# Integrating genomics into breeding for pathogen resistance in American chestnut

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Forest Health Initiative
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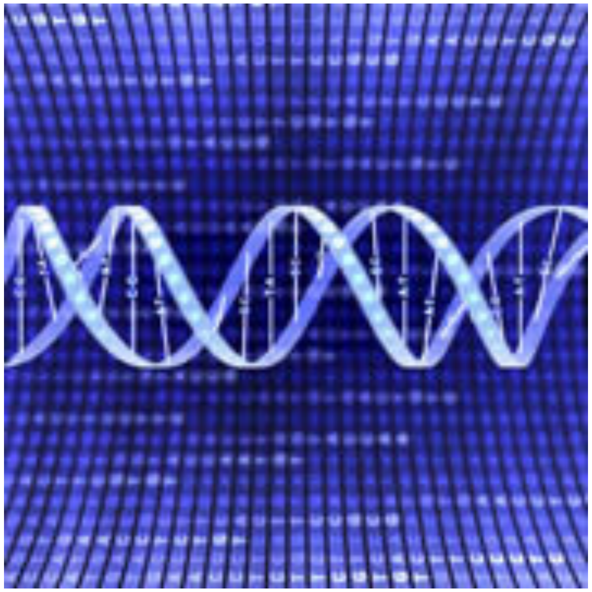
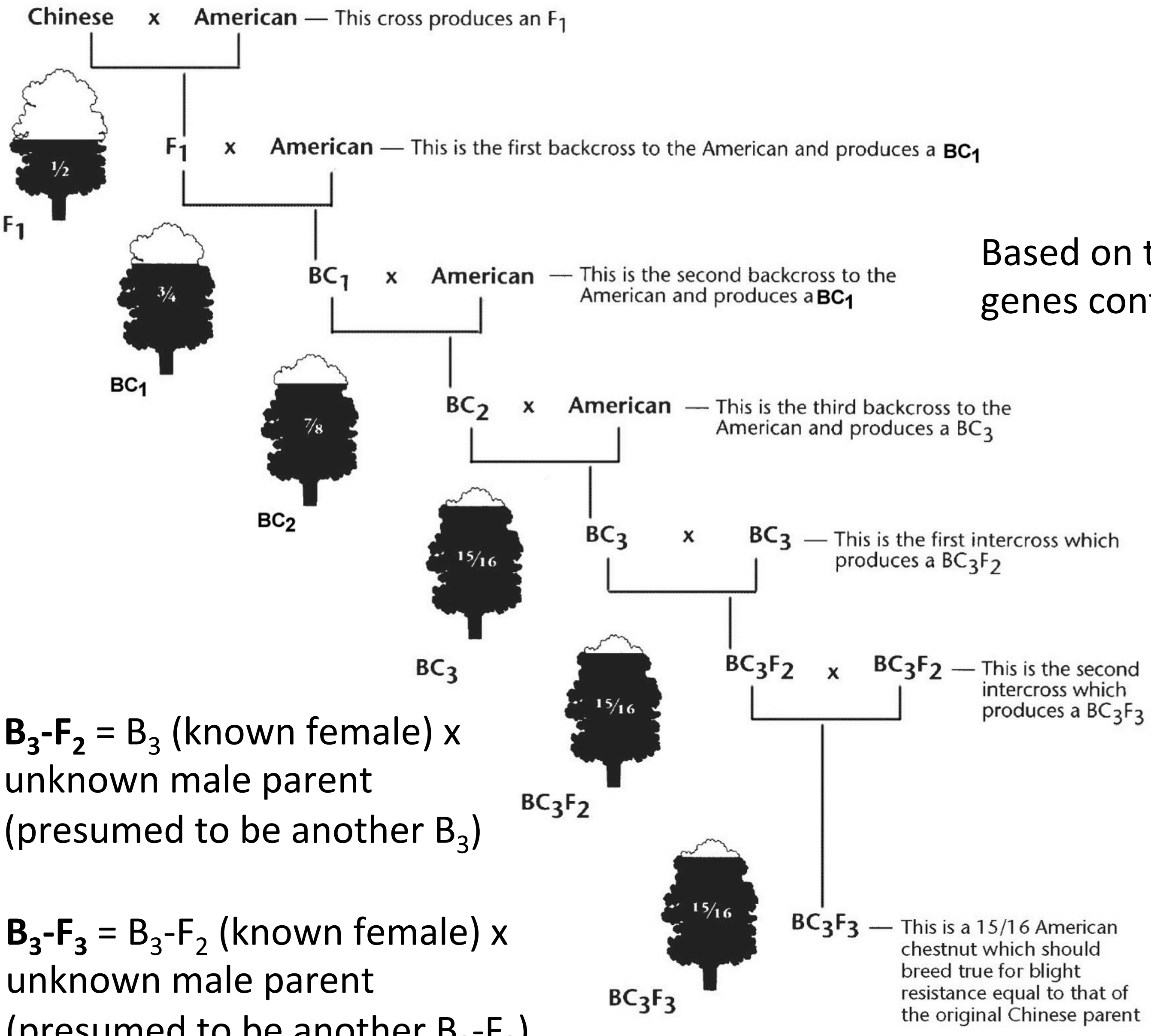


# Backcross breeding for blight resistance



Chinese x American — This cross produces an $F_1$

$F_1$ x American — This is the first backcross to the American and produces a **$BC_1$**

$BC_1$ x American — This is the second backcross to the American and produces a **$BC_1$**

$BC_2$ x American — This is the third backcross to the American and produces a $BC_3$

$BC_3$ x $BC_3$ — This is the first intercross which produces a $BC_3F_2$

$BC_3F_2$ x $BC_3F_2$ — This is the second intercross which produces a $BC_3F_3$

$BC_3F_3$ — This is a 15/16 American chestnut which should breed true for blight resistance equal to that of the original Chinese parent

