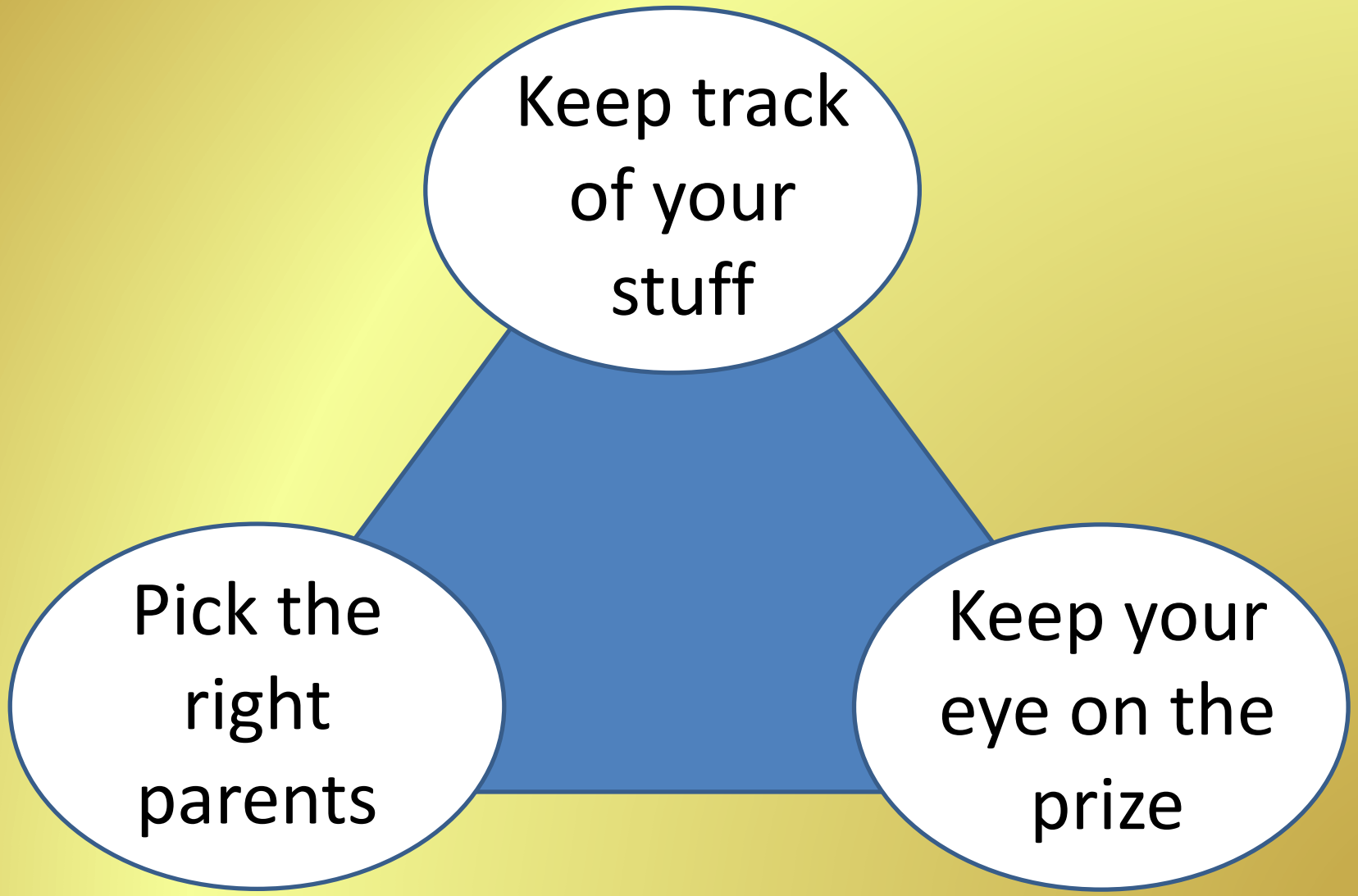

Using DNA Markers to Benefit the Chestnut Industry

How to be a smashing success in plant breeding



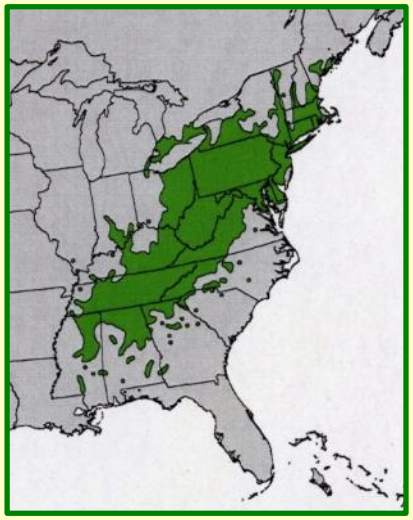
The “Right Set” of DNA markers

- Detect recent ancestry
  - Identify interspecific hybrids
  - Verify parentage

Pick the right parents

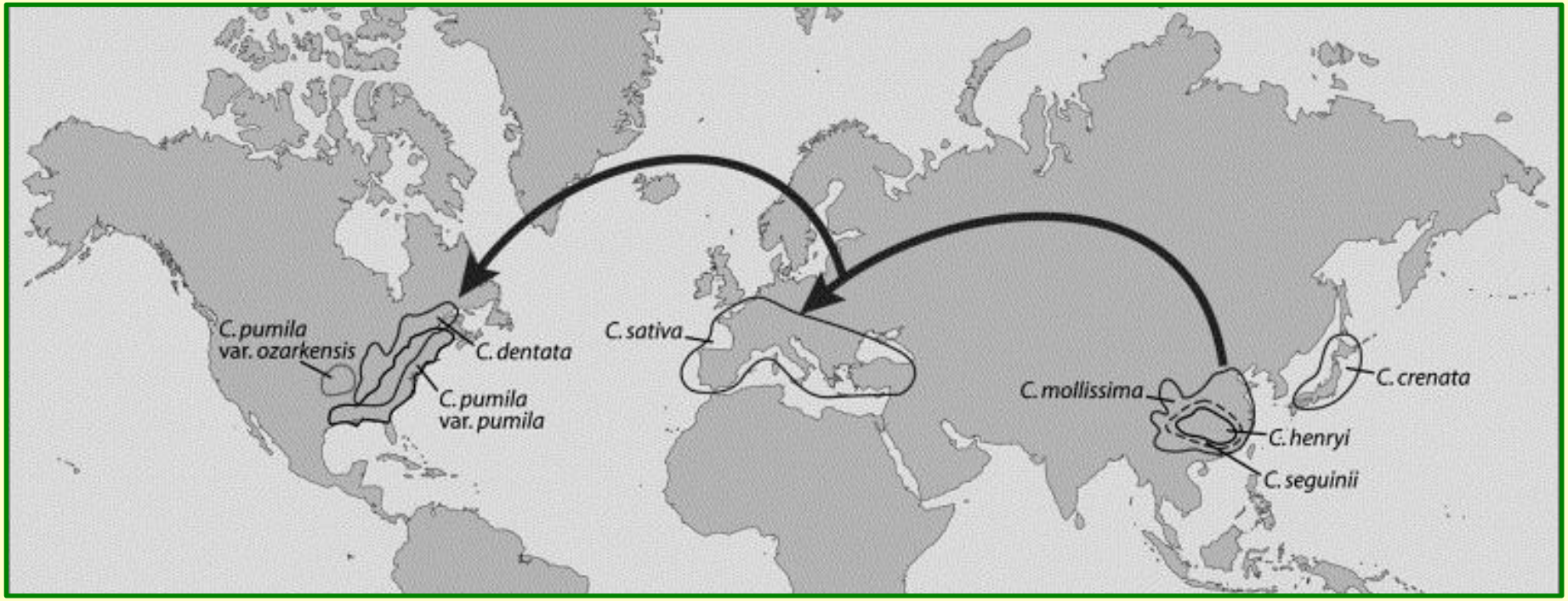
- Verify cultivar identity
  - Provides legal protection
  - Save time and money

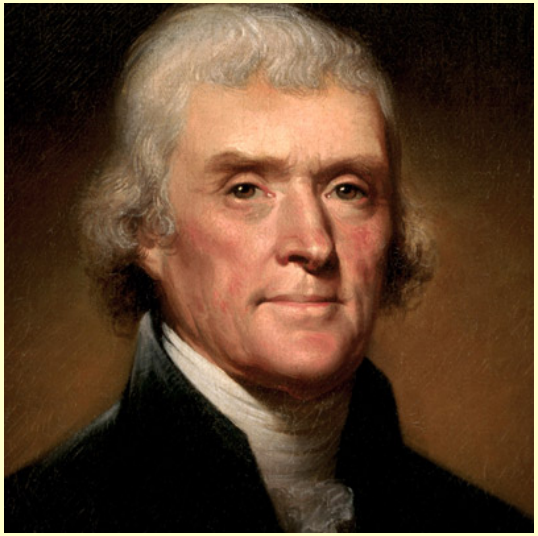
Keep track of your stuff



Castanea is complicated

- Eight *Castanea* species
- Three domesticated species
- All species are interfertile

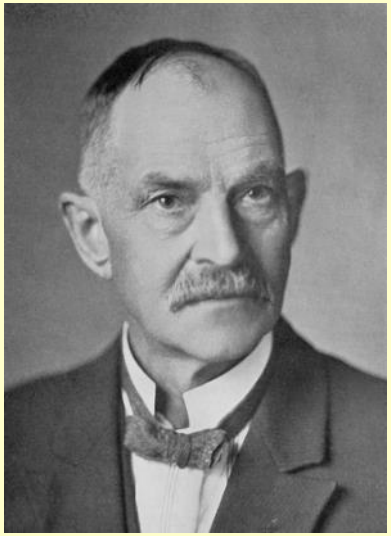




Thomas Jefferson

Eleuthère Irénée du Pont

S. B. Parsons



Dr. Walter Van Fleet

Chestnut germplasm

What happens when you don't or can't keep track of your stuff

- Many species, many "hybrids"
  - European, Chinese, Japanese, American…
- Years of undocumented cultivar development
- Lost or never recorded pedigrees
- Mistakes…..

