Assessing the functional genetic diversity of blight resistance in Chinese chestnut (*Castanea mollissima* Blume) by whole-genome resequencing of a diverse germplasm collection.

Project Summary

Chinese chestnuts vary in their resistance to blight. Understanding the genetic basis for these differences is critical to recovering maximum resistance from Chinese chestnut in backcrosses with American chestnut (*Castanea dentata* (Marsh.) Borkh.). We will use whole-genome resequencing of mature Chinese chestnuts with variable disease responses to assess the functional diversity of DNA sequences associated with blight resistance. This assessment will help isolate candidate genes responsible for chestnut blight resistance, identify variation associated with elevated resistance, and furnish useful information to breeders who need to determine how many donor parents are needed and which parents to use for durable resistance.

Principal Investigators

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Duration of Project

November 2015-June 2017

Funds Requested

$8,000

Short-term Goals

-Sequence the genomes of 20 mature Chinese chestnuts with variable blight resistance

-Sequence the genomes of ‘Clapper’ and ‘Graves,’ resistance donors for the ACF breeding program

-Identify polymorphisms associated with resistant Chinese chestnuts and determine whether they occur in protein-coding regions

-Assess the sequence diversity of QTL regions associated with blight resistance and of candidate resistance genes

-Identify SNP haplotypes associated with elevated blight resistance

Long-Term Goals

-Develop a database of Chinese chestnut genome sequences that will enhance understanding of functional genetic diversity within the species

-Determine the feasibility of using markers to screen uncharacterized Chinese chestnut germplasm for blight resistance

-Enhance blight resistance in the backcross breeding program by allowing more precise selection of high-quality resistance donors and rapid screening of individuals currently in the program for their resistance genotypes.

Narrative

Chinese chestnut (*Castanea mollissima* Blume) has a large native range in temperate eastern and central China and was introduced to the United States in the early 1900s as a nut tree. Chinese chestnut is believed to be a native host of chestnut blight. Because its resistance to blight damage is consistently higher than other *Castanea* species, Chinese chestnut was selected as the resistance donor in the backcross breeding program devised by Dr. Charles Burnham and colleagues (Burnham et al. 1986). In this plan, after three generations of backcrossing hybrids to American chestnut and only advancing trees with elevated resistance and similar appearance to American chestnut, selected trees are intercrossed to produce a population (BC3F2) in which a small percentage of trees breed true for blight resistance.

This strategy was based on a hypothesis that a small number of major genes controlled blight resistance. Since then, quantitative trait locus (QTL) mapping using neutral markers (Kubisiak et al. 1997, 2013) has indicated that three genomic regions account for three-quarters of the variation in blight resistance among Chinese/American chestnut hybrids with minor loci making up the balance. The major QTL regions make up a small percentage of the genome of Chinese chestnut, but the individual genes that confer blight resistance remain unknown.

The success of the American chestnut restoration breeding effort depends on recovering nearly all of Chinese chestnut's blight resistance in advanced backcross progeny. Since there is considerable variation in blight resistance among individual Chinese chestnuts, choosing the best possible resistance donors would increase the likelihood of meeting the program's goals. To this end, the American Chestnut Foundation added a number of resistant Chinese parents to its breeding program at Meadowview; incorporating more resistance donors remains a priority for ACF's breeding program (Hebard 2005). The Chinese grandparents of the program's original resistance donors (the BC1 trees 'Clapper' and 'Graves') were not characterized as having better-than-average blight resistance. Since the original resistance donors were BC1 trees, they contained at most one Chinese chestnut allele at each resistance locus. Many state chapter breeding programs use only 'Clapper' or 'Graves' as resistance donors. With a single BC1 resistance donor, true-breeding offspring of crosses between BC3 trees will be homozygous at resistance loci with two copies of a single allele from the Chinese grandparent of 'Clapper' or

'Graves'. If both 'Clapper' and 'Graves' were included in a pedigree, most of the offspring would be heterozygous (C/G), but only two Chinese sources of resistance would be present.

