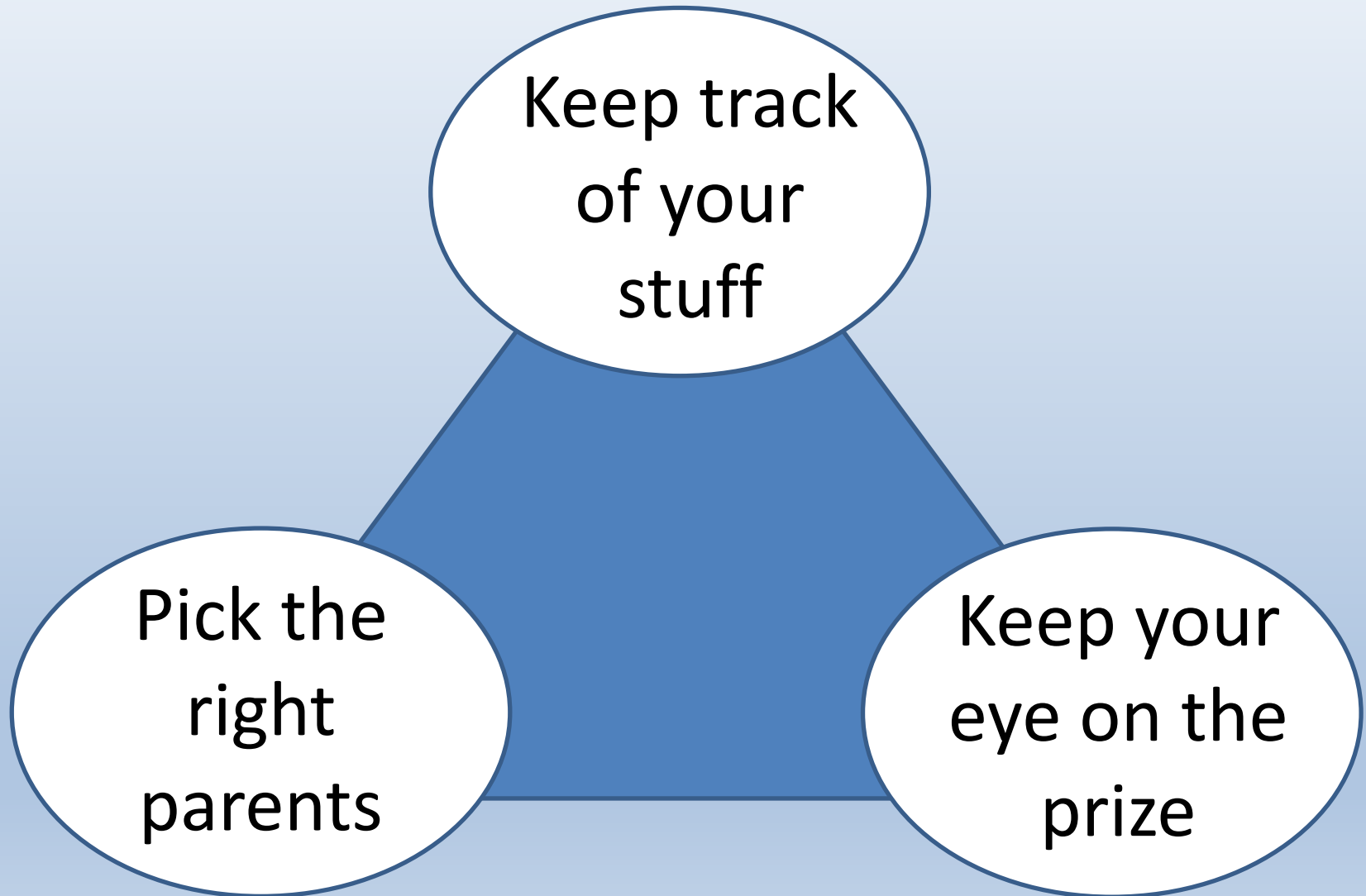
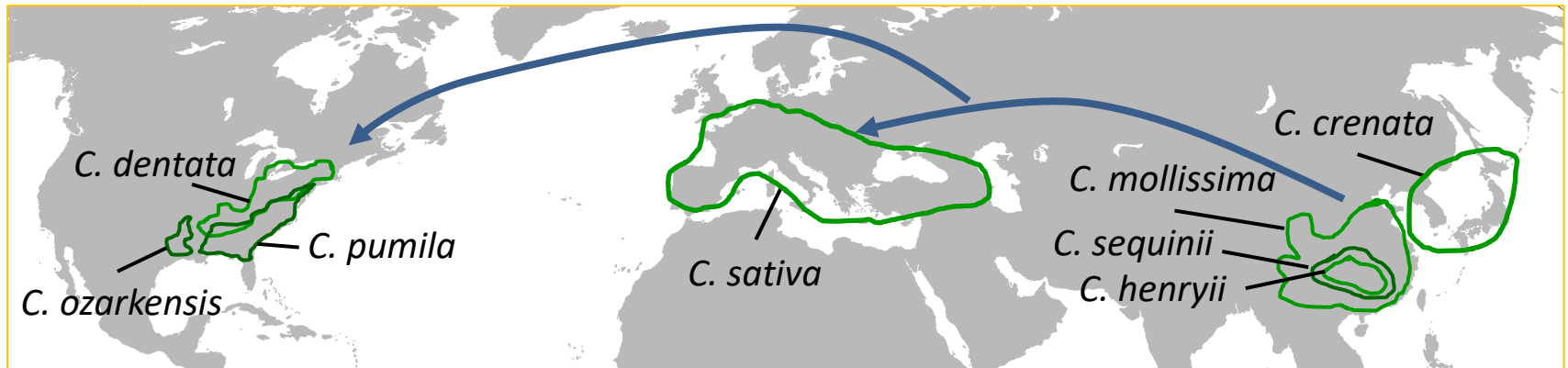




# How to be a smashing success in chestnut breeding



# Castanea: origin and migrations



Approximations of the native ranges of *Castanea* species (green lines).  
Arrows show the migration of chestnuts out of before humans appeared.