Based on the hypothesis that few genes control blight resistance

**$B_3$-$F_2$** = $B_3$ (known female) x unknown male parent (presumed to be another $B_3$)

**$B_3$-$F_3$** = $B_3$-$F_2$ (known female) x unknown male parent (presumed to be another $B_3$-$F_2$)

# What is a source of resistance?

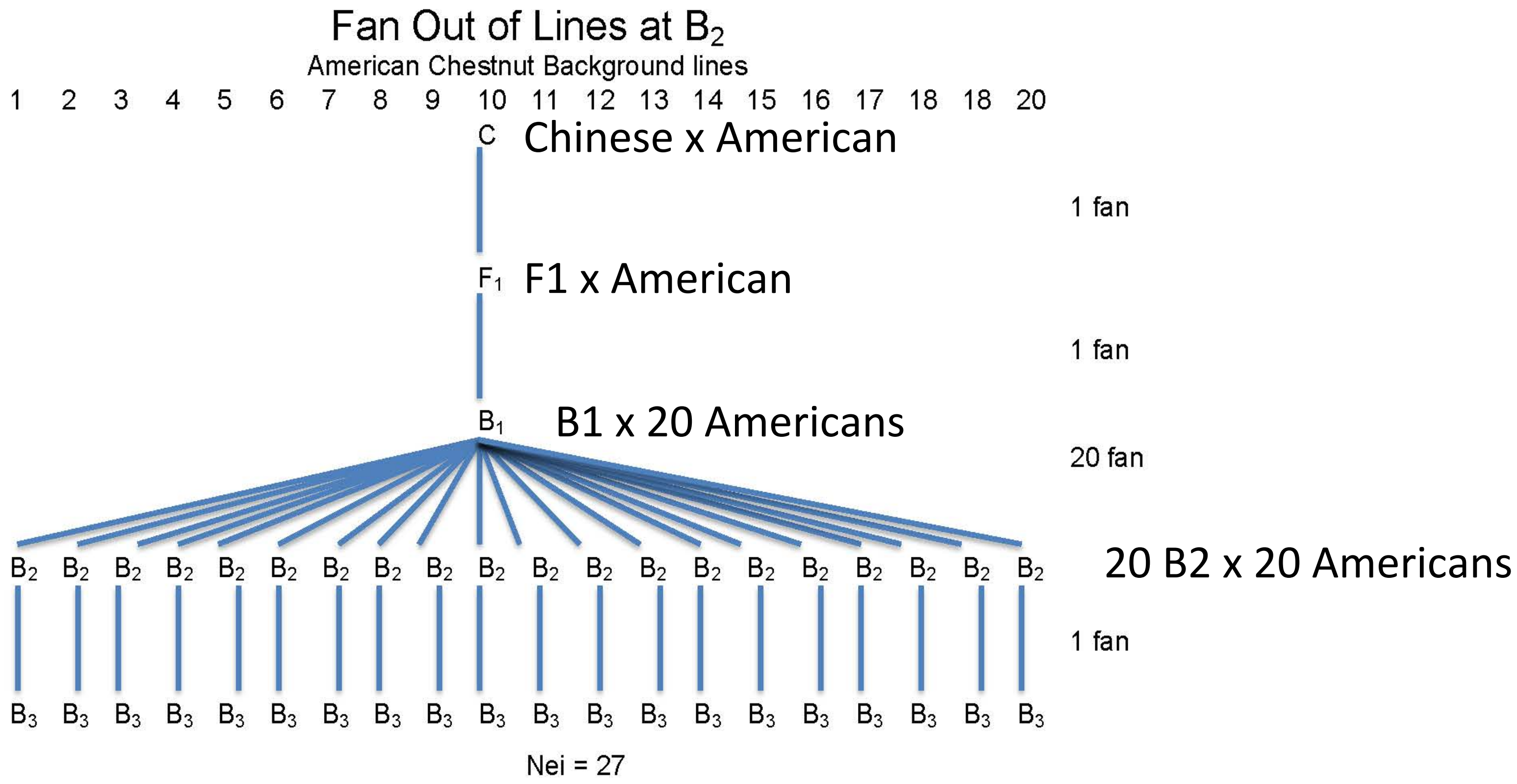
An individual *C. mollissima* or backcross hybrid that contributed resistance alleles to backcross populations of American chestnut

Arthur H. Graves

Russell B. Clapper

- ‘Graves’ and ‘Clapper’ are first backcross (B1) hybrids with different Chinese chestnut grandparents

# Incorporating genetic diversity and regional adaptation into American chestnut backcross hybrids



Fan Out of Lines at $B_2$

American Chestnut Background lines

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 18 20

C Chinese x American

1 fan

$F_1$ F1 x American

1 fan

$B_1$ B1 x 20 Americans

20 fan

$B_2$ $B_2$ $B_2$ $B_2$ $B_2$ $B_2$ $B_2$ $B_2$ $B_2$ $B_2$ $B_2$ $B_2$ $B_2$ $B_2$ $B_2$ $B_2$ $B_2$ $B_2$ $B_2$ $B_2$ 20 B2 x 20 Americans

1 fan

$B_3$ $B_3$ $B_3$ $B_3$ $B_3$ $B_3$ $B_3$ $B_3$ $B_3$ $B_3$ $B_3$ $B_3$ $B_3$ $B_3$ $B_3$ $B_3$ $B_3$ $B_3$ $B_3$ $B_3$

Nei = 27

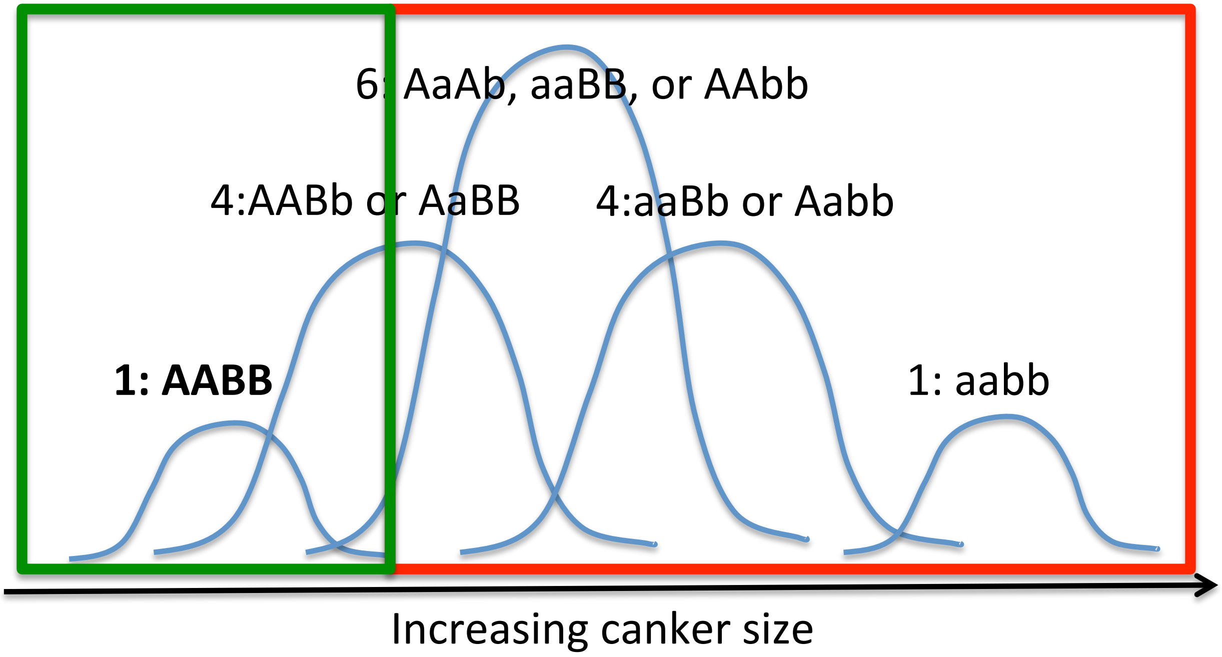
- Selected B2s and B3s are crossed with regionally adapted American chestnuts
- Over 200 Graves and 300 Clapper backcross lines have been advanced by TACF's chapters

