Materials and Methods

Introduction

Despite its location on the edge of the natural range of American chestnut (*Castanea dentata*), Indiana developed one of the earliest American chestnut breeding programs after The American Chestnut Foundation (TACF) began backcrossing hybrids of American chestnut to the resistant Chinese chestnut (*Castanea mollissima*) in the early 1980s. The goal of the program is to develop blight-resistant chestnuts that fulfill the currently empty niche of American chestnut in the central hardwood forest. At several locations around Indiana, third-backcross (BC3) seed orchards of individuals selected for high blight resistance and *dentata*-like features are producing seed. Their offspring, BC3F2 trees, are currently being screened and selected as parents for the BC3F3 generation, which should display consistently high levels of blight resistance and American chestnut-like form. We present a plan for my dissertation research, which will use next-generation DNA sequencing to examine the genetic basis of blight resistance in Indiana’s breeding program, and evaluate the potential use of DNA markers to make early selections and speed up the selection process. I will also map quantitative trait loci (QTL) for traits related to seed dispersal (such as seed size) in the BC3F2 generation of trees. These ecologically important traits are important to understand because they will govern the relationship between restored chestnut and native seed dispersers, and are interesting from an evolutionary standpoint because of the coevolution of mast-bearing trees in the Fagaceae with mammalian and avian seed dispersers.

Methods

**Tree Material**
- A large trial of BC3F2 sapling trees growing at the Southern Indiana Purdue Agricultural Center (SIPAC) will be used for blight resistance QTL mapping and development of marker-assisted selection models.
- BC3F2 seed from the Jackson-Washington State Forest (JWSF) in southern Indiana will be used for seed trait QTL mapping.
- Chinese chestnut selections to be resequenced will be drawn from the diverse collection at Empire Chestnut Company in Carrollton, Ohio.

**Methods**
- A new leaf-inoculation method will be tested on the SIPAC BC3F2 population, with conventional stem inoculations to test the accuracy of the leaf technique. These inoculations will start in Summer 2014.
- DNA will be extracted from seeds prior to dispersal trials using a few milligrams of cotyledon tissue. A hole needs to be punched in the seeds to insert a wire tag (Xiao et al. 2013), so waste material will be used to extract DNA.
- Dispersal trials will take place in the field October-December for three consecutive years (2014-2016). Populations of squirrels accustomed to chestnut (in and around seed orchards and test plantings) will be used to measure the differences in dispersal distance and consumption behavior among American, Chinese and hybrid chestnut and within BC3F2 hybrid seeds.
- RAD-seq genotyping-by-sequencing (Baird et al. 2008), with 100-bp Illumina paired-end reads and samples multiplexed 96/lane, will be used to derive genotypes for QTL mapping.
- A detached-branch inoculation assay will be used to test resistance of pure Chinese chestnuts.
- Illumina sequencing, with two barcoded individuals per lane, will be used to sequence Chinese chestnut (genome size ~770 Mbp).

Project Objectives

**Marker-assisted chestnut breeding**
- Map blight resistance quantitative trait loci (QTL) in the Indiana breeding population using a large number of single-nucleotide DNA markers (~5K) derived from genotyping-by-sequencing (GBS).
- Use whole-genome regression to make phenotype predictions; assess accuracy of this technique for making early selections.

**Mapping ecological traits in hybrid trees**
- Using GBS-derived markers, map QTL for seed-dispersal and survival traits
- Validate these QTL in multiple years
- Correlate QTL related to squirrel dispersal behavior with those for seed nutritional and size traits

**Assessing the range of variability and gene diversity in blight-resistant Chinese chestnut germplasm**
- Investigate the diversity of blight resistance haplotypes within 10 individuals of a diverse germplasm collection of Chinese chestnut (*Castanea mollissima*) using whole-genome resequencing.
- Use resequencing data to examine the seed trait QTL within Chinese chestnut, identifying whether or not seed traits have undergone strong recent selection in Chinese chestnut due to human cultivation.

References

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