# Performance-verified DNA markers

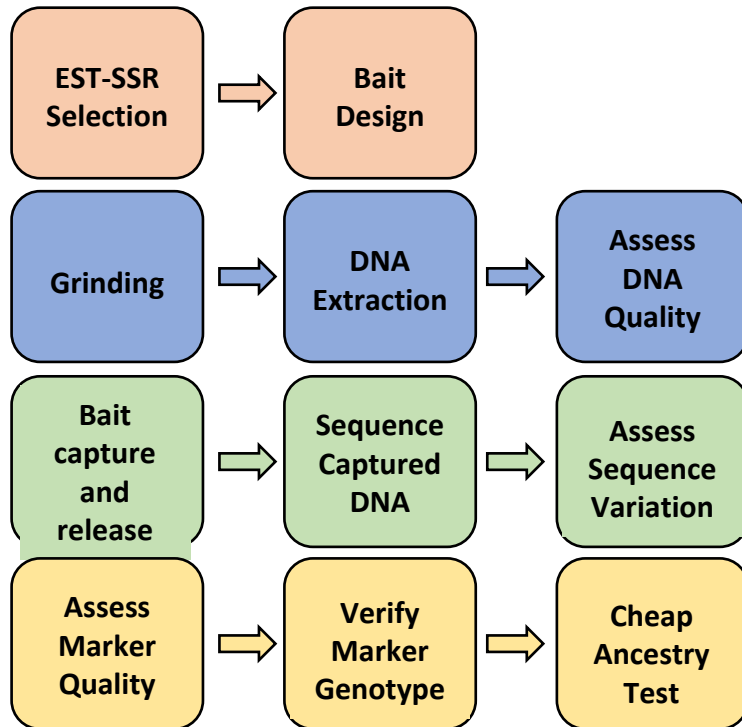
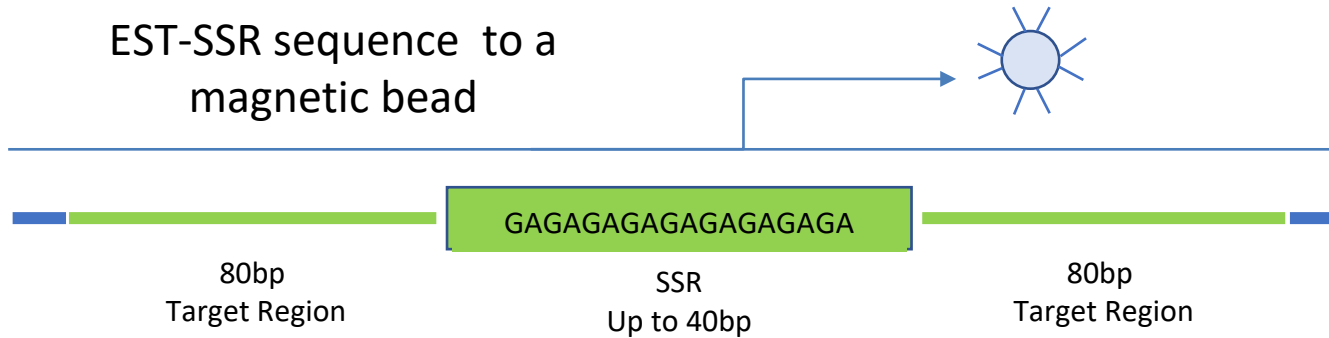
- Increases the odds of picking the right parents
- Best tool for keeping track of your stuff

# Performance-verified DNA markers

- Reproducible
- Informative across *all species* of *Castanea*
  - Distinguishes “pure species”
  - Detects complex interspecific ancestry
  - Identifies the species of the ancestors



EST-SSR sequence to a magnetic bead



- The AIMs project uses a type of DNA marker called an EST-SSR.
- “Candidate” EST-SSR sequences were stuck to magnetic beads.
- The beads were used to capture these EST-SSR markers from a set of 45 *Castanea* representing all eight species
- The captured sequences were then released and sequenced.
- We have verified that this approach produces very high quality genotypes that distinguish all chestnut species and identifies related individuals.

