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The Chinese Chestnut Genome V2.0

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Chinese chestnut (*Castanea mollissima*) genotypes are serving as the source of genes for developing resistance to chestnut blight fungus (*Cryphonectria parasitica*) in American chestnut. We sequenced the genome of Chinese chestnut, to gain a better understanding of the genetic basis of blight resistance and to provide tools to assist in selecting for the blight resistance QTL and against the C. mollissima genetic background, in The American Chestnut Foundation's backcross breeding program. A draft genome (v.1.1) covering app. 90% of the genome of TACF's Chinese chestnut cultivar 'Vanuxem' was released to the public in January, 2014 (www.hardwoodgenomics.org website). In addition, recombinant DNA clones covering the three major blight resistance QTL in the Vanuxem cv. were sequenced to great depth. Hundreds of genes were identified in the resistance QTLs, including 15 known "defense response" genes. The v.1.1 draft genome has served as reference for genome-wide genetic diversity studies among and within chestnut species and for preliminary studies on the progress of backcross breeding at the BC3 generation. We will report progress towards a new version (v.2.0) of the Chinese chestnut genome featuring chromosome-scale sequences, taking 2 approaches. In one approach, very long sequence reads from the PACBio technology were used to produce longer genome fragments, of which over four thousand were placed in proper order along the 12 genetic linkage groups. In a new genome assembly approach, the Dovetail Genomics company is using chromatin-interaction sequence data to assemble more continuous sequence for each of the 12 chromosome pairs in the Mahogany chestnut genotype. This project was supported by The Forest Health Initiative (www.foresthealthinitiative.org), The American Chestnut Foundation, the USDA AFRI program, and The Schatz Center for Tree Molecular Genetics at Penn State University.

Evaluation of an Alternative Small Stem Assay for Blight Resistance

Cipollini, M, L[1]; Moss, JP[1]; Walker, W[1]; Bailey, N[1]; 2018.
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We tested an alternative "small stem assay" (SSA) for blight resistance in chestnut (*Castanea* spp.) seedlings. Whereas most SSAs are done by inoculating vertical incisions in the stem, our assays were done by cutting off ~4.5-5 mm diameter stems, inoculating the cut ends with 5 mm disks of *Cryphonectria parasitica* inoculum, and covering them with plastic sleeves. This alternative SSA was designed to be relatively rapid and reproducible, and to allow seedlings sufficient time to develop lower stem shoots (standard SSAs delay removal of blighted stems until after canker evaluations). We conducted two assays with seedlings of *Castanea dentata* (susceptible), *C. mollissima* (resistant), and hybrids expected to harbor good blight resistance. In the first assay, started on June 27, canker lengths at 21 and 34 days after inoculation clearly distinguished *C. dentata* and *C. mollissima* lines (virtually no overlap), with hybrid progeny overlapping considerably with *C. mollissima*. Seedlings quickly formed new shoots from the lower stems. The second assay, started on July 31, showed no significant difference among lines at 21 and 34 days after inoculation, but was compromised by a contaminant discovered inhibiting growth of the blight fungus on an agar plate; we suspect that inadvertent spread of this contaminant contributed to variation in canker growth. Relative to the first assay, this set showed reduced regrowth, supporting the notion that cutting stems early in the season may result in seedlings better prepared for fall out-planting. Our conclusion is that this technique should be applicable to seedlings that have woody stems 4.5-5 mm in diameter at a point above the first leaf node by the end of June. Results of tests on stems of two adult *C. dentata*, suggest that this technique might also be useful in blight-testing saplings or adults using side branches, rather than inoculating the main stem.

Georgia offers unique opportunities to incorporate greater genetic diversity in *Castanea* breeding programs