What “ought to be true”…..

Grafted trees from the same tree are the same.

All cultivars with the same name are the same.

All cultivars with different names are different.

ISN’T……

What was never true……

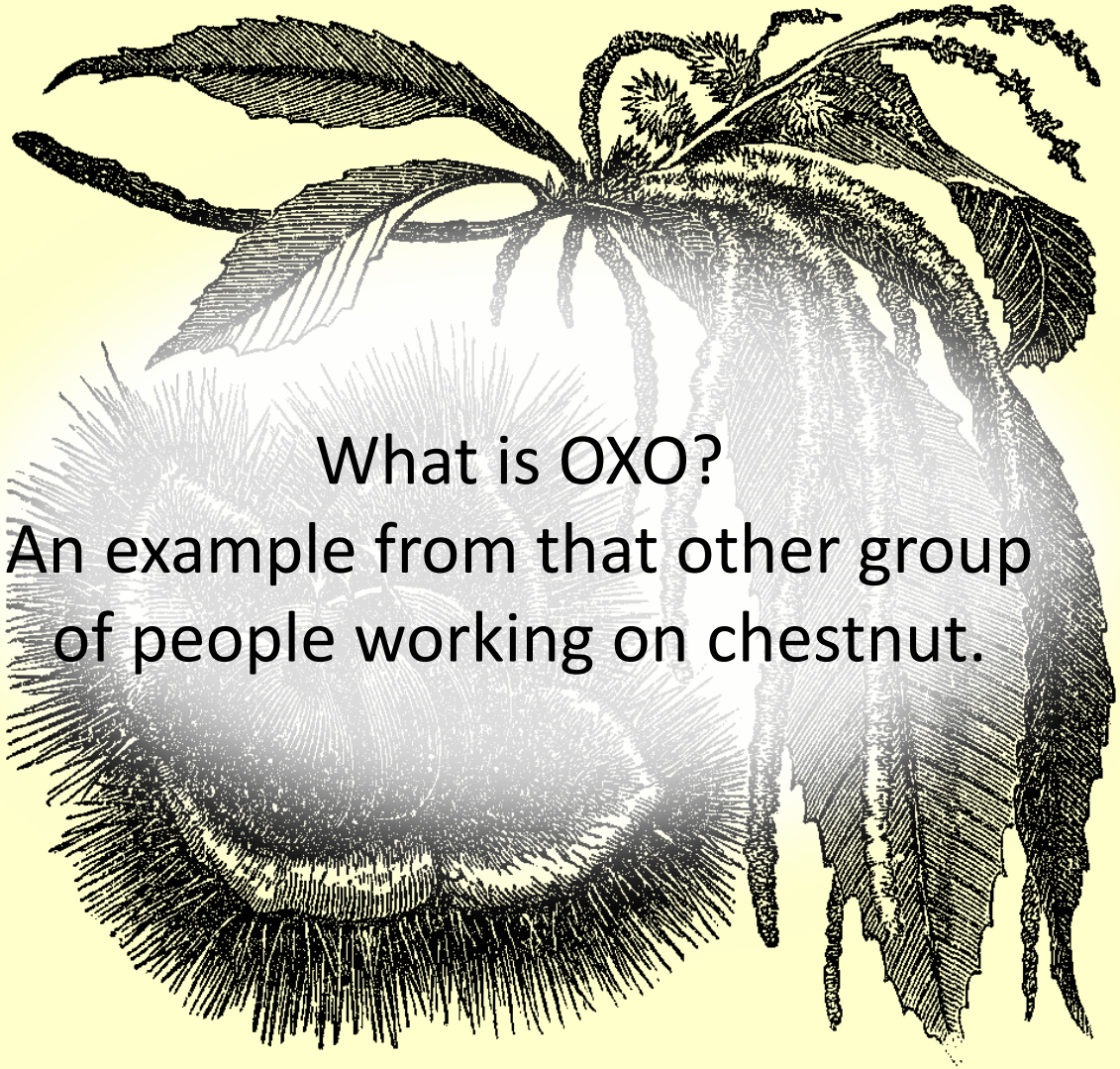
The seed of a given tree is the same as the tree itself.

All the seeds on a given tree are the same.

All hybrids are equivalent.

All trees of the same species are the same.

Still isn't…..



What is OXO?
An example from that other group of people working on chestnut.

Castanea dentata.

OXO

The blight–resistant transgenic chestnut

What species is the OXO parent?

Is it “pure” American chestnut?

What is the ancestry of Clapper?

Is it only Chinese and American?

What is the ancestry of Graves?

Is it only Chinese and American?

What is the ancestry of Nanking?

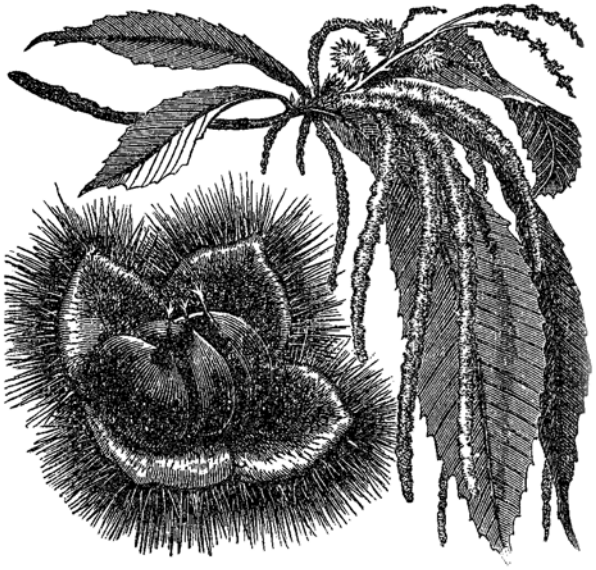
Is it “pure” Chinese chestnut?

What is the ancestry of the B_3F_1s?

What is a “pure “ American chestnut?

THE AIMS PROJECT

Ancestry Informative Markers for Chestnut



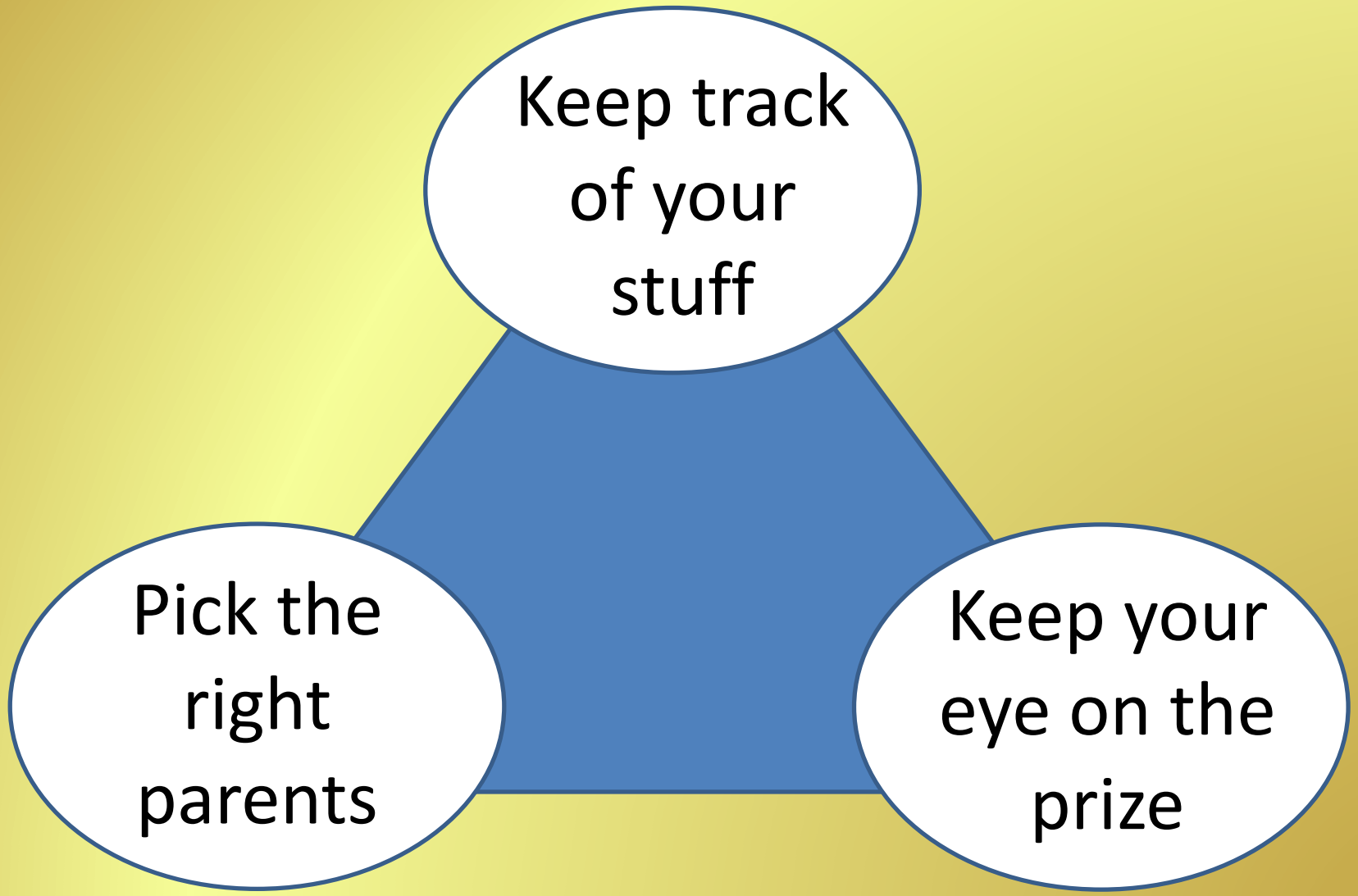
Castanea dentata.