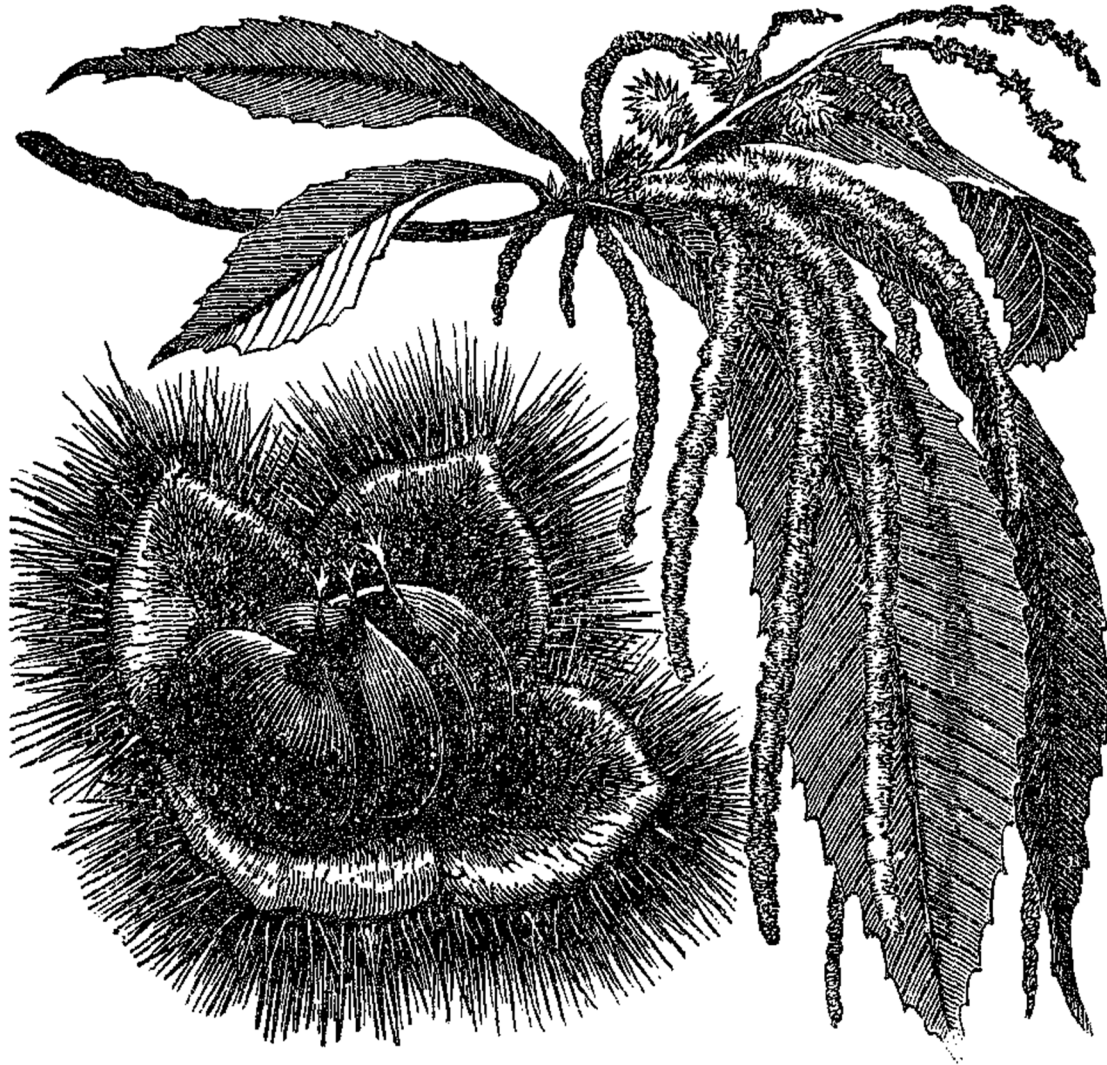
# Process invented to lower the cost of AIMS genotyping

- Use PCR rather than sequence capture.
- Design multiplex PCR reactions.
- Test multiplex PCR reactions for function.
- Resdesign multiplex PCR and test again.
- Extract first set of 96 chestnuts.
- Use multiplex PCR to produce 70 amplicons for each of the 96 chestnuts.
- Clean-up for sequencing.
- Sequence
- Extract second set of 96 chestnuts.
- Repeat