Whether or not reliance on a single version (allele) or a few versions of resistance genes is a liability for the restoration of American chestnut depends on the molecular basis of the differences in blight resistance observed among Chinese chestnuts. Since blight resistance has a strong effect on fitness, it is possible that resistance loci have evolved under purifying or negative selection. If so, intense selection for the most resistant trees in populations would result in a limited set of "best" resistance genes that are found in the vast majority of Chinese chestnuts: less-effective forms of the genes would have been purged from the population. If this is true, only a few resistance donors would be needed, but selecting the most resistant Chinese chestnut parents available would still be essential.

The other possibility is that blight resistance loci have undergone balancing, or positive, selection. Positive selection leads to high sequence diversity in coding regions, and would result in blight resistance genes with diverse sets of variant alleles, like the major histocompatibility complex (MHC) loci of animal immune systems. In studies of rice and *Arabidopsis* disease resistance loci, very high nucleotide diversity was found within some disease resistance genes (Rose et al. 2004, Thakur et al. 2013). Positive selection would occur if unique alleles conferred an advantage against certain strains of the fungus, or in specific growing environments. In this case, incorporating a large number of Chinese parents would be essential to successfully developing blight-resistant chestnuts for restoration to eastern U.S. forests.

We plan to investigate the functional diversity of blight resistance loci in Chinese chestnut by sequencing the genomes of 20 Chinese chestnut individuals, 2 American chestnuts, and several interspecific hybrids with variable blight resistance. By aligning sequences from these individuals with the published Chinese chestnut blight QTL and whole genome reference sequences, we will be able to examine sequence (haplotype) diversity in regions linked to blight resistance (the QTL) and within candidate genes themselves. To accomplish this, we have selected a set of mature Chinese chestnuts and interspecific hybrids of partial Chinese ancestry with variable blight resistance phenotypes growing at the Empire Chestnut Company in Carrollton, Ohio. The blight resistance of these individuals has been determined by at least 15 years of natural inoculation in an orchard setting. They include trees from the major northern and southern chestnut-growing regions of China. 'Nanking' has already been sequenced, and we

hope to sequence 'Clapper' and 'Graves'.to compare their DNA sequences across the blight resistance QTL with sequences from a diverse collection of resistant and susceptible Chinese chestnuts.

The draft genome sequence of Chinese chestnut (http://www.hardwoodgenomics.org/chinese-chestnut-genome) makes it possible to investigate haplotype diversity within blight resistance QTL. The chestnut genome is estimated to be just under 800 million base pairs in length, moderately sized for a plant genome and comparable to sorghum (*Sorghum bicolor*) and tomato (*Solanum lycopersicon*); several times larger than peach (200 Mb), and much smaller than maize (*Zea mays;* 2300 Mb) or spruce (*Picea glauca;* 20 Gb, or 20,000 Mb). With a genome this size, whole-genome resequencing is a reasonable approach.

Resequencing has revolutionized plant breeding over the past decade, and its importance is expected to expand in the future (Huang et al. 2009, Ingvarsson and Street 2011). Large-scale resequencing is already being applied in breeding programs for non-model organisms, including apple, peach, walnut, and almond, and there are no theoretical limitations to doing so in chestnut. Resequencing has been applied to landscape genetics of poplar (Slavov et al. 2012) and the published reference genome of watermelon (*Citrullus lanatus*) came with genome sequences of 20 diverse accessions that revealed sequence variants associated with important fruit traits (Guo et al. 2013). Whole genome resequencing results in datasets containing hundreds of thousands of single nucleotide polymorphisms (SNPs). Examining patterns of variation in these SNPs across the genome can reveal regions of the genome where the frequency of polymorphisms is decreased due to selective sweeps, or increased due to positive selection. Investigations of disease resistance genes in rice and soybean (Lam et al. 2010) have found haplotype sets adjacent to important disease resistance loci. By resequencing a diverse set of Chinese chestnuts with variable blight resistance, we expect to identify haplotypes associated with elevated disease resistance and to generate markers to screen uncharacterized Chinese germplasm for potential as a blight resistance donor. This is important because Chinese donors that cannot be distinguished in terms of their resistance may have important differences at the DNA level. Understanding the diversity of haplotypes at blight resistance loci in Chinese chestnut would also shed light on the co-evolution of chestnut and blight. Additionally, regions of suppressed or elevated recombination can be revealed by resequencing data (Lexer and Stoelting 2012); identifying these regions in chestnut will empower hybrid and backcross breeding efforts.