# Selection in $B_3$-$F_2$ seed orchards

- 2 sources x 20 to 30 lines x 9 reps per line x 150 trees per rep = **60,000 trees**
- Aim to select 1 – 2 of the most blight resistant individuals per rep = **500 - 1000 trees**
- Selected trees will be a seed source for restoration or recurrent selection

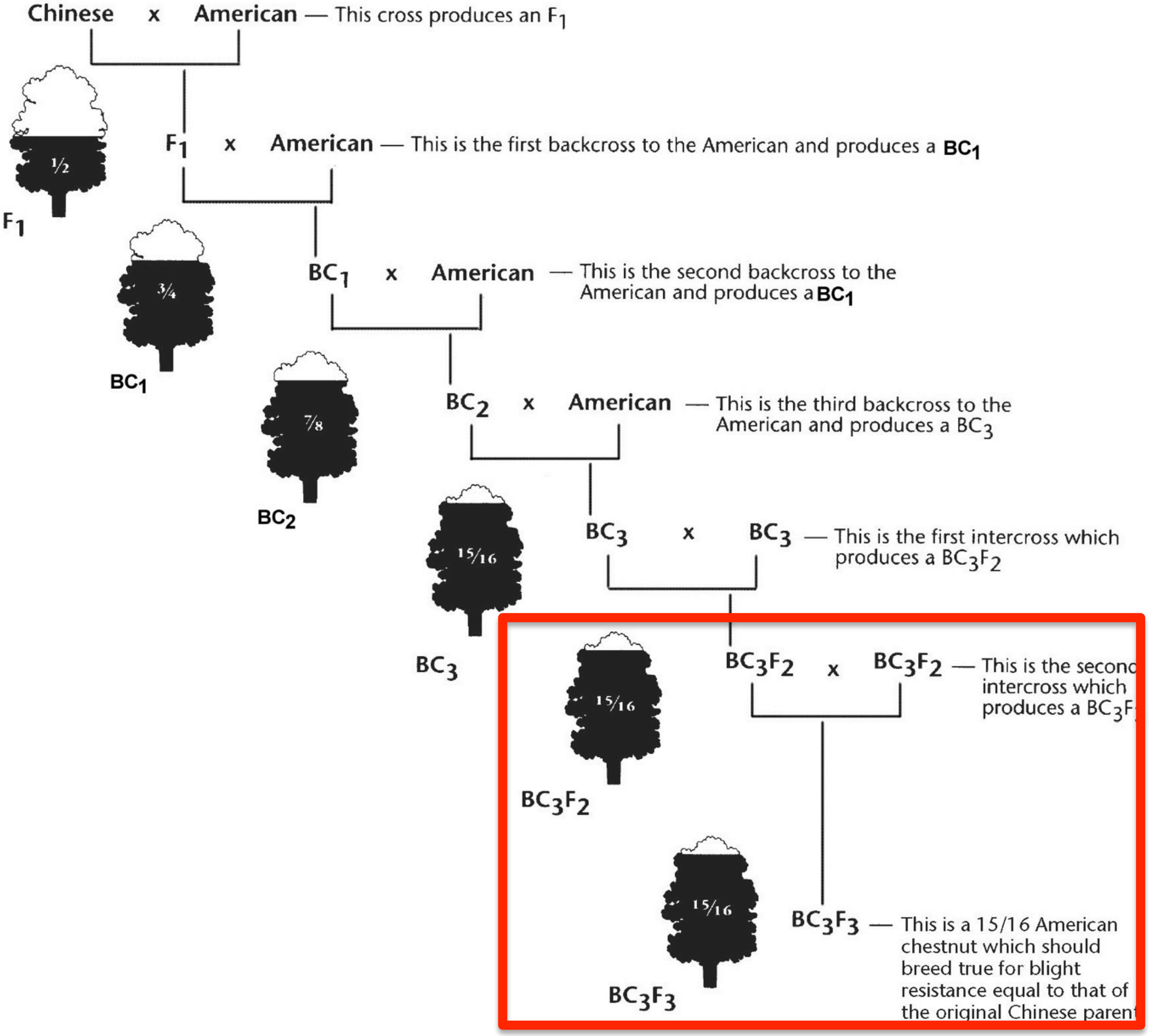
Expected segregation of blight resistance genotypes under a 2 locus model

How to select the most blight resistant individual from among the remaining trees in from each rep?



75% - 90% of trees have significant canker expansion after artificial inoculation at age 3 and are culled from the seed orchard

# Progeny testing for blight resistance



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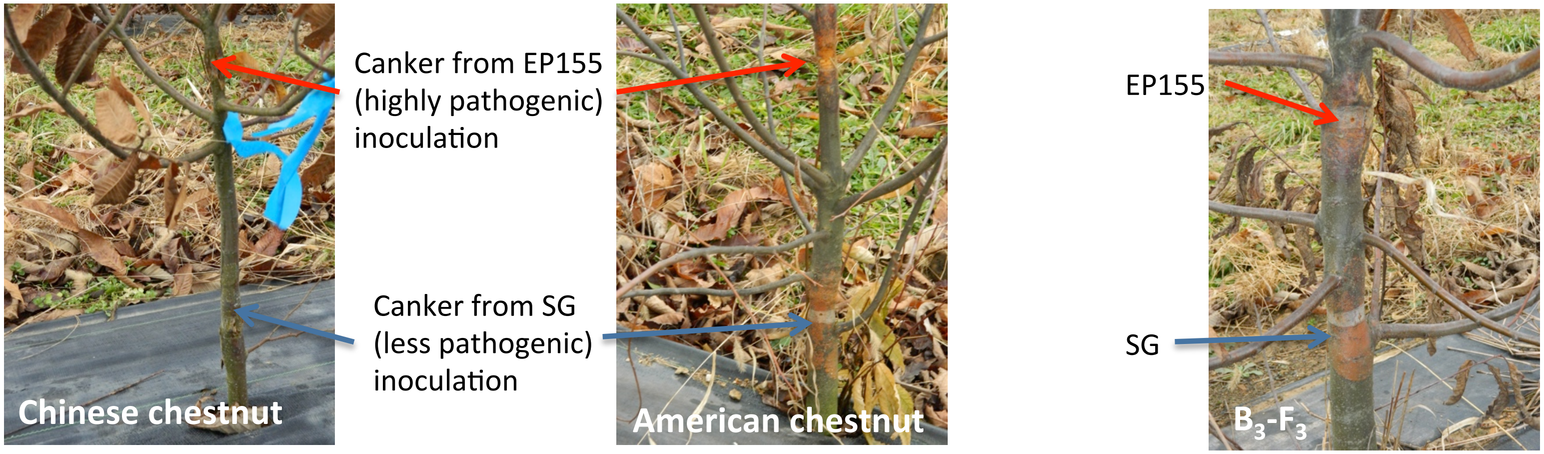
A **$B_3$-$F_2$** parent's genetic resistance to blight is evaluated from the average canker size of its open pollinated $B_3$-$F_3$ progeny relative to other $B_3$-$F_2$ parents

