a. Project Title: Mapping of resistance to *Phytophthora cinnamomi* (*Pc*) in interspecific American/ Chinese chestnut populations

b. Summary: Root rot caused by *Phytophthora cinnamomi* is one of the major factors affecting restoration of American chestnut in the southeast. Our goal is to identify genomic regions in Asian chestnuts that control resistance to *Pc* in progeny of Asian/American chestnut hybrids. We propose two specific objectives: 1) generate sequence-based markers and construct a genome-wide linkage map for cross HB2 (KY115 × AD98) segregating for resistance and 2) determine the genomic intervals conferring *Pc* resistance introgressed to this cross from Chinese chestnut ' Mahogany'. We expect providing usable markers for marker-assisted selection for resistance to *Pc* in American/Asian hybrid backcross progenies.

c. Principal Investigator(s) and Institutional Affiliation(s):

Principal Investigator: Tetyana N. Zhebentyayeva, Department of Genetics & Biochemistry, and Laboratory for Genomics & Computational Biology, Clemson University

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d. Duration of project – 10/17/2014-10/16/2015

e. Total amount requested - $10,000

f. Short and long-term goals of the project
Short-term goal: Delineation of genomic region(s) conferring resistance to *Phytophthora cinnamomi* in the BC_1F_1 cross HB2-2012 (KY115 × AD98) deriving resistance originally from the Chinese chestnut cultivar 'Mahogany'.
Long-term goals: Delineation of genomic regions conferring resistance to *Pc* in the most common Asian sources of *Pc* resistance in the US breeding programs, *C. molissima* ('Mahogany' and 'Nanking') and Japanese chestnut *C. crenata* ('Morrow Mountain' and 'Fort Defiance'). Incorporation of marker-associated QTLs into TACF's breeding pipeline will speed up selection of individuals resistant to both *Cp* and *Pc.*

g. Narrative (5 pages)

Introduction

The American chestnut, *Castanea dentata*, was at one time the dominant tree species throughout the forests of the eastern United States (Russell, 1987). With the introduction of the chestnut blight fungus *Cryphonectria parasitica* (*Cp*), the American chestnut was rapidly reduced from the major component of the eastern forest to an understory shrub represented by root-collar sprouts of blight-killed trees (Anagnostakis, 1987; Paillet, 2002). The outbreak of the soil-borne oomycete pathogen *Phytophthora cinnamomi* (*Pc*), causing *Phytophthora* root rot (PRR), contributed to an almost complete elimination of the American chestnut from its southern range (Jacobs et al., 2013). Thus, restoration of American chestnut in the southeast, where climatic conditions are favorable for the life cycle of *Pc*, unequivocally requires pyramiding resistance to both *Cp* and *Pc*. Fortunately, substantial levels of resistance to both pathogens have been found in Asian species of *Castanea*, in particular Chinese chestnut (*C. mollissima*) and Japanese chestnut (*C. crenata*) (Crandall et al., 1945; Graves, 1950; Anagnostakis, 1992). To address the need for screening *Cp*-resistant hybrid material for resistance to *Pc*, a collaboration among the USDA Forest Service, Forest Health Research and Education Center (FHREC), The American Chestnut Foundation (TACF), Clemson University and The Chestnut Return Farm was established and centered on an effective screening method for detecting *Pc* resistance (Jeffers et al. 2009; Jeffers et al., 2012). In parallel, a study was initiated to determine the genetic basis for *Pc* resistance and to develop markers to identify and predict *Pc* resistance in advanced breeding material. The project proposed here on mapping of resistance to *Pc* is the central genetic component of this collaborative effort.

Background and significance

Development of a set of transcriptome-based markers and construction of the reference *Castanea* genetic maps improved the delineation of the genomic regions underlying three QTLs for resistance to *Cp* (Kubisiak et al., 2013). As a first step, species-specific SNP markers for differentiation the Chinese (resistance sources) and American chestnut parents (recurrent parents) were designed for immediate use in the TACF's backcross breeding program (Georgi et al., 2014). Identification of markers associated with three QTL intervals for resistance to *Cp* (*Cbr1*, *Cbr2*, and *Cbr3*) was initiated through whole-genome resequencing of resistant vs. susceptible individuals that originated from the F_2 segregating cross (A.G. Abbott, unpublished). Thus, a number of SSR and SNP markers have been generated in support of the TACF backcross breeding program for *Cp* resistance. Unfortunately, identification of genetic regions controlling resistance to *Pc* is far from being implemented in breeding. So, the **ultimate goal** of this project is to address the construction of a genetic linkage genetic map and delineate the genomic regions conferring resistance to *Pc* derived from the four resistant genotypes - two from the species *C. molissima* ('Mahogany', 'Nanking') and two from the species *C. crenata* ('Morrow Mountain', 'Fort Defiance'). Delineation of genetic intervals conferring *Pc* resistance will facilitate the identification of markers associated with this important trait and their practical implementation in

breeding for resistance to both chestnut pathogens, *Cp* and *Pc*. Due to limited funding available through this call for proposals, we narrowed our tasks and selected for study an extended BC_1F_1cross - KY115 × AD98 ('Mahogany' background) phenotyped for PRR resistance in 2011-2014 (Table 1). Our **specific objectives** are to: 1) construct a genome-wide genetic linkage map for the KY115 x AD98 cross (HB2-2012 in Table 1) using the Genotyping-by-Sequencing (GBS) approach and 2) delineate and identify DNA sequence-based markers for the genomic intervals that contain the QTLs for resistance to *Pc* introgressed from 'Mahogany'.