- 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

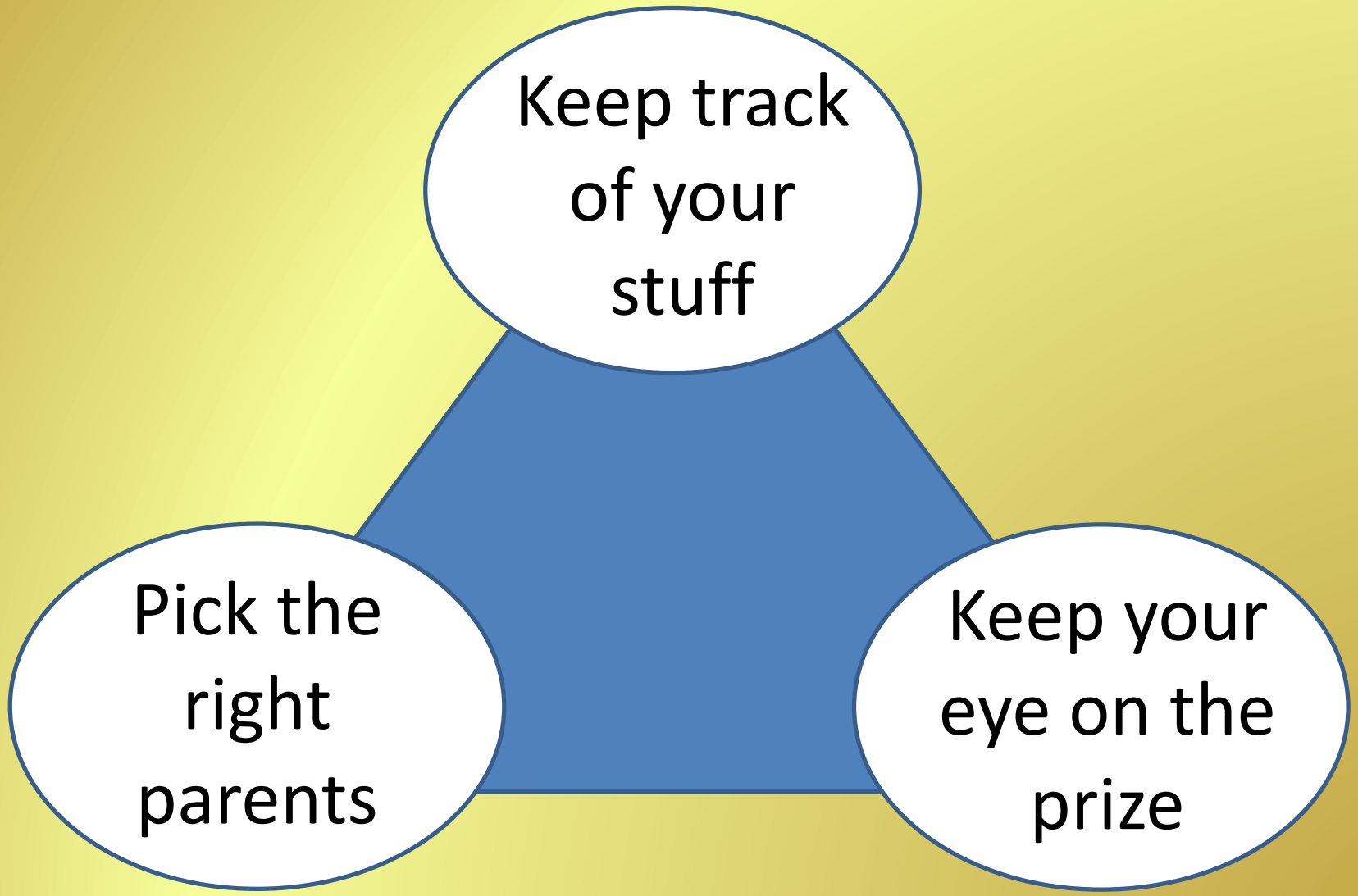
Project Details

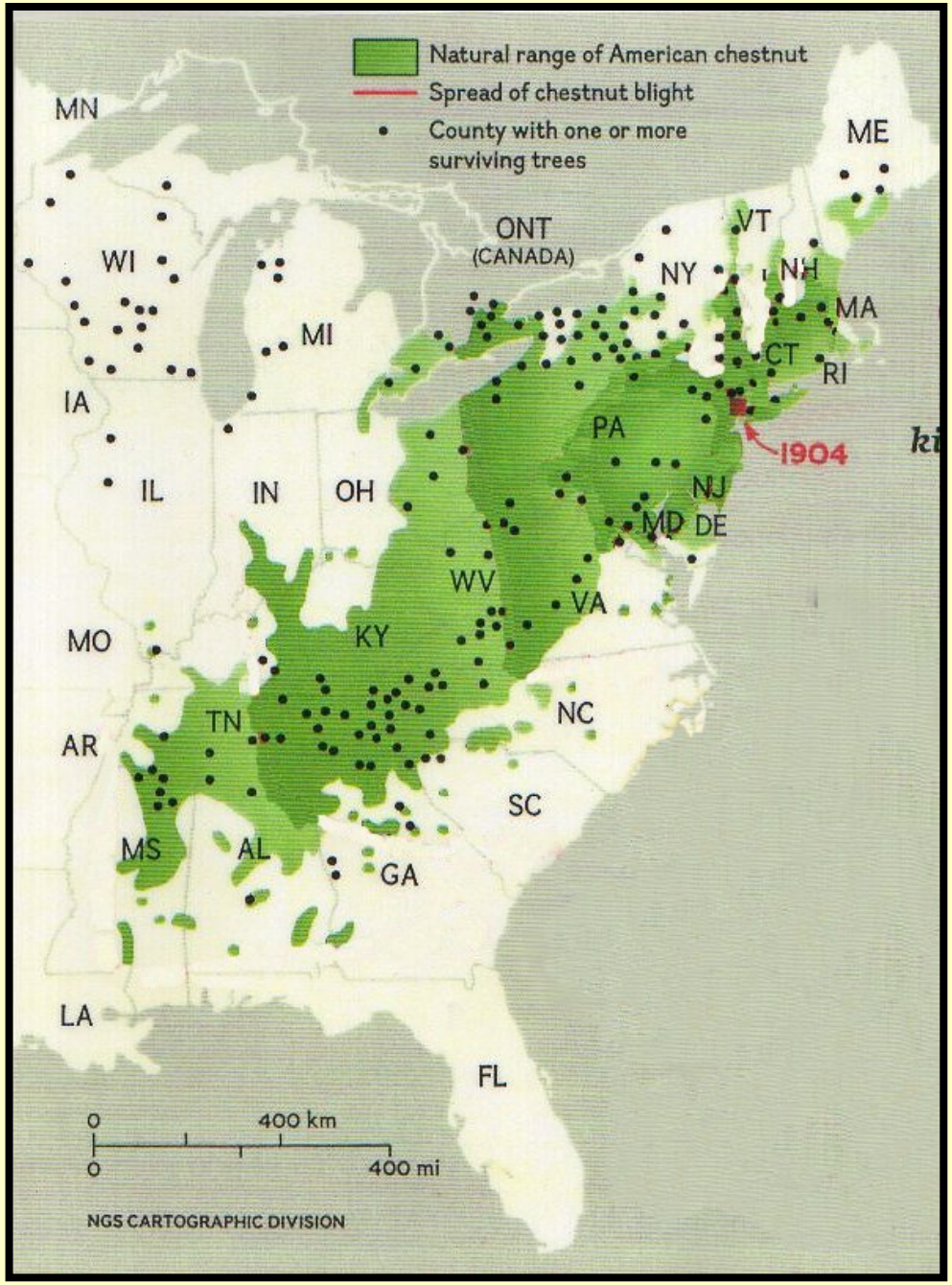
- All eight species required
- > 30 *unrelated* trees per species
- 30-50 EST-SSR DNA markers
- Technical specs
  - Detect interspecific hybrids >95% confidence
  - Identify the parental species of hybrids
  - Uniquely identify every tree
  - Platform independent
  - Cost less than $100/tree

