Fagaceae Genomics and American Chestnut

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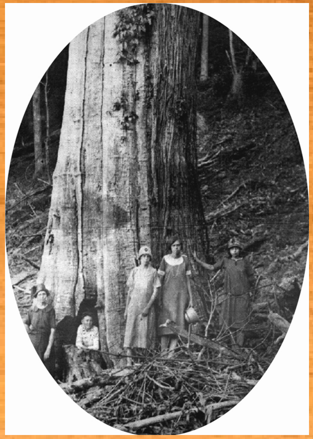


NSF Genomic Tool Development for the Fagaceae

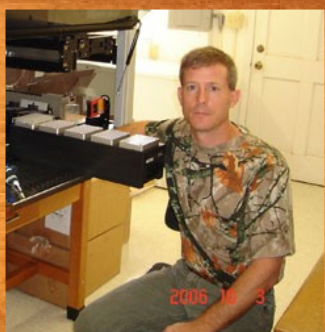
Who we are

Ron Sederoff, NCSU

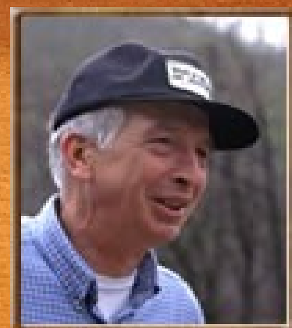
John Carlson, Penn State

Jeff Tomkins, Clemson

Bill Powell, SUNY-ESF



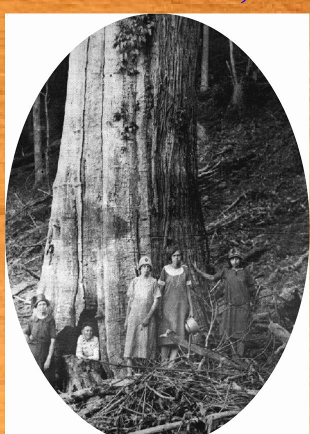
Tom Kubisiak, SIFG



Fred Hebard, TACF

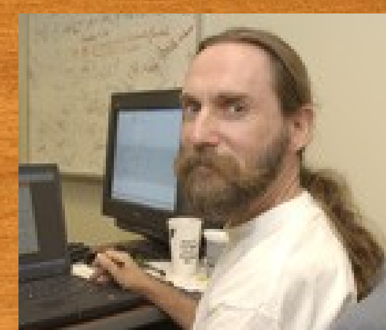
Sandra Anagnostakis, CAES

Nick Wheeler, NCSU



Dahlia Nielsen NCSU

Paul Sisco, TACF



Chris Smith, NCSU

Comparative Genomics.
The Fagaceae (beech family) contains 7 genera and ~1000 species.
Main interest: Beech, chestnut and oak

The species we study are:

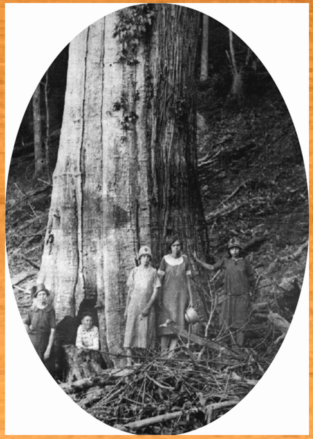
Chinese chestnut: *Castanea mollisima*

American chestnut: *Castanea dentata*

Northern Red oak: *Quercus rubra*

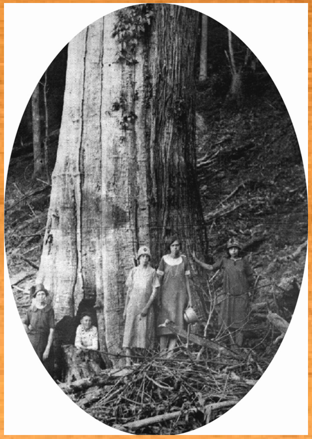
White oak: *Quercus alba*

American beech: *Fagus grandifolia*



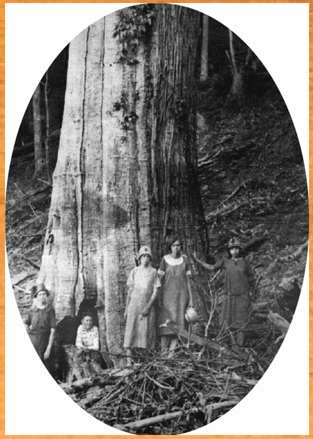
Some research objectives

- Gene discovery
- Genetic marker development
- Large new mapping populations
- New genetic maps
- Physical maps
- Comparative genomics
- Web site (Clemson).