Cipollini, M.L.[1]; Metaxas, A.M.[2]; Klaus, J.[3]; Klaus, N.[4]; Moss, JP.[1]; Walker, W.[1]; Bailey, N.[1] 2018.
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Since 2005, GA-TACF has mapped living *Castanea dentata* trees in Georgia. Most reports have come from volunteers and scientists via Tree Locator forms, and a number of recent reports have come via Tree Snap App uploads. GA-TACF currently lists ~200 *C. dentata* (confirmed by morphological analysis) in 40/159 counties. In 2018, leaf samples were collected from *C. dentata* for DNA analysis, as part of a landscape genomic analysis carried out by TACF and its state chapters in collaboration with Virginia Tech. Of ~1,000 trees to be sampled, approximately 50 originated from Georgia. The southernmost trees sampled were on Pine Mountain, Harris County with the northeastern-most and northwestern-most trees in Rabun County and Dade County, respectively. We compare the distribution of currently known and sampled locations with a map of the current distribution of *C. dentata* based on USFS-FIA data, and with maps derived from early 1800's witness tree records. This comparison will highlight locations under-represented in the TACF database and the genomic analysis. Georgia represents the southernmost range of *C. dentata* and includes known genetic variation not found elsewhere. Southern-climate- and soil-adapted genotypes may be particularly useful to TACF's breeding program if the range of *C. dentata* shifts northward, as is predicted by climate change models. Georgia is expected to experience shifts in five major ecosystem types, not all of which are currently thought to support chestnut growth. Species-habitat and genomic-based models may differ if more chestnuts can be included from underrepresented locations where they occurred historically. We suggest that the search for surviving trees ought to focus more on the Piedmont and Coastal Plain, where *Phytophthora* root rot likely decimated *C. dentata* prior to the onset of blight. Crucially, surveys of this nature are time-sensitive, because *C. dentata* individuals have a high likelihood of impermanence due to diseases and pests.

First and second year survivorship of large-scale American chestnut planting driven by soil moisture

Culver, L[1]; Saielli, T.[2]; Madritch, M.[1] 2018.
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The American Chestnut Foundation's breeding program is focused on developing blight resistant *Castanea dentata* hybrids. However, it is unknown how specific crosses will respond under different planting regimes that simulate large-scale reforestation efforts. We planted 636 trees from 13 different crosses under two different site treatments: old-field and forest understory. Plantings were conducted at W. Kerr Scott Dam and Reservoir in Wilkesboro, North Carolina. During the first two years of growth we tracked seedling survivorship, soil moisture content, photosynthetically active radiation, and leaf nitrogen content. The effects of cross type on survivorship were minimal. However, after two years seedlings planted in the old-field treatment had significantly higher survivorship (~75%) than did those seedlings planted in the forest treatment (~50%). The difference in survivorship was likely driven by water stress in the forest treatment, as reflected by differences in soil moisture content between the two treatments. The combination of relatively dry summers with the water demands of the forest overstory likely led to increased mortality of seedlings regardless of cross identity, highlighting the importance of soil moisture during the first year of growth.

Accelerated, graft-based, germplasm conservation targeting under-sampled and genetically diverse American chestnut populations allows rapid introduction of rare adaptive alleles into TACF breeding program

Deason, T.[1] and Craddock, J.H.[1] 2018.
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The American chestnut, *Castanea dentata*, has been devastated by the exotic invasive pathogens *Cryphonectria parasitica* and *Phytophthora cinnamom*i to which it has no resistance. The American Chestnut Foundation (TACF) has developed an interspecific backcross breeding program to introgress disease resistance from Asian chestnut species, primarily *Castanea mollissima*, into *C. dentata* populations. The genetic base of this program must be expanded to be successful in the long run. Southern populations harbor greater genetic diversity and possibly rare alleles. Conservation of these diverse populations will widen the genetic base of the TACF breeding program and strengthen efforts to restore the species. We propose vegetative propagation through grafting in order to collect and conserve southern populations of *C. dentata*. We have located and collected scionwood from 33 American chestnuts (19 of which are new to the breeding program) across 9 sites in Tennessee and Alabama. Four types of rootstocks (*C. dentata, C. mollissima*, and F1 and B3F2 hybrids) were chosen to account for possible graft incompatibility, although compatibility was not measured in this study. The whip-and-tongue and bark-flap grafting techniques were used depending on scion-rootstock diameter. Fourteen of the 33 individuals were grafted to 155 rootstocks, and 40 grafted plants have survived the first growing season. These container-grown grafted plants will be conserved *ex situ* in a nursery where, released from competition for light, they can produce flowers. To date, one individual has produced male flowers three months after grafting and its pollen is in cold storage for use next year. The eventual production of female flowers from these novel Southern sources will enable us to capture cytoplasmic genes as well.

