TACF Science Review, August 9-11, 2006

Science Review Agenda

All meetings will be at the 4-H Center, unless otherwise specified. All meals will be at the 4-H Center, at 7:30 am, 12:00 and 6:00 pm, unless otherwise specified.

Tuesday, August 8, 2006, 7:00 pm	Dinner at The Tavern in Abingdon (222 East Main Street, Abingdon, VA)
Wednesday, 8:00 am	Proceed from 4-H Center to Jefferson National Forest to observe current chestnut biology
Wednesday, 10:00 am	Return to Wagner Farm and tour
Wednesday, 12:00 pm	Box lunch at Wagner Farm office
Wednesday, 12:30 pm	Visit local Chinese chestnut tree to discuss
Wednesday, 1:00 pm	Tour Price Farm
Wednesday, 3:00 pm	Tour Clapper Seed Orchard (Price Farm Annex)
Wednesday, 5:00 pm	Return to 4-H Center for supper
Wednesday, 6:40 pm	Kim Steiner on future of scientific decision making in TACF
Wednesday, 7:00 pm	Fred Hebard on Meadowview science to date
Wednesday, 7:45 pm	Sara Fitzsimmons on regional (chapter) breeding program
Wednesday, 8:00 pm	Paul Sisco on molecular mapping results to date
Wednesday, 8:20 pm	Bob Paris on his planned research and research currently underway
Wednesday, 9-9:30 pm	Adjourn
Thursday, 8:00 am	Committee meets to discuss and formulate questions
Thursday, 10:00 am	Committee interviews group and individuals as they see fit
Thursday, 12:00 pm	Lunch at 4-H Center
Thursday, 1:00 pm	Committee meets to write report and interview group and individuals as they see fit
Thursday, 6:00 pm	Dinner at local restaurant or ordered into 4-H Center
Thursday, 7:00 pm	Committee meets to finish report
Friday, 8:00 am	Committee give oral report and recommendations
Friday, 9:00 am	Committee answers questions from general members of the foundation
Friday, 11:30 am	Adjourn
Friday, 12:00 pm	Lunch at 4-H Center

Reviewer Accommodations:
Southwest Virginia 4H Center, 25236 Hillman Highway, Abingdon, VA 24210. (276) 676-6180. Directions to the center:
http://www.ext.vt.edu/resources/4h/southwest/reachus.html

TACF Staff and Board Accommodations:
Holiday Inn Express, 940 East Main Street, Abingdon, VA 24210 (276) 676-2829

Meadowview Research Farms – Wagner Farm
14005 Glenbrook Avenue, Meadowview, VA 24361 (276) 944-4631

TACF Science Review Panel 2006

Glen Stanosz, Professor of Plant Pathology, University of Wisconsin-Madison
grs@plantpath.wisc.edu

Lauren Fins, Professor of Forest Genetics, University of Idaho
lfins@uidaho.edu

Robert McIntosh, Senior Research Fellow, University of Sydney
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Ron Phillips, Regents Professor of Plant Genetics, University of Minnesota
Phill005@UMN.edu

The reviewers are:

Dr. Glen Stanosz
Professor of Plant Pathology
Department of Plant Pathology
University of Wisconsin - Madison

Dr. Lauren Fins
Professor of Forest Genetics
Department of Forest Resources
University of Idaho

Professor Robert MacIntosh
Senior Research Fellow
Plant Breeding Institute – Cobbitty
The University of Sydney
AUSTRALIA

Phillips, Ronald L., Ph.D.
Regents' Professor
Research and education; crop tissue culture, genomics, and cytogenetics
Department of Agronomy and Plant Genetics, University of Minnesota

The first Science Review was held in 1999 and the final report of which can be found here: http://www.acf.org/ScienceR.pdf

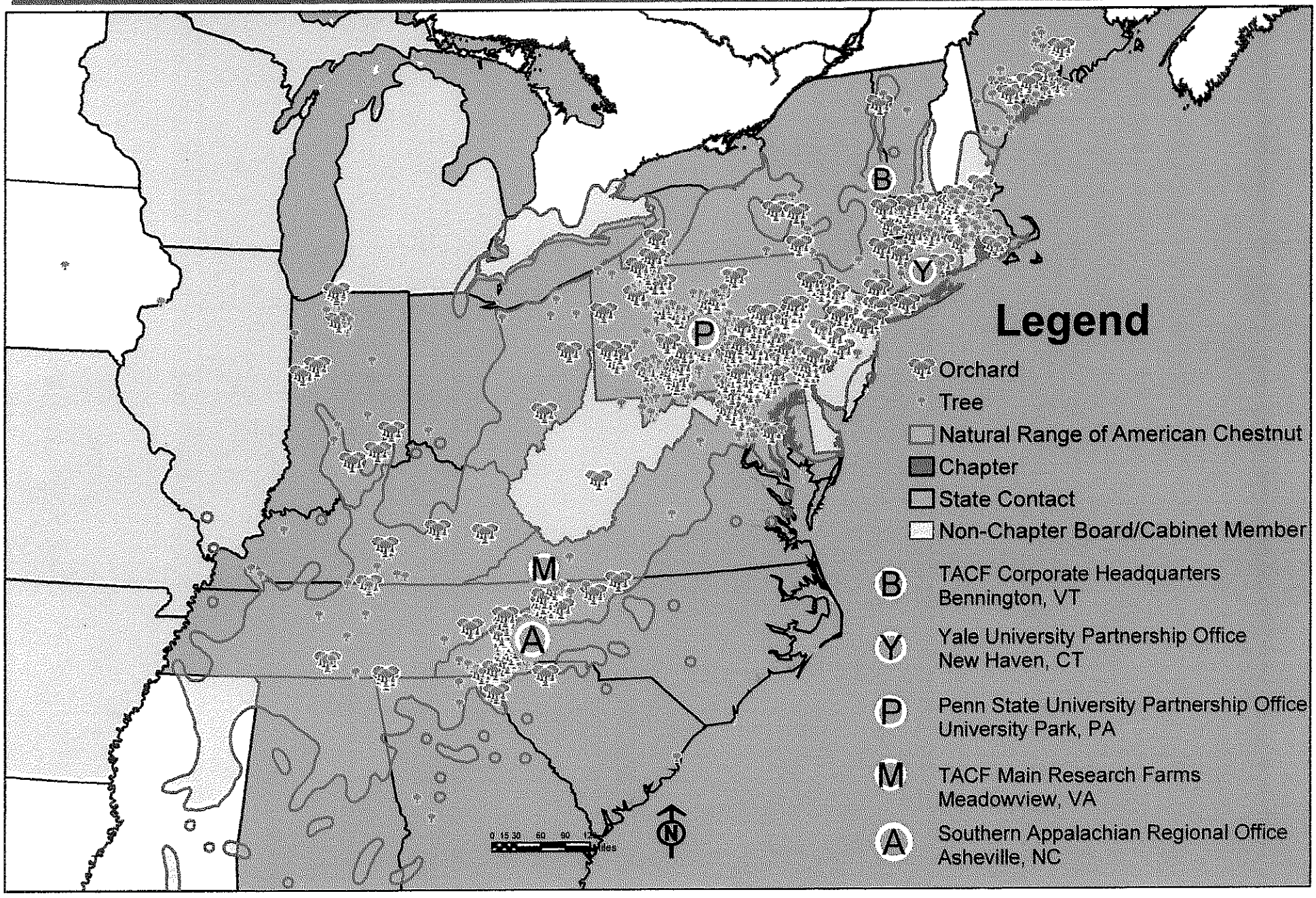
The nearest airport is the Tri-Cities Airport in Bristol, Tennessee.

Area accommodations:
The Holiday Inn Express, 940 East Main Street, Abingdon, VA, Tel: 276-676-2829 ($88-104)

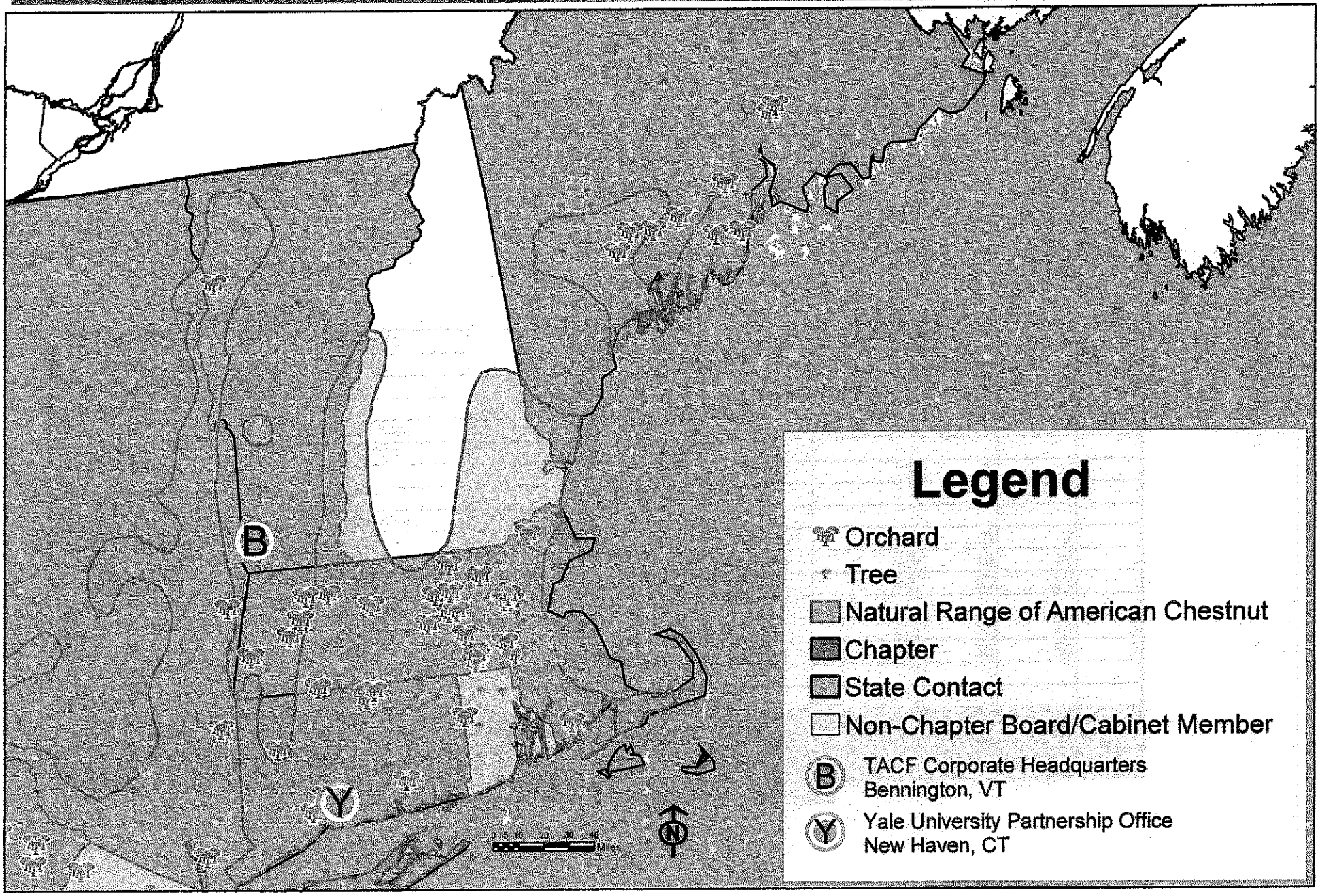
If you are going to attend, please let me know for space planning purposes.