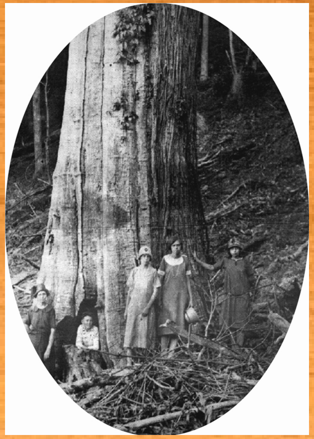
DNA Sequencing

- All EST based: cDNA libraries from different species and tissues. (Powell)
- Traditional ABI sequencing (Tomkins).
- 454 sequencing. 2 million reads total on 5 species (Carlson).
- Annotation (Smith).



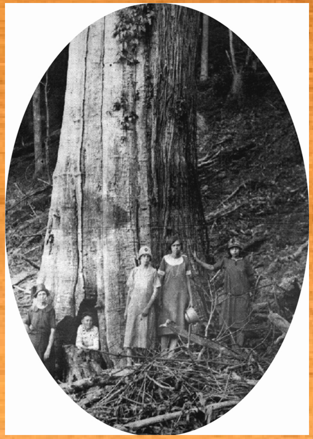
Gene discovery
Predicted proteins

- There are 10,562 of hits to Populus
  - (at e-15).
- Of these, 8266 also hit Arabidopsis.
- Many hits are known only by their "gene model" predictions.
- So our data verify their predictions as real genes.

