



6/22/2014



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



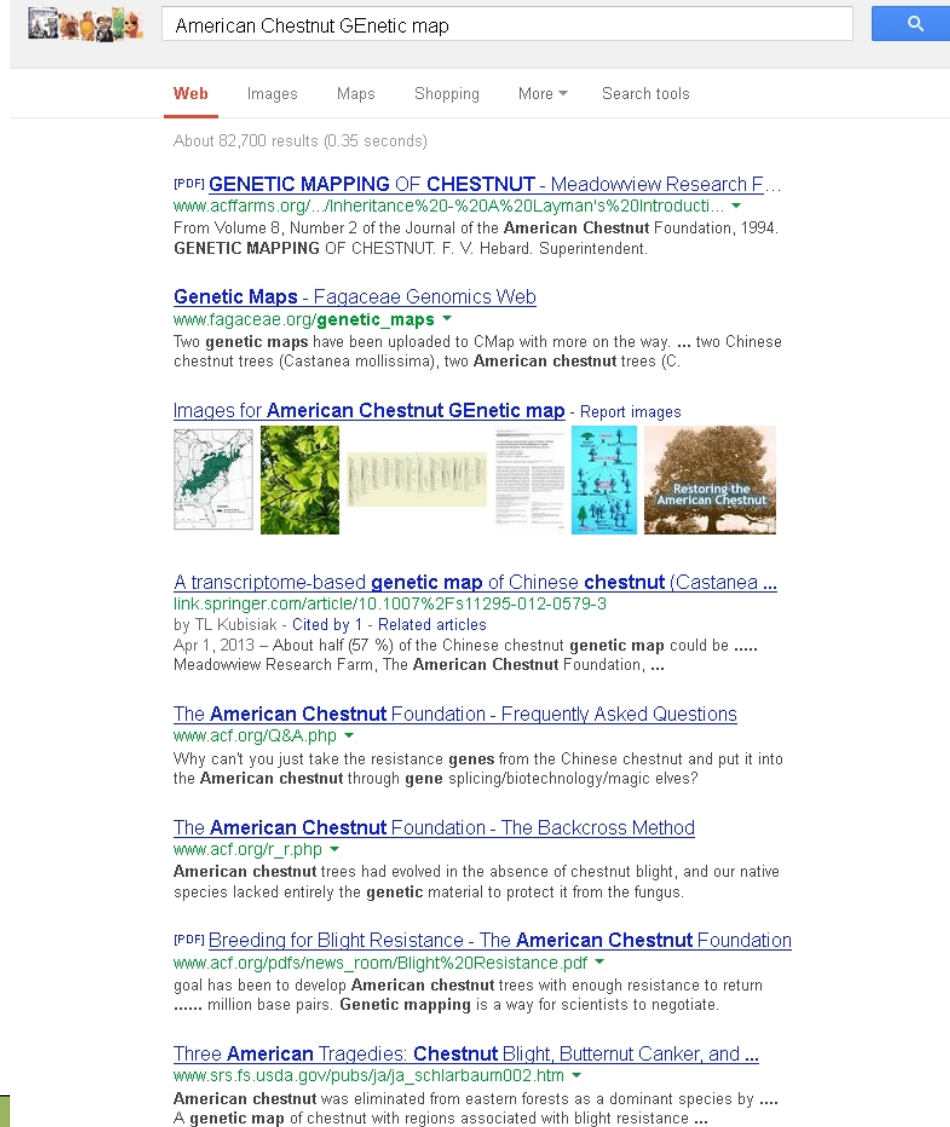
Chestnut PCR products

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.



American Chestnut Genetic map


Web Images Maps Shopping More Search tools

About 82,700 results (0.35 seconds)

[\(PDF\) GENETIC MAPPING OF CHESTNUT - Meadowview Research F...](#)
www.acffarms.org/.../inheritance%20-%20A%20Layman's%20Introducti...
From Volume 8, Number 2 of the Journal of the **American Chestnut** Foundation, 1994.
GENETIC MAPPING OF CHESTNUT. F. V. Hebard. Superintendent.

[Genetic Maps - Fagaceae Genomics Web](#)
www.fagaceae.org/genetic_maps
Two **genetic maps** have been uploaded to CMap with more on the way. ... two Chinese chestnut trees (*Castanea mollissima*), two **American chestnut** trees (C.