Comparisons in drought response between American chestnut, Chinese chestnut, and assorted BC3F3 hybrids

Fredericksen, B, W[1]; Rosenthal, D, M[1]; 2018.
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Physiological responses to stresses other than blight is a notable knowledge gap in chestnut reintroduction efforts. To address this gap, we tested if BC3F3 hybrid chestnuts performed similarly to pure American chestnuts under a drought stress and tested their ability to respond to drought through osmotic adjustment. Several studies have speculated on chestnut's capacity for osmotic adjustment as a drought tolerance strategy but only one directly tested for it with a negative result. Here we subjected American chestnut, Chinese chestnut, and BC3F3 hybrids from varying families to multiple drought cycles over a growing season. We measured gas exchange rates to determine the magnitude of drought stress and estimated osmotic adjustment by measuring calculating osmotic potential and turgor loss points (wilting point) from cellular solute concentration at full saturation.

Results show that American chestnuts maintain higher rates of photosynthesis and stomatal conductance both in non-droughted controls and under drought compared to Chinese chestnuts. Hybrids showed intermediate gas exchange rates under control conditions but surprisingly, showed the lowest gas exchange rates under drought. Preliminary results suggest that Chinese, American, and hybrid chestnuts all use osmotic adjustment under drought stress, which is indicated by a positive relationship between predawn leaf water potential and estimated turgor loss point ($p\text{-value}<0.001$, $R2 = 0.30$). In the control group, Chinese chestnut maintained lower osmotic potential compared to pure Americans ($p\text{-value}<0.05$), while hybrids showed an intermediate response not statistically differing from Chinese or Americans. More data is needed to quantify species' specific capacities for osmotic adjustment under drought. In summary, pure Americans had greater gas exchange rates under drought than both hybrids and Chinese. Hybrids showed surprisingly low rates of gas exchange. This study marks the first evidence for osmotic adjustment as a drought tolerance strategy in American and hybrid chestnuts.

Predictive Ecology: Simulating long-term changes in forest populations, with applications for chestnut reintroduction

Hammond, S. T.[1]; Osborne, S. D.[1]; 2018.
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An emerging goal of ecological theory is to have accurate predictive power with the least number of starting parameters. The predictive accuracy of models across spatial and temporal scales is critically important for ecologists—particularly as research is done on the reintroduction of species. Any planned large-scale reintroduction of resistant American chestnuts to the wild will require detailed understanding of location, introduction method, and interactions with forests that have had nearly 100 years to reach a new equilibrium after the loss to the original, dominant chestnut species.

Here we present proof-of-principle results for a monotypic forest in which computer simulations hindcast complex population dynamics responding to abiotic (fire) influences. The results show that population patterns over hundreds of years are predictive, and provide a framework for examination of multi-species successional changes associated with the chestnuts loss...and future recovery.

Early Screening to Detect Resistance to *Phytophthora cinnamomi* in Backcross Chinese-American Chestnut Hybrids

Hein, K.[1]; Perkins, M.T.[1]; Craddock, J.H.[1] 2018.
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Phytophthora root rot (PRR), caused by the oomycete *Phytophthora cinnamomi*, is one of the greatest obstacles to survival of American chestnut (*Castanea dentata*) in the southeastern United States. Developing early and reliable PRR screening methods can facilitate the efficient introgression of PRR resistance from Chinese chestnut (*C. mollissima*) into the populations of blight-resistant trees under development by The American Chestnut Foundation (TACF) and collaborators. We tested the efficacy of a method for early identification of PRR-resistant hybrid chestnuts in a greenhouse/nursery setting. During the summer of 2016 and 2017, container-grown seedlings were inoculated with *P. cinnamomi*, prepared on a clarified V8 agar medium, and rice-grain or vermiculite inoculum. Root necrotic lesions were rated in the first winter after inoculation using the symptom severity scale of Jeffers et al. (2009), where "0" represents an asymptomatic plant, "1" corresponds to lesions on the lateral roots only, "2" corresponds to lesions on the lateral and on tap roots, and "3" corresponds to plant death caused by severe root rot. Results of 2017 were inconclusive because of inoculum failure and winter freeze damage to roots that confounded symptom severity rating. Results of the 2016 trial showed significant differences in the average root rating between *C. mollissima* and *C. dentata*, as expected. Average symptom severity ratings of six of the backcross families were not significantly different from that of the PRR-resistant Chinese chestnut controls. Families that were interpreted to have any degree of PRR resistance are assumed to have inherited PRR resistance alleles from *C. mollissima*. Trees identified as PRR-resistant have been transplanted into orchard settings for further observation, and represent a population of individuals that will be utilized for future breeding and restoration efforts.