Because trees have long generation times and long juvenility, the cost of evaluating a cross is much higher for tree breeders, and accurate characterization of germplasm is more important than it is in other plant breeding programs. Understanding the molecular basis of differences in blight resistance within Chinese chestnut will help breeders plan for the number of resistant parents needed to capture in the restoration breeding population a significant portion of the natural diversity of blight resistance genes. Additionally, we will be able to identify whether the most resistant Chinese chestnuts tend to be heterozygotes at disease resistance loci or are homozygous for a single strong resistance haplotype. This information will help breeders plan crosses and design seed orchards to ensure maximum resistance in backcrossed trees that contain Chinese blight resistance genes. Our resequencing dataset, made available to the wider research community, will allow the examination of sequence diversity for traits other than blight resistance that are of interest to chestnut breeders, including stem form and nut characteristics. A resequencing dataset for Chinese chestnut would be the first of its kind for a nut-producing, long-lived hardwood tree and would augment ongoing research on the genomics of trees in the ecologically and economically important Fagaceae family.

Methods

Based on other researchers' experience and our own, genomic DNA extracted from dormant chestnut twigs results in a higher quantity and quality of sequence data. We have determined, through discussions with a maize breeder familiar with resequencing, that resequencing 20 individuals will allow us to adequately assess sequence diversity at resistance loci.

We have determined that sequencing two individuals in each Illumina lane results in enough genome coverage to accurately call SNPs across most of the genome (Table 1). However, if we continue to obtain high-quality DNA preparations, we could include 3 individuals in each lane and still obtain 15-17x coverage. Sequences will be obtained in 100 bp paired-end reads. Sequencing will be performed by the Purdue Genomics Core Facility. We have obtained blight resistance QTL and whole genome reference sequences from the hardwoodgenomics.org website, and we will use the program Geneious 7.0 (Kearse et al. 2012) to assemble quality-filtered reads to the reference sequences and to call SNPs. We also plan to use the freely available software packages Samtools and Bcftools (Li et al. 2009) to assemble-to-reference and call SNPs because they are easier than Geneious to integrate with other analysis

programs. Their package popgenome (Pfeifer et al. 2014) will be used to conduct nucleotide diversity analyses across the blight resistance QTL sequences. The freely available program AUGUSTUS (Stanke and Morgenstern 2005) will be used to predict genes in the blight resistance QTL sequences, along with the predictions already available on the hardwoodgenomics.org website. We will use Fastphase (Scheet and Stephens 2006) to conduct haplotype phasing across the blight resistance QTL sequences. Other analyses and intermediate steps will be performed using custom Perl scripts.

Status of project

We have sequenced the genomes of 10 Chinese chestnuts and 4 interspecific hybrids (Table 1). Two American chestnuts from Indiana and two additional Chinese chestnuts are currently being sequenced at the Purdue Genomics Core Facility. We assembled the reads from each individual to the three known blight resistance QTL, and identified large numbers of polymorphisms (SNPs and indels). As a preliminary analysis, we identified loci that displayed a strong segregation pattern between the resistant (n=6) and susceptible (n=8) individuals sequenced to date by calculating an *ad hoc* score that is highest for a locus where resistant and susceptible trees differ the most. The score was calculated, over all nucleotides N:

$$score = \sum (N\ susceptible - N\ resistant)(N\ resistant - N\ susceptible)$$

When we calculated this score over all loci on the three blight resistance QTL (Figure 1), the highest scores tended to cluster in a few regions of each QTL sequence. We used Augustus software to predict genes across the blight resistance QTL, and used these predictions to determine the relationship of polymorphisms to predicted coding sequences. Many of the SNPs most highly-associated with resistance were located near or within predicted genes, and several of these genes have functions associated with disease response (senescence-associated protein and several cupredoxin proteins on cbr1, a ubiquination protein on cbr2, and an epoxide hydrolase/lipase on cbr3). Especially interesting were the numerous peaks near oxidoreductase genes on cbr1 (Figure 1). We hope to further test and extend these preliminary results with help from TACF.

Table 1. Samples sequenced to date. Names in parentheses are accession codes from Empire Chestnut Company.