Thank you,

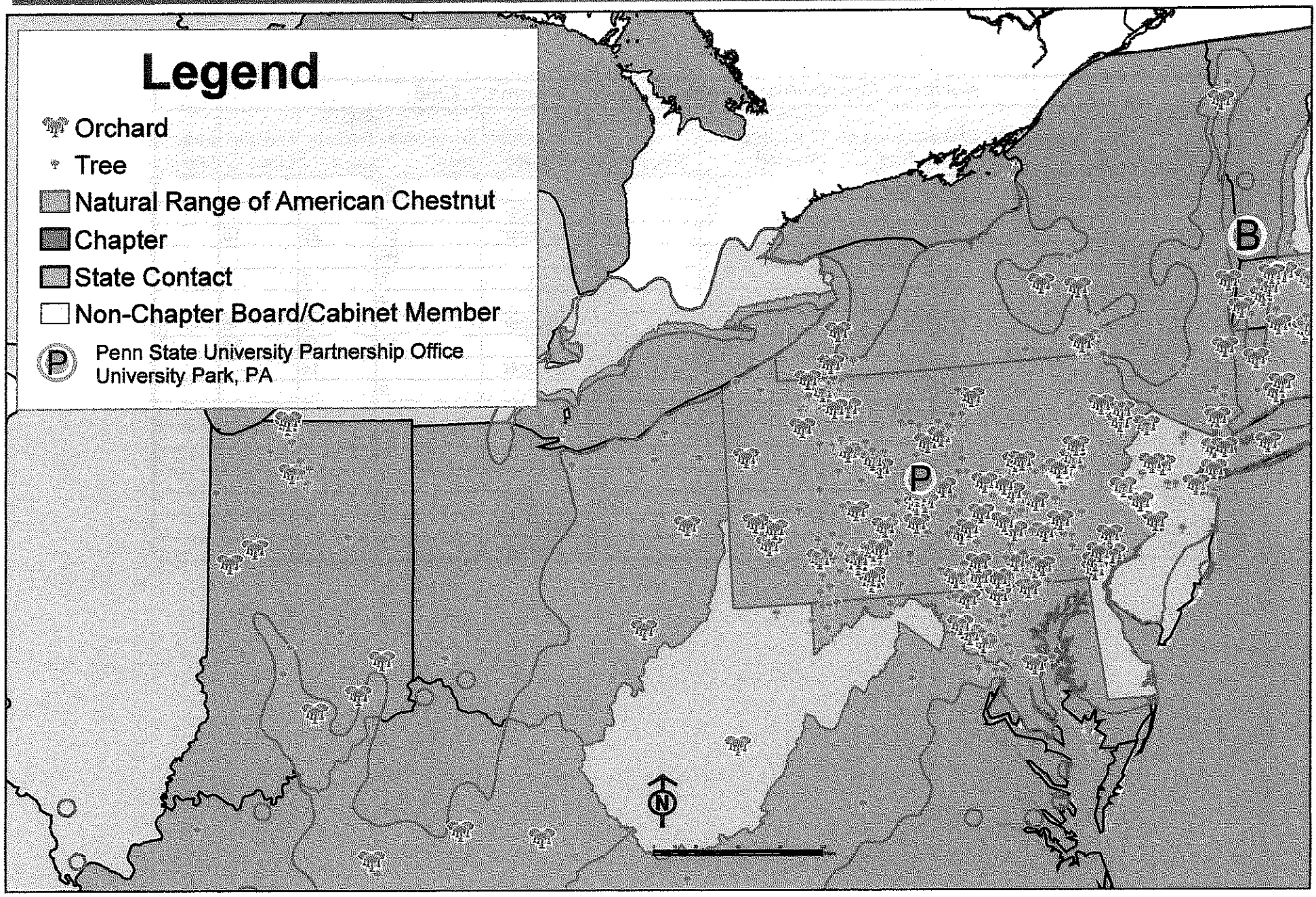
TACF's Regional Breeding Program Resources



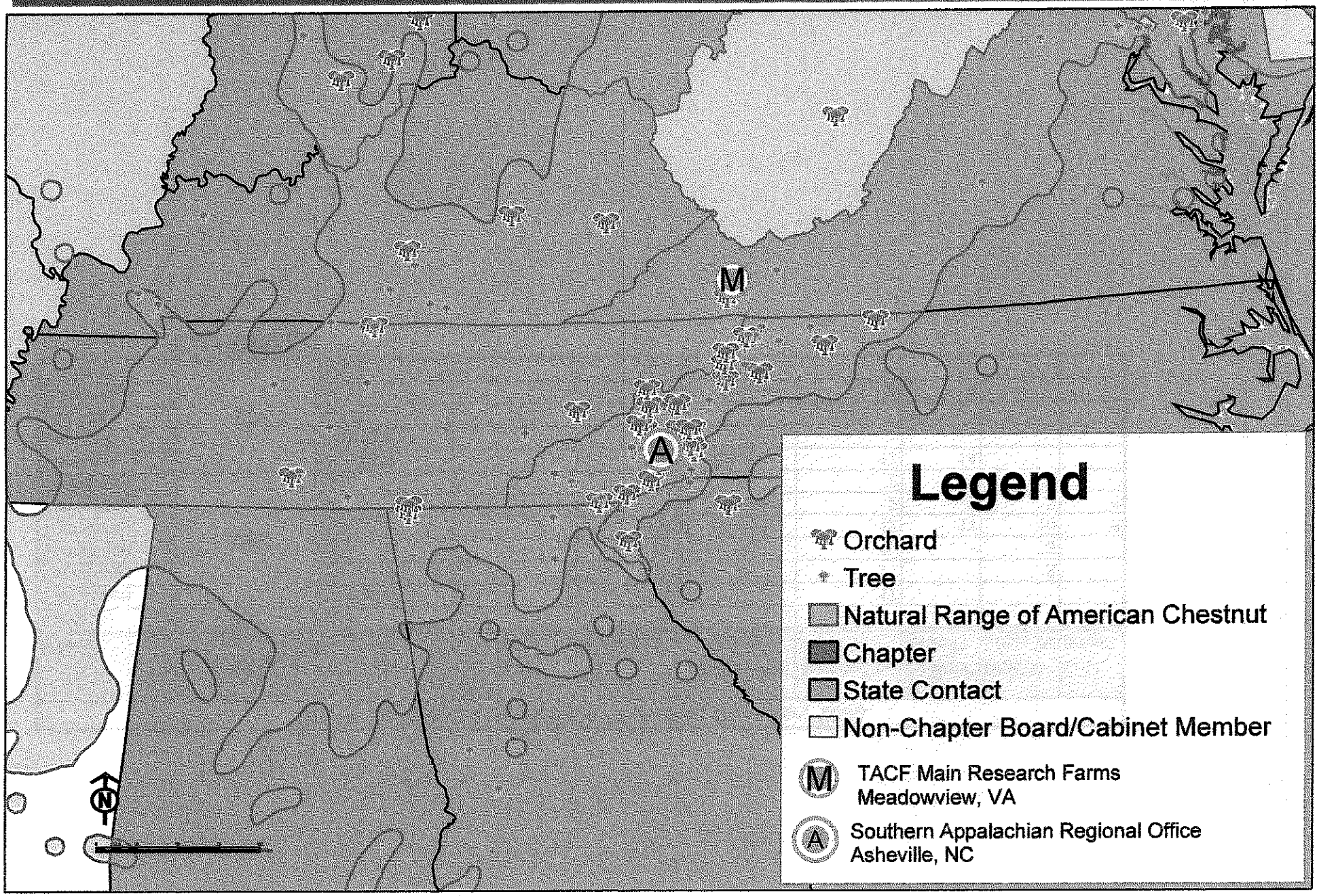
TACF's New England Region's Breeding Resources



TACF's Northern Appalachian Region's Breeding Resources



TACF's Southern Appalachian Region's Breeding Resources



Welcome to the American Chestnut Foundation's research farms. The purpose of the farms is to breed American chestnut trees for resistance to the blight disease, so that they might be grown again in the mountains to provide a reliable source of nuts for wildlife and to provide nuts and timber for people.

The primary approach we are using is to introduce the blight resistance of the Chinese chestnut tree into the American chestnut tree. The only characteristic we want from the Chinese tree is its blight resistance, so we are using the backcross method to dilute out the undesirable characteristics of the Chinese parent in hybrids. The backcross method entails crossing the two trees to obtain a tree which is 1/2 American, 1/2 Chinese. This first hybrid is then backcrossed to American chestnut to obtain a tree which is 3/4 American, 1/4 Chinese, on average. First backcrosses which are blight resistant are then backcrossed again to American chestnut, to obtain trees which are 7/8 American, 1/8 Chinese. A third cycle of selecting and backcrossing produces trees which are 15/16 American, 1/16 Chinese. Plant breeders have found that third backcrosses are indistinguishable from the recurrent parent, in this case American chestnut. A final step is to intercross third backcrosses with each other to produce trees which have a chance of inheriting the genes for blight resistance from both parents; they will breed true for those genes, and will serve as the mother trees to produce nuts for reforestation.

Among our most advanced crosses, the Price Research Farm has about 6300 15/16-American chestnut trees. These are derived from about 2700 7/8-American chestnut trees at the Wagner farms. The parents of the 15/16-American trees were selected from amongst the 7/8-American trees for moderate levels of blight resistance, comparable or better than that found in the first hybrid between Chinese and American chestnut. In addition we selected for American chestnut traits, such as a columnar growth habit and non-hairy leaves and twigs. We screen trees for blight resistance by inoculating them with the blight fungus and measuring canker size, picking the trees with the smallest cankers. We grow such large numbers of backcross trees in order to avoid inbreeding and preserve genetic diversity in the resistant trees; one cannot restore the species with only a few trees. Additionally, we are working with more than one source of blight resistance, in order to reduce the possibility that the blight fungus will be able to evolve in the future to overcome our blight resistance.