How to be a smashing success in plant breeding



How to be a smashing success in plant breeding





Chestnut blight introduced in the late 19TH 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**

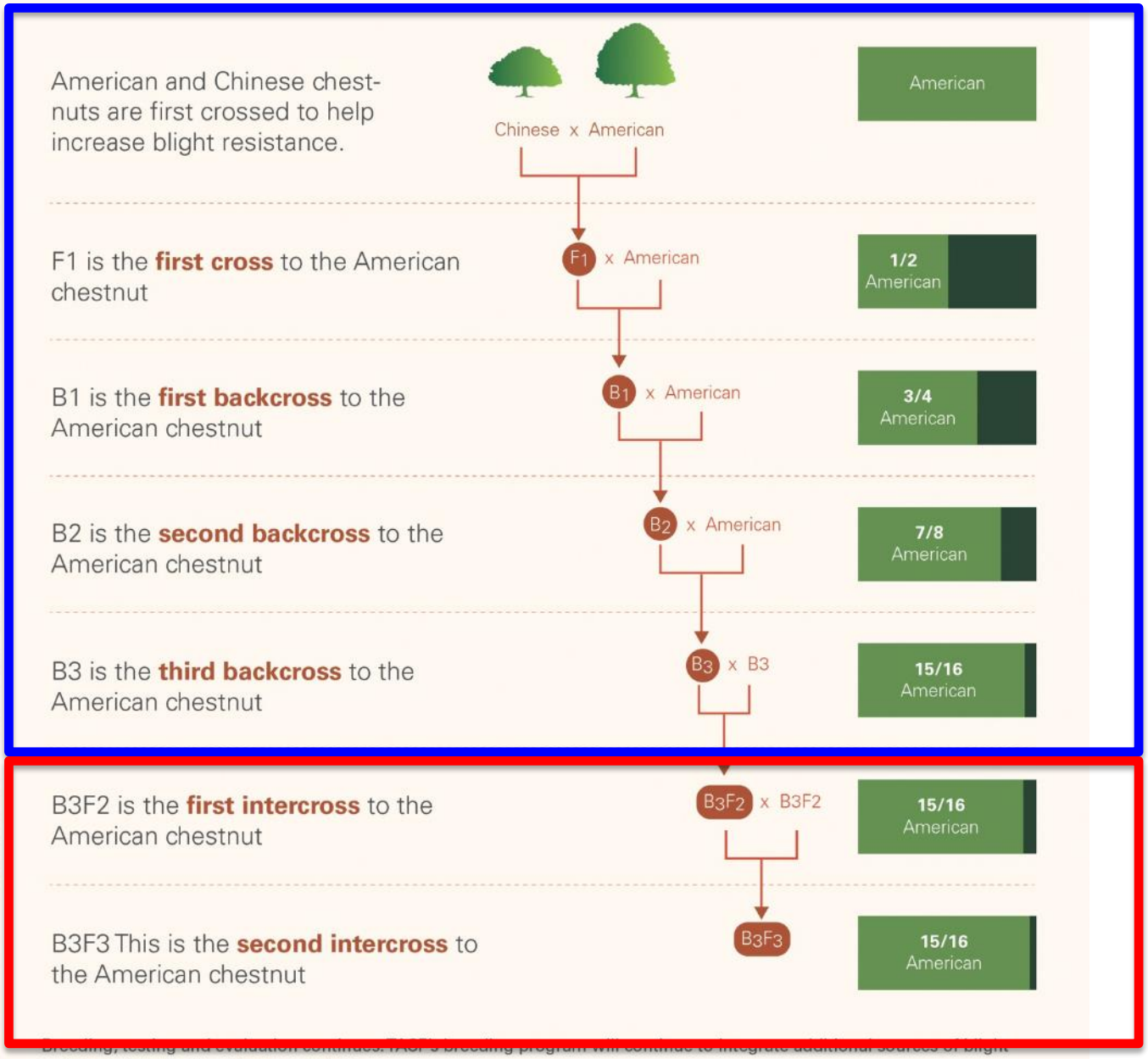
Chinese chestnut (*C. mollissima*)

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

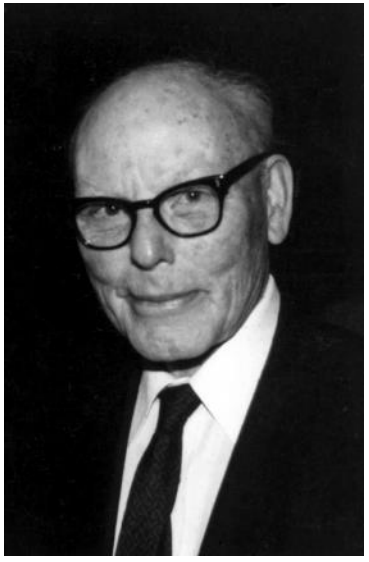
F_1

Intermediate blight resistance, not competitive in the forest

The American Chestnut Foundation's Backcross breeding program



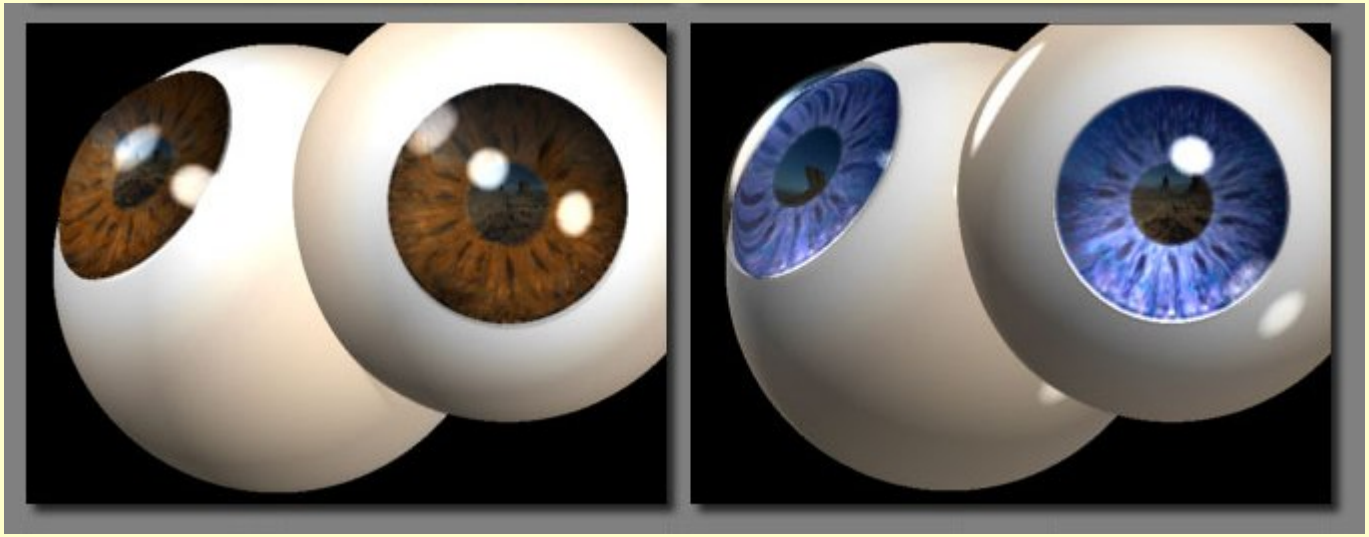
Backcrossing to recover American chestnut forest morphology



