling the species.



Progress Report on Small Stem Assays at PSU Orchard

By Stephen Hoy, PSU Orchard Manager

2020 is a milestone for me as the chestnut orchard manager. My first field season in 2014 began with seedling plantings in the arboretum. Those first trees were large enough to inoculate in 2019 and now in 2020 the trees have been rated and rogued, by 2023 these trees should be inoculated with EP155 and ready for another round of rating and rogueing.

Steve Hoy evaluating SSA in PSU shade house.

2020 marks the first year the PA/NJ Chapter has utilized the Small Stem Assay method to prescreen B3F2 lines that in a normal year would have been planted in the arboretum orchard plots of 150 individuals. Complications prevented the annual spring planting at the Arboretum chestnut orchards as well as several other events, however, we found this to be an opportunity to trial the small stem assay with the seedlings that would have been transplanted. The 4 groups of B3F2 seedlings were kept in pots, inoculated in June with the SG2,3 strain of chestnut blight (*Cryphonectria parasitica*) and monitored for wilt. As seedlings succumbed to the infection they were removed. At the end of the growing season the expectation is to reduce the number of individuals in each of the 4 groups by 50% (from 150 down to +/-75). Reducing the population will allow the seedlings to be planted at a wider spacing (seedlings are usually planted at 1 foot spacing within rows) reducing stress from competition and improving annual growth.

Penn State Graduate and summer intern Sean Freidhof preparing small stem assays in the shade house this summer.

The 4 lines included in the SSAs are some of the last lines to be represented in the arboretum orchards, bringing the chapter a step closer to completing the planting portion in the B3F2 orchard. In 2020 we were able to inoculate 7 plots with the SG2,3 strain, perform ratings on 17 plots, removed more than 500 stems with inferior levels of blight resistance, and are now making preparations for the harvest season at the end of September.

A B3F2 seedling from ku00-08-14 inoculated with SG2,3 of chestnut blight showing elevated blight resistance.

A B3F2 seedling from ku00-08-14 inoculated with SG2,3 of chestnut blight showing low levels of resistance.

A B3F2 seedling from ku00-13-1 inoculated with SG2,3 of chestnut blight showing elevated blight resistance.

A B3F2 seedling from ku00-13-1 inoculated with SG2,3 of chestnut blight showing low levels of resistance.

ACTION ALERT:
Please Voice Your Support to the USDA for Saving the American Chestnut Tree!

The American Chestnut Foundation (TACF) and the State University of New York College of Environmental Science and Forestry (ESF) have been leading an unprecedented mission to restore the American chestnut tree to its native range. Researchers at ESF have developed Darling 58, a transgenic American chestnut tree with enhanced blight tolerance and have submitted a petition to the USDA's office of Animal and Plant Health Inspection Service (USDA-APHIS) to clear the way for restoration planting programs.

The public comment period, an important step in the USDA-APHIS review, is now open through October 19, 2020. Please submit a comment to help demonstrate your support for the Darling 58 so together we can save the beloved American chestnut tree. If approved by USDA-APHIS, Darling 58 can be planted for general use in restoration programs.

For Resources and More:

Visit TACF's Public Comment Period webpage to review helpful resources prior to submitting your comment:
www.acf.org/science-strategies/biotechnology/documents-for-public-comment-period/

Check out the recording of September 4th, TACF Chestnut Chat on the Public Comment Period by visiting:
https://www.acf.org/resources/chestnut-chat-series/

You may submit comments by either of the following methods:

- *Federal eRulemaking Portal:* Go to *http://www.regulations.gov/#!docketDetail;D=APHIS-2020-0030*.
- *Postal Mail/Commercial Delivery:* Send your comment to Docket No. APHIS-2020-0030, Regulatory Analysis and Development, PPD, APHIS, Station 3A-03.8, 4700 River Road Unit 118, Riverdale, MD 20737-1238.