The prospects for success in our backcross breeding program are bright if only a few genes control blight resistance. However, if numerous genes are needed to confer resistance, then either some of those probably would be lost during backcrossing, leaving the trees with inadequate resistance or else so many associated Chinese chestnut traits would be retained that the trees will not resemble American chestnut.

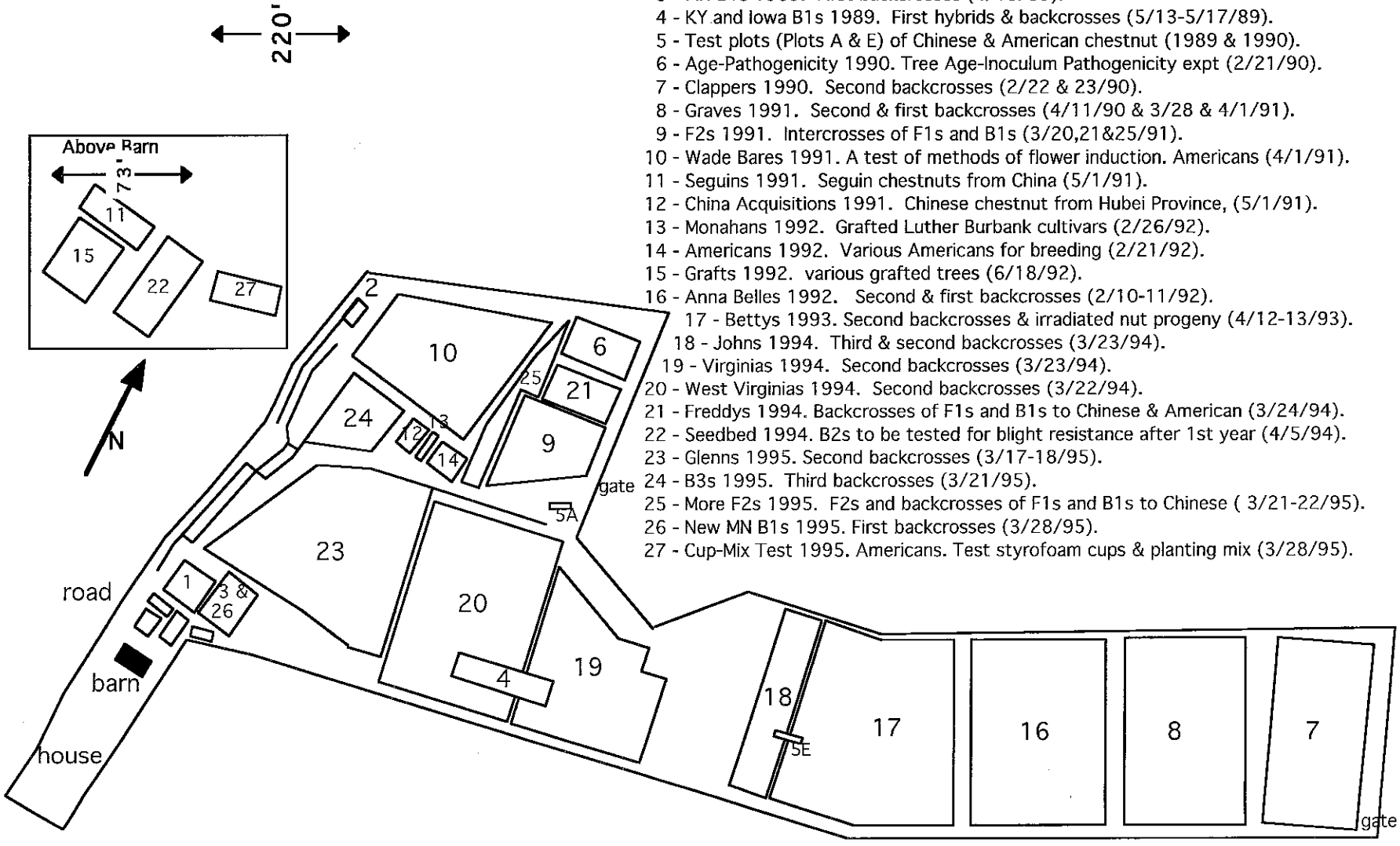
Results over the last 10 years indicate that only a few genes control blight resistance, and that we should be able to backcross it into American chestnut. First, we were able to recover highly blight-resistant progeny when we intercrossed with each other three types of crosses; these were: 1/2-American, 1/2-Chinese trees; 3/4-American, 1/4-Chinese; and 7/8-American, 1/8-Chinese. The 1/2-American and 3/4-American trees are in Plot 9 at the Wagner Farm while the 7/8-American trees are in Plots 2, 25 & 32 at the Price Farm. Secondly, we have genetically mapped some of our trees using molecular and morphological markers, and blight resistant mapped to only a few gene locations.

We have begun intercrossing 15/16-American, 1/16-Chinese backcross chestnut trees derived from the Clapper tree in Plots 1,5,13, & 27 at the Price Farm and planting them at a third farm. Similar nuts derived from the Graves tree in Price Farm Plot 6,19, & 43, and Wagner Farm Plot 24 are being planted back into the Wagner Farm. We began selecting highly blight-resistant progeny from these in 2004, and some of those highly blight-resistant progeny should began bearing nuts in 2005. After trees from those nuts have grown for 50 years in the forest, our children will be able to see whether we succeeded in producing trees that grow like the American chestnut tree of old.

American Chestnut Foundation

Wagner Research Farm

Boundaries and Plots

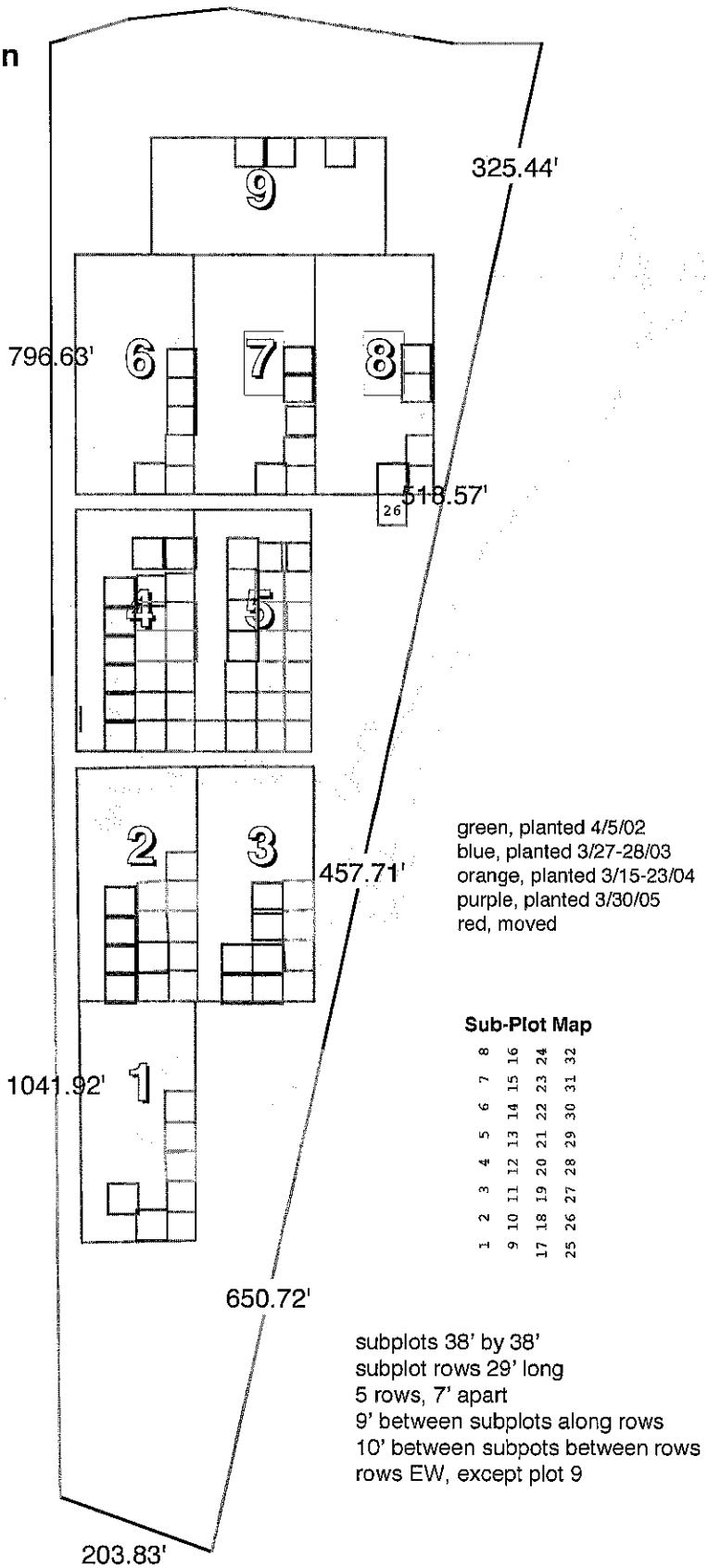


Chestnut Plots: Name, Description & Date Planted.

1 - Chinese Demonstration 1989. Chinese cultivars (4/15/89).
2 - Exotics 1989. Various cultivars of complex parentage (4/15/89).
3 - MN B1s 1989. First backcrosses (4/19/89).
4 - KY and Iowa B1s 1989. First hybrids & backcrosses (5/13-5/17/89).
5 - Test plots (Plots A & E) of Chinese & American chestnut (1989 & 1990).
6 - Age-Pathogenicity 1990. Tree Age-Inoculum Pathogenicity expt (2/21/90).
7 - Clappers 1990. Second backcrosses (2/22 & 23/90).
8 - Graves 1991. Second & first backcrosses (4/11/90 & 3/28 & 4/1/91).
9 - F2s 1991. Intercrosses of F1s and B1s (3/20,21&25/91).
10 - Wade Bares 1991. A test of methods of flower induction. Americans (4/1/91).
11 - Seguins 1991. Seguin chestnuts from China (5/1/91).
12 - China Acquisitions 1991. Chinese chestnut from Hubei Province, (5/1/91).
13 - Monahans 1992. Grafted Luther Burbank cultivars (2/26/92).
14 - Americans 1992. Various Americans for breeding (2/21/92).
15 - Grafts 1992. various grafted trees (6/18/92).
16 - Anna Belles 1992. Second & first backcrosses (2/10-11/92).
17 - Bettys 1993. Second backcrosses & irradiated nut progeny (4/12-13/93).
18 - Johns 1994. Third & second backcrosses (3/23/94).
19 - Virginias 1994. Second backcrosses (3/23/94).
20 - West Virginias 1994. Second backcrosses (3/22/94).
21 - Freddys 1994. Backcrosses of F1s and B1s to Chinese & American (3/24/94).
22 - Seedbed 1994. B2s to be tested for blight resistance after 1st year (4/5/94).
23 - Glenns 1995. Second backcrosses (3/17-18/95).
24 - B3s 1995. Third backcrosses (3/21/95).
25 - More F2s 1995. F2s and backcrosses of F1s and B1s to Chinese (3/21-22/95).
26 - New MN B1s 1995. First backcrosses (3/28/95).
27 - Cup-Mix Test 1995. Americans. Test styrofoam cups & planting mix (3/28/95).

