a. Project Title: Conservation, collection, and characterization of *Castanea dentata* germplasm in the South

b. Summary:

An accelerated, graft-based, germplasm conservation method incorporating predictive GIS habitat models targeting undersampled and genetically diverse American chestnut populations allows rapid introduction of genes into the breeding program. Grafting promotes precocious blooming, and facilitates genomic sampling. We will target geographic areas of known high genetic diversity and populations thought to harbor alleles with adaptive value. Grafts will be grown in a high-light environment to expedite flowering. Preliminary trials include 11 individuals successfully grafted; one individual produced catkins three months from grafting, and leaves were collected from all 11 genotypes in support of the TACF landscape genomics study.

c. Principal investigator and institutional affiliation:

Dr. J. Hill Craddock
Trent Deason
Erin Taylor
University of Tennessee Chattanooga
Department of Biology, Geology, and Environmental Science

d. Duration of project:

October 2018 – October 2019

e. Amount requested:

$5,230

f. Short and long term goals:

We request funds to advance an accelerated, graft-based, germplasm conservation method incorporating predictive GIS habitat models targeting undersampled and genetically diverse American chestnut populations. Graft propagation will allow for the rapid introduction of genes into the TACF breeding program. Grafting can promote precocious blooming, and it will facilitate genomic sampling. We will target geographic areas of known high genetic diversity and populations thought to harbor alleles with high adaptive value. Grafted plants will be grown in high-light environment growth chambers to expedite flowering. Results of preliminary trials include 11 individuals from Alabama and Tennessee locations successfully grafted to a variety of rootstocks. One individual produced catkins three months from grafting, and leaves were collected in July 2018 from all 11 genotypes in support of the TACF landscape genomics study.

The short term goal of this proposal is to capture new and/or under sampled sources of American chestnut throughout the Southeast and accelerate flower induction for breeding. Pollen will be collected and processed for use in hybridization. Collection data will be used to to train the GIS habitat models to better predict chestnut occurrence. Grafting can promote precocious blooming, and it will facilitate genomic sampling because many grafted accessions can be grown together in a single location. And all grafted accessions will be sampled to support the ongoing TACF range-wide genomics study. The long term goal is to conserve as much as possible of the presumed adaptive variation present in the southernmost populations of *Castanea dentata*. Container-grown grafted plants will will be available for TACF Germplasm Conservation Orchards and maintained in containers at UTC. GIS habitat models can be used in future projects and applications to aid in locating naturally occuring American chestnut populations.

g. Narrative:

Introduction: The American chestnut, *Castanea dentata*, is susceptible to a fungal blight (*Cryphonectria parasitica*) as well as to Phytophthora root rot (PRR) caused by *Phytophthora cinnamomi*. Conversely, the Chinese chestnut (*Castanea mollissima*) has shown resistance to both (Anagnostakis 2001, Burnham 1988). Previous research has produced viable Chinese-American chestnut hybrids that capture the disease resistance from the Chinese, while maintaining the morphological characters of the American (Burnham 1988, Diskin 2006). However, the primary breeding method requires locating flowering American chestnuts, which is difficult, effectively limiting the number of individuals in the gene pool (Fei 2007). Most individuals in surviving *C. dentata* populations never bloom because they are in the understory (Paillet 2002). Even when conditions are ideal, breeding wild trees is time consuming and involves repeated visits to the field in order to place and retrieve pollination bags to collect pollen and/or seeds. This method is complicated when field conditions, such as terrain and distance from roads, make it difficult to collect pollen and nuts efficiently. We propose a graft-based method that focuses on collecting scionwood, rather than pollen or seeds, from naturally occurring American chestnut trees. Collection of scionwood is not dependent on the sexual maturity of the plant; even trees that are not blooming can be brought into the breeding program. Grafted American chestnuts can be planted in the field, in a germplasm conservation orchard (GCO), or maintained in containers. Container grown trees can be manipulated in ways that may accelerate the development of flowers. The increased photoperiod conditions in a lighted greenhouse, or growth chamber may expedite flowering and shorten the time to pollen collection (Baier et.al. 2012, Sanz-Pérez 2008). If these Southern American clones produce seeds, we also capture their cytoplasmic genes, and possibly rare alleles, that will further enhance the genetic diversity of the breeding program and conservation efforts.

