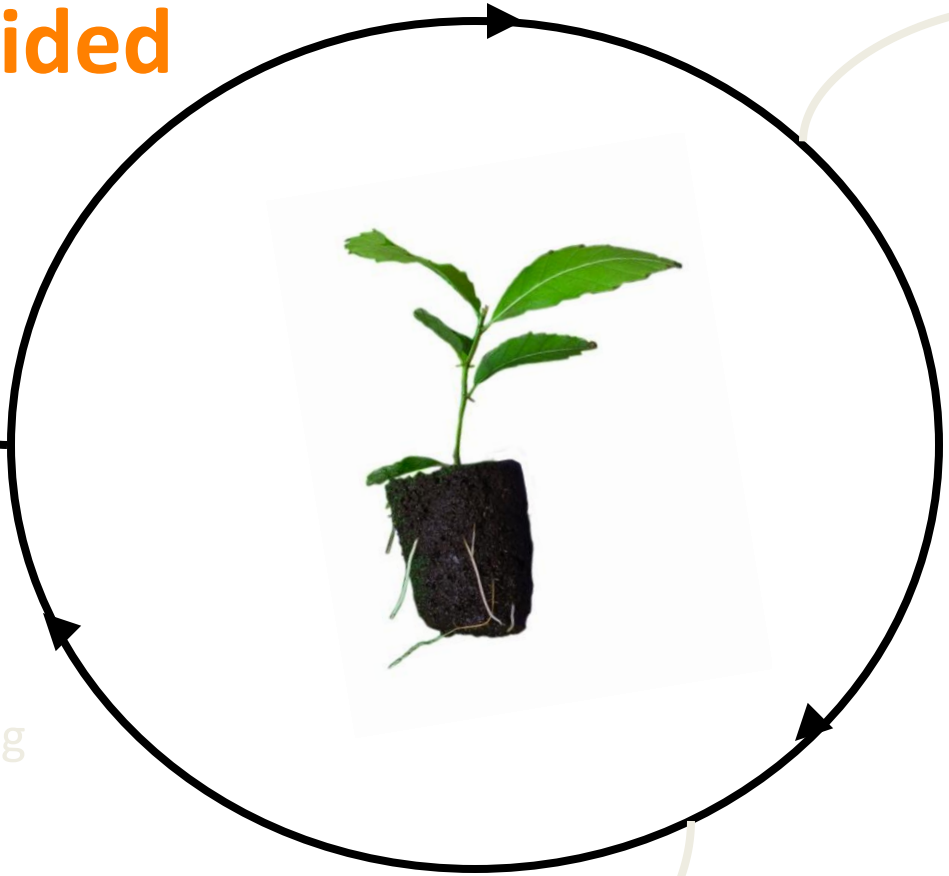
FHI Biotechnology Approaches

Clonal testing

New varieties

Marker-aided breeding



Transgenics

Genome sequencing

GE trees

Personnel

- Germplasm (material for clonal testing, transformation)
  - Fred Hebard (The American Chestnut Foundation, TACF)
  - Sandra Anagnostakis (Connecticut Agr Expt Station, CAES)
  - Jerre Creighton (Virginia Dept of Forestry, VDF)
  - Gary Griffin (Virginia Tech & Am Chestnut Cooperators' Foundation, ACCF)
- Breeding (crosses for resistance gene mapping)
    - Fred, Sandra
  - Sara Fitzsimmons (TACF & Penn State Univ)
- Testing (phenotyping, mechanisms of resistance)
      - Blight (Fred, Sara), Resistance mechanisms (new post-doc with Fred)
      - Early screening (SUNY Team & Josh Bronson (USFS, Resistance Screening Center)
    - Phytophthora (Joe James, Steve Jeffers) with Clemson Univ
    - Field (developing now and year 3)
- Mapping (genotyping, gene discovery, marker selection)
    - Tom Kubisiak(left USFS in Feb), Dana, SIFG lab (Chuck, Casey, Thomas, Kristel)
  - Clemson Team, Bert Abbott's Lab and CUGI
    - Bode Olukolu (leaving now for NC State)
    - Meg Staton, Ali Barakat, Eric Feng