Development of an *in vitro* screen for *Phytophthora cinnamomi* resistance in hybrid and transgenic chestnut trees

Merkle, S.A.[1]; MacKnight, M.G. [1]; Gladfelter, H.J. [1]; Jeffers, S.N.[2]; 2018.
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The lack of resistance to *Phytophthora cinnamomi*, an oomycete pathogen that causes Phytophthora root rot disease in American chestnut (*Castanea dentata*), may pose a major barrier to introducing the products of The American Chestnut Foundation's (TACF) breeding program to the southern portion of the American chestnut's original range. The integration of genes for *P. cinnamomi* resistance from Chinese chestnut (*Castanea mollissima*) into TACF's hybrid backcross chestnuts is now proceeding. Combining somatic embryogenesis-based propagation of chestnuts with a reliable *in vitro* assay for resistance to *P. cinnamomi* would help to more rapidly evaluate hybrid backcross chestnut clones thought to carry resistance genes, accelerating the production of elite chestnut varieties with resistance to both chestnut blight (*Cryphonectria parasitica*) and *P. cinnamomi*. Our goal was to define a quantitative *in vitro* screening approach that could be applied to identify *P. cinnamomi*-resistant hybrid backcross chestnuts (B3 and BC3F3), and to test transgenic American chestnuts carrying the candidate *P. cinnamomi* resistance genes *RPH* and *NPR3/4*, using pure American chestnuts and Chinese chestnuts as susceptible and resistant controls, respectively. Clones were screened using micropropagated shoots "planted" into agar gel and inoculated with a locally-collected isolate of *P. cinnamomi*. In three experiments, the expansion rate of a necrotic lesion from the base of the shoot to the tip was used to compare resistance among the different chestnut genotypes. Within 30 days of inoculation, Chinese chestnuts showed significantly shorter stem lesions compared to pure American chestnuts and hybrid backcross chestnuts. In addition, it appeared that some of the hybrid backcross chestnuts displayed intermediate resistance between the susceptible American chestnut and Chinese chestnut genotypes tested, as might be expected, although others were no more resistant than pure American chestnuts. None of the tested transgenic chestnut shoots showed evidence of enhanced resistance compared to pure American chestnut.

A small stem assay for early detection of blight resistance in full-sib F2 hybrid chestnut families

Miller, M.J. [1]; Craddock, J.H. [1] 2018.
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Restoration efforts of the American chestnut began in the 1920s with attempts to introduce blight resistance into the American chestnut species by hybridizing it with the Asian species. In 1981 Charles Burnham hypothesized that three generations of backcrossing and selection for blight resistance would be sufficient to recover trees with American chestnut morphology and Asian chestnut levels of disease resistance. The American Chestnut Foundation was founded in 1983 to test Burnham's proposal. The TACF state chapters that are currently participating the breeding program have been challenged by the difficulty and long time periods required to screen hybrid seedling progeny for blight resistance – a process that can take from to 5 to 7 years and requires large commitments of land and other resources. Small stem assays offer an attractive alternative in that they can be completed in one or two years, using container-grown plants in a greenhouse or nursery setting. We report here the results of the inoculation of 600 chestnut seedlings during their first growing season. We used two full sibling F2 hybrid families derived from 'Nanking' Chinese, and open pollinated seedlings of *Castanea dentata* and *C. mollissima* as controls in a randomized complete block design. All seedlings were inoculated with *Cryphonectria parasitica* strain EP155 in early summer when stem diameters were greater than 5mm. Seedling survival and canker lengths were measured eight weeks post inoculation. The American and Chinese exhibited significant differences in resistance. The F2s displayed a full range of resistance from highly resistant to low resistance.