Charles Burnham

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

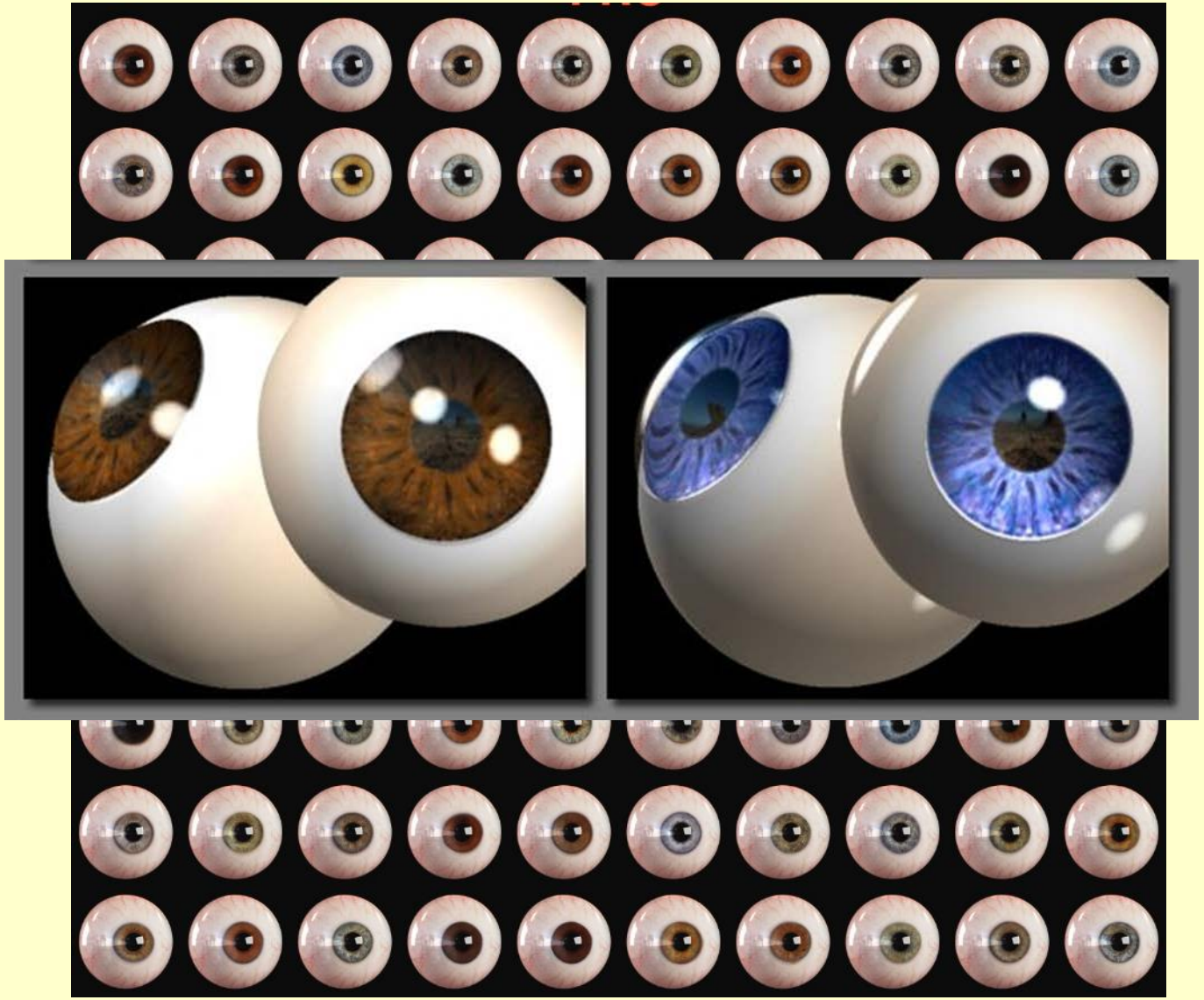
The backcross breeding program was based on a simple genetic model



BB bb

What we thought we knew

Actual eye colors



KNOW YOUR PARENTS

There is genetic variance in Chinese chestnuts for the level of “resistance”.

The genetics of blight resistance is more complicated than previously thought.

Inheritance of blight resistance genes in the first intercross generation (B3-F2): 3 locus model

AaBbCc x AaBbCc

1 of 64 B3-F_2 homozygous for resistance at 3 loci

Target of selection

	ABC	ABc	aBC	AbC	Abc	abC	aBc	abc
ABC	AABBCC	AABBCc	AaBBCC	AABbCC	AABbCc	AaBbCC	AaBBCc	AaBbCc
ABc	AABBcC	AABBcc	AaBBcC	AABbcC	AABbcc	AaBbCc	AaBBcc	AaBbcc
aBC	AaBBCC	AaBBCc	aaBBCC	AaBbCC	AaBbCc	aaBbCC	aaBBCc	AaBbCc
AbC	AABbCC	AABbCc	AaBbCC	AAbbCC	AAbbCc	AabbCC	AaBbCc	AabbCc
Abc	AABbCc	AABbcc	AaBbCc	AAbbCc	AAbbcc	AabbCc	AaBbcc	Aabbcc
abC	AaBbCC	AaBbCc	aaBbCC	AabbCC	AabbCc	aabbCC	aaBbCc	aabbCc
aBc	AaBBCc	AaBBcc	aaBBCc	AaBbCc	AaBbcc	aaBbCc	aaBBcc	aaBbcc
abc	AaBbCc	AaBbcc	aaBbCc	AabbCc	Aabbcc	aabbCc	aaBbcc	aabbcc

Design of B_3F_2 seed orchards

- Plant each backcross line in 9 blocks
- 150 trees per line in each block
- 30,000 B_3F_2s to screen for resistance per source
- Select the most blight resistant tree per 150 trees
- 250 B_3F_2 selections per source

Select against blight susceptibility in B_3F_2 seed orchards

Artificially inoculate stems at age two with slightly pathogenic strain of *C. parasitica*

Trees with small cankers

Trees with moderately sized cankers

Trees with large and sunken cankers

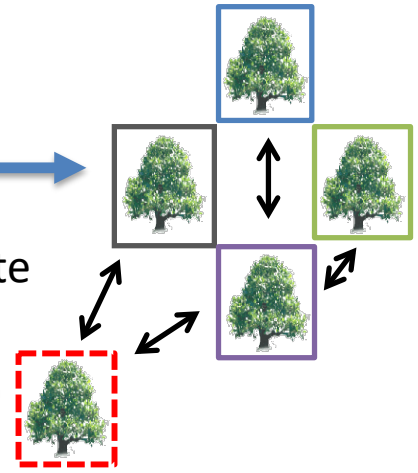
Rogue 80% - 90% of trees based on canker phenotype

Progeny test BC_3F_2 trees remaining after initial culling

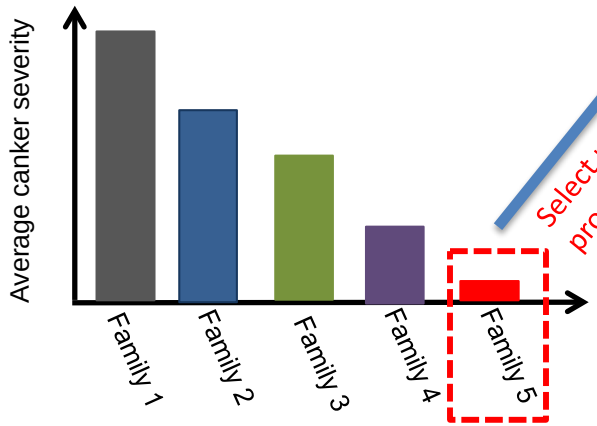
Unselected BC_3F_2 seed orchard

Artificially inoculate & cull susceptible

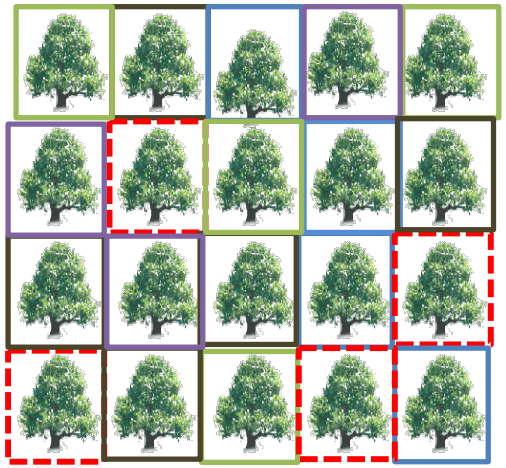
Partially selected seed orchard



Open-pollination among BC_3F_2 survivors



Select parent whose progeny have smallest cankers



Artificially inoculate BC_3F_3 progeny from each selection candidate

Phytophthora cinnamomi extirpated American chestnut from the Southeastern coastal plain prior to chestnut blight

Selection and breeding for *P. cinnamomi* resistance is now a secondary objective of TACF's breeding program