[Images for American Chestnut Genetic map](#) - Report images



[A transcriptome-based genetic map of Chinese chestnut \(Castanea ...](#)
link.springer.com/article/10.1007%2Fs11295-012-0579-3
by TL Kubisiak - Cited by 1 - Related articles
Apr 1, 2013 – About half (57 %) of the Chinese chestnut **genetic map** could be
Meadowview Research Farm, The **American Chestnut** Foundation, ...

[The American Chestnut Foundation - Frequently Asked Questions](#)
www.acf.org/Q&A.php
Why can't you just take the resistance **genes** from the Chinese chestnut and put it into the **American chestnut** through **gene** splicing/biotechnology/magic elves?

[The American Chestnut Foundation - The Backcross Method](#)
www.acf.org/r_r.php
American chestnut trees had evolved in the absence of chestnut blight, and our native species lacked entirely the **genetic** material to protect it from the fungus.

[\(PDF\) Breeding for Blight Resistance - The American Chestnut Foundation](#)
www.acf.org/pdfs/news_room/Blight%20Resistance.pdf
goal has been to develop **American chestnut** trees with enough resistance to return million base pairs. **Genetic mapping** is a way for scientists to negotiate.

[Three American Tragedies: Chestnut Blight, Butternut Canker, and ...](#)
www.srs.fs.usda.gov/pubs/ja/ja_schlarbaum002.htm
American chestnut was eliminated from eastern forests as a dominant species by ...
A **genetic map** of chestnut with regions associated with blight resistance ...

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

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Putative species identification

Primers that amplified the intergenic spacer region between *trnT* (UGU) and the *trnL* (UAA) 5' exon of the chloroplast genome (primers a and b: 5'-CATTACAAATGCGATGCTCT-3' and 5'-TCTACCGATTTGCCATATC-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				
CsCAT01 ^a	F ^b :AGAATGCCCACTTTTGCA R:CTCCCTTATGGTCTCG	(AC) _n AT(AC) _n	167-211	31
CsCAT14	F:GAGGTGTGTTTCATCATTAC R:ATCTCAAGTCAAAAGGTGTC	(AC) _n	121-151	15
CsCAT15	F:TCTGCGACCTCGAAACCGA R:CTAGGGTTTCATTCTAG	(AG) _n	115-141	15
QaCA022	F:AACAATAGGAGTTGGTTTGAG R:GTTAGGGTTTGGAAAATAGGA	(AC) _n	160-188	13
QaGA068	F:GCTTTTCTTCCAGGGCTAC R:GTGGGACAGTGAGGCAGAG	(AG) _n	156-192	17
QaGA209	F:CAAGCAGTATTGTTTTATCTC R:GTTGCCCTGTGAACACTAC	(AG) _n	227-265	15
RAPDs				
106	CGTCTGCCCC	NA	500 525 650 700 800	2 2 2 2 2
184	CAAACGGCAC	NA	450 1150 1800	2 2 2
213	CAGCGAACTA	NA NA	900 1000	2 2
225	CGACTCACAG	NA	800 1450	2 2
237	CGACCAGAGC	NA	825 1000 1250	2 2 2
423	GGGTCTCGAA	NA	600 875	2 2
500	TTGCGTCATG	NA	775	2
514	CGGTTAGACG	NA	575	2

^aLocus 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.

^bF=forward primer, and R=reverse primer

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

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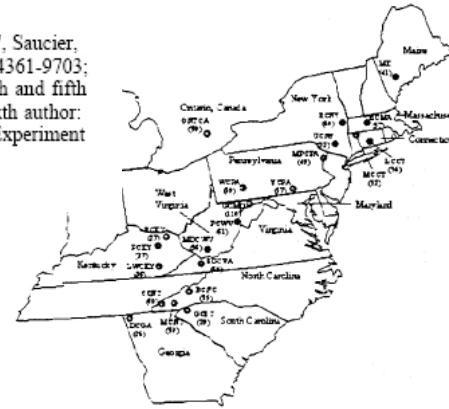


Figure 1. Map of the geographic origin of the 22 *Castanea dentata* Borkh. populations sampled in this investigation. The number in parentheses refers to the number of trees sampled at each location.

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.

