

The American Chestnut Foundation

Our Vision

To restore the American Chestnut to its Native Range

Develop trees that will thrive and synergize with other forest flora and be able to adapt to the challenges of an ever-changing environment

Our major challenges to this vision

- The **blight** that devastated nearly all American chestnuts—yes, the blight's still here!
(*Castanea parasitica*---an Ascomycete fungus)
- **Root rot** that is also a major pathogen in areas disturbed by development, particularly in Southern Appalachia. (*Phytophthora cinnamoni*---an oomycete)
- Integration of disease resistance into native **trees that can continue to display American chestnut character and grow sustainably** in their every-changing native habitats
- Development and implementation of a **clear plan for wide-spread restoration** efforts---with estimated timelines
- Secure critical **partners and funds** to support all these efforts

“3-Burr”: Major tools and how we employ them to meet these challenges

- Biotechnology

- Genomics---American and Chinese chestnut (the latter contains genes for blight and root rot resistance)--- with partners, we have sequenced and annotated these genomes to create high quality reference genomes

- Characterize the genetic diversity in existing American chestnuts for use in breeding
- Distinguish between Chinese and American sequences through DNA sequence comparisons
- Develop molecular markers for key genes to support breeding program
- Genome-wide association studies (GWAS) for discovery of approx. 4 major genes in Chinese chestnut genome that confer resistance to root rot. With breeders, attempt to transfer these genes to American chestnut to create root rot resistant trees. We already may have some resistant Americans from traditional breeding, and they can be analyzed to determine what DNA sequences encode these genes. **Note that we don't propose a similar strategy for blight because we have found that too many genes are involved for this approach to work.**

- Transgenic and gene editing technologies 1.0

- Our partners at SUNY have created a transgenic American chestnut with a gene called OXO that is showing promising resistance to chestnut blight. It is now passing through the US regulatory system.
- We aim to create 4 new transgenic founder lines for the OXO gene inserted into 4 genetically diverse native American Chestnuts and will initiate detailed characterization of the best events
- With the help of experts, explore new approaches to enhance efficiency of delivery of DNA/RNA constructs—especially important for the new approach of gene editing
- Identify resistance genes for root rot through use of informatics and knock-outs of candidate genes in Chinese chestnut using gene editing

“3-Burr”, continued

- **Breeding. Our staff and our trained regional chapter volunteers will lead these efforts!**
 - **Preserve genetic diversity** in existing specimen trees in special orchards---with special emphasis on preserving adaptation to climate change
 - Promote use of high light conditions to induce early flowering and **create “fast plants” with short generation times** for breeding and propagation purposes---create a plan involving chapters AND private sector nurseries for large-scale propagation of “fast plants” as we move toward restoration
 - Facilitate **deregulation by US gov’t of the OXO event** in Darling variety of chestnut
 - Conduct **multi-location field trials** for the oxalate oxidase gene (**OXO**) to better quantify its efficacy against blight
 - Assess results of first trials with OXO event in Darling
 - Have chapter volunteers backcross OXO gene into a set of 4 genetically diverse American chestnut trees
 - Use these in standardized multi-location trials; quantify effectiveness of gene
- **Biocontrol**
 - Deploy super donor **hypovirulent strains** of the chestnut blight fungus to keep blight susceptible American chestnut trees healthy for use in breeding with blight tolerant varieties

On the horizon.....

1. **Make breeding easier!!** Make a gradual shift from traditional transgenics to **gene editing** that is potentially faster, more precise and has less regulatory issues. (pdf of review of gene editing attached in email). Improve use of state-of-the-art gene editing tools to create new events that have the OXO gene linked to 3-4 genes for root rot resistance so all genes will segregate together and make breeding much easier.
2. With gene editing, we can upregulate a number of different promoters for multiple “good alleles” for new genes of importance we identify, downregulate bad alleles; we could regulate flowering time/onset of juvenility/control plant architecture----**every day there are more ways to use gene editing to improve plants!**
3. **Better assay for blight resistance!!** Through use of RNA-seq and metabolomics, try to identify a few key biomarkers highly expressed during very early stage of blight or root rot infection. Develop improved resistance assays based upon expression of one or two definitive markers.

On the horizon.....

Collaborate more!! Sponsor a convening event that will bring together groups using molecular approaches to plant disease resistance and adaptation to climate change----with focus on oak, chestnut, beech, maple, ash, poplar. Purpose is to form a consortium that might be funded by large foundations, NSF, USDA Forest Service, DOE, private philanthropists, wood, paper, and tourist industries.

1. Develop collaborations with top-tier, cutting edge molecular **phytopathologists** aiming to develop robust durable resistance to complex traits through use of as few genes as possible. Example: for blight (a canker disease that induces necrosis), try manipulation of key NLP genes (e.g NPP1) and their receptor(s) that are believed to control necroses. Amazingly the same few genes can be effective in fungi, oomycetes and microbes. **(I've also attached a pdf of a brief review of this kind of work and cloning of one such gene in chestnut!!)**
2. Develop collaborations with the few **groups taking genomics approaches in other hardwoods** for addressing resistance to diseases similar to blight (e.g necrotic diseases such as Cercospora in hybrid poplar). And trees such as Eucalyptus are also susceptible to root rot (Phytophthora cinnamoni). Other Phytophthera species cause sudden oak death (P. ramorum), bleeding canker in maple (P. cactorum), and stem rot in soybean (P. sojae). Such tree research groups are found in the US Dept of Forestry labs, private sector, and academia). It seems that there are reference genomes now for hybrid poplar, beech, Chinese and American chestnut, Eucalyptus,
3. Develop collaborations with **forest restoration scientists**; new technologies for hi-throughput propagation of trees; explore value of restoration of mixed hardwood forests; dig deep into the importance of mycorrhizal interactions; other pests and pathogens

Some relevant journal articles