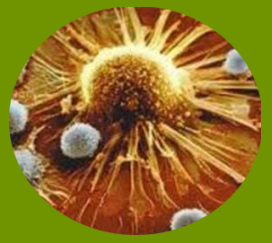
Cryphonectria parasitica tendrils on chestnut tree bark (Photo: Ministry of Agriculture and Regional Development Archive, Ministry of Agriculture and Regional Development, Bugwood.org)



6/22/2014

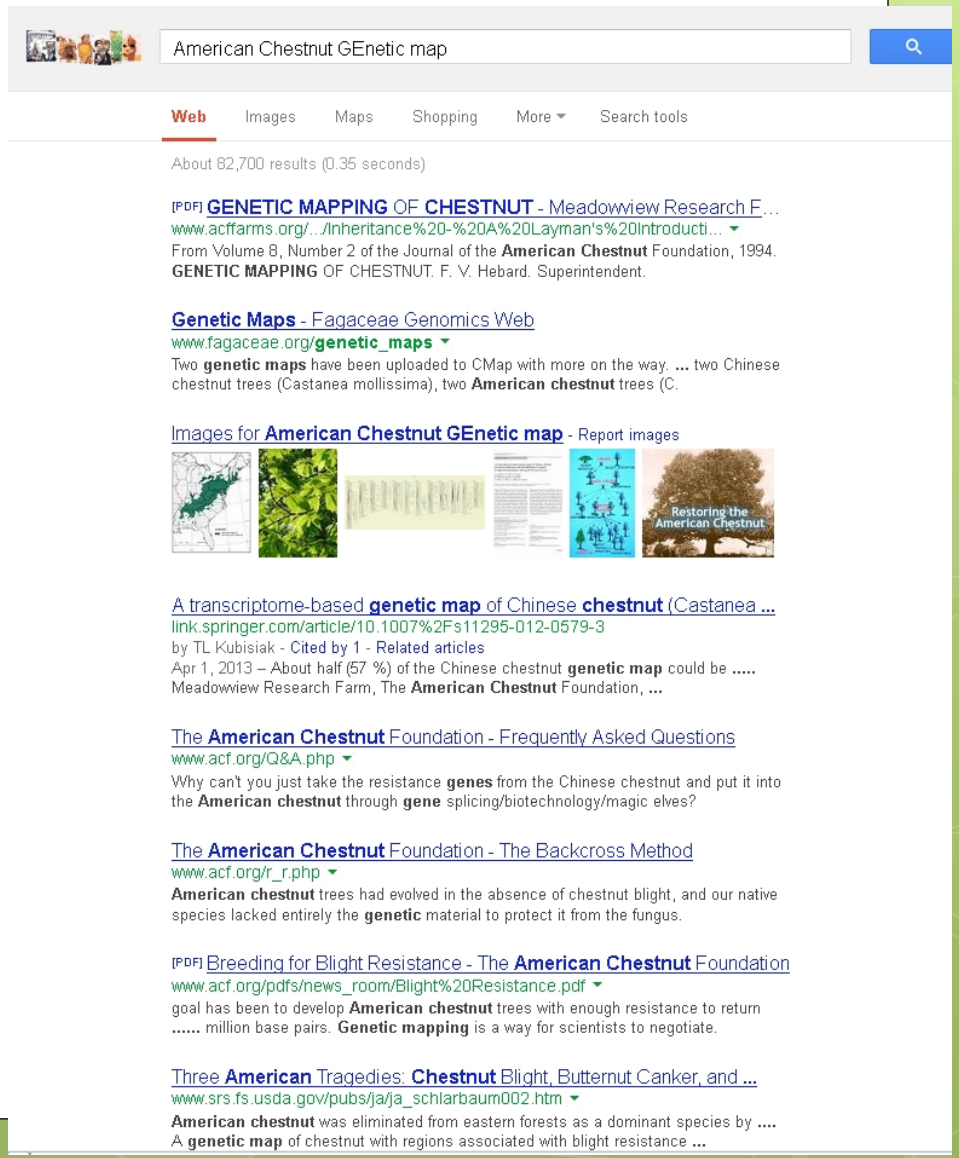
Chestnut PCR products

B3 Summer Science Camp at Olympic High School

Dr. Jennifer Weller

How did I know about the chloroplast gene difference?