Sample*	**Description/origin**	**Bases of sequence**	**Coverage**
EC06 (72-49.5)	Susceptible *Cm*	9,260,381,051	~11x
EC10 (SC3)	Susceptible *Cm* (Southern China)	8,222,958,309	~10x
EC14 (NC6)	Resistant *Cm* (Northern China)	10,409,768,009	~13x
EC19 (NC2)	Susceptible *Cm* (Northern China)	5,550,828,877	~7x
EC23 (B32)	Susceptible (*Cd x Cs) x Cm* "Paragon" seedling	10,541,019,369	~13x
EC26 (Nanking)	Resistant *Cm*	10,356,181,466	~13x
EC09 (B21)	Resistant (*Cd x Cs) x Cm* "Paragon" seedling	20,592,332,404	~25x
EC13 (Schmucki)	Resistant *Cm* (Northern China)	22,014,846,268	~27x
EC15 (SC2)	Susceptible *Cm* (Southern China	20,232,385,484	~25x
EC16 (SC1=B66)	Resistant *Cm* (Southern China; red leaf)	21,367,008,759	~26x
EC17 (NC5)	Resistant *Cm* (Northern China)	19,972,882,115	~24x
EC18 (NC1)	Susceptible *Cm* (Northern China)	21,931,104,690	~27x
EC22 (Paragon)	Susceptible *Cd x Cs* hybrid	20,060,894,569	~25x
EC24 (Paragon-1)	Susceptible (*Cd x Cs) x Cm* "Paragon" seedling	20,592,382,508	~25x

Submitted for sequencing June 19, 2015

EC20 (NC3)	Susceptible *Cm* (Northern China)
EC21 (NC4)	Resistant *Cm* (Northern China)
IAC1	*C. dentata* (state champion; northern Indiana)
IAC2	*C. dentata* (southern Indiana ; native range)

Collected but not submitted

EC01 (72-132)	Susceptible *Cm*
EC02 (72-139)	Resistant *Cm*
EC03 (72-41.5)	Susceptible *Cm*
EC04 (81-16)	Resistant *Cm*
EC05 (72-124)	Resistant *Cm*
EC07 (72-226)	Resistant *Cm*
EC08 (72-211)	Susceptible *Cm*
EC12 (SC4)	Resistant *Cm*
EC25 (B25)	Resistant *Cm x Cd* hybrid