Plant material

The proposed study is based on hybrid plant material generated by different TACF's chapters for evaluating resistance to *Pc*. Phenotyping for severity of root rot symptoms (PRR test) was established in 2003 by J.B. James and S.N. Jeffers at the Chestnut Return Farm, Seneca, SC (Jeffers et al, 2009; James 2011a; 2011b). Following their protocol, plants were inoculated and phenotyped for four phenotypic classes of disease presence in the end of the growing season. Inheritance of resistance to *Pc* was detected in interspecific crosses derived from American chestnut (susceptible) and Chinese chestnut (resistant). From 2004-2010, 197 hybrid families were tested from generations that ranged from F_1 to BC_4 (Jeffers et al., 2012). Since 2011, extended hybrid crosses were developed specifically for statistical analysis, genetic mapping and QTL detection. These mapping populations segregating for resistance to *Pc* are hybrid lineages derived from crosses of two resistant individuals of the Chinese chestnut (*C. molissima),* 'Mahogany' and 'Nanking' with susceptible American accessions. Recently, a third source of resistance, the Japanese chestnut (*C. crenata),* was incorporated into the PRR-phenotyping as well (Table 1). To satisfy the requirement of increased progeny size for high resolution map construction, the BC_1F_1 cross HB2 (KY115 × AD98) that has a combined progeny size of 684 individuals was included into phenotyping for four consecutive vegetative seasons. For a more comprehensive QTL study, altogether 1408 phenotyped individuals (5 crosses, *C. molissima* background) are now available for high-throughput genotyping using Next-Generation Sequencing platforms. Together, these materials provide a solid foundation for a genetic study of resistance to *Pc* in *Castanea* and identification of the genomic regions underlying this important trait.

Table1. Interspecific hybrid populations developed for linkage analysis and QTL mapping of the resistance to root rot disease in chestnut

Hybrid population code-year	Total plants	Root rot symptoms severity classes				Type of family	Source of resistance
		0	1	2	3		
HB2-2011	41	1	3	20	17	BC_1F_1	*C. mollissima* 'Mahogany'
HB2-2012	179	0	2	122	55	BC_1F_1	
HB2-2013	230	0	18	146	66	BC_1F_1	
HB2-2014	234	in progress				BC_1F_1	
JB1-2013	115	2	20	37	56	BC_3	
JB1-2014	187	in progress				BC_3	
NK1-2012	20	1	13	6	0	BC_1F_1	*C. mollissima* 'Nanking'
NK2-2012	83	2	32	42	7	BC_1F_1	
NK4-2014	319	in progress				BC_1F_1	
MJ1-2014	75	in progress				BC_1F_1	*C. crenata* 'Morrow Mountain'

Genetic linkage mapping and QTL detection (preliminary results)

Using limited progeny sets for some of the backcross families in Table 1, we conducted initial low resolution linkage mapping and QTL detection for the *Pc* resistance introgressed from Chinese chestnut. Utilizing a genome-wide SNP array, a low density genetic map was constructed for a limited number of individuals issued from a cross of AdairKY1 × GL158 ('Nanking' background) (Olukolu et al., 2012; Zhebentyayeva et al., 2014). Resistance to *Pc* was mapped to linkage group E (LG_E). Using a set of the LG_E SSR markers from the Chinese chestnut reference map, we also genotyped additional hybrid populations and generated local LG_E maps for half-sib crosses NK1, NK2 ('Nanking' background) and KY115 × AD98 ('Mahogany' background). Overlapping QTLs for *Pc* resistance were detected in both Chinese chestnut resistance sources (Fig.1). Additionally, QTL signal on LG_E was found in the third source of the *Pc* resistance, *C. crenata*, using an interspecific cross with *C. sativa* (R. Costa and C. Santos, personal communication). Due to the low density of markers in these preliminary LG_E maps only a broad QTL interval on the bottom

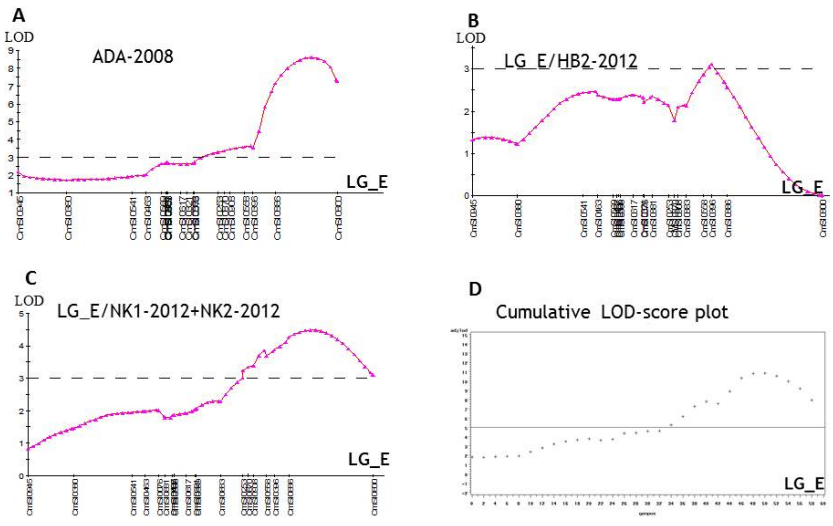

Fig. 2. Major QTL for Phytophthora root rot resistance on LG_E using Multiple QTL mapping (1 cM screen). A,B, C – local maps for LG_E in three BC_1F_1 crosses; D - Cumulative LOD-score over the three crosses.

half of the LG_E was detected. Moreover, a large gap in the *C. molissima* physical map (M. Staton, personnel communication) exists for the same reason, thus limiting the utility of this resource for further marker develoment directed at narrowing down QTL interval (s) in this important region. Becuase of the limited number of individuls available (n=48) for QTL detection in the cross AdairKY1 × GL158, only one broad major QTL on the LG_E was detected. For this reason, the extended mapping populations in Table 1 are critical for higher resolution QTL mapping and for evaluating QTL stability across four years of phenotyping.

General conclusion: The above preliminary work demonstrates that the proposed study has a solid foundation (hybrid material and genotyping platforms) to significantly advance the delineation of genomic regions controlling resistance to *Pc* in *Castanea*. The large mapping population HB2 (KY115 × AD98) phenotyped for four years and composed of 684 individual is ideally suited for application of high-throughput genotyping platforms and genome mapping

Approach

Specific Objective 1: Determine and characterize the genomic intervals conferring resistance to *Pc* in segregating family HB2 phenotyped in 2012, 2013 and 2014 (resistance source *C. molissima*, 'Mahogany') (potentially years 1, 2 and 3 of the project). We will start with the cross HB2-2012 that was used previously for construction of a low-resolution SSR map for LG_E.