Background/Significance: The natural history of American chestnut, *Castanea dentata*, and its demise, have been thoroughly reviewed (Roane et al. 1986; Anagnostakis 1987). In the eastern hardwood forests, and particularly in the mountains of the Southeast, American chestnut was important economically and culturally, possibly more than any other one tree in its range (Ashe 1911). In 1904, a fungal blight, later described as *Cryphonectria parasitica*, was documented on American chestnut in the Bronx Zoological Park, New York. Despite prevention measures, chestnut blight spread rapidly, decimating American chestnut throughout the entirety of its range (Anagnostakis 1987; Roane et al. 1986). Early efforts to find blight-resistant cultivars failed, which gave way to a backcross breeding program developed by TACF designed to introgress disease resistance from *C. mollissima* into surviving *C. dentata* (Burnham 1988). This ongoing program has had considerable success in development of both potentially blight resistant lineages and maintaining morphological characteristics of American chestnut (Diskin 2006).

In recent years, ecological niche modelling has been used in assessing the potential geographical distribution of species, and can applied to several fields, such as ecology, biogeography, and conservation (Elith et al. 2006; Fei et al. 2007; Levine et al. 2004; Wang et al. 2010). One particular use from this research will be to identify where there are likely to be *C. dentata* stands in bloom. Disturbances such as burns and clear-cuts have shown to be a strong indicator of where *C. dentata* blooms are likely to be found. The principal objective of these disturbances is to regenerate the forest with healthier trees. However, timber harvesting is a secondary objective when it comes to clear-cutting. Boring et al. (1981) showed that *Castanea* comprised the leading component of woody biomass on clear-cuts in the first 5 years after cutting. These results indicate that chestnut is surviving and perhaps thriving despite the chestnut blight, and that *Castanea* survival could be related to its ability to regenerate stems by sprouting (Paillet 2002). Along with this aspect, we could use these models to assist in scionwood collection for asexual propagation. Including graft-based propagation allows the conservation of individuals previously excluded due to reproductive immaturity and/or geographic inaccessibility. Expanding the current breeding program to include regionally sourced, grafted American chestnuts will provide potential development of additional resistant lineages as well as conserve regional variation..

Research Plan and Methods:

We seek to continue our regionally focused, graft-based propagation method for collecting American chestnut. We will target individuals not suited for traditional pollination breeding and individuals not easily pollinated due to factors such as equipment access, geography and/or sexual immaturity. The addition of GIS habitat modeling will help target areas of collection. A minimum of four selection sites will be chosen using the Forestry Inventory Analysis (FIA) data, university herbarium data, and GIS prediction models based on a variety of environmental factors, in addition to TACF members and landowner connections. Current literature states that *C. dentata* prefers sites that are well drained and have a pH of 4-6, slopes of 0-10%, and greater than 75% soil sand content (Wang et al., 2013). Elevation ranges associated with the Southern Appalachian Mountains for *C. dentata* range from 1,300 to 4,500 feet (Braun 1950). Climate variables include 40-48 inches of precipitation, a mean annual temperature of 40-60 degrees Fahrenheit, and 240 frost-free days (Wang et al., 2013). There has been a record of low *C. dentata* presence in the bottoms of ravines and valleys because frosts are common on wet, protected soils. While *C. dentata* is believed to be one of the most cold-hardy species of the *Castanea* genus (Huang 1998; Wang 2013), it is still prone to frost damage (Parker et al. 1993; Wang 2013). These factors will form the initial parameters for creating both models.

Two different types of models will be generated in order to obtain the most accurate information. A comparison of both presence-only modelling techniques indicates that the Maxent and the GARP models have better predictive accuracy than other methods (Elith et al. 2006, Wang et al. 2010) and will be used for this study. The GARP model utilizes both presence and absence data, and has been widely used to model the potential geographic distribution of species. The Maxent model uses presence and continuous data, that incorporates interactions between different variables (Phillips et al. 2006) and has a better performance when only a limited amount of information is available (Elith et al. 2006, Wang et al 2010). Access to the modeling software and ArcGIS will be needed. Through the University of Tennessee Chattanooga, we will have access to the required resources to create both models of the *C. dentata* distribution. Trips will be made to each identified location in fall of 2018 to confirm locations and species identification, and again in winter of 2018-2019 to collect scionwood. Scions will be grafted and grown the following spring 2019.