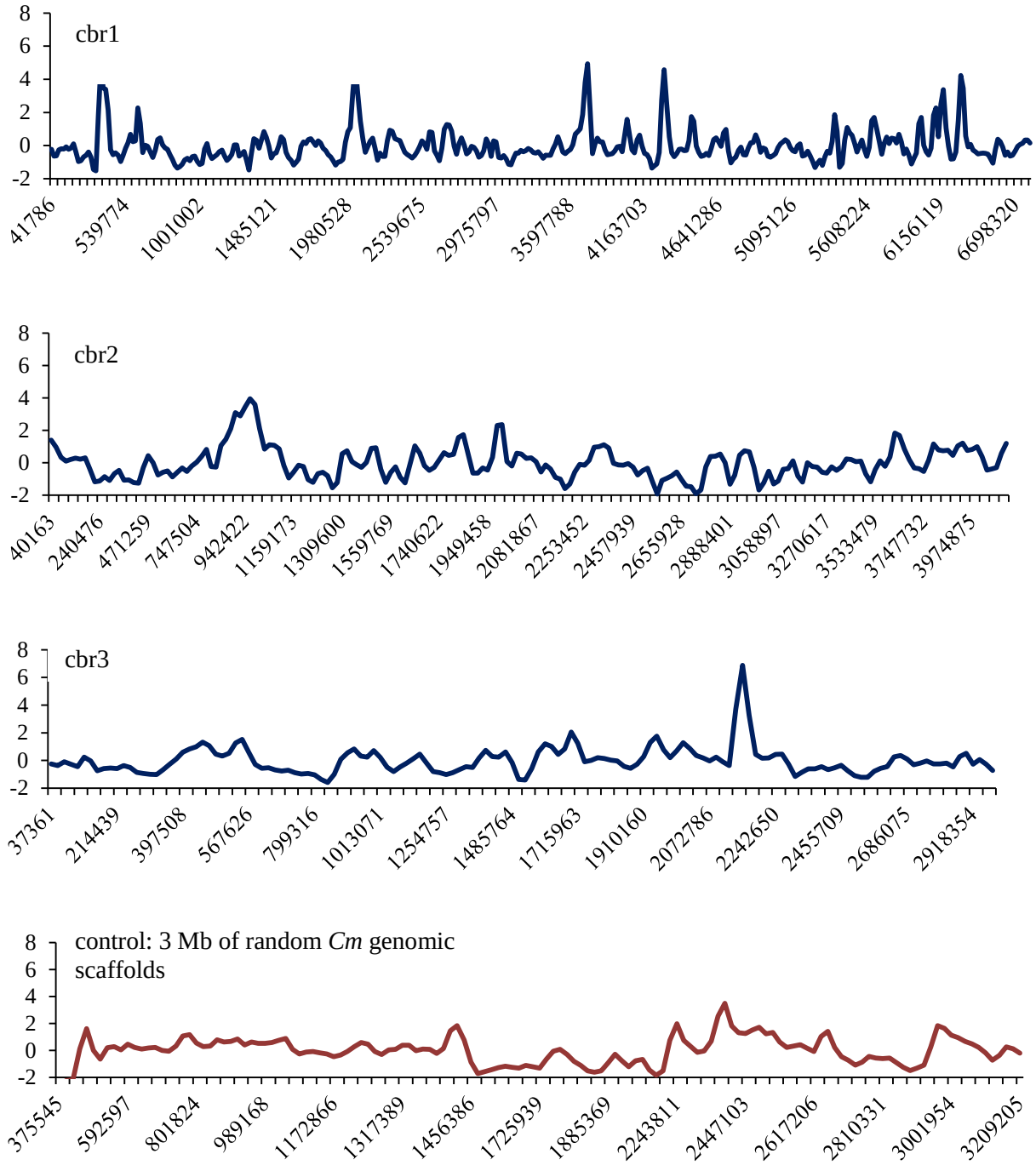

Figure 1. Association of DNA polymorphisms across three blight resistance QTL sequences (cbr1, cbr2, cbr3) and a 3 Mb set of randomly selected chestnut genome scaffolds (control). The scores charted were calculated as the average *ad hoc* association score (described in text) of all SNPs across a 500-locus window. Values displayed are standardized to the mean and standard deviation of all scores within the sequence.

Timeline

DNA will be extracted from selected trees in November 2015 and sequenced as soon as possible. Once sequences are generated (January 2016), quality and coverage analysis and sequence alignment will be completed by the end of March 2016. We anticipate the remaining analysis--quantification of haplotype diversity, identification of selective sweeps, and identification of SNPs and haplotypes associated with elevated blight resistance--will take an additional 6-8 months with a goal of June 2017 for completion of analysis.

Measurement and Reporting of Results

We plan to provide updates of the sequence generation and analysis to be published in the Journal of the American Chestnut Foundation in the summer of 2016. A final manuscript detailing the results of the blight resistance functional diversity analysis should be submitted for publication by the end of 2017. At this time, we also plan to make the generated sequences publicly available to the wider research community. Results measured will include a map of SNP diversity across the entire Chinese chestnut genome, with detailed maps of SNP variation across the blight resistance QTL regions. We will also report the sequences of any haplotypes identified that are associated with elevated or decreased blight resistance.

Allocation of Funds

All funding by TACF will be used to pay for Illumina sequencing of 'Clapper,' 'Graves,' and four additional Chinese chestnuts, and will be spent in December 2015 or January 2016 when sequencing is completed. The rate for sequencing at Purdue is $2500 per lane, and sample preparation (quality control, cleanup, library preparation) costs $100-$200/sample depending on the work needed. These prices are highly competitive with other sequencing centers, and the Purdue genomics facility includes preliminary filtering and trimming of reads in the sequencing cost. Therefore, the total cost to sequence 6 individuals (2 per lane) is about $8000. N. LaBonte will be supported by a van Eck endowment scholarship during his work on the project. This work is also being supported by the Northern Nut Growers' Association (Chinese chestnut sequencing) and the Indiana Academy of Sciences (American chestnut sequencing).