Environmental Interactions with Transgenic American Chestnuts

Newhouse, A.E.[1]; Powell W.A.[1]; with gracious acknowledgement to many research collaborators. 2018.
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'Darling 58' transgenic American chestnuts have shown significantly enhanced tolerance to the chestnut blight fungus, and will soon be evaluated by US federal regulatory agencies before potential public distribution. The oxalate oxidase enzyme expressed in 'Darling' transgenic American chestnuts is naturally found in many food crops and wild plants, and it protects the tree by degrading a toxin rather than by killing the fungus, so it is unlikely to present novel risks when expressed in American chestnut. However, it is valuable for regulators and prudent for environmental scientists to carefully evaluate potential environmental risks before deploying new restoration material. This poster summarizes several comparative risk assessments of environmental interactions with Darling transgenic American chestnuts compared to non-transgenic controls.

Starting with interactions at or below ground level, assessments included colonization of chestnut roots by mycorrhizal fungi, potential effects of chestnut leaves on seed germination from nearby native plants, persistence of transgene activity in drying leaves, leaf litter decomposition rates, and effects of chestnut leaf litter on amphibians in vernal pools. Moving up the tree, additional experiments evaluated insect herbivory of chestnut leaves, bumblebee use of chestnut pollen, transgene inheritance by chestnut seedling offspring, and growth rates of those offspring. The data from these interaction experiments overwhelmingly support a conclusion that OxO-expressing Darling American chestnuts are not noticeably different from wild-type or traditionally-bred controls, apart from their tolerance to chestnut blight. Some of these tests showed differences between non-transgenic American and Chinese chestnuts, but in experiments where backcross chestnuts were included, they also did not generally show significant differences from American chestnuts.

American Chestnut Reintroduction in Kentucky; a GIS Approach

Pease, J, R[1]. 2018.
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To address optimization of American chestnut (*Castanea dentata*) reintroduction efforts and efficient use of resources in the reintroduction process, we will conduct a large-scale project focusing on Kentucky's public lands and site suitability. We will derive site suitability criteria from literature to predict chestnut densities in both Land Between the Lakes National Recreation Area (LBL) and Daniel Boone National Forest (DBNF). Both of which are locations in Kentucky but with different geological conditions. Determining areas where chestnuts are most prevalent on these landscapes should detail locations where reintroduced chestnuts will have greater chances of survival. Two papers, one specifying site characteristics in Mammoth Cave National Park (KY) and the other in the national capital region, will be analyzed for their site characteristics. Suitability maps based on those criteria will be ground-truthed in LBL and DBNF to determine accuracy in those specific locales and adjusted for managers in each area if necessary. It is our hope that these maps and criteria can be used to more efficiently utilize saplings and seedlings to expedite large-scale reintroduction projects across vast landscapes. By adding to the framework that will allow land managers across *C. dentata's* range to reintroduce chestnuts with higher survival rates: it is our hope that American chestnuts can reclaim their place in Appalachian forests sooner rather than later.

Morphological and DNA evidence support recognition of Alabama chinquapin, *Castanea pumila* var. *alabamensis*

Perkins, M.T.[1]; Zhebentyayeva, T.N.[2]; Sisco, P.H.[3]; Craddock, J.H.[1]. 2018. [1]Department of Biology, Geology, and Environmental Science, The University of Tennessee at Chattanooga, 350H Holt Hall, Chattanooga, TN; [2]The Schatz Center for Tree Molecular Genetics, Department of Ecosystem Science and Management, The Pennsylvania State University, 322 Forest Resources Building, University Park, PA; [3]The American Chestnut Foundation (Carolinas Chapter), 85 Stoney Hill Court, Asheville, NC

Characterizing the genetic diversity of species within the North American *Castanea* is critical to restoration of the American chestnut because such efforts provide clues regarding past patterns of hybridization between American chestnut and its sister species, North American chinquapin (*C. pumila*), as well as information regarding the partitioning of genetic diversity within the genus. Historical reports based on morphology and recent studies involving small numbers of molecular markers have posited that natural hybridization between American chestnut and Allegheny chinquapin has produced phenotypically intermediate populations in the Southeast. These reports have resulted in description of multiple putative hybrid taxa, including *Castanea alabamensis*, a taxon from northern Alabama. We investigated the hypothesized hybrid origin of *Castanea alabamensis* by performing sequence-based genotyping of plants representing American chestnut, Allegheny chinquapin, Ozark chinquapin, Japanese chestnut, Chinese chestnut, and several populations matching the taxonomic description of *C. alabamensis*. Alignment of Illumina reads to the Chinese chestnut reference genome v1.1 yielded 190,656 single nucleotide polymorphism (SNP) loci for analyses. Phylogenetic analysis indicated that *C. alabamensis* samples cluster in a group that is sister to Ozark chinquapin within the broader chinquapin clade. Bayesian analyses of population structure provided evidence of limited hybridization between American chestnut and Allegheny chinquapin and extensive admixture among different chinquapin varieties; however, *C. alabamensis* exhibited no signature of American chestnut ancestry. Principal components analysis revealed the existence of four genetic clusters in our North American samples: American chestnut, Allegheny chinquapin, Ozark chinquapin, and *C. alabamensis*. Our results do not support the hybridization hypothesis, but instead suggest that *C. alabamensis* is a distinct variety of chinquapin, and we therefore refer to it as *C. pumila* var. *alabamensis*. The results of our study demonstrate the potential of high-throughput sequencing to uncover and characterize cryptic diversity in the North American *Castanea* species.