*Castanea dentata.*

What we know

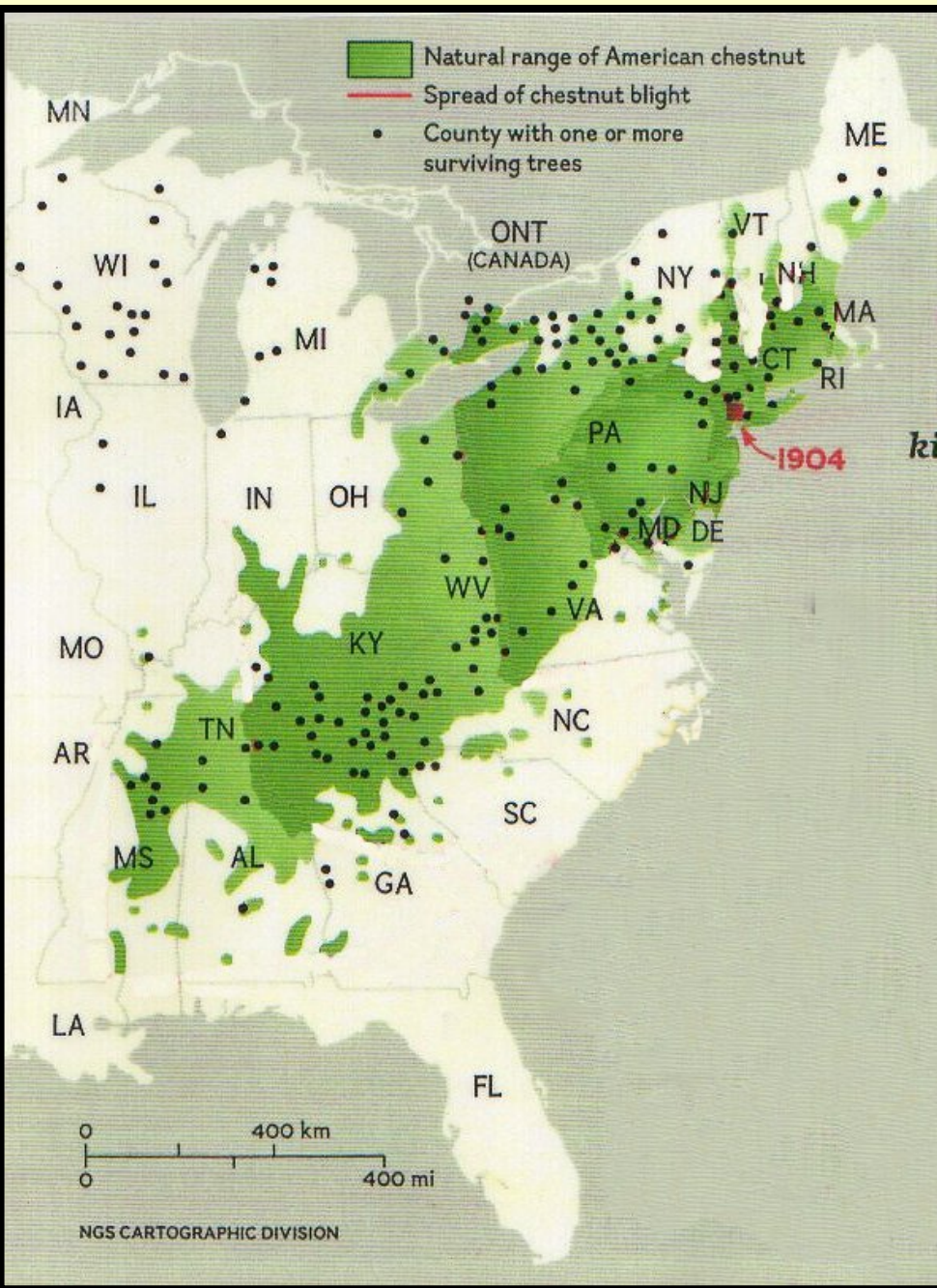
What we thought we knew

What we should have known

What we never know

A new direction

A reminder



Chestnut blight introduced in the late 19<sup>TH</sup> Century.

Spread throughout the entire native range by 1950.



*Cryphonectria parasitica*

A necrotrophic fungal pathogen that kills and eats the cambium.

# Transfer blight resistance from Asian *Castanea* spp. into American chestnut

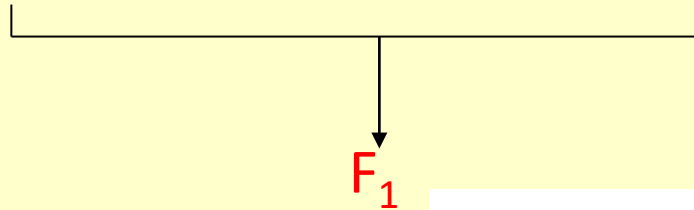
## American chestnut (*C. dentata*)

- Not resistant to blight
- Height: 80 – 100 feet
- **Dominant canopy tree**



## Chinese chestnut (*C. mollissima*)

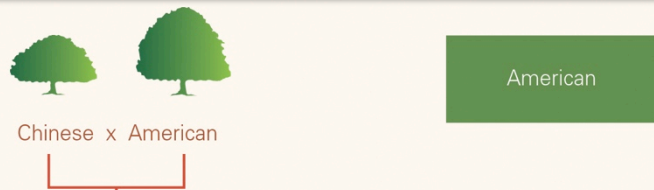
- **Resistant to blight**
- Height: 40 – 60 feet
- Orchard tree



Intermediate blight resistance, not competitive in the forest

# The American Chestnut Foundation's Backcross breeding program

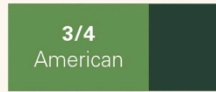
American and Chinese chestnuts are first crossed to help increase blight resistance.



F1 is the **first cross** to the American chestnut



B1 is the **first backcross** to the American chestnut



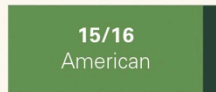
B2 is the **second backcross** to the American chestnut



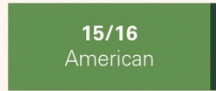
B3 is the **third backcross** to the American chestnut



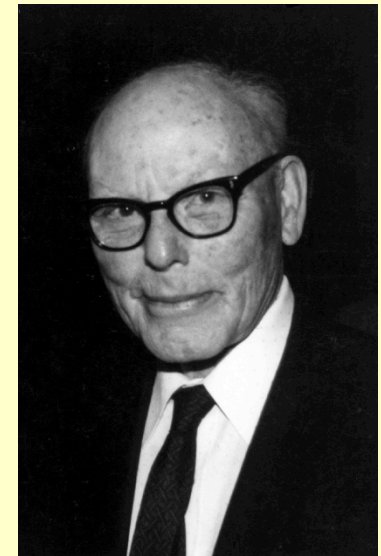
B3F2 is the **first intercross** to the American chestnut