Activity a: Phenotyping: All seedlings derived from the families described in Table 1 as phenotyping "in progress" will be subjected to tub-based phenotyping assay (PRR test) according to a robust protocol that has been proven reliable over 10 years of testing (Jeffers et al., 2009; James, 2011a; 2011b, Jeffers et al., 2012).

Methods: Tub-based PRR-test: Stratified seeds from controlled pollination are planted outside in plastic tubs in April at a field site at the Chestnut Return Farm in Oconee County, SC. A randomized block design was used for planting the hybrid families and control plants, *C. molissima* (resistant) and *C. dentata* (susceptible). In July, 2-month-old plants were inoculated with two strains of *Pc* originally recovered from the study site. Seedlings are watered throughout the study period. To stimulate disease development, the container mix in each tub will be brought to water saturation at least once while plants are actively growing. Plants will be evaluated in December when fully dormant. Each seedling will be scored for mortality and *Pc* severity as shown in preliminary results section (Fig 1).

Activity b: Genotyping-by-sequencing (GBS) approaches: Genome-wide mapping study of a small BC_1F_1 family derived from the Chinese cultivar 'Nanking' identified a single QTL on LG_E (Olukolu *et al.,* 2012; Zhebentyayeva *et al.,* 2014). The location on LG_E was confirmed using low density SSR maps for hybrid families HB2 and NK2, NK3 originated from Chinese chestnut cultivars 'Mahogany' and 'Nanking' respectively (see preliminary results above). To improve resolution of QTL intervals using increased marker density and progeny sizes, we propose utilizing of highly informative, sequence-derived markers generated using the GBS approach (Elshire et al., 2011). This relatively inexpensive method is currently being implemented in our laboratory for both single-family mapping and genome wide association studies in tropical yam and cotton respectively (C. Saski, unpublished).

Methods: GBS-based SNP marker identification will employ the pipelines currently used in the Clemson University Laboratory for Genomics & Computational Biology. Briefly, progeny DNA samples from each mapping family will be prepared by methods we have optimized for DNA extraction from chestnut leaves (Kubisiak *et al.,* 2013). DNA will be quantified using the Qubit fluorometer (Life Technologies). The GBS libraries will be constructed for each individual and sequenced using the HiSeq2500 (Illumina) using single-ended reads and 101 cycles. With a genome size of ~750 Mb (chestnut), we will multiplex 48 individual DNA samples per HiSeq lane thus outputting approximately 0.8X genome coverage. The raw reads will be subjected to the SNP calling bioinformatic pipeline TASSEL-GBS (Glaubitz et al. 2014). Sequenced parental genomes (J. Carlson, personal communication) will be used for imputation of missing SNPs.

Activity c: Genome-wide SSR genotyping: To align the GBS maps against Chinese chestnut reference map by Kubisiak et al. (2013), we also propose genotyping of 3 to 4 SSRs evenly spanning each linkage group for integration of the SSRs into the GBS maps. We expect that with this strategy we will refine the QTL interval on LG_E while determining whether or not additional QTL loci on other linkage groups have been introgressed from Asian chestnut species. Of note, the SSR genotyping for HB2-2012 cross has been completed and local LG_E map was constructed (see preliminary results above).

Methods: The progeny DNAs will be screened with mapped SSRs from all linkage groups of the *C. mollissima* map following published protocol by Kubisiak et al. (2013). We are planning the use of the same set of primers shared by C.D. Nelson's Lab at SIFG (USDA Forest Service). Fragment size determination of multiplexed SSRs will be done using the ABI3700 instrument (Applied Biosystems) and help from experienced personnel at SIFG.

Specific Objective 2: Linkage genetic mapping and QTL detection

Activity a: Linkage map construction: Genetic linkage mapping will be executed as published previously for two crosses segregating for resistance to *Cp* (Kubisiak et al., 2013).

Methods: Linkage analyses will be performed with JoinMap v4.0 (van Ooijen 2006). Data will be coded separately for each parent and loaded into single JoinMap session. Maps will be first constructed for each parent with LOD >3.0 and used for QTL analysis. Map graphics will be generated with MapChart v2.1 (Voorrips 2002)

Activity b: Whole genome QTL analyses: QTL analyses will be performed essentially as published previously (Kubisiak et al. 2013).

Methods: MapQTL v5 (Van Ooijen, 2004) with the same phenotypic scoring scale employed previously for QTL mapping of *Pc* resistance using a low resolution maps (see preliminary results) will be utilized. The *Pc* resistance will be investigated using non-parametric analysis (Kruskal-Wallis test), interval mapping and composite interval mapping implemented in MapQTL5. Permutation tests will be done to determine the 5% genome–wide error for declaring QTL (Churchill and Doerge, 1994).

Pitfalls and limitations to proposed procedures: The most serious pitfall of the proposed work is the possibility that we will not identify strong-effect QTLs in the HB2 cross (source of resistance Chinese chestnut 'Mahogany'). However, plant material generated and phenotyped for this study represents advanced backcross generations of the 'Mahogany' lineage and different sources of resistance as well--Chinese chestnut 'Nanking" and Japanese chestnut 'Morrow Mountain'. Thus, we have the flexibility to switch our genotyping and mapping efforts to other hybrid populations listed in Table 1 if necessary.

h. Timeline, showing start and completion dates for each goal.

Specific Objectives	**3m***	**6m**	**9m**	**12m**
1- genotyping	All progeny DNAs quantified and phenotyping complete. GBS sequencing initiated	GBS sequencing completed. SNP analyses and SSR analysis underway	SNP and SSR analyses completed and mapping completed	
2-QTL Mapping			Mapping results compiled and QTL intervals identified. Comparative mapping initiated	Mapping completed, QTL defined and manuscripts in preparation

* Denotes months completed

i. How results will be measured and reported.