# Chestnut blight canker sizes are evaluated five months after artificial inoculation of $B_3$-$F_3$ trees with two strains of *Cryphonectria parasitica* with high and low pathogenicity



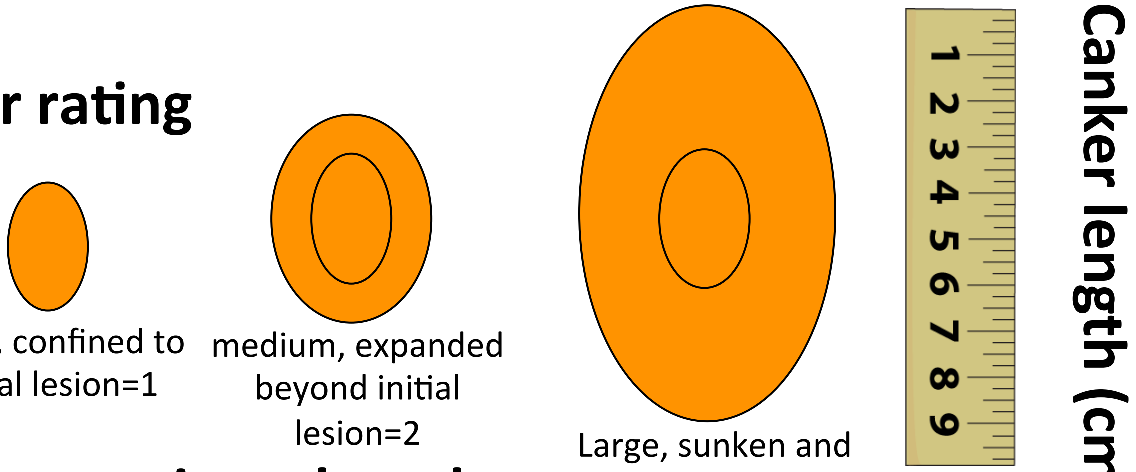
Canker from EP155 (highly pathogenic) inoculation

Canker from SG (less pathogenic) inoculation

Photos by
Dr. Laura Georgi

## Canker rating

Canker phenotyping methods and data by Dr. Fred Hebard



Small, confined to initial lesion=1

medium, expanded beyond initial lesion=2

Large, sunken and sporulating=3

## Canker severity = rating + length

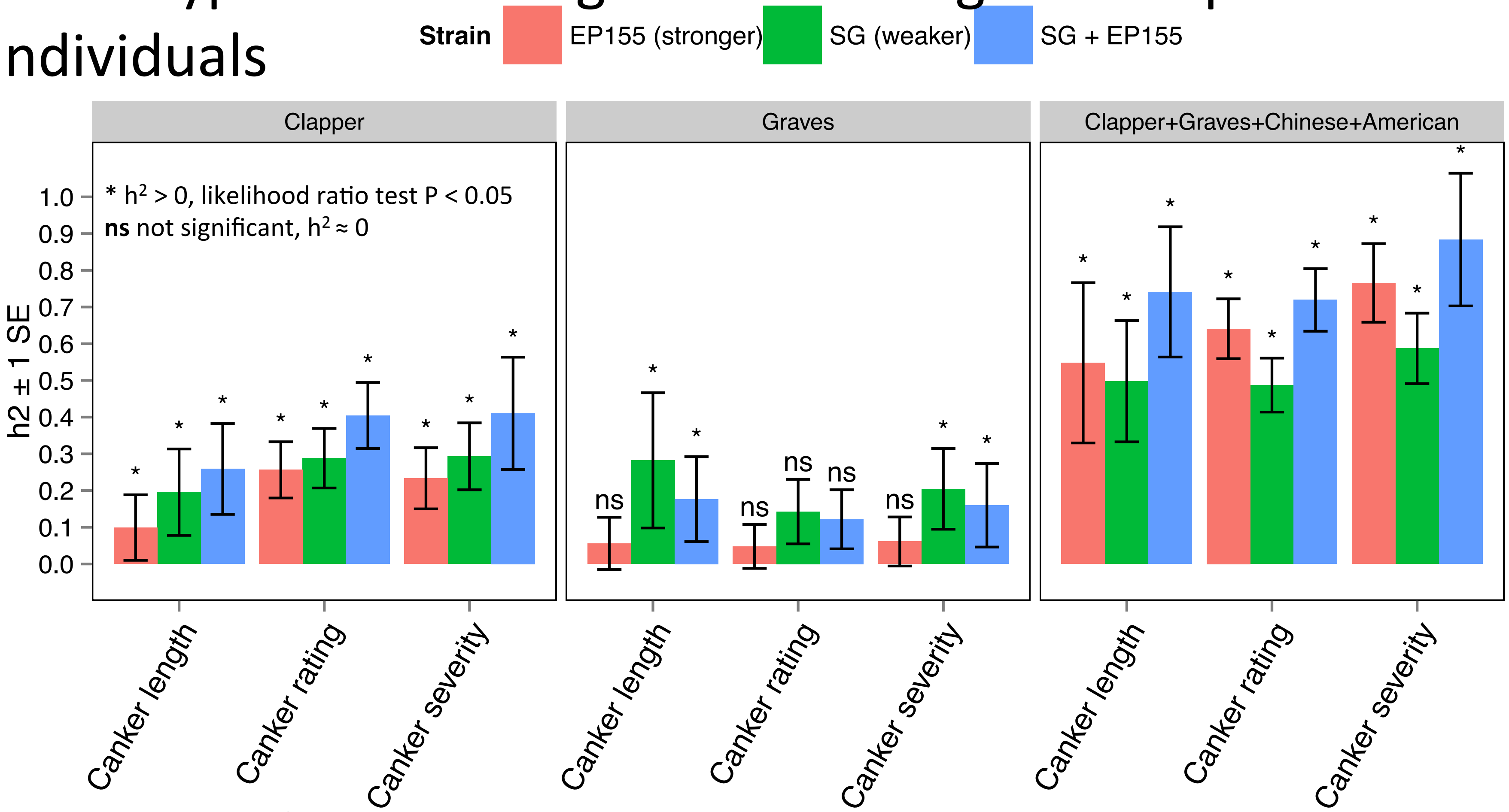
(rating and length normalized to mean=0 and standard deviation = 1 prior to summing)