B3F3 This is the **second intercross** to the American chestnut



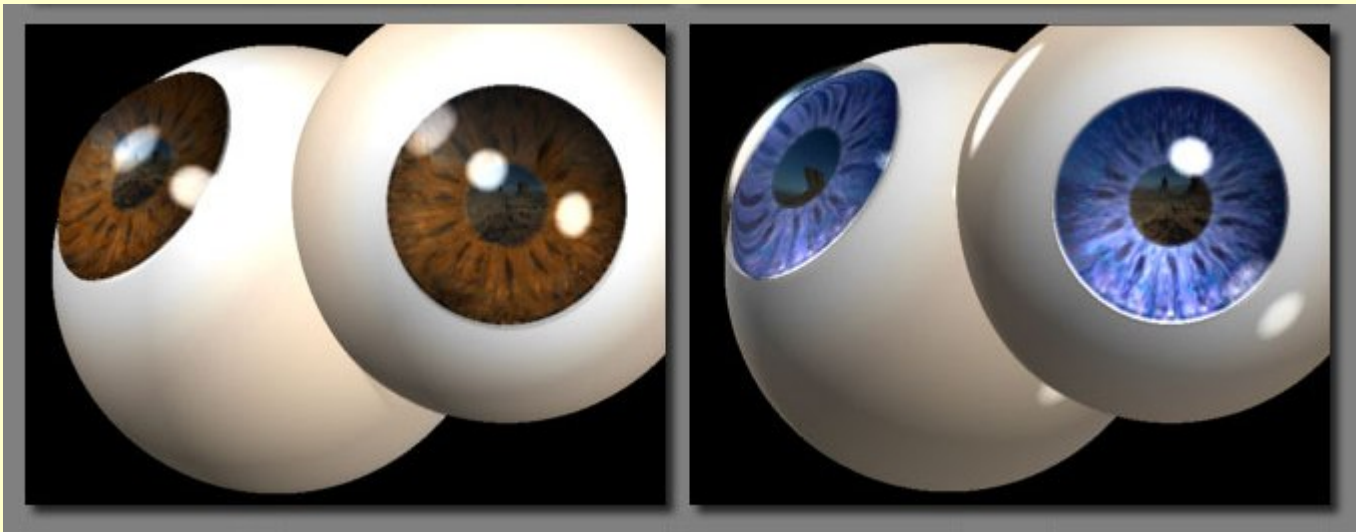
Backcrossing to recover American chestnut forest morphology



Charles Burnham

Intercrossing to combine Chinese chestnut alleles for blight resistance into a homozygous state