At the end of the grant period, final financial and final performance reports will be delivered. A summary of research for a general audience will be submitted to the Journal of The American Chestnut Foundation. All results from the project are expected to be included in peer-reviewed journal articles and disseminated publically at conferences and scientific meetings through poster and oral presentations.

Exploitation of the expected results: Once we have identified markers whose specific alleles associated with resistance to *Pc*, we will utilize these markers in TACF's disease-resistance breeding program to screen for *Pc* resistance.

j. **Breakdown of how and when funds will be spent**

TableI.Project(Line(Items/Cost			
Task	Amount	Cost per item	Totals
*192 samples**			
DNA quantification (Qubit® 2.0 Fluorometer)	*191*	$2	$382
GBS Library Construction	192	$15	$2,880
2x100bp Illumina HiSeq Sequencing (per sample) 48 Samples /Lane	3	$1,900	$2,700
SSR genotyping using ABI3700 (181x12 LGsx3 SSRs per linkage group	6516/4per lane=1629	$2	$3,258
Bioinformatic Analysis Hours	7	$110	$770
		Total	$10,000
* including 1 BLANK per plate, duplicated samples and parental/grandparental controls in each plate			

Budget Narrative: Supplies requested include but are not limited to the preparation of DNA, quantification, genotyping-by-sequencing library preparations and SSR genotyping. The requested salary/fringe for the CU GCBL's technician is in line with current trends in salary for similar positions in this specialty and the amount of time required completing the outlined tasks.

k. References

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l. Brief C.V. for Principal Investigators:

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Leningrad State University, USSR, combined BS- MS (1977) Biochemistry
Leningrad State University, USSR, PhD (1984) Plant Biochemistry

Appointments (current in bold)

2013 Research Associate Professor, CU Genomics & Computational Biology Laboratory
2011 Research Associate Professor, Genetics & Biochemistry, Clemson University
2003-2011 Research Scientist, Genetic & Biochemistry, Clemson University
2000 – 2003 Visiting Scientist, Department of Horticulture, Clemson University
1987 – 2003 Staff Senior Research Scientist, Nikita Botanical Garden, Crimea, Ukraine
1981 –1987 Staff Research Scientist, Nikita Botanical Garden, Crimea, Ukraine

Other Experience and Professional Memberships

2006 - EUCARPIA (European Association for research in plant breeding)
2014 – The American Chestnut Foundation *(C60EB64)*

Ongoing Research Support

USD- AFRS (58-5430-2-313) 8/1/2014-7/31/2016
Data needed for foreign regulatory packages for the PPV resistant transgenic *Prunus domestica* cv. 'HoneySweet'. PI: C.Saski; co-PI: T. Zhebentyayeva

Completed Research Support

NIFA USDA CRIS **(**SC-1700363) 7/1/2008-12/31/2010
Translational genomics of flavonoid pathway genes for improving nutritional value of stone fruits. PI: A.G. Abbott; co-PIs: T. Zhebentyayeva

Selected Peer-reviewed Publications (Selected from 33 peer-reviewed publications)

Most relevant to the current application (in chronological order):

1. Fang G-C, Blackmon B, Staton M, Nelson CD, Kubisiak TL, Olukolu B, Henry D, Zhebentyayeva T, Saski C, Cheng C-H, Monsanto M, Ficklin S, Atkins M, Georgi L, Barakat A, Wheeler N, Carlson J, Sederoff R, Abbott A (2013) A physical map of the Chinese chestnut (*Castanea mollissima*) genome and its integration with the genetic map. Tree Genetics & Genomes, 9: 525–537.
2. Kubisiak TL, Nelson CD, Staton ME, Zhebentyayeva T, Smith C, Olukolu B, Fang G-C, Anagnostakis S, Hebard FV, Wheeler N, Abbott AG, R.R. Sederoff RR (2013) A transcriptome-based genetic map of Chinese chestnut, (*Castanea mollissima*), and identification of regions of segmental homology with peach (*Prunus persica*). Tree Genetics & Genomes, 9: 557-571.

3. T. Zhebentyayeva, Staton M, Olukolu B, Chandra A, Jeffers S, James J, Sisco P, Hebard F, Georgi L, Nelson CD and Abbott AG (2014) Genetic and Genomic Resources for Mapping Resistance to Root Rot Disease (*Phytophthora cinnamomi*) in Chestnut. Acta Horticulturae (ISHS) 1019:263-270.
4. C.D. Nelson, W.A. Powell, C.A. Maynard, K.M. Baier, A. Newhouse, S.A. Merkle, C.J. Nairn, L. Kong, J.E. Carlson, C. Addo-Quaye, M.E. Staton, F.V. Hebard, L.L. Georgi, A.G. Abbott, B.A. Olukolu, T. Zhebentyayeva (2014) The Forest Health Initiative, American chestnut (Castanea dentata) as a Model for Forest Tree Restoration: Biological Research Program. Acta Horticulturae (ISHS) 1019:173-178.
5. Islam-Faridi N, Majid MA, Zhebentyayeva T, Georgi LL, Staton ME, Hebard FV, Sisco PH, Carlson JE, Nelson CD (2013) Developing a cyto-molecular map of chestnut (*Castanea* spp.) using genetically and physically mapped BACs. BMC Proceedings (accepted).

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4. Fan S., Bielenberg D., Zhebentyayeva T., G.Reighard G., Okie W.R., Holland D. and Abbott A.G. (2010) Mapping quantitative trait loci associated with chilling requirement, heat requirement and blooming data in peach. New Phytologist, 185: 917-930.
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6. Soriano JM, Domingo ML, Zuriaga E, Romero C, Zhebentyayeva T, Abbott AG, Badenes ML (2012) Identification of simple sequence repeat markers tightly linked to plum pox virus resistance in apricot. Molecular Breeding, 30:1017–1026.
7. Sajer O., Scorza R, Dardick C, Zhebentyayeva T, Abbott AG, Horn R (2012) Development of sequence-tagged site markers linked to the pillar growth type in peach [*Prunus persica* L. (Batsch)]. Plant Breeding,131: 186—192.
8. Arus P, Verde I, Sosinski B, Zhebentyayeva TN, Abbott AG (2012) The peach genome. Tree Genetics & Genomes, 8: 531-547.\
9. International peach genome Initiative (2013) The high-quality draft genome of peach (*Prunus persica*) identifies unique patterns of genetic diversity, domestication and genome evolution. Nature Genetics, 45: 487–494
10. Zhebentyayeva T., Fan S., Chandra A., Bielenberg D.G., Reighard G.L., Okie W.R., Abbott A.G. (2014) Dissection of chilling requirement and bloom date QTLs in peach using a whole genome sequencing of sibling trees from an F2 mapping population. Tree Genetics & Genomes, 10: 35-51.