American Chestnut Foundation
Clapper Seed Orchard Map

Boundaries and Plots



green, planted 4/5/02
blue, planted 3/27-28/03
orange, planted 3/15-23/04
purple, planted 3/30/05
red, moved

Sub-Plot Map

1	2	3	4	5	6	7	8
9	10	11	12	13	14	15	16
17	18	19	20	21	22	23	24
25	26	27	28	29	30	31	32

subplots 38' by 38'
subplot rows 29' long
5 rows, 7' apart
9' between subplots along rows
10' between subpots between rows
rows EW, except plot 9

Table 1. Type and number of chestnut trees and planted nuts at TACF Meadowview Research Farms in May 2006, with the number of sources of blight resistance and the number of American chestnut lines in the breeding stock.

Type of Tree	Number of		
	Nuts or Trees	Sources of Resistance	American Lines*
American	2162		235
Chinese	692	51	
Chinese x American: F_1	523	22	90
American x (Chinese x American): B_1	425	15	33
American x [American x (Chinese x American)]: B_2	1559	10	91
American x {American x [American x (Chinese x American)]}: B_3	3818	9	77
Am x (Am x {Am x [Am x (Ch x Am)]}):B_4	9	1	1
(Ch x Am) x (Ch x Am): F_2	710	6	6
[Ch x Am) x (Ch x Am)] x [Ch x Am) x (Ch x Am)]:F_3	6	1	1
[Am x (Ch x Am)] x [Am x (Ch x Am)]: B_1-F_2	769	4	8
{Am x [Am x (Ch x Am)]} x {Am x [Am x (Ch x Am)]}:B_2-F_2	341	5	5
(Am x {Am x [Am x (Ch x Am)]}) x (Am x {Am x [Am x (Ch x Am)]}):B_3-F_2	12376	2	29
B_3-F_3	121	1	2
Chinese x (Chinese x American): Chinese B_1	191	3	4
Chinese x [American x (Chinese x American)]	41	1	1
European x American: F_1	3	1	1
Japanese	3	2	
American x Japanese: F_1	11	2	2
(American x Japanese) x American: B_1	10	2	2
(American x Japanese) x American] x American: B_2	23	1	1
Castanea seguinii	48	1	
Chinese x Castanea pumila: F_1	9		
Large, Surviving American x American: F_1	328	13	29
(Large, Surviving American x American) x American: B_1	403	7	11
[(Large, Surviving American x American) x American] x American: B_2	94	2	3
Large, Surviving American x Large, Surviving American, and similar: I_1	689	13	12
Large, Surviving American: $F_2 = F_1xF_1$, etc, same LS parent	448	6	8
Large, Surviving American Other	175	9	10
Irradiated American x American: F_1	1	1	1
Other	25		
Total	**26013**		

* The number of lines varied depending on the source of resistance. We will have to make additional crosses in some lines to achieve the desired number of 75 progeny per generation within a line. In keeping with past practice, the number of lines for each source of resistance are added separately; thus, progeny from two sources of resistance that share an American parents would be counted as two lines rather than one line (this only occurs rarely).

Genetic Mapping and Molecular Markers in Chestnut

Paul Sisco

The American Chestnut Foundation

Highlights –

Uses of Molecular Markers in TACF's Breeding Program

- To identify genetic loci in Chinese chestnut that are conferring resistance
- To determine the gene action of each locus (dominant, recessive, additive)
- To identify the homozygotes for resistance in the seed production orchard
- To aid in the recovery of as much of the American genome as possible
- To monitor the level of genetic diversity in TACF's released seed
- To aid in protecting TACF's material from unauthorized use (identity)

Genetic Mapping Experiments as of 1999 Science Review

F2 and BC1 Genetic Mapping Populations based on Chinese cultivar 'Mahogany'

R4T92 (*C. mollissima* x *C. dentata*) X R4T31 (*C. mollissima* x *C. dentata*)

4-52 F1 tree as female x 4-31 F1 tree as male = F2 mapping population
Five VA American chestnut females x 4-52 and five x 4-31 = BC1 mapping population

1991: F1 seed planted in "F2" orchard at Meadowview; BC1 seed planted in "Graves" orchard at Meadowview

June 1993: ~185 F2 trees inoculated with two strains of blight
Aug and Sept 1993: Blight cankers measured on F2 trees

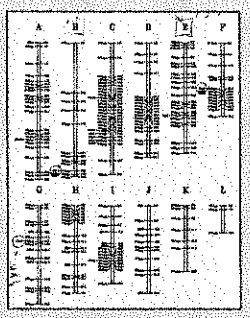
June 1995: ~ 57 BC1 trees inoculated with two strains of blight
Aug 1995: Blight cankers measured in each month

1997: Paper published in *Phytopathology* based on 8 isozyme, 17 RFLP, and 215 RAPD markers mapped in 102 F2 trees selected from the tails of the resistance distribution; 12 linkage groups identified (A - L) 3 *Cbr* (*Chestnut blight resistance*) genes localized to LGs B, F, and G

Old Programs –
CAES + USDA

One source of resistance most thoroughly analyzed –
'Mahogany'

1997 Genetic Map based on 'Mahogany' F2 Mapping Population



Kubisiak et al., 1997. *Phytopathology* 87:751-759

Problems with map as of 1999 Science Review

In the unpublished BC1 map based on 57 trees:

The *Cbr3* locus on LG_G was not present.

An additional *Cbr* locus was on LG_E. *Cbr1* on LG_B and *Cbr2* on LG_F were confirmed.

LGs "B" and "E" could easily form a single linkage group in the BC1 map. If they represented only one chromosome, where was the 12th linkage group?

There was a lot of linkage distortion in both the F2 and BC1 maps. In the F2, 20% of the RAPD markers, 31% of the RFLP markers, and 25% of the isozyme markers were significantly distorted from expected ratios. Both LGs "B" and "E" contained regions distorted toward the Chinese genome. Could this be the reason for their appearing to be on the same chromosome?

Genetic Mapping Experiments 2000 - 2006

2000: 276 AFLP markers were added to the F2 map in a joint project with North Carolina State University (Ron Sederoff and Catherine Clark). This was funded by a Park Foundation Grant.

Conclusion: The AFLP markers were distributed in a similar manner to the RAPD markers. They helped to saturate the genetic map, but they did not help determine whether LGs "B" and "E" represented one or two chromosome pairs.

Spring, 2001: A map of European chestnut is published. Twelve linkage groups are identified. Casasoli et al., TAG 102:1190-1199.

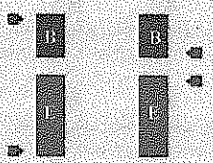
2001-2002: Thanks to another Park Foundation Grant, a cooperative project was initiated between TACF, Tom Kubisiak (SIFG), and scientists in Italy and France (Villani, Kremer, Casasoli). 29 microsatellite and 5S rDNA markers allowed correlation of 11 of 12 linkage groups between the European and the American/Chinese genetic maps. The one missing: LG_B.

Correlating the Chinese/American and European Chestnut Genetic Maps

Linkage Groups

Chinese/American Chestnut Map	European Chestnut Map	Common Loci
A	1	2 SSRs
B	11 ?	None
C	8	4 SSRs
D	10	4 SSRs
E	4	1 SSR and 5SrDNA
F	7	1 SSR
G	3	1 SSR
H	6	3 SSRs
I	5	2 SSRs
J	12	1 SSR and 1 Isozyme
K	2	2 SSRs
L	9	3 SSRs

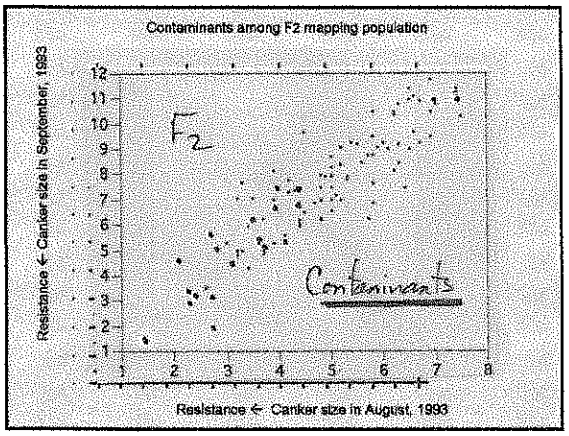
The *Cbr* loci on "B" and "E" appear to be unlinked



Genetic Mapping Experiments 2000 – 2006
Other conclusions from the SSR studies

18 of the 102 F2 trees mapped in the 1997 publication were found to be from contaminating pollen.

Aneuploids



Genetic Mapping Experiments 2000 – 2006
Other conclusions from the SSR studies

Reanalysis of the F2 mapping data eliminated the *Cbr* locus on LG_G, leaving only those on LGs_B and F, plus the addition of the locus on LG_E identified in the BC1 mapping study. So the results of the F2 and BC1 analyses were now consistent, with three *Cbr* loci identified on LGs B, E, and F.

2005: Further analysis indicated that the resistance phenotype of LG_F was masked in the F2 offspring of F1 parent 4-31 but present in offspring of 4-52 in the F2 mapping population

In the BC1 mapping population, the effect of the LG_F locus was seen in offspring of both F1 parents.