Chestnut Genomics — Continued from page 5

especially those between *Castanea pumila* (the Allegheny chinquapin) and *Castanea dentata* (the date, almost all of TACFs identification work for wild-type American chestnuts has been performed by morphological analysis on leaf and stem characteristics. While this is usually sufficient, complex hybrids, especially those between *Castanea pumila* (the Allegheny chinquapin) and *Castanea dentata* (the American chestnut) are particularly difficult, and sometimes impossible, to detect morphologically. The *Castanea* reference genomes would allow for a deeper investigation into putative hybrids, detecting introgression which would be indistinguishable by phenotypic analysis alone.

Of particular interest is that Japanese chestnuts, *Castanea crenata*, were particularly difficult to find with the initial call for samples. Once members of the PA/NJ Chapter were informed of the need for trees this fall, more than 20 trees were found in the Chapter's holdings, and volunteers acted quickly to collect and ship samples for sequencing and analysis.

MANY THANKS

None of these projects would be possible without the extraordinary efforts of PA/NJ volunteers to find, document, and collect materials from trees. The Chapter continues to be unique both in the variety and overall number of holdings, offering incredible value to each of the studies detailed in this article.

The Chestnut Newsletter

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RETURN SERVICE REQUESTED

SOME IMPORTANT DATES

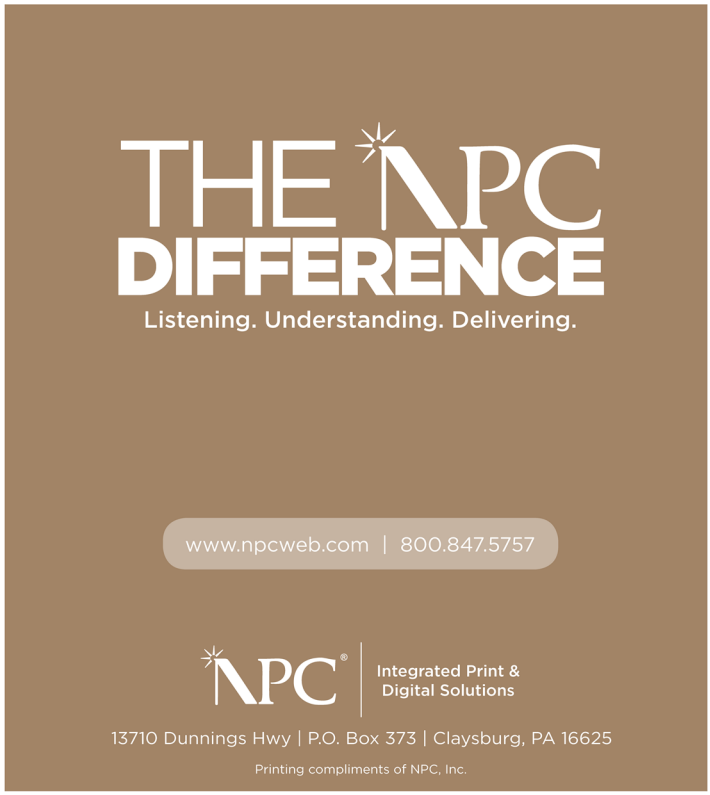
October 4th — Walk In Penn's Woods
Virtual Chestnut Hike / Allegheny National Forest

November 14th — Fall Member Brown Bag Webinar
Zoom

January — Virtual PA Farm Show
Details Coming Soon

Upcoming TACF Chestnut Chats
https://www.acf.org/resources/chestnut-chat-series/
October 2:
Pests and Pathogens: Erin Lizotte, MSU
October 16:
Chestnut Cooking
October 30:
GWAS 101: David Kainer, Oak Ridge National Laboratory
November 13:
Cloning and Embryogenesis: Scott Merkle, UGA

Visit our website for updates and more information about these and other activities of the PA/NJ Chapter.
www.patacf.org



The PA/NJ Chapter of TACF is grateful for the continued support of NPC through their pro bono printing of this newsletter. NPC has freely printed every issue of The Chestnut Tree.