BIOGRAPHICAL SKETCH - C. Dana Nelson

Research Geneticist and Project Leader
USDA Forest Service, Southern Research Station,
Southern Institute of Forest Genetics, 23332 Success Road, Saucier, MS 39574
(228) 832-2747; fax (228) 832-0130; dananelson@fs.fed.us

a. Professional Preparation

Institution	*Major/Area*	*Degree*	*Year*
University of Minnesota	Forest Genetics	Ph.D.	1988
Oklahoma State University	Forest Genetics	M.S.	1984
Iowa State University	Forestry	B.S.	1982

b. Appointments (current in bold)

Oct 2013: Visiting Scientist, Department of Forestry, University of Kentucky
Mar 2008: Adjunct faculty, Department of Ecosystem Management, Texas A&M University
Mar 2004: Adjunct faculty, Department of Plant and Soil Sciences, Mississippi State University
Feb 2004: Project Leader, Southern Institute of Forest Genetics, USDA Forest Service
Oct 2002: Acting Project Leader, Southern Institute of Forest Genetics, USDA Forest Service
Nov 2001: Research Geneticist, Southern Institute of Forest Genetics, USDA Forest Service
Jan 1997: Adjunct faculty, School of Forest Resources and Conservation, University of Florida
Aug 1994: Research Scientist/Project Leader, Forest Resources Division, International Paper Comp.
Oct 1993: Adjunct faculty, School of Forestry, Wildlife and Fisheries, Louisiana State University
Apr 1989: Research Geneticist, Southern Institute of Forest Genetics, USDA Forest Service
1988-89: Postdoctoral Associate, Southern Institute of Forest Genetics, USDA Forest Service

c. Synergistic Activities

d. Dr. Nelson developed high-throughput genetic mapping labs at both the SIFG (1992-94) and International Paper Company (1994-1996). The labs support research on the applications of genetic marker technologies in forest genetics and tree breeding research.

e. Dr. Nelson is Supervisory Research Geneticist and Project Leader of a newly formed project within the Southern Research Station. The new project, resulting from the merger of two projects (SIFG in Saucier, MS and "Biological Foundations of Forest Productivity and Sustainability" in Research Triangle Park, NC), is allowing for the integration of forest genetic and physiologic research to an unprecedented degree with a goal of developing a program that spans from the gene/genome to the forest stand/ecosystem level.

f. Dr. Dr. Nelson served as the lead Principal Investigator for the Forest Health Initiative (FHI) project. This was a three-year research program designed to test the hypothesis that biotechnology and genomics can lead to a plantable American chestnut (*Castanea dentata*) tree capable of resisting chestnut blight disease (caused by *Cryphonectria parasitica*). Some of the project's accomplishments include: development and integration of genome-wide genetic and physical maps for Chinese chestnut (*C. mollissima*), identification of candidate genes for resistance and their transformation into American chestnuts, development of an early screening method for blight resistance, and field testing under APHIS permits.

g. Dr. Nelson is the lead Principal Investigator for a new research partnership between the Southern Research Station and the University of Kentucky. This research program is being developed to some degree in the spirit of the FHI, where additional forest tree health issues will be addressed.

h. Publications

i. Recent refereed publications

Quesada, Tania, M.F.R. Resende Jr., P. Muñoz, J.L. Wegrzyn, D.B. Neale, M. Kirst, G.F. Peter, S.A. Gezan, **C.D. Nelson**, J.M. Davis. 2014. Mapping fusiform rust resistance genes within a complex mating design of loblolly pine. Forests (in press).

Nelson, C.D., W.A. Powell, S.A. Merkle, J.E. Carlson, F.V. Hebard, N. Islam-Faridi, M.E. Staton, L. Georgi. 2014. Chestnut. In: K. Ramawat, editor, *Tree Biotechnology, Chapter 1,* CRC Press, in press (August 2013).

Westbrook, J.W., M.F.R. Resende Jr., P. Munoz, A.R. Walker, D.B. Neale, J.L. Wegrzyn, **C.D. Nelson**, M. Kirst, D.A. Huber, S.A. Gezan, G.F. Peter, J.M. Davis. 2013. Association genetics of oleoresin flow in loblolly pine: discovering genes and predicting phenotype for improved resistance to bark beetles and bioenergy potential, New Phytologist 199:89-100.

Nelson, CD, GF Peter, SE McKeand, EJ Jokela, RB Rummer, LH Groom and KH Johnsen. 2013. Pines. In: B.P. Singh, editor, *Biofuel Crops: Production, Chapter 20*, CAB International, Wallingford, UK, pp. 427-459.

Korecký, J, J Klápště, M Lstibůrek, J Kobliha, **CD Nelson**, YA El-Kassaby. 2013. Comparison of genetic parameters from marker-based relationship, sibship, and combined models in Scots pine multi-site open-pollinated tests. Tree Genetics and Genomes (DOI 10.1007/s11295-013-0630-z).

Vose, JM, DN Wear, AE Mayfield, III, **CD Nelson**. 2013. Hemlock woolly adelgid in the southern Appalachians: Control strategies, ecological impacts, and potential management responses. Forest Ecology and Management 291:209-219.

Jacobs, D.F., H.J. Dalgleish, **C.D. Nelson**. 2013. A conceptual framework for restoration of threatened plants: the effective model of American chestnut (*Castanea dentata*) reintroduction. New Phytologist 197: 378-393.