Probabilities of non-correlation of loci with blight resistance:

F2 Map > 93 trees

LG	4-31 1:1 All 93 trees	4-52 1:1 All 93 trees	3:1, Ch "1" All 93 trees
B	.08 423_1150	.01 590_1200	<.0001 A11_1100
E	.04 256_2700	.002 F16_0300	.005 256_0875
F	.22 e40m51_388.9	.001 EMC38-1f	.08 e37m56_205.1

BC1 Map > 57 trees

LG	Marker	4-31 1:1 30 trees	4-52 1:1 27 trees	1:1 All 57 trees
B	660_0350	.029	.001	.0001
E	038_0600	.008	.007	.0002
F	723_0600	.003	.014	<.0001

In F2, 4-31 always male

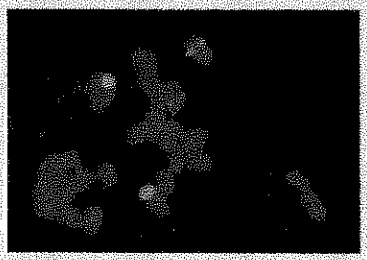
B
- 423_1150 46:39
- 590_1200 53:30
- A11_1100 55-30

E
- 256-2700 29-54
- F16-0300 26-40
- 256.0875 53-30

F
- e40m51_388.9 30-43
- EMC38-1f 49-28
- e37m56_205.1 62-10

660-0350 4-31 18:12 4-52 17:9
038-0600 16-14 9-16
723_0600 18-12 13-12

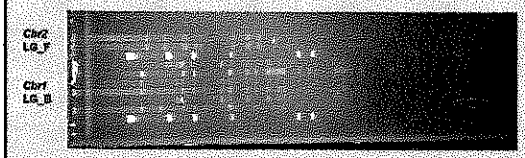
Other Accomplishments 2000 – 2006 in genetic mapping and marker development:
Flourescent In-situ Hybridization by Nurul Islam-Faridi of SIFG/Texas A&M



18S ribosomal RNA sites (green) and telomeres (red)

Other Accomplishments 2000 – 2006 in genetic mapping and marker development:

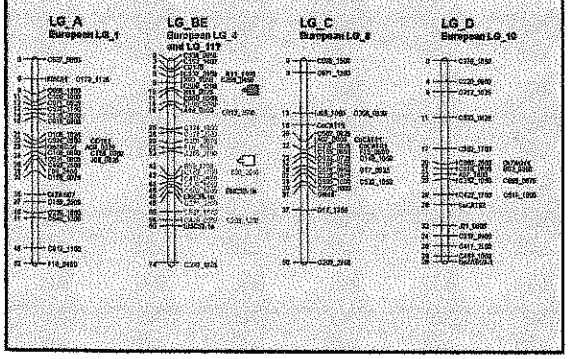
Creation of a Bacterial Artificial Chromosome Library from the Chinese cultivar 'Mahogany'

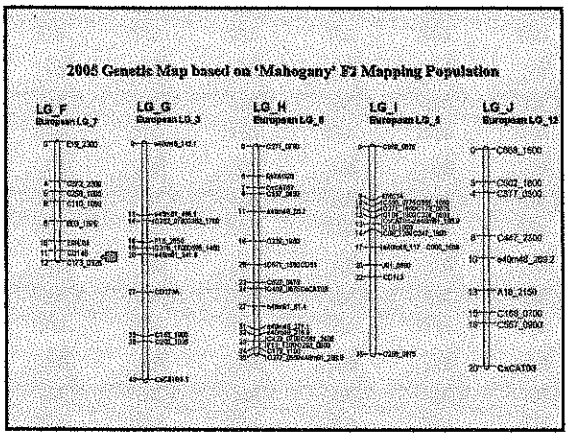


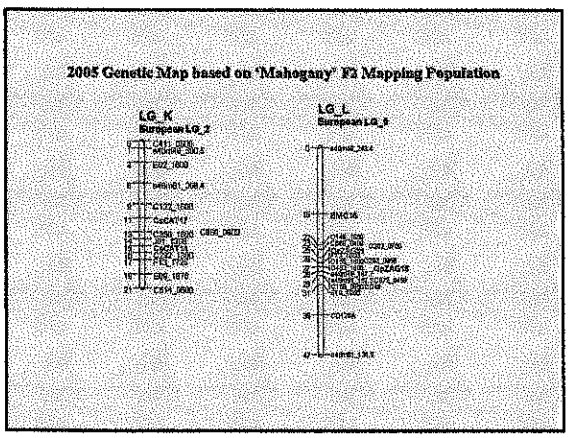
Laura Georgi, lab of Albert Abbott, Clemson University

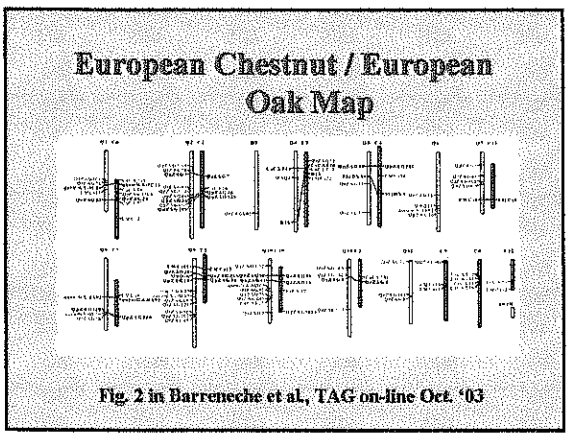
Purpose - saturate the regions around the Chr loci with more co-dom markers.

2005 Genetic Map based on 'Mahogany' F2 Mapping Population









Koerner-Ollenberg Prize

NE-1015

The Next Steps: NSF and JGI Projects

National Science Foundation Award # 0820135: "Genomic tool development for the Fagaceae"

Project Period: Sept. 1, 2008 – Aug. 31, 2010

P.I. Ron Sederoff, North Carolina State University
co-P.I.'s Jeff Tomkins, Clemson University Genome Institute
Paul Sisco, The American Chestnut Foundation

DOE/Joint Genome Institute Community Sequencing Program:

"Sequencing 400 Mb of *Cryphonectria parasitica*"

Project Period: 2006 - 2007

P.I. Don Nuss, The University of Maryland
co-P.I.'s Alice Churchill and Michael Milgroom, Cornell University

Goals of NSF Project

Isolate RNA, make cDNAs, and at least partially sequence:
1. Chinese chestnut challenged and unchallenged by the blight fungus
2. American chestnut challenged and unchallenged by the blight fungus
3. *Quercus rubra*, *Quercus alba*, and *Fagus grandifolia*

Create a "base map" for Chinese chestnut using 384 progeny from the cross 'Mahogany' x 'Nanking' and 500 co-dominant markers (SNPs and SSRs)

Determine segments of Chinese genome remaining in advanced breeding populations (BC3 and SC3F2)

Anchor the genetic map to the physical (BAC contig) map of Chinese chestnut cv. 'Mahogany'

Establish a Fagaceae database at Clemson University Genome Institute

Advisory Board

Douglas Cook, UC-Davis, *Medicago* genomics
Susan McCouch, Cornell, *Oryza* genomics
Antoine Kremer, INRA, *Fagaceae* genomics
Jeanne Romero-Severson, Notre Dame, *Quercus* genomics
Jennifer Koch, USDA/ARS, *Fagus* genomics

Papers in NPS Proceedings (Steiner and Carlson, 2006)

Liu, Song, and John Carlson. Selection for Chinese vs. American genetic material in blight resistant backcross progeny using genomic DNA. pp. 139-150.

Powell, W.A., Merkle, S.A., Liang, H., and C.A. Maynard. Blight resistance technology: transgenic approaches. pp. 79-89.

Kubisiak T. and J. Roberds. Genetic structure of American chestnut populations based upon neutral DNA markers. pp. 109-122.

Papers re: European chestnut and oak

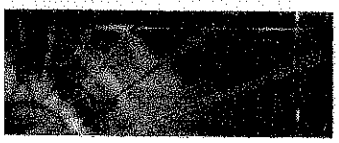
Casasoli, M., Mattioni, C., Cherubini, and F. Villani. 2001. A genetic linkage map of European chestnut (*Castanea sativa* Mill.) based on RAPD, ISSR, and isozyme markers. TAG 102:1190-1199.

Barreneche, T., Casasoli, M., Russell, K., Akkak, A., Meddour, H., Plomion, C., Villani, F., and A. Kremer. 2004. Comparative mapping between *Quercus* and *Castanea* using simple-sequence repeats (SSRs). TAG 108:558-566.

Casasoli, M., Pot, D., Plomion, C., Morstavedt, M.C., Barreneche, T., Lauteri, M., and F. Villani. 2004. Identification of QTLs affecting adaptive traits in *Castanea sativa* Mill. Plant Cell and Environment 27:1093-1101.

Casasoli, M., Derory, J., Morera-Dutrey, C., Brendel, O., Porth, I., Guehl, J-M., Villani, F., and A. Kremer. Comparison of quantitative trait loci for adaptive traits between oak and chestnut based on an expressed sequence tag consensus map. Genetics 172:533-546.

Awards



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Award Abstract #0605135

Genomic tool development for the Fagaceae

NSF Org:	DBI
Initial Amendment Date:	July 22, 2006
Latest Amendment Date:	July 22, 2006
Award Number:	0605135
Award Instrument:	Continuing grant
Program Manager:	Jane Silverthorne DBI Division of Biological Infrastructure BIO Directorate for Biological Sciences
Start Date:	September 1, 2006
Expires:	August 31, 2007 (Estimated)
Awarded Amount to Date:	$706548
Investigator(s):	Ronald Sederoff ron_sederoff@ncsu.edu(Principal Investigator) Paul Sisco (Co-Principal Investigator) Jeffrey Tomkins (Co-Principal Investigator)
Sponsor:	North Carolina State University CAMPUS BOX 7201 RALEIGH, NC 27695 919/515-2444
NSF Program(s):	PLANT GENOME RESEARCH PROJECT
Field Application(s):	
Program Reference Code(s):	BIOT,9109
Program Element Code(s):	1329

ABSTRACT

PI: Ron Sederoff, North Carolina State University CoPIs: Jeff Tompkins, Clemson University; Paul Sisco, The American Chestnut Foundation Senior Personnel: Frederick Hebard, The American Chestnut Foundation; Chris Smith, Dahlia Nielsen, North Carolina State University; Sandra Anagnostakis, Connecticut Agricultural Experiment Station; John Carlson, Pennsylvania State University; William Powell, State University of New York, Syracuse. The family of forest trees (the Fagaceae) that includes the chestnuts, oaks and beeches, dominate the hardwood forests of the northern hemisphere. This project will

study the genomes of this family of trees because the species have significant economic value and represent a major natural resource. Chestnut will be used as the model to advance the production of an American chestnut (Castanea dentata) resistant to chestnut blight through physical identification of genes for resistance. Chestnut blight, caused by Cryphonectria parasitica, was among the greatest ecological disaster in this nation's history. This project would be the first genome project directed to ecosystem restoration and would provide a model for addressing ecological crises caused by pathogens and pests that threaten the world's forest resources. This project's work will focus on mapping of the chestnut genome and the comparison of chestnut to oaks, beeches and other forest trees. A major objective is an integrated genetic and physical map of chestnut that would become the basis for future targeted sequencing or whole genome sequencing. The work proposed would accelerate breeding programs for chestnut for urban and rural forestry, food crops, timber, high quality wood products and ecological restoration. Scientific progress would be accelerated for all species of the Fagaceae. Breeding would be advanced for oak and beech species, as well. The selection of a disease resistant chestnut would have dramatic value for rural communities in the Appalachian Mountains, a region of the USA that is in great need of economic development. Resistant chestnut trees could restore a vast damaged ecosystem, provide nutrients for wildlife, and create major new economic resources. The Project will help organize an international working community for genomics of the Fagaceae. A series of work shops developed with the Institute of Forest Biotechnology will be held for the Heritage Tree Program. Undergraduates and faculty from St. Augustine's, a Historically Black College, will take part in the bioinformatics analysis as summer interns. Genomics and Proteomics training will be provided to high school teacher as part of an ongoing program at Clemson University. Access to project outcomes: Sequence data will deposited in GenBank (http://www.ncbi.nih.gov/Genbank/) within 60 days of analysis. A web site for the Fagaceae community has been set up at Clemson University (http://www.genome.clemson.edu/projects/fagaceae/). An archive of EST and BAC clones will be maintained at Clemson through the Clemson University of Genomics Institute (CGUI).