It is important to note that this study is not designed to measure graft compatibility, but only to employ the most widely accepted chestnut grafting techniques. Results from our previous grafts will be used to guide grafting during the spring of 2019. Scionwood collected from naturally occurring *C. dentata* (ortets) will be grafted to a range of rootstocks (*C. dentata, C. mollissima*, F1 *C. dentata X C.* mollissima, and BC3 of the same pedigree) and grown *ex situ*. Additional rootstocks planted in the spring of 2018 will be used in the spring 2019. We will use the whip and tongue and/or bark-flap grafts (Craddock and Bassi, 1999). Container-grown, grafted plants (ramets) will be grown under increased temperature (heated greenhouse/growth chamber) and photoperiod (artificial lighting) in order to expedite budburst and development of inflorescence (Baier et.al. 2012, Huang 1994, Sanz-Pérez 2008). Of the scions collected, a portion of the ramets will be used in a control group to be grown under standard greenhouse/nursery conditions. This will serve as a baseline comparison for the effectiveness of increased temperature and light exposure on reducing the number of days to produce flowers and pollen. We hope to collect scionwood from at least ten ortets from four sampling regions, and to graft at least ten rootstocks per ortet for a total of 400 grafted plants.

We predict that increased photoperiod of red and blue spectrum light will produce flowers and pollen on successful grafts sooner than the control group. Data will be collected through regular observations of both groups and due to the potential for small sample sizes, it will be analyzed through a Fisher's exact test to show any statistical significance on reduction of flowering time.

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h. Timeline

October – November 2018	December 2018 – February 2019	March – May 2019	June – October 2019
Determine locations and visit for species ID and model training. Previously grafted plants begin temperature and photoperiod treatment	Revisit locations for scionwood collection; record data on temperature and photoperiod treatment	Graft recently collected scions to rootstocks, monitor success.	Review GIS model for accuracy, continue graft monitoring, and manuscript preparation

i. How results will be measured and reported
A financial report and final outcomes will be submitted to TACF upon completion of the project. The results will, we hope, will be presented scientific conferences as well as published in reviewed scientific journals.

j. Breakdown of how funds will be spent
Identified American chestnut habitat/locations will be visited once each during the summer or fall of 2018 in order to locate individuals and confirm species identification, then each site will be revisited in winter of 2018-2019 to collect scionwood. Mileage reimbursement is to offset the cost of travel based on two trips per collection region (one for scouting and one for scion collection) at an estimated 300 mi roundtrip, at the University of Tennessee Chattanooga mileage rate. Lighting equipment will be purchased, including bulbs, and fixtures by the SEARCH grant (formerly Provost grant) awarded by UT Chattanooga. Though on site light equipment exists, the available space is not sufficient to conduct this research. Grafting supplies will be required, such as grafting tape and wax. Consumables, such as ice/ice packs and sealable plastic bags will be required on collecting trips in order to maximize scion survival. UTC student salary will support two graduate students at $12/hr for 125 hours each (250 hrs total) for tasks including GIS model building/analysis, growth chamber setup and monitoring, plant care in nursery/growth chamber, and grafting.

Budget:

Item	Cost
Mileage	**$1,150**
Pots	**$475**
Potting Medium	**$550**
Grafting Supplies	**$25**
Consumables	**$30**
UTC Student Salary	**$3,000**
TOTAL	**$5,230**

k.   Brief C.V. for each Principal Investigators

Curriculum Vitae for J. Hill Craddock
UC Foundation Davenport Professor in Biology
The University of Tennessee at Chattanooga
Department of Biology, Geology, and Environmental Science
615 McCallie Ave. Chattanooga, TN 37403

a. Professional Preparation

Università di Torino	Turin, Italy	Pomology	Dott. Ric. 1992
Oregon State University	Corvallis, OR	Horticulture	M.S, 1987
Indiana University	Bloomington, IN	Biology & Fine Arts	B.A., 1983

b. Appointments
2008 – present Professor, University of Tennessee at Chattanooga
1999 – 2007   Associate Professor, University of Tennessee at Chattanooga
1996 – 1998   Assistant Professor, University of Tennessee at Chattanooga
1994 – 1996   Postdoctoral Research Associate, Tennessee State University
1993 – 1994   Postdoctoral Fellow, Università di Torino
1989 – 1992   Graduate Research Assistant, Università di Torino
1984 – 1987   Res. Assistant, USDA-ARS National Clonal Germplasm Repository

c. Publications *(relevant to current proposal)*
1. Georgi, L., **Craddock, J.H.**, Bevins, D., Kling, R., and Hebard, F. (2014) Grafting chestnuts. *J. Amer. Chestnut Foundation* 28(1): 20-23.