# Scope of progeny testing for blight resistance

| | Clapper | Graves |
|---|---|---|
| Number of $B_3$-$F_2$ parents progeny tested 2011-2014 | 180 | 85 |
| Number of additional $B_3$-$F_2$ parental lines to be inoculated in 2015-2016 | 204 | 119 |
| Number of $B_3$-$F_2$ that have produced seed from 2002 - 2014 | 557 | 268 |
| Remaining trees to select from in $B_3$-$F_2$ orchards at Meadowview | 5500 | 7500 |
| N to select | 300 | 300 |
| Approximate N of trees to select from in in TACF state chapters | 30000 | 25000 |

Progeny testing too slow and laborious make final selections

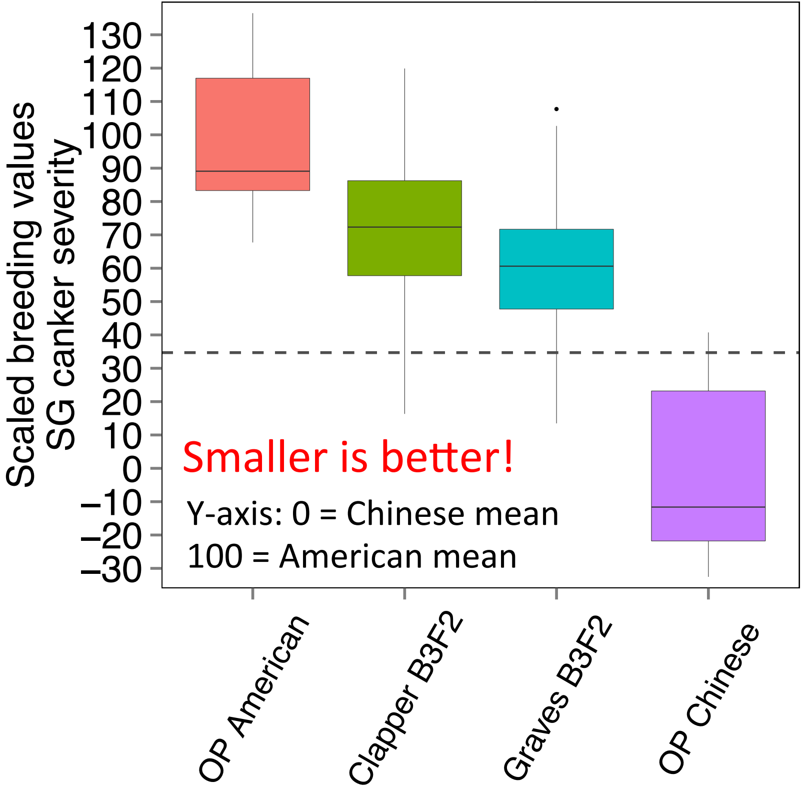
# Significant heritable variation canker size remains after phenotypic selection against most blight-susceptible individuals



**Heritability ($h^2$)** – proportion of variation canker size among $B_3$-$F_3$ trees that is inherited from their $B_3$-$F_2$ female parents

Phenotypic selection of remaining $B_3$-$F_2$ based on canker ratings may not be sufficiently accurate to select the most blight resistant individuals

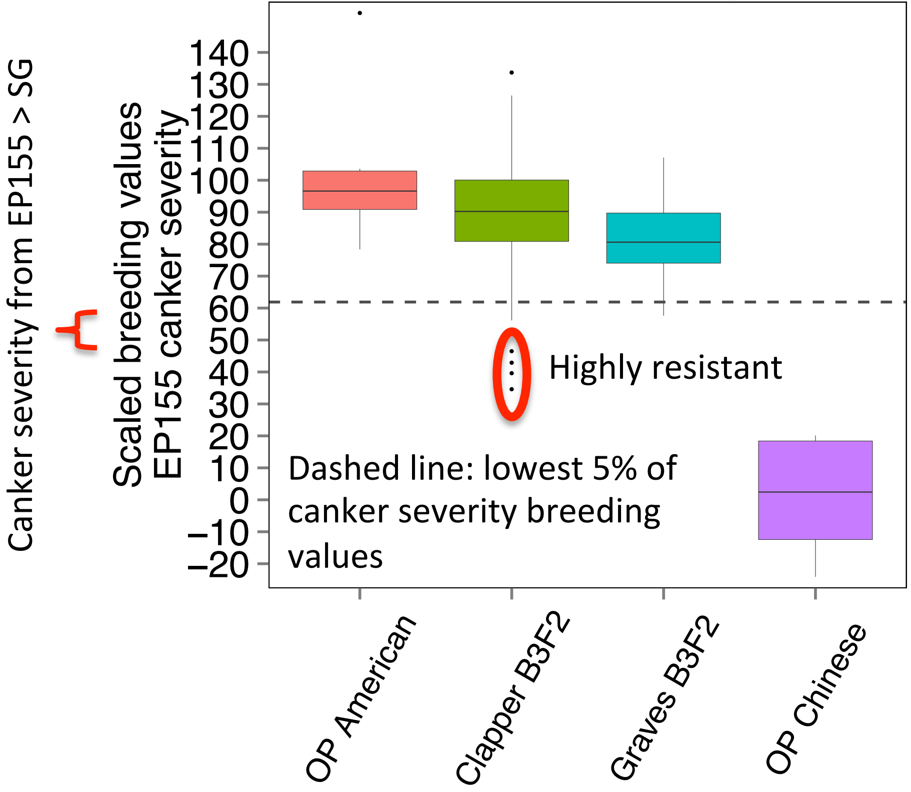
# Canker severity ***breeding value*** estimates for $B_3$-$F_2$ trees are intermediate between Chinese chestnut and American chestnut



**SG (less pathogenic)**

Smaller is better!