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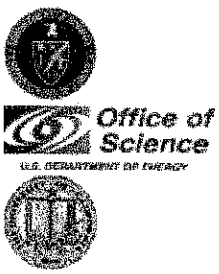
Why Sequence Chestnut Blight Fungus?

Cryphonectria parasitica, the chestnut blight fungus, is responsible for epidemics that caused the destruction of hundreds of millions of mature chestnut trees in forests of North America and Europe during the first part of the 20th century. The discovery of a group of RNA viruses, now classified in the family *Hypoviridae* (hypoviruses), that reduce the virulence (hypovirulence) of this pathogen stimulated intensive research into the potential of using fungal viruses for the biological control of fungal disease. Subsequent epidemiologic and population genetic studies have established the chestnut/*C. parasitica*/hypovirus pathosystem as the textbook example of both the consequences of accidental introduction of an exotic organism and of hypovirulence-mediated biological control of fungal pathogens.

Interest in *C. parasitica*, hypoviruses, and their interactions now extends well beyond disease control potential. The development of a robust *C. parasitica* transformation protocol and of hypovirus reverse genetics led to the establishment of a biologically relevant experimental system with the rare capacity for efficiently manipulating the genomes of both a eukaryotic virus and its host. Scientists studying this system have also made significant contributions to the current understanding of mycovirus/host interactions, fungal population genetics, mechanisms underlying fungal pathogenesis, and fungal signal transduction pathways. Very recent advances with this system are providing important new insights into the role of RNA silencing as an antiviral defense mechanism in fungi and the impact of viruses on cell death associated with fungal vegetative incompatibility systems and secondary metabolism. Thus, the availability of the *C. parasitica* genome sequence, the first for an Ascomycete tree pathogen, will greatly accelerate the efforts of an active and growing research community to address a broad range of important fundamental and applied research topics.

Principal Investigators: Donald L. Nuss (Univ. of Maryland Biotech. Inst.) and Alice Churchill and Michael Milgroom (Cornell Univ.)



2nd TACF Science Review

Preliminary Oral Report
August 11, 2006

Review Panel

- Glen Stanosz, Professor of Plant Pathology, University of Wisconsin-Madison
- Lauren Fins, Professor of Forest Genetics, University of Idaho
- Robert Macintosh, Senior Research Fellow, University of Sydney
- Ron Phillips, Regents Professor of Plant Genetics, University of Minnesota

Process

- Extensive documents received in advance
- Visited chestnut sites in forest as well as Wagner, Price, and Duncan Research Farms
- Oral presentations by key staff
- Consultations with other key people
- Discussions among the review panel

Outline of Report Presentation

- Current strengths – Ron Phillips
- Breeding – Robert Macintosh
- Deployment – Lauren Fins
- Efficiency – Glen Stanosz
- Genetics – Ron Phillips
- Scientific Communication – Lauren Fins
- Resources – Glen Stanosz
- Release – Robert Macintosh

Current Stengths

- Presence of several talented scientists with notable enthusiasm toward the TACF mission
- Development of B3F3 materials
- Initiation of major analysis of new disease resistance sources
- Generation of considerable molecular marker mapping information

Current Strengths

- Careful decisions on artificial innoculation procedures and modifications
- Development of pertinent research projects by outside scientists
- Significant NSF grant to advance the molecular genetics
- Publication of progress
- Maturation of the chapter concept

Genetics

Encourage use of molecular markers

Uses of Molecular Markers

- Mapping of genes for disease resistance and other traits for selection at the seedling stage
- Detection of outcrosses
- Recovery of recurrent parent
- Identification of recombinants
- Fingerprinting for ID and protection purposes

Genetics

- Test the translocation hypothesis through cytology and pollen sterility studies
- Test for translocation(s) in interspecific crosses with new disease resistance sources
- Employ molecular markers to maximize detection of recombinants
- Use BACs with linked markers present to determine homologies in new resistance sources

The American Chestnut Foundation
2nd Science Review
August 8-11, 2006

Introduction

Over 150 years ago, Louis Pasteur said that chance favors the prepared mind. A review of a scientific program prepares the minds of those involved to not only have a well-formulated plan but to also recognize those opportunities that present themselves along the way. Much of the value of a scientific review is in the preparation - summarization of the pertinent information obtained to date on which to base future work, development of a plan for the next steps in the process, projection of needs in terms of information and resources, and presentation of the pertinent points to a review team. This advanced information helped us to focus on the mission of TACF which is to restore the American Chestnut to the original range in the eastern United States by developing, testing, and distributing a chestnut tree with the basic American Chestnut traits and a reasonable degree of resistance to chestnut blight.

The TACF Science Review Team was composed of Lauren Fins, Professor of Forest Genetics, University of Idaho, Robert McIntosh, Honorary Professor, Plant Breeding Institute, University of Sydney, Ron Phillips, Regents Professor of Plant Genetics, University of Minnesota, and Glen Stanosz, Professor of Plant Pathology, University of Wisconsin-Madison. TACF staff included Dr. Frederick V. Hebard, Dr. Robert L. Paris, Dr. Paul H. Sisco, Ms. Sara F. Fitzsimmons, Ms. Cornelia C. Pinchot and Mr. William White. TACF members included Dr. Kim Steiner, Dr. Albert Ellingboe, Dr. Cameron Gundersen, Dr. J. Hill Craddock, Mr. K.O. Summerville, Dr. William MacDonald, Dr. Safiya Samman, Dr. C. Dana Nelson, and Mr. Richard S. Will. Their participation was of great assistance to the review team.

The process for this review of the American Chestnut Foundation's science activities involved the delivery in advance of extensive documentation of progress to date and several publications that reviewed the history of TACF and goals for the future. We were privileged to visit a forest site with mixed forest species, including chestnuts in various stages of their life cycle. We also learned about canker formation caused by the chestnut blight and examined the growth pattern of the fungus under the bark. We then observed the research material at the three primary research sites (Wagner, Price, and Price-annex farms). This was followed by key staff orally presenting their research program largely from a breeding and genetics perspective. We also learned about the activities of the various TACF state chapters and how they relate to the mission of TACF. In addition, the review panel informally interviewed Richard Will, Finance Committee Chair, Al Ellingboe, Science Director, and Kim Steiner, Vice Chair Science. The Review Panel greatly appreciated the excellent review of the program presented by the TACF staff and volunteer members.

The format of this report is to provide a brief listing of some of the current strengths that represent significant progress in the program since the 1st TACF Science Review in 1999, to present a list of our recommendations, and then to discuss various issues related to breeding, genetics, adaptation and environmental variation, efficiency, scientific communication, resources, and germplasm release and commercialization.

Current strengths: The Review Panel was encouraged by the presence of several talented scientists with noticeable enthusiasm toward the TACF mission, the development of third backcross F2 materials (called B3F2), initiation of a study on new sources of resistance, generation of considerable molecular marker mapping information, the careful decisions on artificial inoculation procedures and modifications based on experience, the development of pertinent research projects by outside scientists, the significant NSF grant to advance the molecular genetics of the chestnut species, the publication of progress, and the maturation of the chapter concept. The enthusiastic state chapters are important in many ways, including the evaluation and contribution of genetic materials.

Recommendations

The Review Panel recommends:

1. Continuation of the current breeding program to the B6 generation, and the interim limited distribution of B3-derived populations as a means of assessing American Chestnut characteristics, variability and adaptation, and blight assessment levels over a range of environments.

2. The collection of molecular genetic information that will support identification of current and future resistance sources and the selection of the American Chestnut genetic background in future breeding endeavors.

3. TACF and its Chapters formalize a Germplasm and Commercialization Strategy for identification, distribution, release and benefit-sharing of genetically improved populations.

4. A close collaboration of the TACF staff with the NSF grant participants in order to encourage research that will advance the breeding program.

5. That the TACF staff follow the literature dealing with species that are collinear with the Chestnut since much of that information will be as valuable as if acquired with the Chestnut.

6. Disease resistance genes be mapped from current and future genetic sources with flanking markers identified in order to follow the resistance genes in a limited marker-assisted selection program.

7. To the extent possible, long-term maintenance of mapping populations be a priority to facilitate subsequent gathering of data on other traits deemed important.

8. The advanced backcross material be used in mapping studies since mapping procedures can be more efficient with such materials.

9. Involvement of molecular markers for detecting outcrosses, accelerating the backcross program, and fingerprinting for ID and protection purposes.

10. Testing for the presence of one or more chromosome translocations in the current breeding materials and the new sources of resistance.

11. Testing for population differences across species range and use of the information to inform breeding and deployment strategies.

12. Establishing field tests to determine levels of field resistance to blight and other potential problems using the most advanced generation of trees.

13. That TACF continues to engage members by asking them to be involved in the testing program and to provide feedback, similar to beta sites and/or rose testing.

14. Adding at least a half-time position for database and web management and a lab technician for molecular genetics applications.

15. That TACF utilize the broader scientific community's meetings, such as AAAS, to network and disseminate information on the "Chestnut Story."

16. That TACF host internal "working" meetings for everyone involved in hands-on work in all chapters.

17. The use of small non-overlapping independent factorials (SNIFs) for mating design in breeding program.

18. The use of clonal material when appropriate for seed orchard establishment and for other studies that support the research objective of TACF.