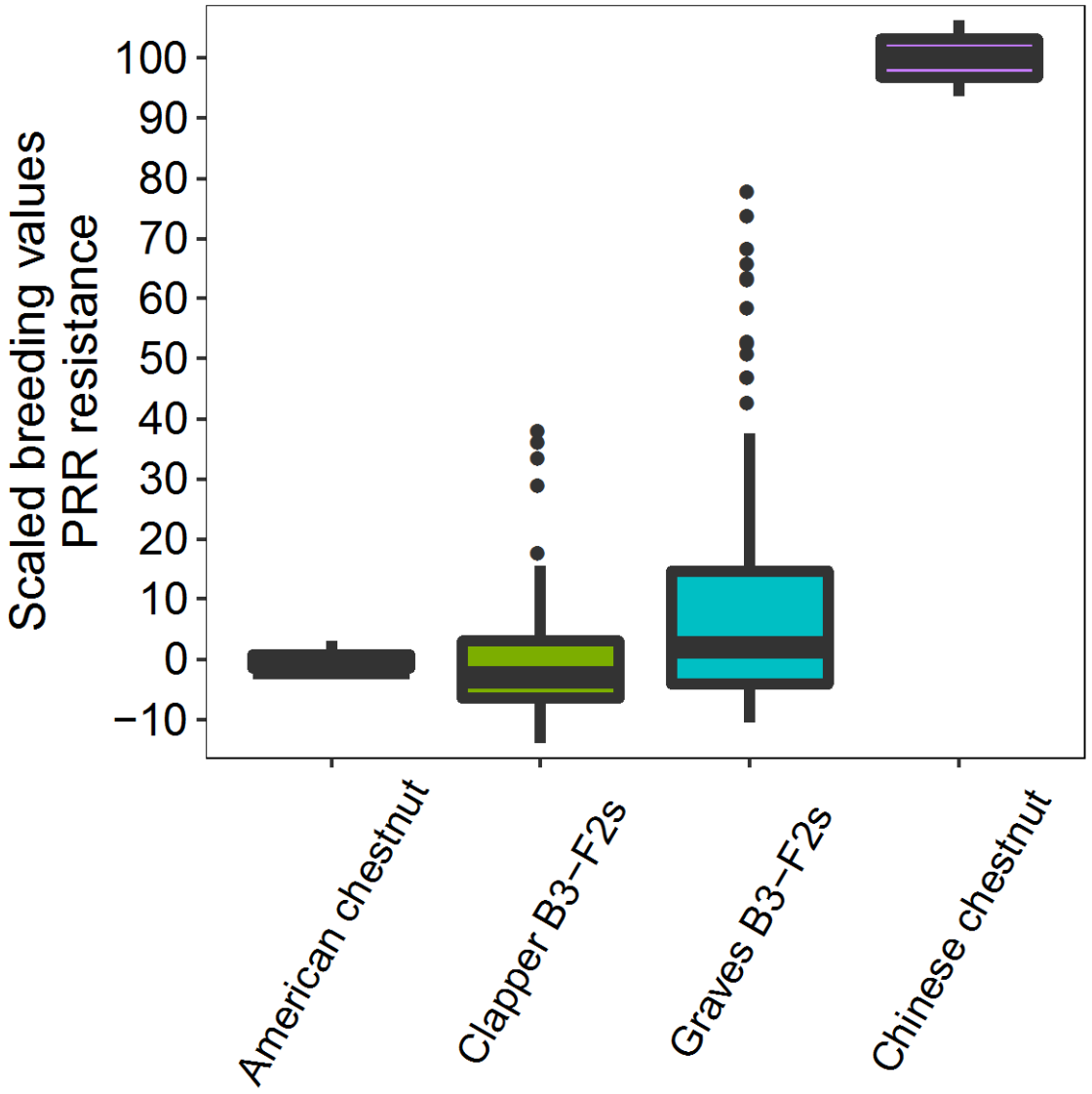
Range over which *P. cinnamomi* affected American chestnut (Crandall, 1945)

Joe James

Steve Jeffers

What we should have known

Genetic variation in PRR resistance



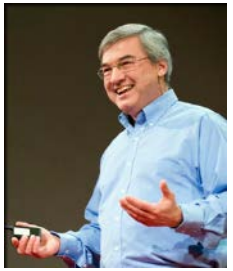
KNOW YOUR PARENTS

There is genetic variance in Chinese chestnuts for the level of “resistance”.

The genetics of blight resistance is more complicated than previously thought.

Some of the Graves B_3F_2 lines may have some resistance to *P. cinnamomi.*

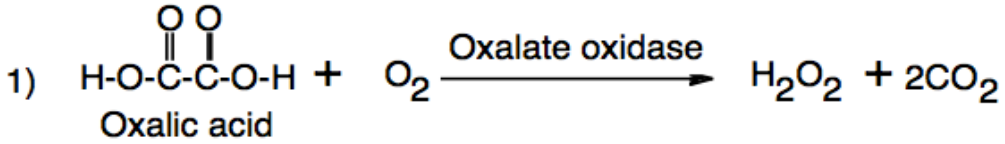
Transgenic insertion of the oxalate oxidase gene from wheat (OxO) enhances blight resistance

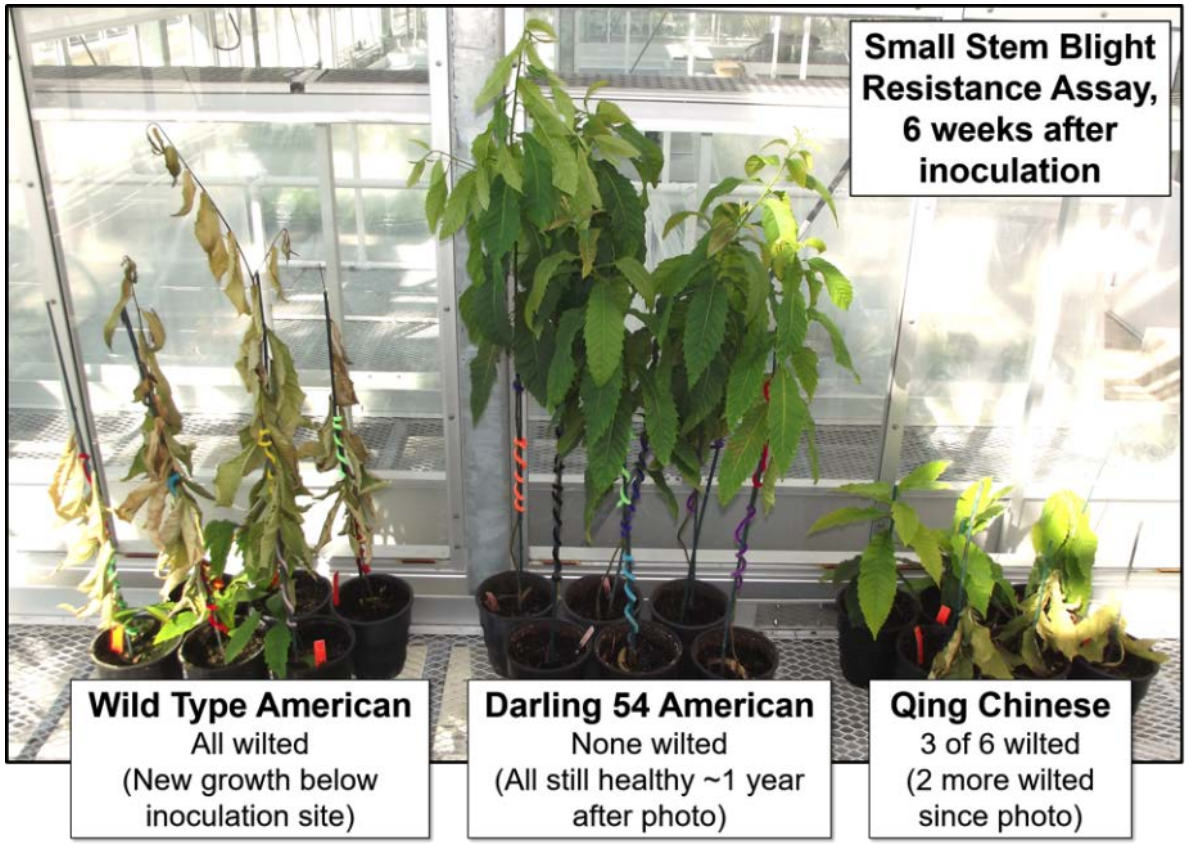


Bill Powell
SUNY-ESF

A ubiquitous enzyme from wheat
also found in: banana, rice, barley, sorghum, strawberry, date palm, beet, cacao, peanut, peach, apricot, and many more…

Detoxifies oxalate (oxalic acid)





Inheritance of OxO blight resistance

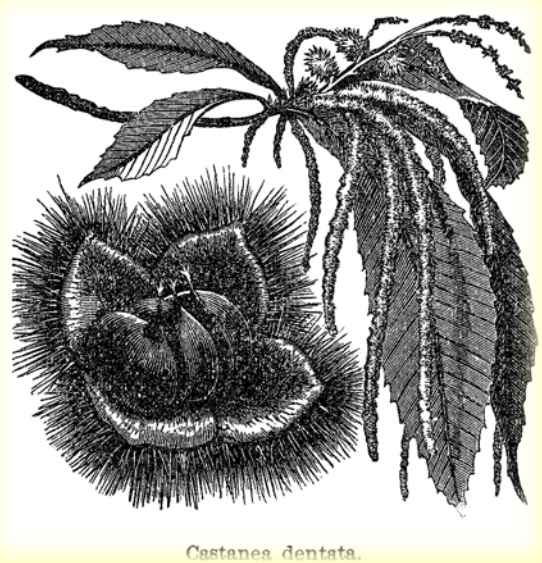
Pollen from ‘Darling’ American chestnut

Pollinate wild-type mother trees

Harvest nuts

~50% of offspring inherit the oxalate detoxifying gene

Photographed 13 weeks after inoculation with *C. parasitica* strain EP155



KNOW YOUR PARENTS

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The genetics of blight resistance is more complicated than previously thought.