References

Burnham, C.R., Rutter, P.A., French, D.W. 1986. Breeding blight-resistant chestnuts. Plant Breeding Reviews 4: 347-397.

Cao, J., Schneeberger, K., Ossowski, S., Guenther, Bender, S. et al. 2011. Whole-genome sequencing of multiple *Arabidopsis thaliana* populations. Nature Genetics 43(10):95.

Ellis, J., Dodds, P., Pryor, T. 2000. Structure, function and evolution of plant disease resistance genes. Current Opinion in Plant Biology 3: 278-284.

Guo, S., Zhang, J., Sun, H., Salse, J., Lucas, W.J. et al. 2013. The draft genome of watermelon (*Citrullus lanatus*) and resequencing of 20 diverse accessions. Nature Genetics 45(1):51.

Hebard, F.V. 2005. Meadowview Notes 2004-2005. Journal of the American Chestnut Foundation 19(2): 16-27.

Huang, X., Feng, Q., Qian, Q., Zhao, Q., Wang, L., et al. 2009. High-throughput genotyping by whole-genome resequencing. Genome Research 19:1068-1076.

Ingvarsson, P.K., Street, N.R. 2011. Association genetics of complex traits in plants. New Phytologist 189:909-922.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjies, P., & Drummond, A. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics, 28(12), 1647-1649.

Kubisiak, T.L., Hebard, F.V., Nelson, C.D., Zhang, J., Bernatzky, R., Huang, J., Anagnostakis, S.L., Doudrick, R.L. 1997. Molecular mapping of resistance to blight in an interspecific cross in the genus *Castanea*. Phytopathology 87:751-759.

Kubisiak, T.L., Nelson, C.D., Staton, M.E., Zhebentyayeva, T., Smith, C., Olukolu, B.A., Fang, G.-C., Hebard, F.V., Anagnostakis, S., Wheeler, N., Sisco, P.H., Abbott, A.G., Sederoff, R.R. 2013. A transcriptome-based genetic map of Chinese chestnut (*Castanea mollissima*) and identification of regions of segmental homology with peach (*Prunus persica*). Tree Genetics and Genomes 9:557-571.

Lam, H-M, X, X., Liu, X., Chen, W., Yang, G., et al. 2010. Resequencing of 31 wild and cultivated soybean genomes identifies patterns of genetic diversity and selection. Nature Genetics 42: 1053-1059.

Lexer, C. & Stoelting, K.N. 2012. Whole genome sequencing (WGS) meets biogeography and shows that genomic selection in forest trees is feasible. New Phytologist 196:652-654.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Durbin, R. 2009. The sequence alignment/map format and SAMtools. Bioinformatics 25(16): 2078-2079.
Pfeifer, B., Wittelsbuerger, U., Ramos-Onsins, S.E., Lercher, M.J. 2014. PopGenome: an efficient Swiss army knife for population genomic analyses in R. Mol Biol Evol. 31:1929–1936

Rose, L. E., Bittner-Eddy, P. D., Langley, C. H., Holub, E. B., Michelmore, R. W., Beynon, J. L. 2004. The maintenance of extreme amino acid diversity at the disease resistance gene, RPP13, in *Arabidopsis thaliana*. Genetics 166(3): 1517-1527.

Slavov, G. T., DiFazio, S. P., Martin, J., Schackwitz, W., Muchero, W., Rodgers-Melnick, E., et al. 2012. Genome resequencing reveals multiscale geographic structure and extensive linkage disequilibrium in the forest tree *Populus trichocarpa*. New Phytologist 196:713-725.

Stanke, M., Morgenstern, B. 2005. AUGUSTUS: a web server for gene prediction in eukaryotes that allows user-defined constraints. Nucleic Acids Research 33:W465-W467.

Scheet, P. and Stephens, M. 2006. A fast and flexible statistical model for large-scale population genotype data: applications to inferring missing genotypes and haplotypic phase. American Journal of Human Genetics.

Thakur, S., Gupta, Y. K., Singh, P. K., Rathour, R., Variar, M., et al. 2013. Molecular diversity in rice blast resistance gene Pi-ta makes it highly effective against dynamic population of *Magnoporthe oryzae*. Functional and Integrated Genomics 13:309-322.