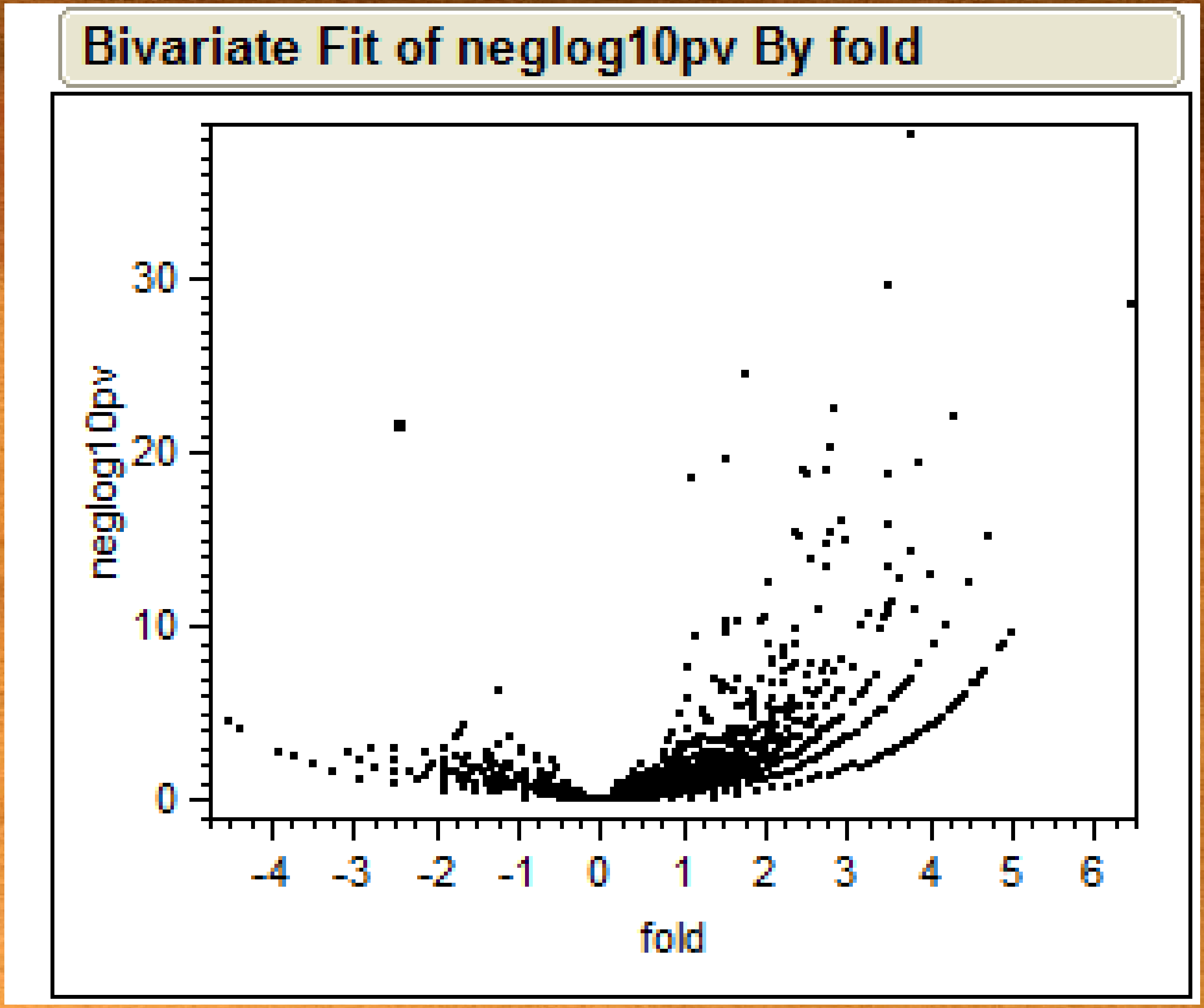


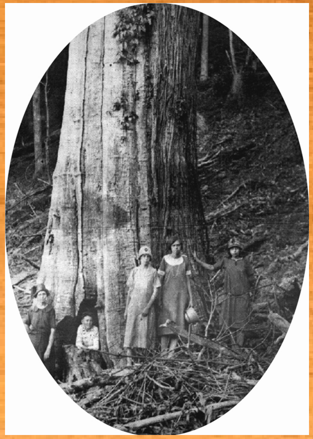
Genes Identified by species

Species:	Predicted Proteins
Chinese chestnut	7719
American chestnut	1067
Red oak	4985
White oak	3525
Total Fagaceae	10,562



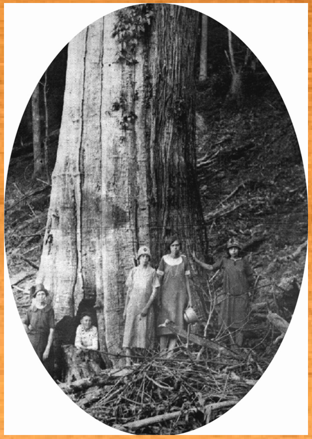
Expression data: electronic northerns





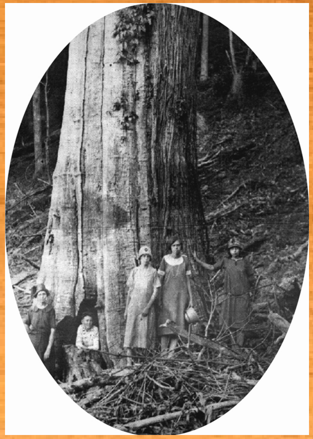
Marker Development
(Kubisiak and Smith)

- Many more flexible informative markers.
- Genotyping
- Solving mapping problems
- Comparative mapping
- Using 454 EST sequence data to find SSRs and SNPs.
- Anchoring ESTs to genetic and physical maps.



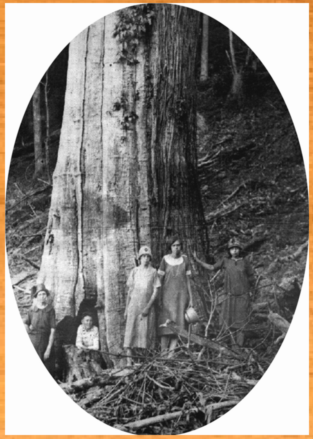
Comparative marker development

- Survey sequence data for SSRs and SNPs for polymorphisms that vary within species, and between species.
- Find Chinese vs American species specific markers to aid mapping in hybrid backcrosses.
- Explore variation within Fagaceae, such as comparative mapping.



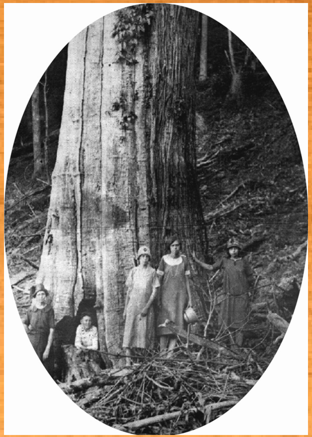
Looking for SSRs

- Looking within species for SSRs in 454 contigs.
- Ran scripts to search for repeated patterns and variation in the repeats within the contig.
- Found 104 in the first pass and have successfully tested a third (so far).
- SSR genotyping has turned up some misidentified parents.



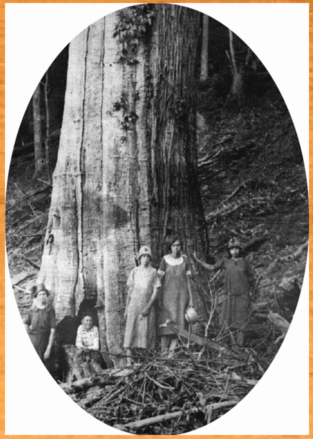
Looking for SNPs

- BLAST against Populus transcripts (N).
- Group all reads that match the same gene.(e-10 cutoff).
- Toss out genes with less than 4 reads to get 12,158 genes.
- Assemble the reads for each gene (PHRAP).
- Run PolyBayes on each assembly to find SNPs.
- Parse output for SNPs with >0.95 probability.