Some of the Graves B_3F_2 lines may have some resistance to *P. cinnamomi.*

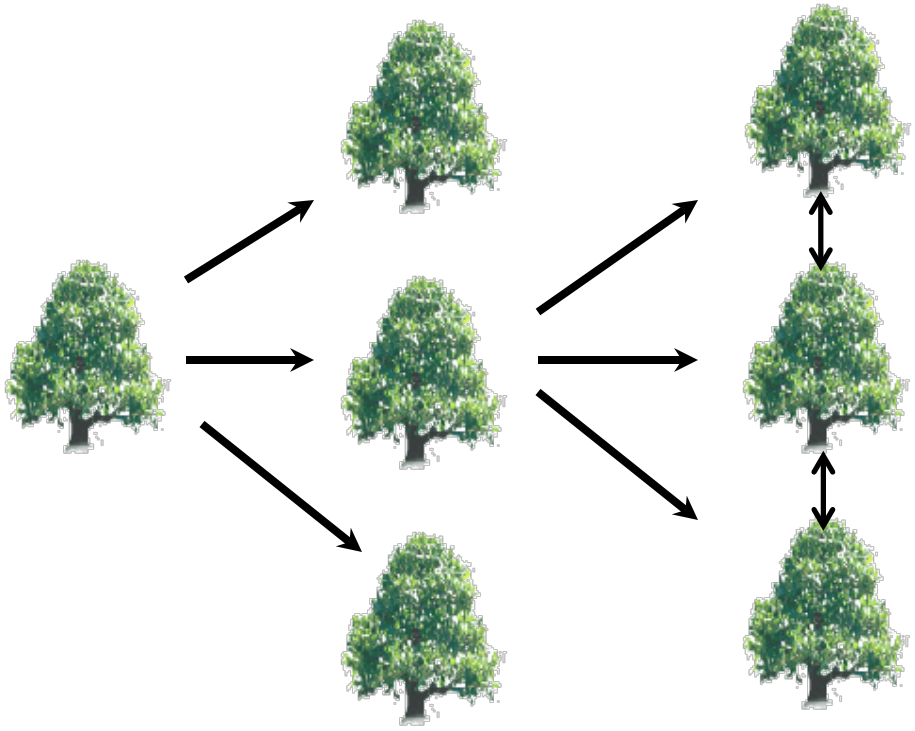
The OXO parents have monogenic genetic resistance to blight.

Combining resistance to chestnut blight and *Phytophthora* root rot

Transgenic American chestnut

Phytophthora resistant hybrids

Intercross progeny with OxO



Screen for resistance to *Phytophthora*

Offspring
50% OxO resistance

Just when you thought we were done…

What species is the OXO parent?

Is it “pure” American chestnut?

What is the ancestry of Clapper?

Is it only Chinese and American?

What is the ancestry of Graves?

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What we never know

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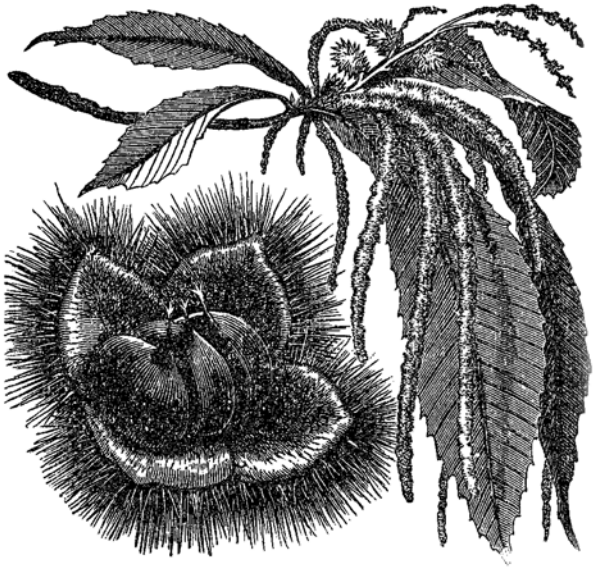
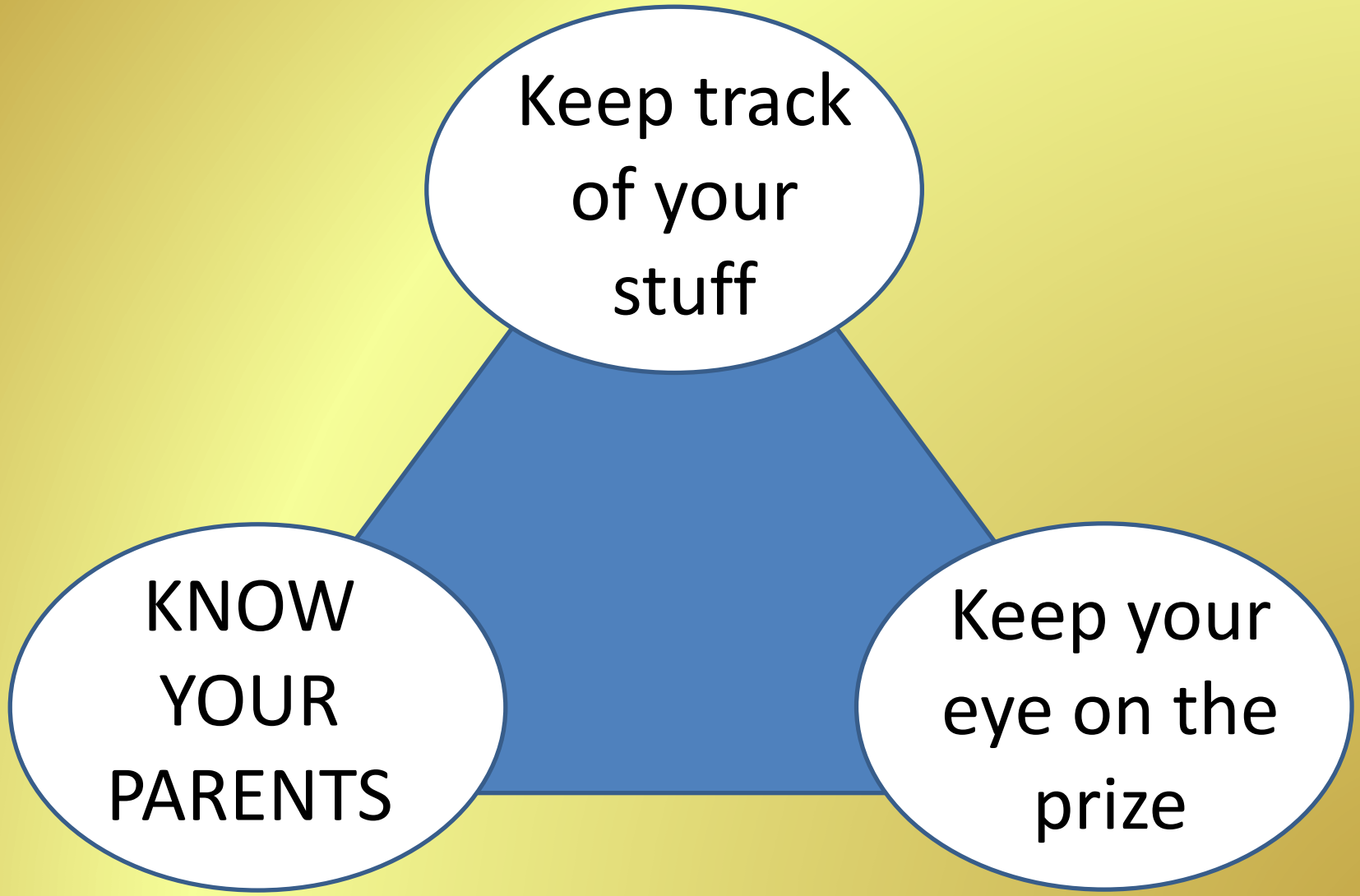


- Verify identity of breeding stock
  - Valid performance evaluation
  - Predicable characteristics
  - Firm legal protections
  - Avoidance of inbreeding
  - Accurate estimate of effective population size
- Detect recent ancestry
  - Identify interspecific hybrids
  - Predict genetic value in descendants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