Y-axis: 0 = Chinese mean
100 = American mean



**EP155 (more pathogenic)**

Canker severity from EP155 > SG

Highly resistant

Dashed line: lowest 5% of canker severity breeding values

The lowest 5% of **SG** canker severity breeding values vary from 13 to 35 (closer to Chinese than American)

The lowest 5% of **EP155** canker severity breeding values vary from 35 to 62 (Intermediate between Chinese and American)

# Where the breeding program stands

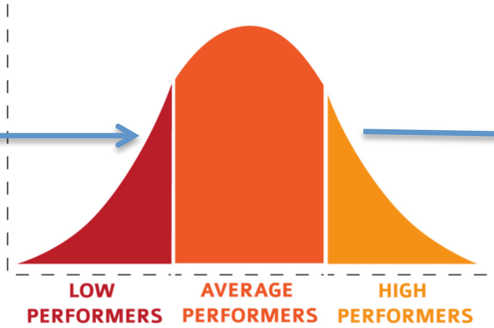
- Intermediate blight resistance at $B_3$-$F_2$ suggests that *some* blight resistance alleles from Chinese chestnut sources have been lost or are not combining in a homozygous state
- The observation that some $B_3$-$F_2$ trees are highly resistant to blight and also have a ~ 94% American chestnut genetic background suggests that major genes for blight resistance have been retained through backcrossing
- We expect average blight resistance at $B_3$-$F_3$ to increase after selections at $B_3$-$F_2$ are complete

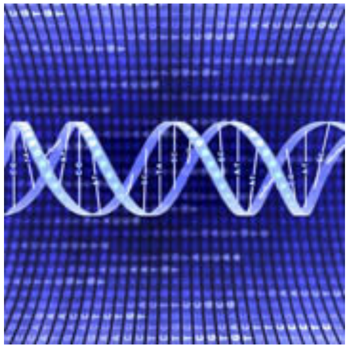
# Genomic selection will enable TACF to finish selecting the most blight resistant $B_3$-$F_2$ individuals in the next 5 years

Progeny test
(2011 – 2018)

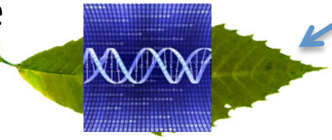
Estimate blight resistance breeding values values in a training population

*Canker size = **genetics** + environment*





Build a genomic prediction model by estimating how DNA sequence variation in this population relates to breeding values for blight resistance



For surviving $B_3$-$F_2$ individuals that have *not* been progeny tested, predict blight resistance from the marker genotypes
(2016 – 2020)

# Genomic selection is expected to exceed the accuracy of progeny testing

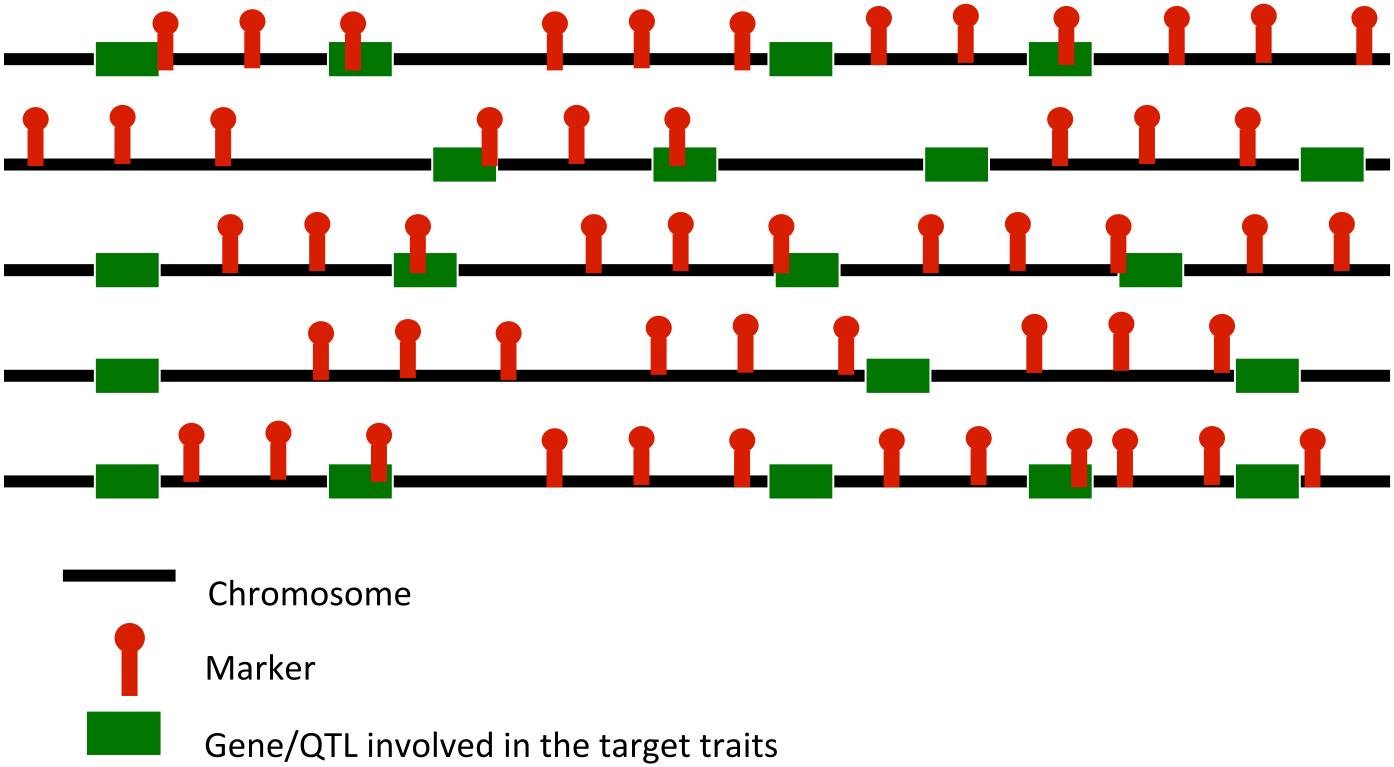
Accuracy = correlation between genomic prediction of breeding value and true breeding value

Accuracy of progeny testing ~ 0.68 (Grattipaglia & Resende 2011)

| N individuals in training population | N QTL | $h^2$ | N markers | % genetic variance explained | Accuracy of genomic selection |
|---|---|---|---|---|---|
| 500 | 2 | 0.1 | 22000 | 95% | 0.98 |
| 500 | 20 | 0.1 | 22000 | 95% | 0.84 |
| 500 | 2 | 0.4 | 22000 | 95% | 0.99 |
| 500 | 20 | 0.4 | 22000 | 95% | 0.95 |