Stewart, JF, CG Tauer, JM Guldin, **CD Nelson**. 2013. Hybridization in naturally regenerated shortleaf pine as affected by the distance to nearby artificially regenerated stands of loblolly pine. Southern Journal of Applied Forestry 37(2):102-107.

Nelson, C.D., Powell, W.A., Maynard, C.A., Baier, K.M., Newhouse, A., Merkle, S.A., Nairn, C.J., Kong, L., Carlson, J.E., Addo-Quaye, C., Staton, M.E., Hebard, F.V., Georgi, L.L., Abbott, A.G., Olukolu, B.A., and Zhebentyayeva, T. (2013) The Forest Health Initiative, American chestnut (*Castanea dentata*) as a model for forest tree restoration: Biological Research Program. Acta Hort 1019:179-189.

Zhebentyayeva, T., Chandra, A., Abbott, A.G., Staton, M.E., Olukolu, B.A., Hebard, F.V., Georgi, L.L., Jeffers, S.N., Sisco, P.H., James, J.B., and **Nelson, C.D.** 2013. Genetic and genomic resources for mapping resistance to *Phytophthora cinnamomi* in chestnut. Acta Hort 1019:263-270.

Georgi, L.L., F.V. Hebard, M.E. Staton, B.A. Olukolu, AG. Abbott, **C.D. Nelson**. 2013. Adapting Chestnut Single Nucleotide Polymorphisms for Use in Breeding. Acta Hort 1019:105-112.

Kubisiak, T.L., **C.D. Nelson**, M.E. Staton, T. Zhebentyayeva, C. Smith, B.A. Olukolu, G.-C. Fang, F.V. Hebard, S. Anagnostakis, N. Wheeler, P.H. Sisco, A.G. Abbott, R.R. Sederoff. 2012. 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.

Fang, G-C., B.P. Blackmon, M.E. Staton, **C.D. Nelson**, T.L. Kubisiak, B.A. Olukolu, D. Henry, T. Zhebentyayeva, C.A. Saski, C-H. Cheng, M. Monsanto, S. Ficklin, M. Atkins, L.L. Georgi, A. Barakat, N. Wheeler, J.E. Carlson, R. Sederoff, A.G. Abbott. 2012. A physical map of the Chinese chestnut (*Castanea mollissima*) genome, and its integration with the genetic map. Tree Genetics and Genomes 9:525-537.

Tauer, CG, JF Stewart, R Will, C Lilly, J Guldin, **CD Nelson**. 2012. Hybridization leads to loss of genetic stability in shortleaf pine: Unexpected consequences of pine management and fire suppression. Journal of Forestry (June):216-224.

Kubisiak, T.L., C. Anderson, H.V. Amerson, J.A. Smith, J.M. Davis, and **C.D. Nelson**. 2011. A genomic map map enriched for markers linked to Avr1 *Cronartium quercuum* f.sp. *fusiforme*. Fungal Genetics and Biology 48:266-274.

Nelson C. Dana, Randall J. Rousseau, Joshua P. Adams, M. Cetin Yuceer. 2011. Report on the 31st Southern Forest Tree Improvement Conference (SFTIC). Tree Genetics and Genomes (DOI 10.1007/s11295-011-0454-7) 3 p.

Echt CS, S Saha, KV Krutovsky, K Wimalanathan, JE Erpelding, C Liang, **CD Nelson**. 2011. An annotated genetic map of loblolly pine based on microsatellite and cDNA markers. BMC Genetics 12:17.

Echt CS, S Saha, DL Deemer, **CD Nelson**. 2011. Microsatellite DNA in Genomic Survey Sequences and UniGenes of Loblolly Pine. Tree Genetics and Genomes 7:773-780.

BIOGRAPHICAL SKETCH

A. Personal Data

Name: Albert Glenn Abbott
Institution: University of Kentucky

Address: Department of Forestry Telephone: Office: 859-257-7596
University of Kentucky Fax: 859-323-1031
Lexington, Kentucky
Email: *albert.abbott@uky.edu*

B. Professional Preparation:

1. University of Connecticut, Storrs, CT , Biological Sciences, B.S. 197 2-1976
2. Brown University, Providence, RI, Cell and Molecular Biology, Ph.D. 1980.
3. Plant Breeding Institute, Cambridge, England, Rockefeller Foundation Postdoctoral Fellow in Plant Molecular Biology (Jan. 1981-Jan. 1983). Worked with Dr. R. Flavell.
4. Brown University, NIH Postdoctoral Research Associate in Insect Molecular Biology with Dr. Susan Gerbi, (Jan. 1984-Aug. 1984).
5. Brown University, NSF Postdoctoral Research Associate in Plant Molecular Biology with Dr. S. Beale, (Aug. 1983-Jan. 1984)

C. Appointments:

1. Staff Scientist III, Dept. of Forestry, University of Kentucky. (2014-), Co-director of Forest Health Research and Education Center, UKY.
2. ANR Chaire d' Excellence, INRA Bordeaux France. (2012-2014)
3. Coker Chair in Plant Molecular Genetics, Dept of Genetics and Biochemistry, Clemson University (2011-2013),
4. Professor of Genetics and Biochemistry and Coker Chair in Plant Molecular Genetics, Dept of Genetics and Biochcmistry, Clemson University (2001-2010)
5. Associate Professor of Biological Sciences, Dept. of Biological Sciences, Clemson University (1989-2000)
6. Assistant Professor of Biological Sciences, Department of Biological Sciences, Clemson University (Aug. 1984- 1989).

D. Closely related publications:

Jung S , Jiwan D, Cho I, Lee T, Abbott A, Sosinski B and Main D. 2009. Synteny of *Prunus* and other model plant species. BMC Genomics 10:76

Jung S., Cestaro A., Troggio M., Main D., Zheng P., Cho I., Folta K.M., Sosinski B., Abbott A., Celton J-M., Arus P., Shulaev V., Verde I.,Morgante M., Rokhsar D.S., Velasco R. , Sargent D.J. (2012). Whole genome comparisons of *Fragaria*, *Prunus* and *Malus* reveal different modes of evolution between Rosaceous subfamilies. BMC Genomics , 13:129.