- Look up American Chestnut, molecular markers, genetic map, comparative genomics as keywords in Google.



Steiner, K. C. and Carlson, J. E, eds. 2006. Restoration of American Chestnut To Forest Lands - Proceedings of a Conference and Workshop. May 4-6, 2004, The North Carolina Arboretum. Natural Resources Report NPS/NCR/CUE/NRR - 2006/001, National Park Service. Washington, DC.

GENETIC STRUCTURE OF AMERICAN CHESTNUT POPULATIONS BASED ON NEUTRAL DNA MARKERS

Thomas L. Kubisiak and James H. Roberds
USDA Forest Service, Southern Research Station, Southern Institute of Forest Genetics,
23332 Hwy 67, Saucier, MS 39574 USA (tkubisiak@fs.fed.us)

Putative species identification

Primers that amplified the intergenic spacer region between *trn*T (UGU) and the *trn*L (UAA) 5' exon of the chloroplast genome (primers a and b: 5'-CATTACAAATGCGATGCTCT-3' and 5'-TCTACCGATTTCGCCATATC-3', respectively; Taberlet et al. 1991) were found to uniquely differentiate American chestnut chloroplast DNA from all other *Castanea* (chestnut and chinkapin) species. Based on DNA sequence data (data courtesy F. Dane and P. Lang of Auburn University) this primer pair was found to amplify a band 857 base pairs (bp) in length in American chestnut, and bands ranging from 942 to 945 bp in all other *Castanea* species including the native chinkapin (both *C. pumila* var. *alleghaniensis* and *C. pumila* var. *ozarkensis*). Much of the size difference observed between American chestnut and the other *Castanea* species was due to two unique deletions (one 12 bp and the other 75 bp in length) contained within this region of the American chestnut chloroplast genome. A larger sampling of native chinkapin (specifically *C. pumila*; var. *alleghaniensis* - 48 trees) has yet to show the presence of these large deletions.

Table 1. Microsatellite and RAPD primer sequence, repeat type, allele size, and number of unique alleles identified in samples collected from 18 populations of *Castanea dentata* Borkh. located throughout the species natural range in eastern North America.

Locus	Primer Sequence 5'-3'	Repeat type	Allele size (bp)	Number of unique alleles
Microsatellites				
*Cs*CAT01[a]	F[b]:AGAATGCCCACTTTTGCA R:CTCCCTTATGGTCTCG	$(AC)_nAT(AC)_n$	167-211	31
*Cs*CAT14	F:GAGGTTGTTGTTCATCATTAC R:ATCTCAAGTCAAAAGGTGTC	$(AC)_n$	121-151	15
*Cs*CAT15	F:TCTGCGACCTCGAAACCGA R:CTAGGGTTTTCATTTCTAG	$(AG)_n$	115-141	15
*Qa*CA022	F:AACAATAGGAGTTGGTTTGAG R:GTTAGGGTTTGGAAAATAGGA	$(AC)_n$	160-188	13
*Qa*GA068	F:GCTTTTCTTTCCAGGGCTAC R:GTGGGACAGTGAGGCAGAG	$(AG)_n$	156-192	17
*Qa*GA209	F:CAAGCAGTATTGTTTTATCTC R:GTTGCCCCTGTGAACTAC	$(AG)_n$	227-265	15
RAPDs				
106	CGTCTGCCCG	NA	500	2
			525	2
			650	2
			700	2
			800	2
184	CAAACGGCAC	NA	450	2
			1150	2
			1800	2
213	CAGCGAACTA	NA	900	2
		NA	1000	2
225	CGACTCACAG	NA	800	2
			1450	2
237	CGACCAGAGC	NA	825	2
			1000	2
			1250	2
423	GGGTCTCGAA	NA	600	2
			875	2
500	TTGCGTCATG	NA	775	2
514	CGGTTAGACG	NA	575	2

[a]Locus names beginning with *Cs* were derived from *Castanea sativa* (Marinoni et al. 2003) and those beginning with *Qa* were derived from *Quercus alba* (sequences courtesy of A. David and D. Wagner). RAPD primer sequences were obtained from J. Hobbs at the University of British Columbia, BC, Canada.