# How genomic selection works

Breeding values predicted from summed effects of markers in linkage disequilibrium with causal variants

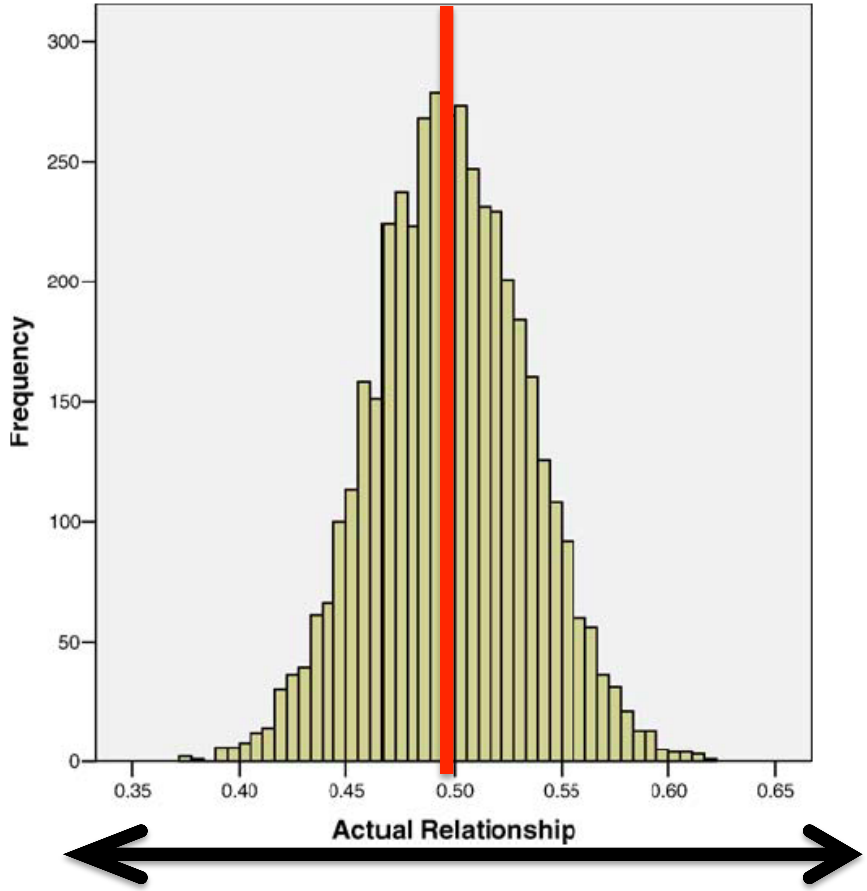


Courtesy: D. Grattapaglia - EMBRAPA

# How genomic selection works

Markers trace relationships & estimate polygenic effects

Full sibs expected to share 50% of genome



N of pairs of individuals

Mendelian segregation around expected relationships

# Genomic selection training populations

**Graves**

- 636 $B_3$-$F_2$ progeny
- 42 $B_3$ mothers
- Between 7 and 15 $B_3$-$F_2$ individuals per family
- Balanced representation of individuals with small, medium, and large cankers
- 205 progeny tested for blight resistance
- 115 also progeny tested for *Phytophthora* root rot resistance

**Clapper**

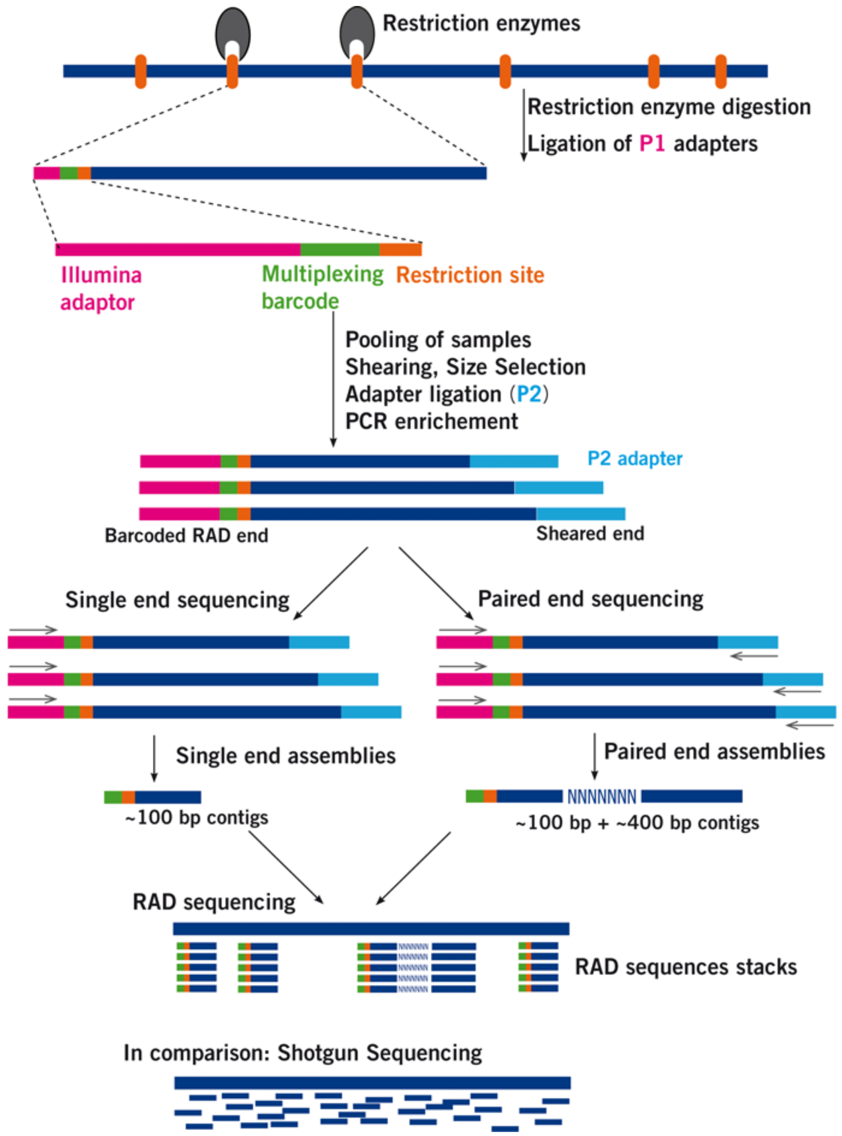
- > 500 $B_3$-$F_2$ progeny
- 83 $B_3$ mothers
- Includes 267 $B_3$-$F_2$ individuals progeny tested for blight resistance
- 131 also progeny tested for *Phytophthora* root rot resistance
- 250 additional individuals will be selected sequencing to represent all backcross lines and full spectrum of blight resistance

Two pathogens for the price of one!