Barakat A., Sriram A., Park J., T. Zhebentyayeva, D. Main, and A.G. Abbott (2012) Genome wide identification of chilling responsive microRNAs in Prunus persica BMC Genomics, 13:481

Barakat A., Staton M., Cheng C-H., Park J., Yassin N.B.M., Ficklin S., Yeh C-C., Hebard F., Baier K., Powell W., Schuster S.C., Wheeler N., Abbott A., Carlson J.E., Sederoff R (2012) Chestnut resistance to the blight disease: insights from transcriptome analysis. BMC Plant Biology, 12:38.

E. Five other publications:

Fang G-C, Blackmon B, Staton M, Nelson CD, Kubisiak TL, Olukolu B, Henry D, Zhebentyayeva T, Saski C, Cheng C-H, Monsanto M, Ficklin S, Atkins M, Georgi L, Barakat A, Wheeler N, Carlson J,

Sederoff R, Abbott A (2013) A physical map of the Chinese chestnut (*Castanea mollissima*) genome and its integration with the genetic map. Tree Genetics & Genomes, 9: 525–537.

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

Verde I, Abbott AG, Scalabrin S, Jung S, Shu S, Marroni F, Zhebentyayeva T, Dettori MT, Grimwood J et al. (2013) The high-quality draft genome of peach (Prunus persica) identifies unique patterns of genetic diversity, domestication and genome evolution. Nature Genetics, published on-line. doi:10.1038/ng.2586

Dardick C, Callahan A, Horn R, Carrasco K-R, Zhebentyayeva T, Hollender C, Whitaker M., Abbott A, Scorza R (2013) Identification of PpTAC1 as a functionally conserved regulator of axillary shoot growth angle in Prunus persica (peach) trees. Plant Journal, 75(4): 618–630.

Zhebentyayeva T., Fan S., Chandra A., Bielenberg D.G., Reighard G.L., Okie W.R., Abbott A.G. (2014) Dissection of chilling requirement and bloom date QTLs in peach using a whole genome sequencing of sibling trees from an F2 mapping population. Tree Genetics & Genomes, 10: 35-51.

F. Synergistic Activities:

1. Involved in teaching and designing summer workshops in genomics and genetics.
2. Service on National and International Rosaceae genomics research steering committees.
3. Serve on Ph.D. committees of students including Ph.D. granting institutions in other countries.
4. Host researchers from multiple countries for genomics and genetics research.
5. Contact person for the development of the Genome Database for Rosaceae as the central Web based databank for Rosaceae Genomics data worldwide.
6. Co-director of the Forest Health Research and Education Center, UKY

G. Collaborators and Other Affiliations:

1. Current Collaborators:
   Dr. P. Arus IRTA, Spain
   Dr. V. Baird, Horticulture, Clemson U.
   Dr. M.Badenes IVIA, Valencia Spain
   Dr D. Bielenberg, Clemson U.
   Dr. A. Callahan, AFRS, WV.
   Dr. V.Decroocq INRA Bordeaux France
   Dr. D. Holland, ARO Israel
   Dr. R. Horn. IMPB, Rostock Germany
   Dr. S. Jung, Michigan
   Dr. D. Main Washington State University
   Dr. W. Okie, USDA/Byron GA
   Dr. G. L. Reighard.
   Dr. R. Scorza AFRS, WV
   Dr. B. Sosinski North Carolina State University
   Dr. I. Verde Fruit Institute, Rome
   Dr. T. Zhebentyayeva, Clemson U
2. Thesis Advisor: Dr. Susan Gerbi, Brown University
   Post-doctoral advisors: Dr. R.B. Flavell (John Innes)
   Dr. S. Beale, Brown University
3. Graduate Students
   B. Levy MS.
   J. Wang, MS
   L. Medlin, MS, SLED, SC Dr. D. Dr.
   C. Zraket, MS
   J. Qin, MS
   S. Medel, MS, Fla.
   D. Gupta, Ph.D
   E. Hiatt Ph.D
   P. Tate Ph.D., Clemson U
   B. Sosinski Ph.D, NCSU
   S. Jung Ph.D. WSU
   F. Teule Ph.D. UWyo
   D. Lalli Ph.D. AFRS/USDA
   S. Hughes-Murphree, MS
   B. Olukolu Ph.D. NCSU
   F. Shenghua Ph.D. GGC
4. Postdoctoral associates
   L. Georgi, Clemon
   A. Lecouls. Montpellier Fr
   S. Rajapakse, Clemson
   K. Sossey, Buffalo, NY

BIOGRAPHICAL SKETCH – Christopher A. Saski

Director, Clemson University Genomics and Computational Biology Laboratory
Research Assistant Professor, Department of Genetics and Biochemistry
310 BRC105 Collings Street, Clemson SC 29634
(864) 656-0973; saski@clemson.edu

i. Professional Preparation

Institution	***Major/Area***	***Degree***	***Year***
Clemson University	Genetics	Ph.D.	2007
Clemson University	Microbiology	B.S.	1999

j. Appointments (current in bold)

2104-Present: Director, CU Genomics and Computational Biology Laboratory, Clemson University

2102-Present: Adjunct faculty, Department of Genetics and Biochemistry, Clemson University

2102-2014: Director, Clemson University Genomics Institute, Clemson University
2008-2012: Assistant Director, Clemson University Genomics Institute, Clemson University
2003-2008: Research Scientist, Clemson University Genomics Institute, Clemson University
2002-2003: Research Specialist, Arizona Genomics Institute, Tucson Arizaon
1999-2002: Research Associate, Clemson University Genomics Institute, Clemson University

k. Synergistic Activities

- Dr. Saski established for the vision, implementation, oversight, and growth of the newly formed genomics and computational biology laboratory designed to interface sophisticated genomics and computational solutions and services with strategic research and educational initiatives in human, agricultural, and environmental fields.
- Dr. Saski is responsible for vision and direction of this unit that includes obtaining funding (research grants and research contracts), management and oversight of all projects (~$1 million per year), staff and students (graduate, undergraduate, high school), daily operations, finances, outreach, research and development
- Dr. Saski is the lead Principal Investigator for the Clemson University genomics and computational biology laboratory which is a species-independent state-of-the-art research facility that operates at the interface of genomics, genetics and computational biology, to link genotype to phenotype in multiple complex systems.