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Education

University of Wisconsin-Madison Cumulative GPA: 3.865	B.S., Forest Ecology	2007-2011
Purdue University	M.S., Forestry	2011-2013
Purdue University Cumulative GPA: 3.800	Ph.D., Forest Genomics	2013-Present

- Fellow in the USDA-AFRI Plant Breeding Partnership Program at Purdue University 2011-2013
- Recipient of Frederick M. van Eck research scholarship, 2013
- Graduate coursework includes: Intro Bioinformatics, Advanced Plant Pathology, Practical Bio Computing, R & Bayesian Analysis in Ecology, Quantitative Methods for Ecologists, Quantitative Genetics, Intro QTL Mapping, Design of Experiments, Intro C Programming, Molecular Ecology and Evolution

Employment

August 2011- Present
Graduate research assistant at Purdue University, West Lafayette, IN; Hardwood Tree Improvement and Regeneration Center (HTIRC)

- Graduate research assistant under Dr. Keith E. Woeste
- Conduct and analyze PCR reactions and genetic data for hardwood trees
- Manage whole-genome sequencing and resulting data

Recent Publications

LaBonte, N.R., Ostry, M.E., Ross-Davis, A., Woeste, K.E. 2015. Estimating heritability of disease resistance and factors that contribute to long-term survival in butternut (*Juglans cinerea* L.). Tree Genetics and Genomes 11:63.

Research Grants

Sequencing the genome of Indiana's largest surviving American chestnut (*Castanea dentata* (Marsh.) Borkh.) .

$2000 grant received from Indiana Academy of Sciences, April 2015

Assessing diversity of blight resistance genes in Chinese chestnut (*Castanea mollissima* Blume) by whole-genome resequencing of a diverse germplasm collection.

Proposal awarded $5000 by Northern Nut Growers Association, August 2014

Recent Presentations and Posters

"Investigating the genetic basis of variation in resistance to chestnut blight among Chinese chestnuts and interspecific hybrids using whole-genome resequencing." (Poster) Presented at:
Center for Advanced Forestry Systems (CAFS) meeting, Asheville, NC 5/20/2015
Southern Forest Tree Improvement Committee (SFTIC), Hot Springs, AR 6/9/2015
Northern Forest Genetics Association (NFGA) meeting, Martinsville, IN 7/15/2015
Northern Nut Growers Association (NNGA) meeting, La Crosse, WI 7/27/2015

"Testing leaf inoculation as a blight resistance screening method for advanced backcross chestnuts"; (Poster) American Chestnut Foundation Annual Meeting, Front Royal, VA 10/18/2015

"Summary of Purdue chestnut blight-resistance breeding"; (Presentation) International Restoring Forests meeting, West Lafayette, IN 10/15/2014

"Estimating the heritability of canker resistance in natural stands of butternut using molecular techniques"; (Presentation) NAFEW Conference, Bloomington, IN 6/17/2013

"Research update: Genetics, environmental factors, and butternut canker disease resistance"; (Presentation) Walnut Council Annual Meeting, Morgantown, WV 7/22/2013

Previous work experience

May 2011-August 2011
- Summer ecological restoration intern, Madison Audubon Society

June 2010-November 2010
- Field research technician, UW-Madison Ecosystem and Landscape Ecology Lab (Dr. Monica Turner, P.I.)

March 2010-June 2010
- Assistant arboriculture consultant, Allison Tree Care

January 2009-May 2011
- Undergraduate GIS research assistant, UW-Madison SILVIS Remote Sensing Lab (Dr. Volker Radeloff, P.I.)

May 2009-August 2009
- Field technician, U.S. Forest Service Northern Research Station, Rhinelander, WI

October 2007-May 2009
- Research Assistant, Potato Germplasm Enhancement Lab, USDA-ARS

KEITH E. WOESTE

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Expertise

Keith Woeste is a forest geneticist; his research focuses on the breeding, molecular and quantitative genetics of deciduous tree species, especially species prized for their high-value timber products or of particular conservation importance. He has extensive experience in the analysis of forest genetic trials and the interaction of genetics and management of timber crops.