The backcross breeding program was based on a simple genetic model



BB

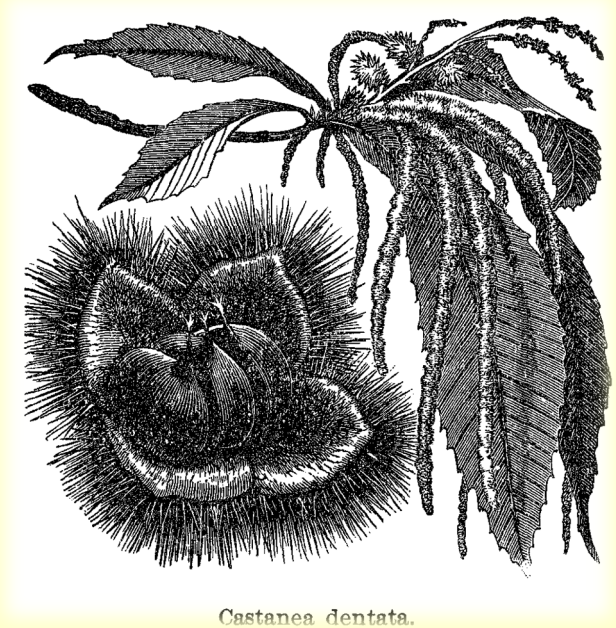
bb

What we thought we knew

# THE AIMS PROJECT

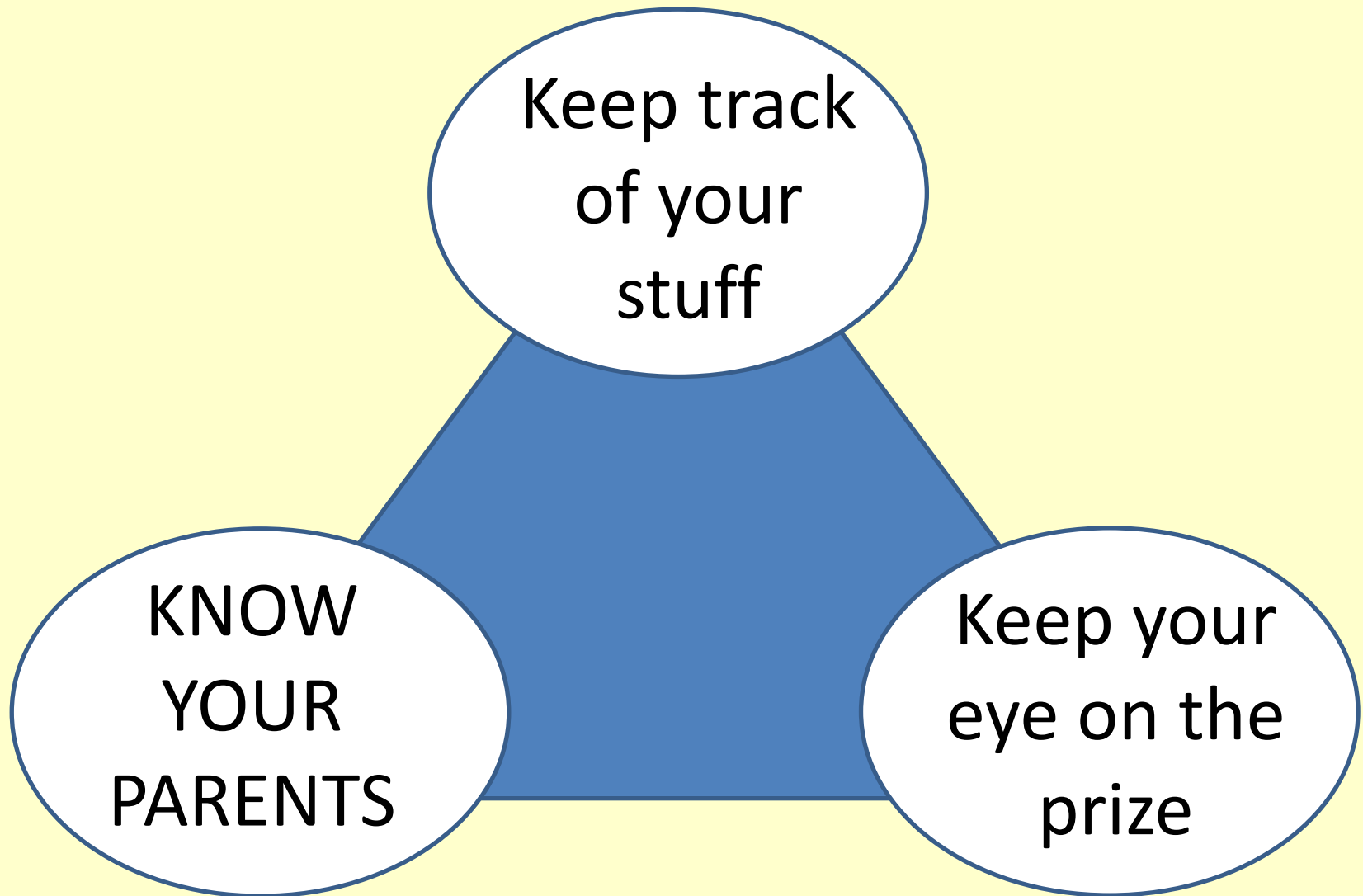
## Ancestry Informative Markers for Chestnut

- Verify identity of breeding stock
  - Valid performance evaluation
  - Predictable characteristics
  - Firm legal protections
  - Avoidance of inbreeding
  - Accurate estimate of effective population size
- Detect recent ancestry
  - Identify interspecific hybrids
  - Predict genetic value in descendants
  - Predict the best parents for crossing
- Infer extent of local adaptation