l. Ongoing Research Support

- Christopher Saski (PI). $14,700. Horn Fly Antennal and Midgut Transcriptomics. Collaborative research agreement with Pia Olafson (USDA-ARS, US Livestock Insects Research Lab). 09.21.2014 – 08.31.2015
- Christopher Saski (PI), Tatyana Zhebhentatyeva (co-PI). $84,000. Data needed for foreign regulatory packages for the PPV resistant transgenic Prumus domestica cv. 'HoneySweet'. Collaborative research agreement with Chris Dardick (USDA-ARS, Appalachian Fruit Research Station). 08.01.14- 07.31.16.
- Christopher Saski (PI). $50,000. *Culicoides sonorensis* transcriptomics of vector competence. Collaborative research agreement with Dana Nayduch (USDA-ARS NPA Center for Grain & Animal Health Res. 05.25.12-6.30.2015
- Sarah Harcum (PI), Christopher Saski (co-PI). $199,999. Transcriptome Analysis of CHO cells to improve productivity and control protein aggregation. NSF EAGER. 04/01/12 –03/31/15.

- Z. Chen (PI), Candace Haigler (co-PI), Brian Scheffler (co-PI), David Stelly (co-PI), Christopher Saski (collaborator) $3,800,000: Cotton Fiber Genomics – Functional and Sequence Analysis of Fiber Development in Tetraploid Cotton.
- Seah Mehlenbacher (PI), Christopher Saski (co-PI). $160,000. A physical map of the highly heterozygous Hazelnut genome and implications for a reference genome sequence.
- Z. Chen (PI), Christopher Saski (co-PI), Jeremy Schmutz (co-PI). $100,000. Sequence and assemble MTP (minimum tile path) BACs on homoeologous chromosomes 11 and 21 in allotetraploid Cotton.

m. Publications

i. Recent refereed publications

1. Nayduch D, Lee M, **Saski C**. (2014) Gene discovery and differential expression analysis of humoral immune response elements in female *Culicoides sonorensis* (Diptera: Ceratopogonidae). Parasites and Vectors.
2. Nayduch D, Lee MB, **Saski CA** (2014) The Reference Transcriptome of the Adult Female Biting Midge (Culicoides sonorensis) and Differential Gene Expression Profiling during Teneral, Blood, and Sucrose Feeding Conditions. PloS one 9: e98123.
3. Pauchet Y, **Saski CA**, Feltus FA, Luyten I, Quesneville H, et al. (2014) Studying the organization of genes encoding plant cell wall degrading enzymes in Chrysomela tremula provides insights into a leaf beetle genome. Insect molecular biology.
4. Motamayor JC, Mockaitis K, Schmutz J, Haiminen N, Livingstone D, 3rd, Cornejo O, Findley SD, Zheng P, Utro F, Royaert S, **Saski C** *et al*: The genome sequence of the most widely cultivated cacao type and its use to identify candidate genes regulating pod color. *Genome Biol* 2013, 14(6):R53.
5. Guang-Chen Fang, BPB, Margaret E Staton, C. Dana Nelson,, Thomas L. Kubisiak BAO, David Henry, Tatyana Zhebentyayeva ,, Christopher A. **Saski C**-HC, Megan Monsanto, Stephen Ficklin, Michael Atkins, Laura L. Georgi, Abdelali Barakat, Nicholas Wheeler, John E. Carlson, Ronald Sederoff, Albert G. Abbott: A physical map of the Chinese Chestnut genome. *Tree Genetics and Genomes* 2012, 9:525-537.
6. Kuhn DN, Livingstone D, Main D, Zheng P, **Saski C**, Feltus FA, Mockaitis K, Farmer AD, May GD, Schnell RJ *et al*: Identification and mapping of conserved ortholog set (COS) II sequences of cacao and their conversion to SNP markers for marker-assisted selection in Theobroma cacao and comparative genomics studies. *Tree Genet Genomes* 2012, 8(1):97-111.
7. McCraney WT, **Saski CA,** Guyon JR: Isolation and characterization of 12 microsatellites for the commercially important sablefish, Anoplopoma fimbria. *Conserv Genet Resour* 2012, 4(2):415-417.
8. **Saski CA**, Feltus FA, Staton ME, Blackmon BP, Ficklin SP, Kuhn DN, Schnell RJ, Shapiro H, Motamayor JC: A genetically anchored physical framework for Theobroma cacao cv. Matina 1-6. *BMC Genomics* 2011, 12(1):413.
9. **Saski CA**, Li Z, Feltus FA, Luo H: New genomic resources for switchgrass: a BAC library and comparative analysis of homoeologous genomic regions harboring bioenergy traits. *BMC Genomics* 2011, 12:369.
10. Feltus FA, **Saski CA**, Mockaitis K, Haiminen N, Parida L, Smith Z, Ford J, Staton ME, Ficklin SP, Blackmon BP *et al*: Sequencing of a QTL-rich region of the Theobroma cacao genome using pooled BACs and the identification of trait specific candidate genes. *BMC Genomics* 2011, 12:379.
11. Ulloa M, Wang C, Hutmacher RB, Wright SD, Davis RM, **Saski CA**, Roberts PA: Mapping Fusarium wilt race 1 resistance genes in cotton by inheritance, QTL and sequencing composition. *Mol Genet Genomics* 2011, 286(1):21-36.
12. Jansen RK, **Saski C**, Lee SB, Hansen AK, Daniell H: Complete plastid genome sequences of three Rosids (Castanea, Prunus, Theobroma): evidence for at least two independent transfers of rpl22 to the nucleus. *Mol Biol Evol* 2011, 28(1):835-847.