Objectives/Deliverables

- Germplasm
  - Provide clonal and transgenic teams with plant material
    - Workhorse lines, experimental controls
    - Diverse Hybrids & Large Surviving Americans (LSAs) for clonal testing
    - Advanced backcross hybrids for clonal increase
- Breeding & Testing (phenotyping)
  - Develop informative populations for trait/gene mapping
  - Collaborate on early disease screening development
  - Collaborate on field testing; clones and transgenics
- Mapping (genotyping)
  - Fine map resistance genes and quantify their effects
  - Collaborate on integrating maps for candidate gene (CG) selection
  - Develop Marker-assisted Selection (MAS) for breeding

Germplasm

- The American Chestnut Foundation (TACF)
  - Germplasm Agreement negotiated & signed with Univ Georgia
    - under MOU between FHI and TACF
- Connecticut Ag Exp Station (CAES)
  - Crosses from CAES's extensive, long-term program
- Virginia Department of Forestry (VDF)
  - Crosses in VDF's operational program
- Am Chestnut Cooperators' Foundation (ACCF)
    - Crosses from ACCF's public domain trees

Breeding & Phenotyping

- Blight resistance mapping resources
  - TACF/NSF experimental 'Mahogany' F2 population
    - Blight resistance phenotyping summer 2011
  - TACF/Meadowview advanced backcross lines
    - Operational blight resistance phenotyping
  - Pennsylvania TACF operational BC3 population
    - Blight resistance phenotyping complete spring 2011
- Phytophthora (ink disease) resistance mapping
  - TACF with Clemson