# THE CARDINAL RULES OF PLANT BREEDING

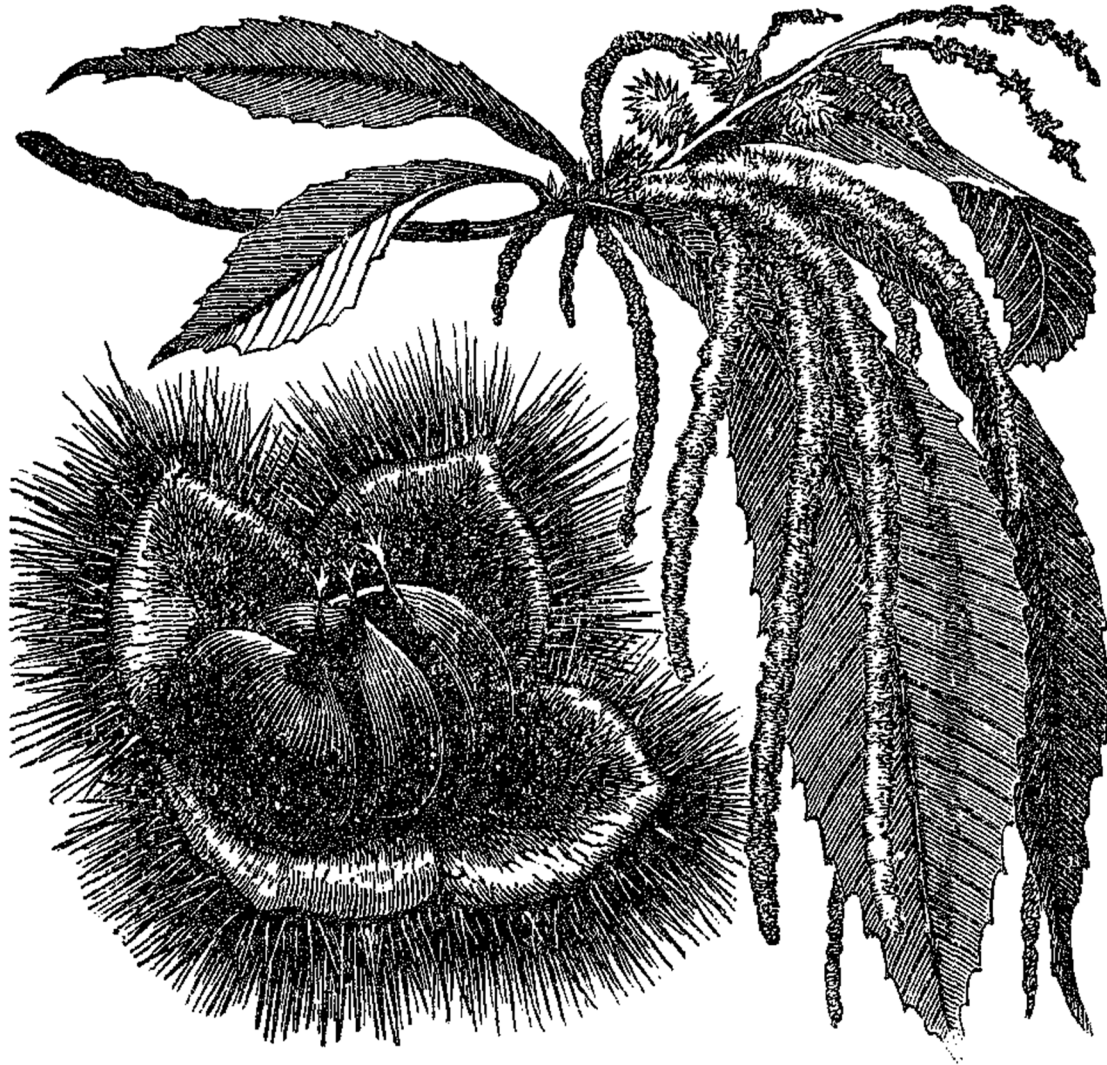


# Restoration of American Chestnut

- Enhancing blight resistance so trees can reproduce in native range
- Combining blight and *Phytophthora* resistance in southern forests
- Having sufficient effective population size to minimize inbreeding