Professional preparation

University of Florida	B.S., Botany	1981
University of California, Davis	M.S., Horticulture	1990
University of California, Davis	Ph.D., Genetics	1994

Appointments

2014	Interim National Program Leader for Genetics, USDA Forest Service.
1999 – present	Research Geneticist (USDA Forest Service), GS-14
1999 – present	Adjunct Assistant Professor, Genetics and Breeding, Department of Forestry and Natural Resources, Purdue University, West Lafayette, IN
1994 - 1998	Post-doctoral Research Fellow, Department of Biological Sciences, University of Illinois, Chicago, Chicago, IL.

Publications-10 most related (116 total)

LaBonte, N., M. Ostry, A. Ross-Davis, **K. Woeste** 2015. Estimating heritability of disease resistance and factors that contribute to long-term survival in butternut (*Juglans cinerea* L.). Tree Genetics and Genomes 11:63.

Worthen-Alexander, L. and **K. Woeste** 2014. Pyrosequencing of the northern red oak (*Quercus rubra* L.) chloroplast genome reveals high quality polymorphisms for population management. Tree Genetics and Genomes. (DOI) **10.1007/s11295-013-0681-1**

Pollegioni, P., I. Olimpieri, **K.E. Woeste**, G. De Simoni, M. Gras, and M. E. Malvolti. 2013. Barriers to interspecific hybridization between *Juglans nigra* L. and *J. regia* L. species. Tree Genetics & Genomes 9:291-305.

Zhao, P., S. Zhang, and **K. Woeste**. 2013. Genotypic data changes family rank for growth and quality traits in a black walnut (*Juglans nigra* L.) progeny test. New Forests 44:357-368. DOI 10.1007/s11056-012-9343-7

Woeste, K., D. Jacobs, and J.R. McKenna. 2011. Half-sib seed source and nursery sowing density affect black walnut (*Juglans nigra* L.) growth after five years. New Forests 41:235-245.

Robichaud, R., J. Glaubitz, O.E. Rhodes and **K. Woeste**. 2010. Genetic consequences of harvest in a mature second growth stand of black walnut (*Juglans nigra* L.). Annals of Forest Science. 67:702-710.

DeWoody, A. J. Bickham, C. Michler, K. Nichols, O. Rhodes and **K. Woeste** (eds.) 2010. Molecular Insights into Natural Resource Conservation and Management. Cambridge University Press, London. 374 p.

Worthen, L.,C. Michler, and **K. Woeste**. 2010. Breeding American chestnut for blight resistance. Plant Breeding Reviews 33:305-339.

Worthen, L., C. Michler, and **K. Woeste**. 2010. Genetic ramifications of restoration of blight resistant American chestnut, (Highlighted Box, p. 307 - 309). In: Chapter 12, Wildlife Reintroductions. DeWoody, A. J. Bickham, C. Michler, K. Nichols, O. Rhodes and K. Woeste (eds.) *Molecular Insights into Natural Resource Conservation and Management*. Cambridge University Press, London.

Pijut, P., **K.E. Woeste**, G. Vengadesan, and C.H. Michler. 2007. Invited Review: Technological advances in temperate hardwood tree improvement including breeding and molecular marker applications. In Vitro Cell Develop Biol - Plant. 43: 283-303

Victory, E. R., Glaubitz, J. C., Rhodes, O.E., and **K.E. Woeste**. 2006. Genetic homogeneity in *Juglans nigra* (Juglandaceae) at nuclear microsatellites. American Journal of Botany 93: 118-126.

Invited presentations: 86; other presentations/ posters at scientific meetings and conferences: 94

Current Students (total, 4 post-docs, 5 PhD, 2 M.S., committee service: 2 PhD, 5 MS)

James Jacobs, PhD student, The etiology of butternut canker disease.
Nick LaBonte, PhD student, Genomic analysis of chestnut.

Synergistic Activities

- Assistant editor, genetics, *Forest Science.*
- The American Chestnut Foundation, Science Advisory Committee.
- Chair, Grand Challenges Committee, USDA Plant Breeding Coordinating Committee-SCC(080)-National Association of Plant Breeders, 2012-2013.
- Principal investigator, NSF/IUCRC Center for Advanced Forestry Systems, 2006-2009.