Key words: genetic diversity, hybridization, chinquapin, *Castanea pumila*

Long-Term Freezer Storage of American Chestnut Pollen

Pilkey, H. C.[1]; Coffey, V.[1]; Matthews, D. F.[1]; Oakes, A. D.[1]; Powell, W. A.[1] 2018.
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Outcrossing transgenic American chestnut trees with wild-type mother trees provides the opportunity to rescue remaining genetic diversity and rare alleles, while producing a blight-tolerant population. The production of transgenic pollen is essential to performing controlled pollinations each year. Currently, floral induction using a high-light treatment occurs indoors and throughout the year. Additionally, seedlings and juvenile tissue-culture produce relatively few catkins. Therefore, transgenic pollen is limited and often must be stored for months until use. It is imperative that transgenic pollen is properly collected, desiccated, and freezer stored to preserve viability for outcrosses. A long-term storage experiment was conducted to determine treatment effect on pollen viability and to develop an effective storage protocol for pollen collection. Two storage containers (20 ml scintillation vials and slide boxes), three desiccation times at 4°C (4, 24, and 48 hours), presence and absence of a desiccation pellet during freezer storage, and two freezer temperatures (-20°C and -80°C) were tested. After 8 months of storage, an in-vitro germination analysis was done to observe pollen tubes and determine pollen viability of the stored samples. Results showed that pollen should be collected and stored within a 20 ml scintillation vial, desiccated for 24 hours, and stored in a -80°C freezer to retain 46.43% viable pollen grains. It was also found that tree genotype and collection date had a significant effect on pollen viability. With these results, we hope to improve future outcross yields.

Chestnut Breeding for Resistance to *Cryphonectria parasitica* and *Phytophthora cinnamomi* at UTC

Smith, W.S.[1] and Craddock, J.H.[1] 2018.
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The American chestnut tree (*Castanea dentata* Borkh.) was once one of the dominant tree species of deciduous forests of the eastern United States. *C. dentata* was an important source of lumber, food, and wildlife fodder. There are two main pathogens affecting *C. dentata*: the ascomycete *Cryphonectria parasitica* and the oomycete *Phytophthora cinnamomi*. At UTC we are mainly focused on backcross breeding to introgress genes for disease resistance from the Asian chestnut species into *C. dentata* in order to get trees that are morphologically identical to American chestnuts, but express the disease resistance found in the Chinese and Japanese species. We have recently begun to explore alternatives to the traditional screening methods that involve large, long-term field trials to identify resistant germplasm. By growing seedlings in containers at UTC's greenhouse we can screen for both *C. parasitica* and *P. cinnamomi* resistance in one or two years. In the Small Stem Assay, young container-grown trees are inoculated with *C. parasitica* in the summer of their first year and selection is completed by the end of the growing season. Similar trials have allowed us to discover novel sources of resistance to *P. cinnamomi*. We are actively searching for new *C. dentata* germplasm from wild specimens to be used in the breeding program. Developing American chestnut populations that are resistant to *C. parasitica* and *P. cinnamomi* will allow natural selection to resume for this valuable tree species that is now functionally extinct. This, in turn, will increase biodiversity in our forests, increase food for wildlife, allow cultivation for nut production, and allow the harvest of high quality timber.