19. The examination of responses of superior selections to a broader range of strains of the chestnut blight pathogen (including hypovirulent strains), as well as responses to less aggressive inoculation techniques and natural infection.

20. Increased availability of land and equipment sufficient to support immediate and future progress in the breeding program and seed orchard development.

Breeding

Currently, there continues to be three presumed sources of resistance in the Clapper, Mahogany/Graves and Nanking sources. While genetic studies indicate that the Mahogany source may contain 2 or 3 partially dominant resistance genes, it is not known if the other sources are genetically different from Mahogany, or from each other. This should be determined by the recently approved NSF grant in which Chestnut will serve as the basic model. In addressing individual resistance sources the review panel endorses the current program and procedures in breeding for resistance using the initial three sources of resistance and to the controlled distribution of B3F3 populations. Feedback of data on the performance of such trees across environments and their blight responses under natural infection conditions, where possible, will be essential.

Because of the limited size of the program, three sources of resistance were as much as could be handled in the past, but with the addition of a second breeder, the search for additional resistance sources should be actively pursued taking advantage of molecular marker information becoming available from the genetics program. Given the varied success of resistance breeding covering a wide range of plant species over the past century, it is likely that no single resistance source will provide the long term durability of resistance needed for the present program aimed at the re-establishment of the American Chestnut to its former geographical range.

In order to maintain a high level of genetic variation in an obligate outbreeding species, particular attention has been given to a strategy of crossing to an array of different local surviving American Chestnut trees in order to preserve genetic variation and avoid inbreeding depression. Considerable attention has also been given to the strategy of detailed partial diallelic intercrossing of the B3F1 and B3F2 plants that will produce the populations of adequate size and genetic variability for future distribution. This strategy could perhaps be simplified by a factorial crossing strategy that targets the maintenance of all genes in the population rather than all genotypes (see later figure). In this case all trees are involved in crosses but not in all combinations. A factorial design is logistically more efficient in that each tree is used only as a female, or as a male, parent.

Pollen of plants developed in the core program has been provided to Chapters for crossing with locally surviving trees in the relevant regions. Eventually comparisons of genotypes developed in the different regions should provide information on both local and more general adaptation.

The Review Panel endorses the continuation of backcrossing to the B6 generation for at least 1 or 2 resistance sources. However as molecular marker data become available

increased emphasis should not only be given to the possibility of using closely linked markers for the resistance genes, but also to the identification of genome-wide "American" Chestnut alleles for selection of the American genetic background (or for selection against Chinese or other genetic backgrounds). As always in the case of linked, rather than 'perfect' markers, field testing is essential to ensure that resistance is present and adequately expressed. It is therefore important that molecular testing strategies be such that data will be accumulated to demonstrate future efficiencies in the discovery and introduction of further resistance sources to the program.

Selection for blight resistance:
The blight response testing strategy is based on the concurrent use of a highly aggressive strain and a less aggressive strain. Differences in the responses allow the determination of different levels of expression. The efficiency of the procedure will be fully assessed only when populations can be grown under "natural' conditions. As this may take several years it is important that selected materials be distributed as soon as possible as the findings will serve as a basis for the future. Widespread testing will provide data on the stability of resistance across environments and also the possibility of pathogenic races in the pathogen.

While hypovirulence caused by infection of the pathogen by viruses leads to reduced aggressiveness by infected pathogen isolates, practical use of the phenomenon currently appears to be limited to horticultural and garden situations where hypovirulent cultures can be used to inoculate existing cankers. There is no evidence for preferential spread of hypovirulent strains, or to widespread infection of existing cankers by hypovirulent types, leading to reduced disease levels and increased survival of individual trees in forest situations.

Transgenics:
In view of the long-term objectives of the program to re-instate the American Chestnut to forests, a watching brief needs to be given to the potential of transgenics. This potential has many dimensions including the cloning and insertion or resistance genes from both related and more distant species, and the up-regulation of degrading enzymes, such as chitinases, and defense-related proteins already present in host plants, but not expressed at sufficient levels to give protection against invading pathogens. In any case such initiatives may not give complete protection and may need to be used in conjunction with partial resistances coming from the conventional breeding program.

Genetics

Molecular genetics knowledge of the Chestnut has greatly increased in the last few years. The development of RAPD and AFLP maps has provided information on the various linkage groups. The development and mapping of more user-friendly markers such as SSRs (Simple Sequence Repeats) is underway and will be important to the program. The identification of six BACs (Bacterial Artificial Chromosomes) with one marker sequence known to be linked to disease resistance and two BACs with another marker sequence also linked to resistance is an important development. The new NSF grant will provide many advances for Chestnut molecular genetics and, with guidance from key TACF staff,

should provide several opportunities to advance the breeding program. The NSF program should provide information on gene expression that would not otherwise be a part of the TACF activities. Special attention should be paid to the literature of species expected to be highly co-linear with the Chestnut; such publications can inform the chestnut program in many different ways. The molecular genetics program at TACF should have objectives that will advance the breeding program.

Mapping:
Applications of the molecular genetics tools to breeding should include the mapping of disease resistance genes in the current and future materials. This activity should result in the tagging of disease resistance loci with flanking markers allowing selection at the seedling stage and information on whether disease resistance genes from various sources are different. Such gene tags should allow the selection of recombination events. Some of the original mapping populations should be maintained since data for additional traits may be obtained later and entered into the database for QTL analysis. The advanced backcross materials available also provide powerful mapping opportunities. Using backcross lines, only a few plants are required to detect linkage of polymorphic markers to the genes for the trait under selection. The concept is that the allele of a marker uniquely present in the donor parent will only be present in backcross lines if it is linked to a trait under selection; the number of backcrosses and the number of independent backcross lines affect the probability of linkage and the numbers required. The results are more complicated, however, when several traits are under selection.

Molecular genetic marker uses:
Molecular markers also should be employed to detect the presence of outcrosses. Such early detection will reduce the amount of confusing results, and in the long run save space in the nursery. Outcrossing in controlled crosses for genetic studies can cause major problems. Outcrossing in the breeding program may or may not be of major importance. However, the elimination of outcrosses is a form of quality control that can only be helpful.

Recovery of the genotype of the recurrent parent (American) can be accelerated by the use of molecular genetic markers. This approach coupled with the backcrossing breeding scheme may give materials that are essentially the equivalent of one or two generations beyond normal expectations via standard breeding. Every generation saved in Chestnut tree breeding represents major savings in time and in accomplishing the ultimate objectives.

Fingerprinting the breeding materials can be important in the description and identification of lines. Such information also will be important in any form of intellectual property protection that TACF decides to pursue. This application of molecular markers requires an understanding of the allelic variation present in Chestnut populations and the selection of a set of clear markers (perhaps 20) that are unique to these Chinese and American materials.

Chromosome translocation tests:
The molecular genetic map derived from Chinese x American crosses indicates the presence of 11 instead of 12 linkage groups. The B and E linkage groups appear to be combined to form a B/E linkage group. The AFLP mapping results of this Chinese x American cross, involving Mahogany as a resistance source, gave evidence for heterozygosity of a chromosome translocation between linkage groups B and E. These linkage groups, known to be independent from earlier studies with intraspecific crosses, formed one linkage group in the Chinese x American interspecific cross. This is not unexpected since these materials represent different species that have become independently diversified through evolution. The new B/E linkage group is quite large and, therefore, could have a major effect on genetic segregation in the progeny.

In addition to the finding of a B/E linkage group, segregation distortion was observed. About 30% of the loci in the above cross showed segregation distortions, and a large portion involved loci on the B/E linkage group. At least one "aneuploid" plant has been observed based on the presence of more than two alleles per locus. These results would be expected if the translocation had a break near the end of a chromosome. The greatest distortion should be near the breakpoints and the most prevalent allele depends on which side of the breakpoints that the locus resides. Analysis of the distortion data may give an indication of the position of the breakpoints.

The third point of interest is that disease QTLs (genes) have been mapped in this cross to linkage groups B, E, and F. The genes on the B/E linkage group are about 25 map units apart. Unusual genetic behavior of the key chromosomes with disease resistance genes may result in unexpected breeding results including difficulty in obtaining highly resistant progeny. Translocation heterozygosity can lead to reduced recombination depending on the chromosome pairing relationships, the transmission of duplicate-deficient gametes and/or the frequency of alternate disjunction in the translocation heterozygote. In addition, if one or more genes for the selected American traits resides on the B and or E linkage groups, then reduced recombination may make it difficult to obtain both disease resistance and the American type. The use of molecular markers to detect appropriate recombination events may be important in such a circumstance. Some of the selected American traits such as pubescence is not on the B/E linkage groups but genes for other American features probably reside on this chromosome making selection of resistance in the progeny more difficult.

Cytology of meiosis in the F1 should reveal the presence of the translocation(s) by exhibiting a ring-of-four or, perhaps more likely in this case, a chain-of-four chromosomes plus 10 chromosome pairs. Pollen sterility also should exist in the heterozygote (F1 or members of later generations) of between 25 and 50%. If duplicate-deficient gametes are produced, one might also observe smaller pollen but well-filled with starch.

Cytological and pollen sterility studies should be done on all new sources of resistance. A cross free of a translocation(s) would be desirable. A written plan would be useful describing the crosses of new resistance sources to be made and the expected results

under various hypotheses. Mapping of the resistance genes in the new sources should be initiated.

Adaptation and Environmental Variation:
High levels of genetic variation among populations are typical of forest tree species that have broad geographic distributions, particularly when their natural ranges occur over steep environmental gradients. Because much of this variation is adaptive, it is critical to understand its distribution and patterns, which should then play a key role in selection and breeding programs and deployment strategies. Phenological traits, such as the date of bud set and the onset of dormancy, are good examples because they are under strong genetic control and can be closely related to adaptation to cold tolerance.

Long-distance movement of trees, particularly from south to north, may place the trees at risk of damage by cold temperatures in late spring or early fall. In reverse, movement from north to south may reduce height growth because trees cease to grow earlier in the season than locally adapted materials.

Although most have been lost to blight, numerous naturally regenerated American Chestnut remnant trees remain in forested areas throughout the original species distribution. These trees can provide the seeds needed for studies of inherent variation across the species range. Such studies can be conducted as *common garden studies* on seedlings over a period of only a few years once the seeds have been collected. Dates of bud break, bud set, and the onset of dormancy, height and diameter growth, and other potentially differentiating traits can be assessed and compared among samples from different populations under relatively uniform environments, such as forest nurseries or farm fields. Such juvenile tests may not have high accuracy in predicting all of the fastest growing trees over a rotation, but they are excellent tools for detecting and differentiating among populations that are genetically different from one another.