2. Sisco, PH, TC Neel, FV Hebard, **JH Craddock**, and J Shaw. (2014) Cytoplasmic male sterility in interspecific hybrids between American and Asian *Castanea* species is correlated with the American D chloroplast haplotype. *Acta Hort.*109:215-222.

3. Perkins, M.T.* and **J.H Craddock.** (2014). The effect of phosphite fungicides on mycorrhiza formation in a family of BC4 Chinese-American chestnut seedlings. Technical Report to USDA NE-1333: Biological Improvement of Chestnut through Technologies that Address Management of the Species, its Pathogens and Pests. 6-5 September 2014, La Crosse, WI.

4. Shaw,J.,**Craddock, J.H**., and Binkley, M.A.* (2012). Phylogeny and Phylogeography of North American *Castanea*Mill. (Fagaceae) Using cpDNA suggests Gene Sharing in the Southern Appalachians (*Castanea* Mill., Fagaceae). *Castanea* 77(2):186-211.

5. **Craddock, J.H.** (2009) USA. Pages 168-174 in: Avanzato, D. (ed.) Following Chestnut Footprints (Castanea spp.) - Cultivation and Culture, Folklore and History, Tradition and Uses :: Sulle Orme del Castagno - Coltura e Cultura, Folclore e Storia, Tradizioni e Usi. *Scripta Horticulturae*(ISHS) Number 9. 175 pp. ISBN 978-90-6605-632-9

6. **Craddock, J.H.** (2007) Chestnut Relatives. In: Bolgiano, C. (ed) Mighty Giants: An American Chestnut Anthology. Images from the Past, and The American Chestnut Foundation. 296 pp. ISBN 978-1884592485

7. **Craddock, J.H.** (2006) Chestnut Breeding in the United States. Pages 109-128 in: Tree Breeding Researches: for the next half century. Proc. International Symposium for the 50th Anniversary of KFGR & the 20th Anniversary of the late Dr. SK Hyun. June 15-16, 2006, Forest Seed Research Center, Suanbo, Korea. KFRI.

8. **Craddock, J.H**., Alexander, M.T.,*. Alexander, S.H.,* Bramblett, J.L., and Worthen, L.M.* (2005) Chestnut cultivar evaluations in Tennessee: orchard establishment and early germplasm characterization. Acta Hort. 693: 465-467.

9. Alexander, M.T.,* Worthen, L.M.,* and **Craddock, J.H.** (2005) Conservation of *Castanea dentata* germplasm of the southeastern United States. *Acta Hort*. 693: 485-490.

10. Bassi, G. and **Craddock, J.H.** (1999) Performance and description of the introduced chestnut cultivar 'Colossal' in Cuneo province, northwest Italy. In: Salesses, G. (ed.) Proc. 2nd International Chestnut Symposium, Bordeaux, France. *Acta Hort.*494: 317-318.

11. **Craddock, J.H.** and Bassi, G. (1999) Effect of clonally propagated interspecific hybrid chestnut rootstocks on short-term graft incompatibility with four cultivars of Italian "Marrone". In: Salesses, G. (ed.) Proc. 2nd International Chestnut Symposium, Bordeaux, France. *Acta Hort.*494: 207-212

12. Craddock, J.H. and **Bassi, G.** (1999) Introduction into Italy of eight *Castanea mollissima* cultivars from China. In: Salesses, G. (ed.) Proc. 2nd International Chestnut Symposium, Bordeaux, France. *Acta Hort.*494: 319-321.

13. **Craddock, J.H.** (1998) Chestnut Resources in North America. *Annual Report of the Northern Nut Growers Association* 89: 19-30.

14. Bounous, G., Paglietta, R., Bellini, E. and **Craddock, J.H.** (1995) Il miglioramento genetico del castagno: situazione, obiettivi e metodi. *Frutticoltura* 57(11): 63-73.