[b]F=forward primer, and R=reverse primer

Molecular Mapping of Resistance to Blight in an Interspecific Cross in the Genus *Castanea*

T. L. Kubisiak, F. V. Hebard, C. D. Nelson, Jiansu Zhang, R. Bernatzky, H. Huang, S. L. Anagnostakis, and R. L. Doudrick

First and eighth authors: USDA Forest Service, Southern Research Station, Southern Institute of Forest Genetics, 23332 Highway 67, Saucier, MS 39574; second author: American Chestnut Foundation Research Farms, 14005 Glenbrook Avenue, Meadowview, VA 24361-9703; third author: International Paper Company, Southlands Experiment Forest, 719 Southlands Road, Bainbridge, GA 31717; fourth and fifth authors: University of Massachusetts, Department of Plant and Soil Sciences, French Hall, Box 32910, Amherst 01003-2910; sixth author: 136 Atwood Research Facility, Kentucky State University, Frankfort 40601; and seventh author: Connecticut Agricultural Experiment Station, Box 1106, New Haven 06504.

Accepted for publication 25 March 1997.

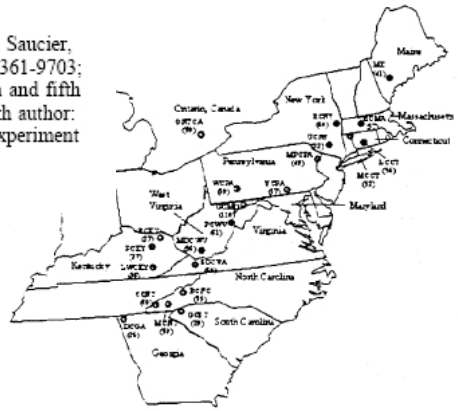


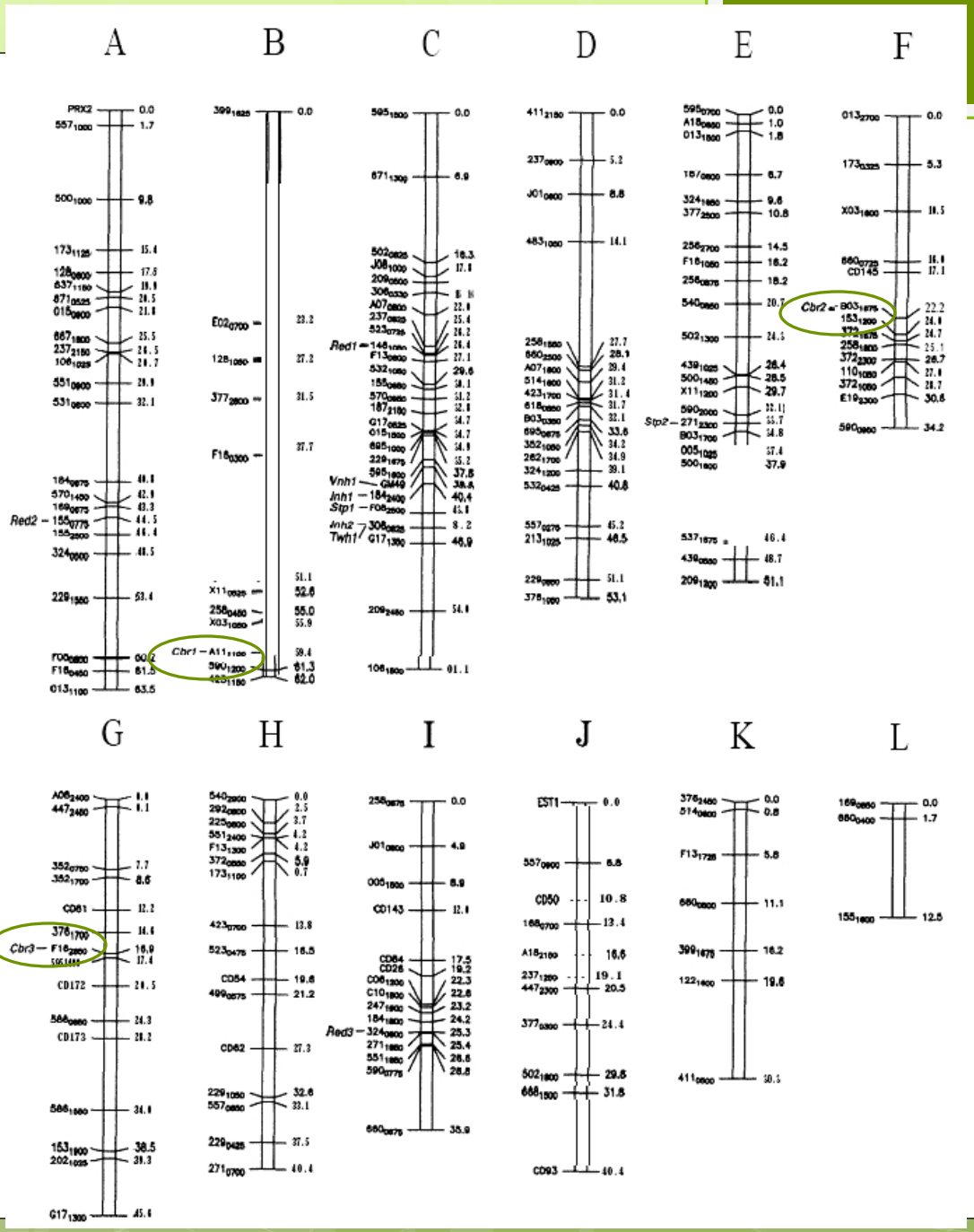
Figure 1. Map of the geographic origin of the 22 *Castanea dentata* Borkh. populations sampled in this investigation. The number in parentheses refers to the number of trees sampled at each location.

Microsatellite PCR amplification and detection