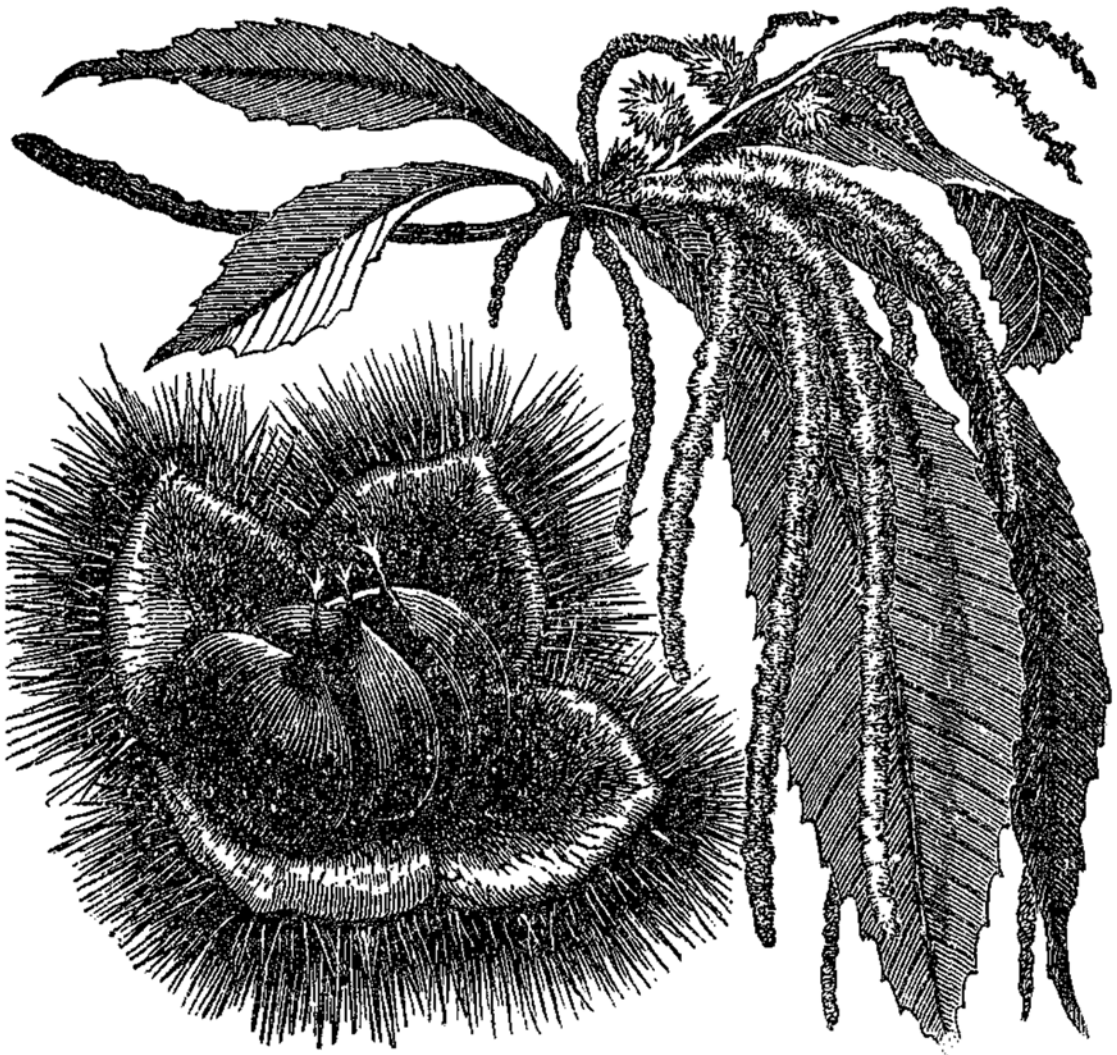
THE CARDINAL RULES OF PLANT BREEDING



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

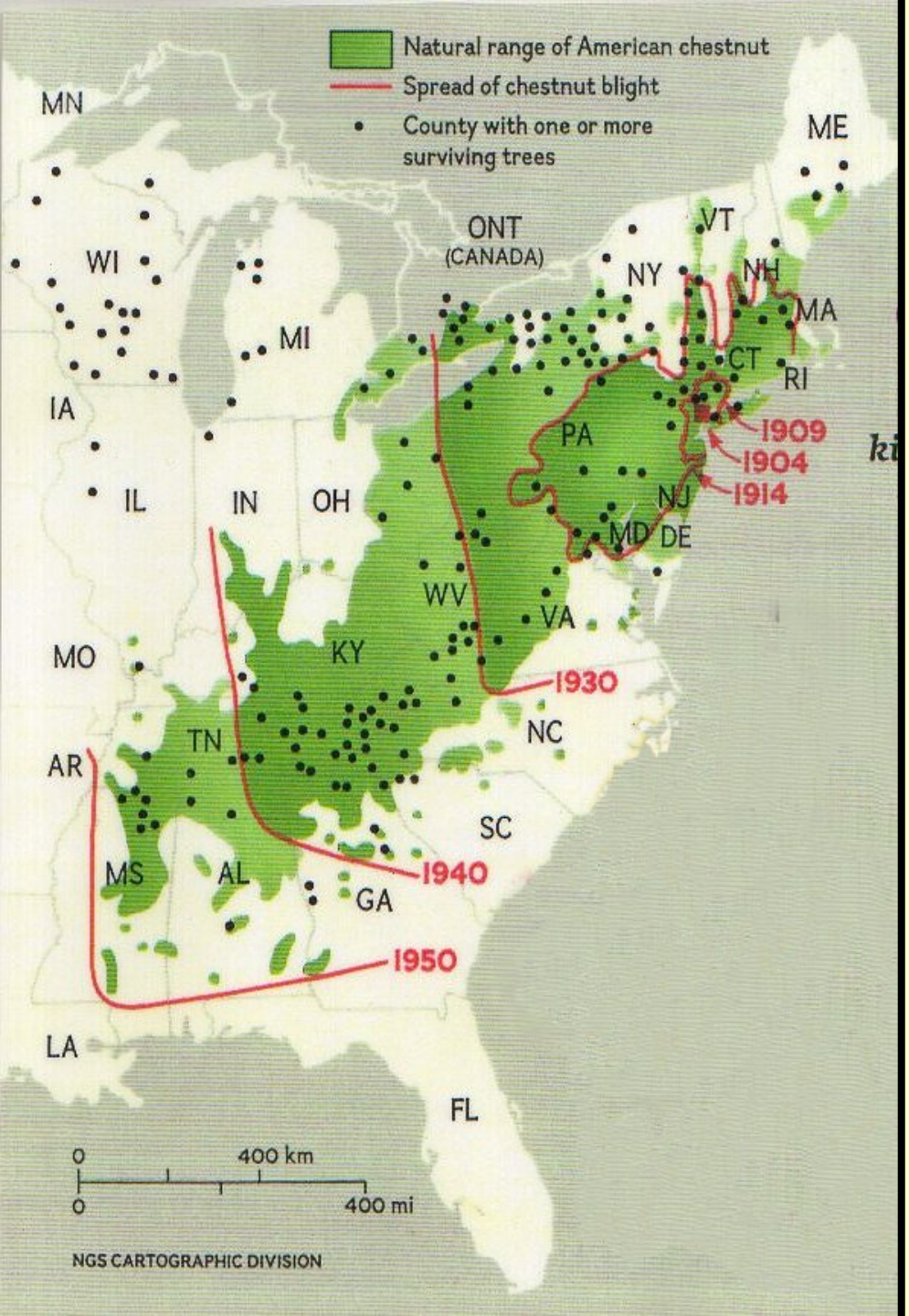
What we thought we knew

What we should have known

What we never know

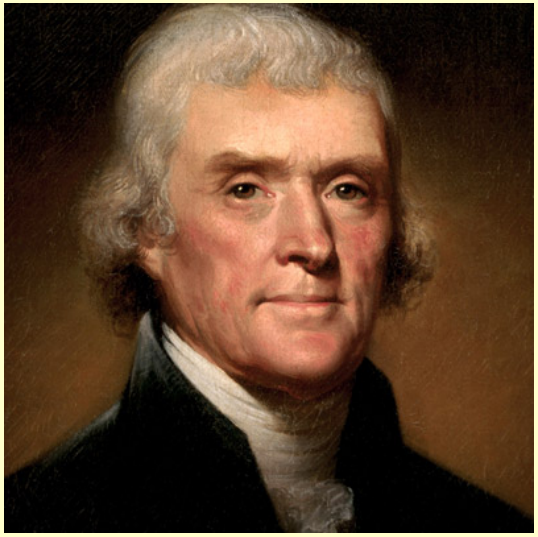
A new direction

A reminder



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 19th Century…

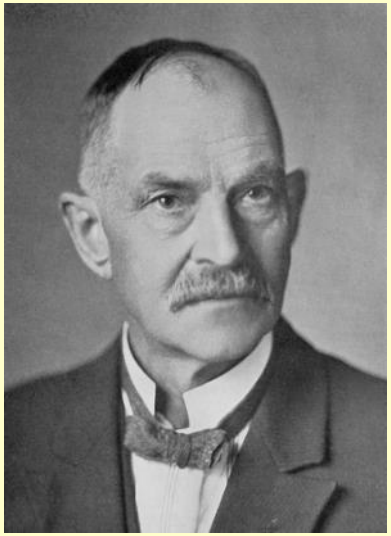
S. B. Parsons

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 19th century and experimented with *C. crenata* ×*C. dentata* hybrids.

In the 20th 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.