15. **Craddock, J.H**., Ferrini, F., Mattii, G.B., Nicese, F.P. and Pellegrino, S. (1991) Ricerche per l'individuazione di impollinatori del "Marrone di Chiusa Pesio." *Frutticoltura* 53(12): 61-63.
*denotes student author

d. Service
· The American Chestnut Foundation, Board of Directors (1997-2014), V.P. and Chair, Science Cabinet (1997-2001), Chair Awards Committee (current)
· Highlands Biological Station, Board of Scientific Advisors (2009-2012)
· Reviewer/referee for *Castanea, Journal of the American Society for Horticultural Science*, *HortScience,*and *J. American Chestnut Foundation*
· Reflection Riding Arboretum and Nature Center, Board of Directors (2000-2014)

· City of Chattanooga Tree Commission (2011-present)
· U.S. Dept. Agriculture Regional Project NE1033: Biological Improvement of Chestnut through Technologies that Address Management of the Species, its Pathogens and Pests (was NE-1015 from 2008-2013), 2017 Meeting Chair (2016 –present)
· U.S. Dept. Agriculture Regional Project NE-140 (Secretary 1999; Chairman 2000)
· International Society for Horticultural Science, Member, Reviewer
· Northern Nut Growers Association, Member, Reviewer, Editor
· Beta Beta Beta Biological Honor Society, Faculty Advisor

3. Public Outreach
· Led public classes at the Reflection Riding Arboretum and Nature Center including "The Fungi" and "Tree ID" as part of the core course offerings for the Certificate of Native Plants program (2013-2014).
· City of Chattanooga Tree Commission, planned and coordinated a series of lectures and volunteer workdays for the "Take-Root Chattanooga Citizen Forester" program (2010-2015).
· City of Chattanooga Tree Commission, helped plan and coordinate the "If Trees Could Sing" installation and launch party, in collaboration with The Nature Conservancy, Coolige Park (2016).
· Coordinated the led the training workshops Appalachian Trail Mega Transect Project, in collaboration with The American Chestnut Foundation, Nantahala Outdoor Center, (2008-2010).
· Numerous public lectures and radio and newspaper interviews about restoration of the American chestnut (1996-present).

Curriculum Vitae for Trent Deason
Education
B.S. Environmental Science, Minor: Biology. University of Tennessee at Chattanooga GPA: 4.0/4.0
A.A.S. Electronic Systems Technology. Community College of the Air Force

Awards and Distinctions
2018	University of Tennessee at Chattanooga Highest Overall GPA
2018	University of Tennessee at Chattanooga Service Award
2017	University of Tennessee at Chattanooga Environmental Science Award
2017	University of Tennessee at Chattanooga SGA Outstanding Senior Award
2015 – 2017	Dean's List
2015	USAF Commendation Medal
2014	NCO of the Quarter
2013	USAF Achievement Medal
2013	USAF Airman Leadership School Academic Achievement Award
2013	NATO Service Medal
2012	USAF Achievement Medal
2011	Airman of the Quarter

Grants
2018	UTC SEARCH Grant Recipient
2017	TACF External Grant Recipient

Publications

2018   Deason, Trent, "Conservation and collection of *Castanea dentata* germplasm in the South"
                    (2018). *Honors Theses.* https://scholar.utc.edu/honors-theses/146

Professional Memberships:
The American Chestnut Foundation

Recent Professional Service
October 2017 - Present TACF Alabama Chapter Intern performing orchard planting and maintenance, data collection, and DentataBase data management.
October 2016 – Present Planting, pollination, assessment of disease severity in chestnut trees, and other work performed in cooperation with the American Chestnut Foundation.
July 2009 – July 2015 Honorable service in the United States Air Force as a Radio Frequency Transmission Systems technician and supervisor, performed and led numerous communication system repairs, maintenance, and operations.

Curriculum Vitae for Erin Taylor
Education
B.S. in Environmental Sciences in Agricultural Systems. Mississippi State University. Graduated May 2017

Awards:
2016   Mississippi State Marketing Assistant of the Year

Professional Memberships:
The American Chestnut Foundation

Professional Development:
January 2018 - University of Tennessee Chattanooga and the American Chestnut Foundation Science
        Meeting
November 2017 - University of Tennessee Chattanooga and the American Chestnut Foundation Winter
        Tree Planting
October 2017 - University of Tennessee Chattanooga and Tennessee Tech University American Chestnut
        Meeting

l. Conflicts of Interest
This project has received partial funding through a UTC SEARCH grant awarded to Trent Deason, in the amount of $1,000. As stated in the budget, those funds will be used to purchase lighting equipment in addition to minimal travel to a single collection site. Dr. J. Hill Craddock, is a member of the Research Advisory Committee and Science and Technology Committee, and will recuse himself from discussion of the present proposal.