Primer sequences and PCR conditions for microsatellite loci developed in European chestnut (*C. sativa*) were obtained from the literature (Marinoni et al. 2003). Primer sequences for microsatellite loci developed in white oak (*Quercus alba* L.) were obtained from A. David and D. Wagner at the University of Kentucky. For each microsatellite, the forward primer was 5'-end labeled with one of three fluorescent dyes to facilitate detection using the Applied Biosystems 3100 Genetic Analyzer and the GENESCAN® version 3.7 fragment analysis software (Applied Biosystems, Inc. Foster City, CA). Microsatellites were PCR amplified and the products post-PCR multiplexed by color and size whenever possible. Allele sizes were determined by including the GENESCAN®-500[TAMRA] internal size standard in each sample lane. The data were scored using GENOTYPER® version 3.7 (Applied Biosystems, Inc. Foster City, CA).

RAPD PCR amplification and detection

RAPD amplification and detection was based on the protocols reported in Kubisiak et al. (1997). RAPD fragments were identified by the manufacturer primer code corresponding to the primer responsible for their amplification, followed by a subscript four digit number indicating the approximate fragment size in base pairs. Markers were chosen based on the intensity of amplification (only intensely amplified bands were scored) and the absence of co-migrating DNA fragments. All markers were found to conform to Mendelian expectations based on their inheritance in at least one of four different interspecific chestnut pedigrees.


A
B
C
D
E
F
G
H
I
J
K
L
Red2
Cbr1
Red1
Vnh1
Inh1
Stp1
Inh2
Twh1
Stp2
Cbr2
Cbr3
Red3
EST1
CD50
CD93