Spatial Habitat Modeling of American chestnut (*Castanea dentata*) and Distribution in the Southeast United States

Taylor, E.[1]; Deason, T.[1]; Craddock, J.H.[1] 2018.
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The American chestnut (*Castanea dentata*) was once a vital component of forests in the eastern U.S., but it was destroyed by the chestnut blight and root rot in the early 20th century. The American Chestnut Foundation (TACF) is working to bring the chestnut back into the forest by a combination of classical plant breeding and state-of-the-art genetic engineering programs. Critical to the success of the TACF mission is a diverse population of regionally adapted trees. There is concern within the chestnut research community that the effective population size in the current breeding populations may not be large enough to allow for species recovery. It is therefore an urgent priority to locate, and capture as a large portion of the existing germplasm as is practically possible. To this end, our project works to locate areas of extant *C. dentata* populations that may harbor alleles for adaptive characteristics not already incorporated in the TACF programs. Characterization of *C. dentata* habitat at the fine scale will also allow predictions of locations for future re-introductions of disease resistant genotypes into areas where *C. dentata* has been extirpated. The Maximum Entropy (Maxent) and Genetic Algorithm for Rule-set Production (GARP) models will be used in predicting where *C. dentata* currently is distributed and where suitable habitats are in order to find previously unknown *C. dentata* specimens. The accuracy of these maps is currently unknown, and should only serve as a jumping off point for future projects and studies relating to *C. dentata.*

Functional genomics analyses of the resistance/ susceptible response in chestnut seedlings to *Phytophthora cinnamomi* infection

Zhebentyayeva, T.N.[1,2]; Noorai, R.E.[2], Gitto, A.J.[3]; Jeffers, S.N.[3]; Sisco, P.H,[4]; Perkins, M.T.[5]; Craddock, J.H.[5]; Saski, C.A.[2]; Carlson, J.E.[1]; Nelson, C.D.6,[6,7]; Abbott, A.G.[7]. 2018.

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Due to availability of resistant Asian and susceptible American and European chestnut species, *Castanea* is ideally suited for studying plant-*Phytophthora cinnamomi* (*Pc*) interactions. In previous years, we employed a genetic approach to map resistance to *Pc* in interspecific crosses using resistant Chinese chestnut, *Castanea mollissima* ('Mahogany' and 'Nanking' resistance donors), and susceptible American chestnut, *C. dentata* (multiple parents). Five cohorts for three hybrid crosses (BC1 and BC3) phenotyped for severity of root symptoms in 2013-2014 were genotyped by sequencing and used for linkage map construction /QTL mapping. Altogether 17 QTLs were detected with eight parental maps. Of these, a major QTL signal in the middle of LG_E (qPcE.2) was associated with resistance to *Pc* in all crosses. Using markers anchored to the *C. mollissima* genome v1.0 assembly, we designed a set of 47 SSR markers within the three LG_E QTL intervals most associated with resistance to *Pc*. These markers are available for testing their predictive power in breeding material by TACF.

We initiated a functional genomics study for candidate gene discovery within QTL intervals and analyzed gene expression and metabolic profiles in chestnut roots interacting with *Pc* zoospores. In a pilot experiment we challenged 1-year old NK5 progeny (Nanking-derived F_2 cross) with *Pc* and harvested root tissue

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at three time-points (2, 4 and 8 days) post inoculation. Non-inoculated plants were used as controls along with inoculated Chinese and American chestnut seedlings. Plants were monitored for progression of root rot symptoms and the three most resistant and three most susceptible plants were chosen for RNAseq analysis and metabolite profiling. In total 24 RNAseq datasets (more than 20 mln sequencing reads each) were generated for expression analysis and metabolic pathway analysis. Currently these analyses are in progress and the most recent update will be presented at the meeting.

Key words: *Phytophthora cinnamomi* resistance, RNAseq analysis, metabolic

Notes

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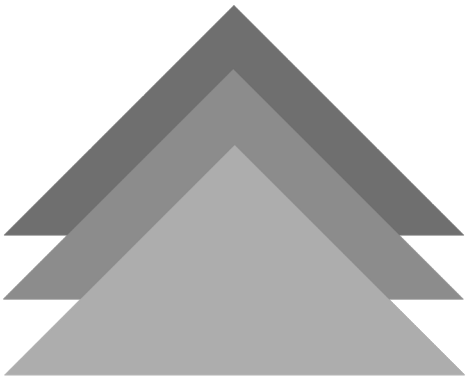
TACF would like to thank these event sponsors for their generous support:



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