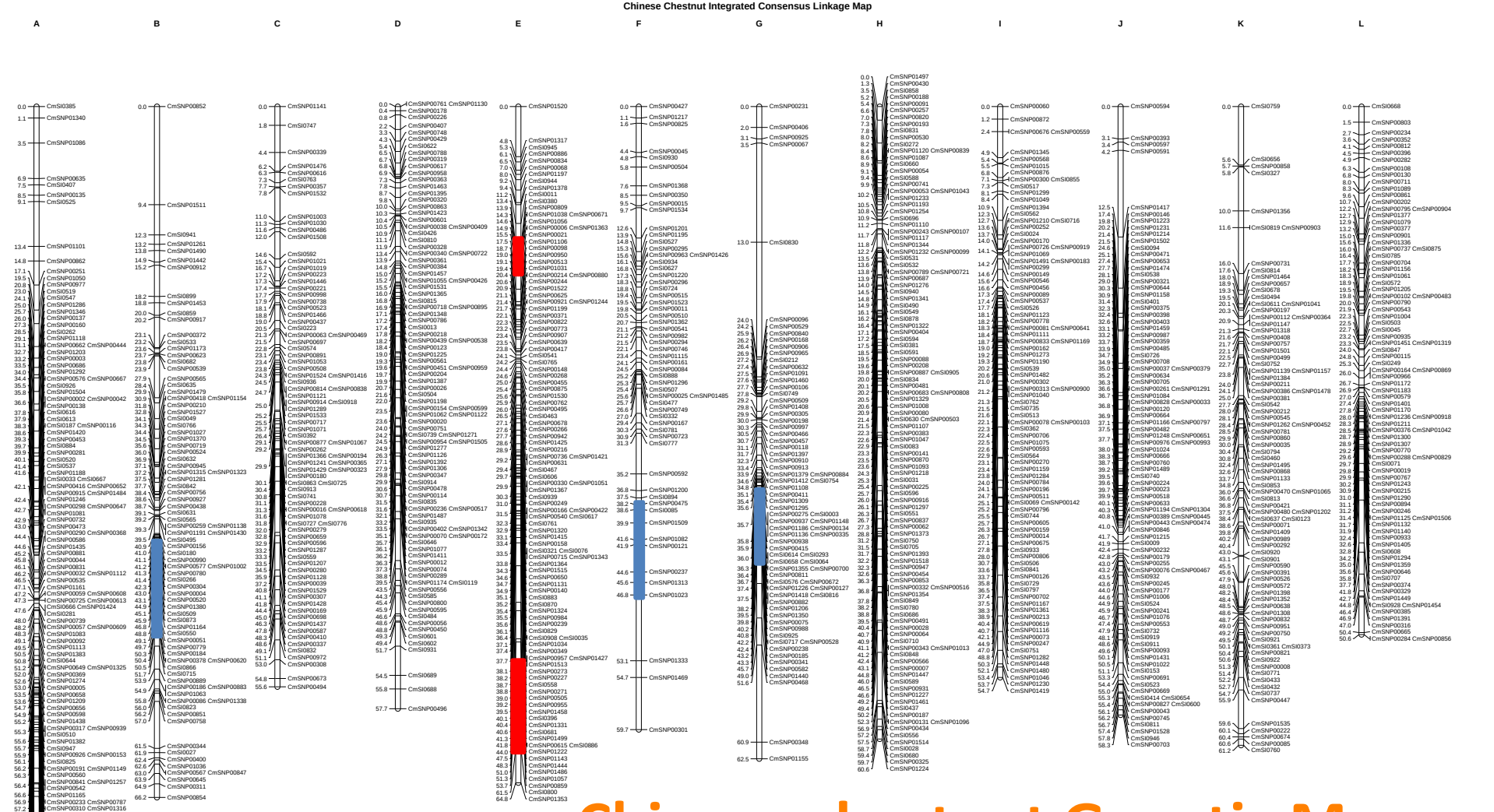
Blight resistance QTLs

- Genetic map— 'Mahogany' F2 cross, 463 markers, 686 cM (92% genome coverage)
  - Two isolates, quantitative scoring
  - Three blight resistance QTLs confirmed (LG-B, LG-F, LG-G), with a fourth one likely (LG-E)
    - Three account for 70% of the genetic variation
    - FHI allows linkage to new, high density Chinese chestnut map, gene sequences and genome sequence
  - 38 Candidate Genes identified for evaluation in transgenics

Ink Disease Resistance QTLs

(*Phytophthora cinnamomi*)

- Genetic map– 'Nanking' BC1 family, 203 SNPs, 575 cM (about 80% genome coverage)
  - markers from Am chestnut, not as efficient for mapping
  - scored for Ink Disease resistance
  - Two major QTLs mapped on LG-E
    - Maternal map: at 12-15 cM, LOD=4.42, R^2=0.35
    - Paternal map: at 46-62 cM, LOD=5.39, R^2=0.40
  - 4 Candidate Genes identified and are being tested in transgenics



Chinese chestnut Genetic Map

1126 markers covering 743 cM

with 3 blight QTLs from F2 map

and 2 ink disease QTL from B1 map

Candidate Gene List (1/3)

order	CCcontig	Uniprot_BestHit	Linkage_Group	cDNA_status	BinaryVector	BV_status	TransPipe
1	CCall-contig8901_v2	**beta-1 3 glucanase**	?	Cloned&sent	pFHI-B13Gluc	received	SUNY-ESF
2	CCall-contig2586_v2	**CBS domain protein**	?	Cloned&sent	pFHI-CBS1	received	SUNY-ESF
3	CCall-contig11269_v2	**UDP glucosyltransferase**	B, G	Cloned&sent	pFHI-UDP	received	UGA
14	CC454_contig41915_v2	**Allene oxide cyclase (AOC)**	?	working	working	waiting	TBD

- Genes selected on multiple sources of information
  - trait mapping (QTL regions of genome)
    - including comparative analysis with Peach
  - gene expression analysis (genes on/off at site of blight infection)
  - gene sequence matching with other resistance-like genes
- Genes are going to Transgenic pipeline for testing
- Markers for these genes will be used for Marker-aided breeding

New Phenotyping Resources

- Expanded ‘Mahogany’ F2 population in VA
  - >50% increase in progeny number
  - Provides higher resolution mapping
- Large ‘Mahogany-Graves’ BC3 population in PA
  - 6x larger than F2 population (even higher resolution)
  - Evaluate in independent environment and genetics
- Operational B3F2 populations in VA
  - Test markers in operational materials

New Genotyping Resources

- 5000 SNP Chip
  - Using scalable, high-density, high-throughput tech
  - Includes validated SNPs from previous chips
    - Chinese (964 SNPs) and American (224 SNPs)
  - Designed new SNPs from Chinese chestnut gene sequences (~3900 SNPs)
  - Higher density markers necessary to take full advantage of larger, higher resolution mapping populations –> more precise gene mapping

Summary

- Our goals and progress are aligned:
  - High-quality candidate genes for transgene (cisgene) testing
  - High-quality DNA markers for marker assisted selection
  - High-quality germplasm for clonal testing and experimental materials
  - Emphasis on early, reliable screening for blight and Phytophthora resistance