This information should then be used to group trees in the breeding program and also in distributing and deploying the material. Ultimately the question that needs to be addressed is how far genetic material can be moved safely, that is, without diminishing adaptation and/or growth and survival potential. This would be an excellent opportunity for a graduate student project.

Testing for Field Resistance:
The inoculation procedure for testing for resistance to blight seems to be working well, ensuring the infection of virtually all trees included in the tests. However, experience with other species suggests that field performance may be considerably different from performance in test environments, potentially better, potentially worse.

We favor the establishment of field tests of the same materials as are included in the inoculation trials. Ideally these materials would be the same genotypes, produced through cloning of embryos or with tissue cultured plantlets, rooted cuttings or grafts as cloning would allow the parallel testing of the same genotypes that are included in the

inoculation trials. We understand that cloning Chestnut is apparently quite difficult, so field testing of the genotypes from the same families is the next best alternative. Family testing would provide information on overall performance of the family as a whole and may be quantified in percentages, for example.

Knowing the levels of field resistance will help the group to provide information to users on real expectations for relatively long-term survival for each generation of selection and breeding. These materials can also serve as archives for genetic variants that may be of use in future studies. Field resistance studies should be well-designed, and replicated in time and space. These tests will also provide opportunities to select individuals for other specific traits that may confer resistance – perhaps some bark characteristics or branching traits.

Engaging Members:
Although we do not believe the genetic materials generated to date are ready to be released to the general public, or even to members broadly, members are likely to be excited about cooperating with TACF to help test their advanced materials for field resistance. The program could be structured similarly to "rose trials" where members are asked to establish seedlings under specified conditions and to collect information for submission to the foundation. This is also similar to the use of Beta Sites in the software industry where co-operators are asked to test the technology and report on problems and/or their satisfaction with the product. Tested materials should be given names that indicate their "experimental" or temporary status until they have been proven to be resistant at high levels and under high risk conditions for infection.

The Beta Site approach has the advantage of engaging members in hands-on work (thereby helping to maintain their enthusiasm) and provides them with materials sooner than if they had to wait for proven resistant genotypes. It also has the advantage of distributing field resistance tests over a broader area for relatively low cost.

The primary challenge with this approach is to maintain contact with the co-operating members and ensure their submission of good data to the project. This may take considerable effort by a staff member and/or by technically competent chapter members.

Establishment:
Although some work has begun to address the challenge of establishment of Chestnuts in prepared areas or forests (two papers in the 2004 meeting proceedings are noted), success is likely to prove very challenging. Nuts will be subject to predation and both nuts and seedlings will suffer damage or death from a variety of naturally occurring and introduced pathogens and insects. Effects of vegetative competition are likely to be severe. Members and supporters of TACF need to develop a realistic appreciation of the practical challenges involved in establishment and recruitment of young trees. Additional applied research on techniques to facilitate establishment under the conditions likely to be encountered in the reintroduction process is greatly needed.

Efficiency

Mating Designs:

The mating design in the Chestnut breeding program is used primarily to produce families from which to select individuals for forward selection. The largest number of independent families that can be generated from X number of individuals is X/2, which is most efficiently accomplished by single pair matings. So 20 parent trees are crossed 1 on 1 in 10 crosses to produce 10 new families, none of which are related to each other unless their parents were related. However, with the low numbers of seeds produced per cross, it might be wise to mate each candidate with more than one other selection. Regardless of how many crosses and combinations are made, the maximum number of unrelated crosses of the 20 parents is still 10, if the starting number is 20 unrelated parent trees.

The most efficient mating design from a logistical standpoint is the SNIF design (Small, Non-overlapping Independent Factorials) using the smallest groups (2 X 2). See example below and comparison with disconnected half-diallels.

Disconnected Half-Diallels

	1	2	3	4	5	6	7	8
1		X	X	X				
2			X	X				
3				X				
4								
5						X	X	X
6							X	X
7								X
8								

Small Disconnected Factorials

	1	2	3	4	5	6	7	8
1								
3	X	X						
4	X	X						
5								
6								
7					X	X		
8					X	X		

For 8 parents, the diallels require 12 crosses to generate 4 independent families and 4 of the trees are used as both males and females, which entails extra field visits to the trees.

For the same number of parents, the factorials require only 8 crosses, results in the same number of independent families (4), but each tree is used as only a female or a male and is crossed with only 2 other parent trees. This arrangement decreases the number of visits to half of the parent trees, reduces the number of pollen lots that must be tracked and reduces the opportunities for error in labeling since each tree is used in a maximum of two crosses. For these reasons, the factorial design is highly superior to the diallel.

Clonal Propagation:

Selective use of clonally propagated Chestnut materials would facilitate achievement of some research objectives and increase efficiency of effort and land use. Although tissue techniques for multiplication of chestnut are not now available, their development by external groups with expertise in that field should be encouraged. In the interim, grafting is possible. Examination of the variation in responses of multiple individuals of the same genotype to inoculation with one or more isolates at one location would be possible. Alternatively, responses of single genotypes in different regions would allow evaluation of genotype by environment interactions (local adaptation and response to local pathogen pressure or "field resistance"). Responses of various genotypes to cultural practices and conditions in establishment would also be facilitated. Most immediately, however, possible development of grafted seed orchards is again suggested. This could allow for consolidation of selections, resulting in more efficient land use and a reduction in the rate of increase of maintenance inputs including labor.

Inoculation:

Effectiveness of any disease resistance screening method is measured by achievement of the differential response between resistant and susceptible plants. The currently used procedure involves wounding and inoculation of field-grown trees with agar plugs bearing mycelia of the pathogen (repeated on the same tree for each of two strains of varying aggressiveness) and appears to be successful. However, this method is very aggressive, tipping the balance heavily in favor of the pathogen and disease development, and often resulting in death. Events that might occur during natural (albeit through small wounds) infection are by-passed and mechanisms that might operate at more natural levels of "inoculum potential" may be overwhelmed.

Given the identification of individuals and families with a range in both response to inoculation and field performance over at least several years, examination of responses of such material to more natural/subtle inoculation methods should be pursued, using field tests, as recommended above, or by monitoring infection and survival in operational plantings. Results might allow more rapid identification of younger material for further breeding. Responses might also reveal more information about speed or variability in successful response of trees to a broader range of pathogen strains, and better reflect field resistance under natural infection and realistic inoculum pressure.

Characterization of pathogen populations:
Performance of material that is ultimately deployed will be influenced by the population of the pathogen encountered. Effective resistance at one location may not be expressed when other populations of the pathogen are encountered at another. Also, superficial canker development and survival of trees at some locations may be a function of hypovirulence in the Chestnut blight fungus population at a given site. Provision of host material with a degree of resistance (that supports hypovirulence effectively) may very well be the key to a greater contribution of hypovirulence as a natural and perhaps long-lasting means of suppression of damage. Thus, consideration should be given to providing or encouraging support for appropriate investigators to characterize pathogen populations both across the range of Chestnut and particularly in areas of deployment of materials from the TACF breeding program.

Scientific Communication

Database:
The Panel support the establishment and maintenance of a web-based database. We are concerned that current staff will not have time or resources to do this work and suggest that a second half-time position be added. The primary responsibility of that half-time person would be to build and maintain the web-based database and ensure its user-friendliness.

Scientific Meetings and Symposia:
While enthusiasm for Chestnut remains high, this is an opportune time to showcase the TACF program. One possibility that would cost little, but provide great exposure is a 90- or 180-minute symposium on Chestnut at AAAS meetings. As a large cadre of reporters generally attends the meetings, a line-up of 3-6 good presentations on the history of Chestnut and the genetic work that has been conducted to date, would well serve the objective of excellent public relations and public education. Other ideas for dissemination of information and good exposure are to "piggy-back" with other forest science or forest genetics and tree improvement meetings, including the Southern Forest Tree Improvement Committee meetings, National Science Foundation, North American Forest Biology Workshop, and SAF Conventions.

Working meetings:
In addition to the scientific meetings suggested above, it might be useful to hold an annual meeting that would include all of the hands-on people employed by or involved with TACF and the state chapters. Logistical and operational issues would be the focus of the meeting. This will help to maintain relative uniformity of test establishment, data collection and entry, and general operations.

Resources

Land:

Land must become increasingly available to advance the breeding program and further develop seed orchards. Although some increased efficiency in current land use is possible, additional land of appropriate qualities for good Chestnut establishment and growth appears to be needed. Whether this can be obtained by land swap, long-term lease, or purchase, it should be acquired soon. This land needs to be as convenient to existing farms as possible to allow efficient use of equipment and personnel.

Personnel:

The current research team consists of very capable personnel who are both passionate and serious about their role in achieving the ultimate goal of TACF. Other personnel will be required to exploit progress in internal and external research. Collaboration with external groups might be most appropriate to achieve a specific objective for which specialized expertise and equipment are required and available in an external program (e.g., cytological research). Routine utilization of molecular marker technology, however, could be accomplished on site by TACF personnel. Addition of a skilled laboratory technician with experience in molecular methodology and designation of the appropriate team member as their supervisor is recommended.

Equipment:

A significant improvement in the rudimentary laboratory space and equipment would also greatly enhance the activities of the research team, and they should be allowed to develop a lab modernization and equipment plan. The laboratory, not for basic research, should allow exploitation of results from molecular studies as the latter provide information and tools useful to the breeding program. Though it need not be large or elaborate, a clean, well-lighted, adequately powered, climate controlled space is required. Equipment should include a laminar flow hood, incubator(s)/freezers, and modern dissecting and compound microscopes. To exploit the benefits of molecular marker analysis, equipment and space for DNA extraction, and polymerase chain reaction amplification, and gel documentation are required. Laboratory space for pollen work is required, and provisions might be necessary for separation of this activity from other laboratory space to avoid DNA contamination.

Although other major equipment needs appear to be met, continued progress in provision of space for equipment storage is necessary. In addition, provision of an additional bucket truck should be considered. Whether by purchase, long-term lease, or recurring seasonal lease, availability of this equipment would increase efficiency personnel at critical times in the breeding program which is core to the goal of the foundation.

Germplasm release and commercialization

While not part of the formal presentation, the Review Panel saw it imperative that TACF develop a deployment policy on release and distribution of blight resistant Chestnut material. The panel was later given a policy statement that addressed many of its concerns; we fully endorse the six guidelines outlined in the document.

The questions of adherence to such guidelines, ownership of germplasm, and benefit sharing between TACF and the Chapters must be more clearly resolved. A simple, perhaps sequential population/cultivar naming system should be adopted. Naming systems that attempt to identify pedigree histories and other details quickly become abbreviated and confused.