A new direction



*Castanea dentata.*

What we know

What we thought we knew

What we should have known

What we never know

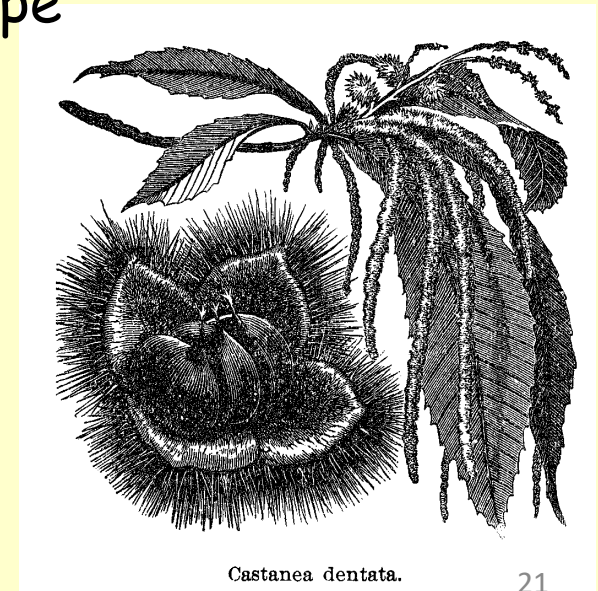
A new direction

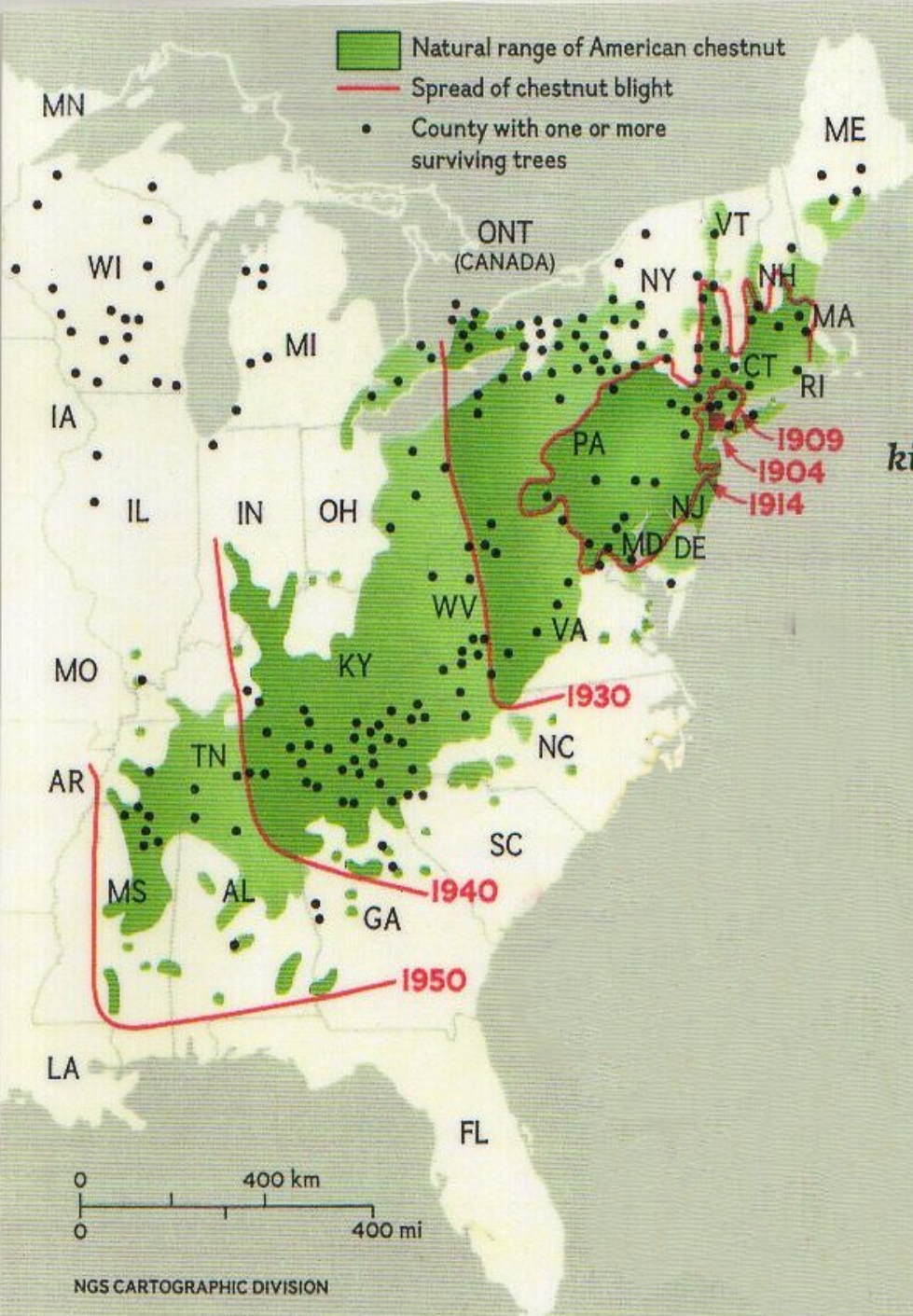
A reminder



# Humans domesticated chestnuts

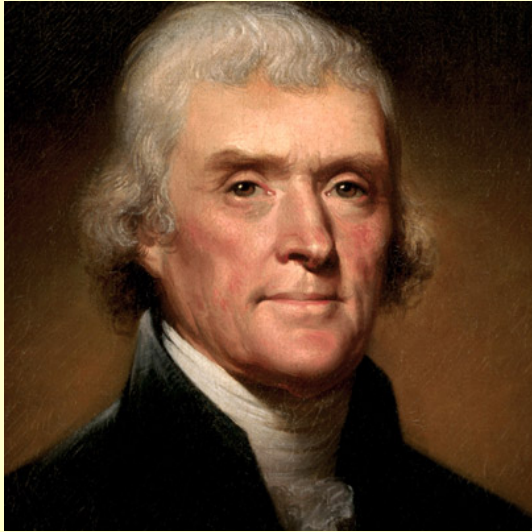
- Japanese Chestnut (*C. crenata* [Siebold & Zucc.](#)) ~ 5500 BP
- Chinese chestnut (*Castanea mollissima* Blume) in central China ~ 4600 BP
- "European" chestnuts (*C. sativa* Mill.) in Asia minor ~5000 BP
- European chestnuts transported to Europe ~2500 BP, then to Britain and Ireland
- American chestnut (*C. dentata* (Marsh.) Borkh.) not domesticated but probably "assisted"





By 1950 blight had spread throughout the species range

Then the story in the U.S. gets complicated



Thomas Jefferson planted *C. sativa* in the orchard at Monticello in 1773.

Jefferson hybridized these with American chestnuts.

Then the French get involved...



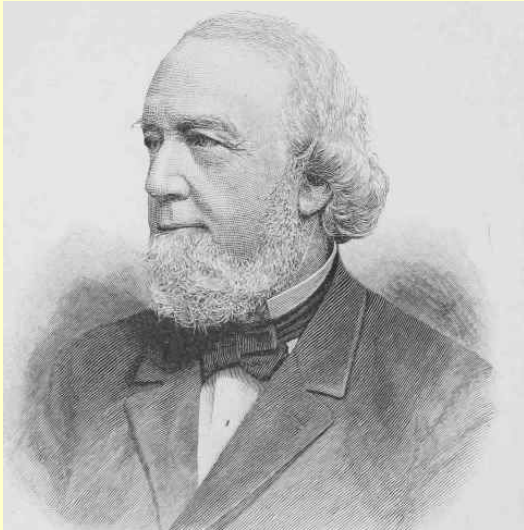
Eleuthère Irénée du Pont de Nemours moved to the United States from France in 1799, planted *C. sativa* in Delaware, imported many cultivars over the years and made many hybrids with *C. dentata*, one of which, 'Paragon', still exists.

Jefferson and DuPont were only two of many who imported chestnuts and experimented with hybridization.

This tradition of citizen science (chestnut breeding) continues to this day.



## Moving on to the 19<sup>th</sup> Century...



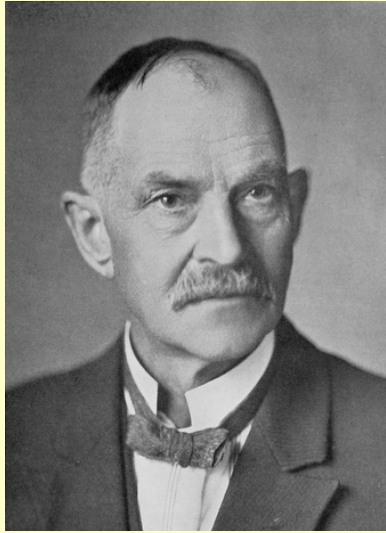
S. B. Parsons

*C. crenata* introduced into America by the S. B. Parsons Company of Flushing NY in 1876 and by Luther Burbank of Santa Rose CA in 1886 .

Two of the Japanese chestnuts planted by Parsons in Connecticut still survive .

George W. Endicott of Villa Ridge, Illinois, grew 'Japan Giant' at the end of the 19<sup>th</sup> century and experimented with *C. crenata* × *C. dentata* hybrids.

In the 20<sup>th</sup> Century hybridization got serious...



Dr. Walter Van Fleet

Between 1900 and 1921, USDA botanist Dr. Walter Van Fleet made thousands of interspecific crosses with native chinquapins, European, Japanese, and Chinese chestnuts.

The USDA expanded this hybridization program during 1925-1949.

This program produced ~ 6000 hybrids involving all the known *Castanea* species.

These hybrids were widely distributed to anyone who wanted them, across the range of the American chestnut.