SNP frequency

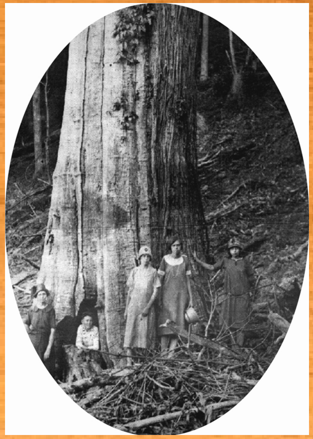
- Total SNPs found by PolyBayes: 17,748.
- Substitutions: 15,563.
- Deletions: 2,185.
- Number of aligned bases screened: 10,153,440.
- Potential SNP frequency: 1/ 572.



Genetic Mapping Objectives

1. Genetic mapping in parent Chinese and American chestnut and derived hybrids.
- Looking for "species specific" markers to track genome segments in hybrids.
- Solve some problems with distortion and linkage (missing LG-B).

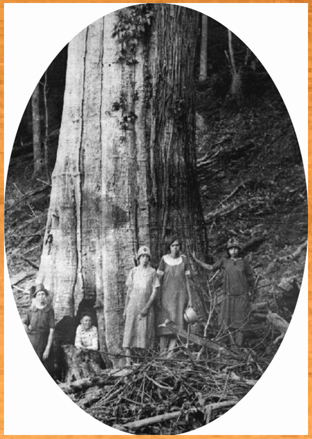
4. QTL mapping for resistance to chestnut blight to confirm a three gene model.



Problems with Current Genetic Maps (Sisco, Kubisiak)

Comparison of European and AC/CC hybrid maps found one linkage group missing: LG-B

LGs "B" and "E" could easily form a single linkage group in the BC_1 map.

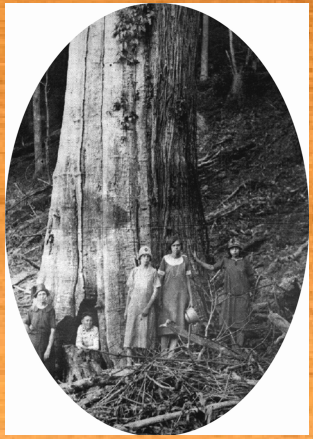


High segregation distortion in both the F_2 and BC_1 maps. (20 to 31%).

Translocations between American and Chinese hybrids could affect breeding and map based cloning.

Identification of resistance genes

- Confirm or extend the three QTL model.
- Use the BC3 populations to “fine map” the QTLs by association in very large populations.
- Identify genomic regions associated with resistance.
- Need resistance to Phytophthera too.



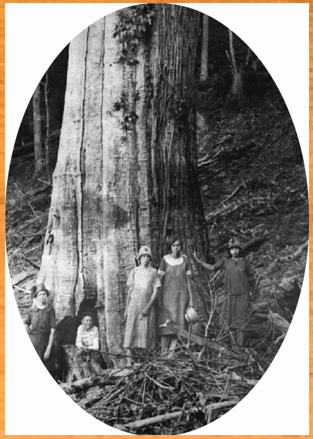
Mapping focus is in Chinese chestnut because that is where the resistance genes are.

To facilitate map based cloning of resistance genes, we need:

A high resolution genetic map.

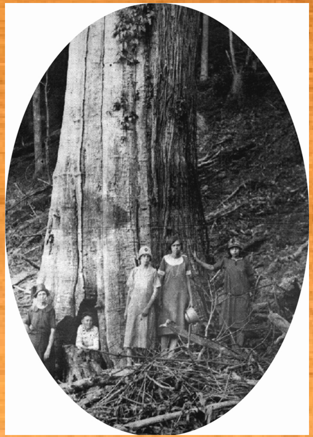
A high quality physical map (BACs)

And to integrate them.



Physical Mapping (Tomkins)

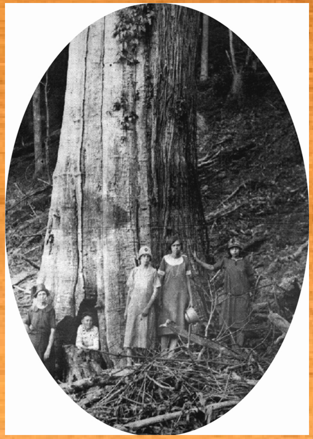
- Construction of 20X BAC libraries of Chinese chestnut.
- Construction of a physical map for Chinese chestnut by fingerprinting and BAC end sequencing.
- Integration of the genetic and physical maps.
- Investigate micro and macrosynteny in the chestnut and oak.



5. Platform for map based cloning.

The Fagaceae Website and Database

- Physical mapping data.
- Genetic mapping data.
- Sequence data.
- Marker development data
- Integration of physical and genetic maps.
- All reports and presentations by project members.



A different kind of forest biotechnology

- This project shows how genome technology could contribute to rescue endangered tree species.
- Substantial social benefits are possible.
- Model for future efforts to conserve and protect endangered tree species through genetics and biotechnology.