Sequencing funded by FHI 2014 - 2015

Pending funding by FHI

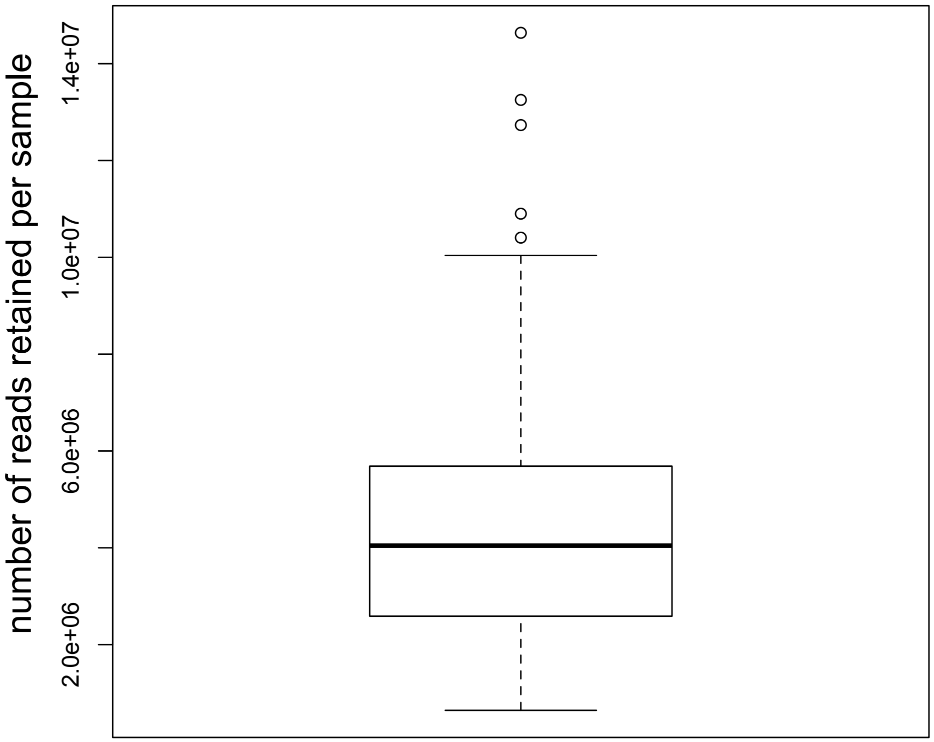
# Restriction associated digestion sequencing (RAD-seq): Complexity reduction for genotyping



1. Digest genome with a pair of restriction enzymes to isolate DNA fragments in vicinity of cut sites for sequencing
2. Ligate barcode sequences to fragments, PCR amplify, and multiplex
3. Sequence (96 individuals per lane)
4. Alignment to reference genome for SNP calling

# Initial sequencing (Holliday VA Tech)

- One lane of Illumina sequencing of 96 Graves individuals complete
- ~ 5 million paired-end reads retained per sample
- Currently aligning to the reference genome
- 5-6 more sequencing lanes pending



# Deliverables

FHI funding will be used to develop genomic selection models and conduct comparative association genetic analysis of pathogen resistance in Clapper and Graves

# 1. Estimate the accuracy of genomic selection for blight and PRR resistance

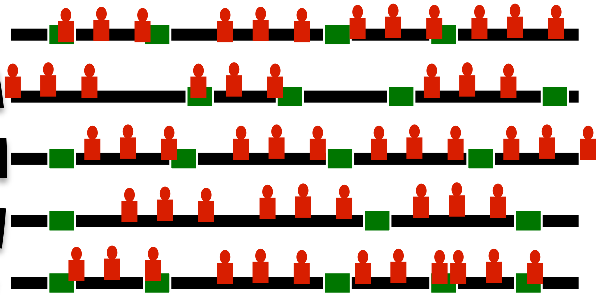
9/10th of training population

Measured pathogen resistance

$$= \mu + X\beta + \begin{bmatrix} -0.1 \\ +0.5 \\ +0.9 \end{bmatrix}$$

Experimental design

Estimated marker effects



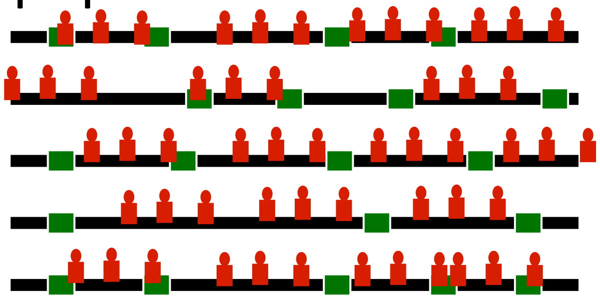
Genome-wide markers

Repeat 10 x

Accuracy = correlation

1/10th of training population

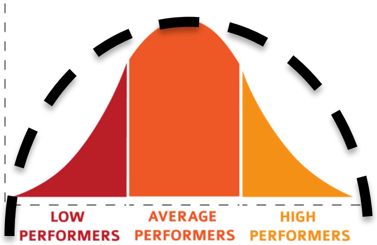
$$\sum_{i=1}^{N\ \text{markers}} \begin{bmatrix} -0.1 \\ +0.5 \\ +0.9 \end{bmatrix} \times$$



Marker effects

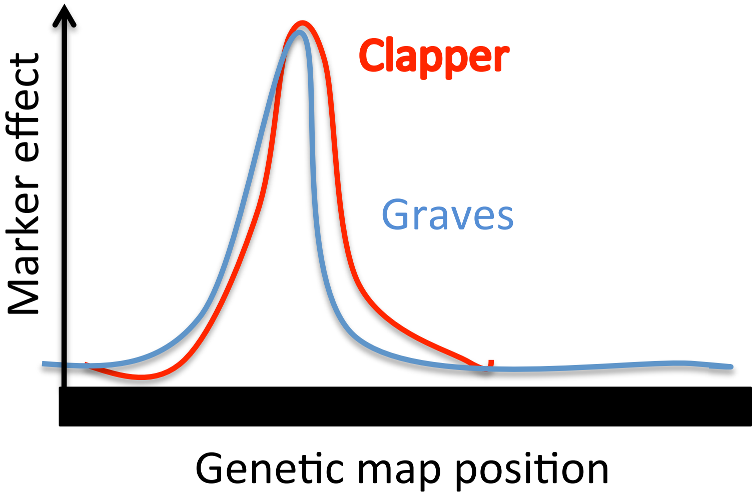
Marker genotypes

=

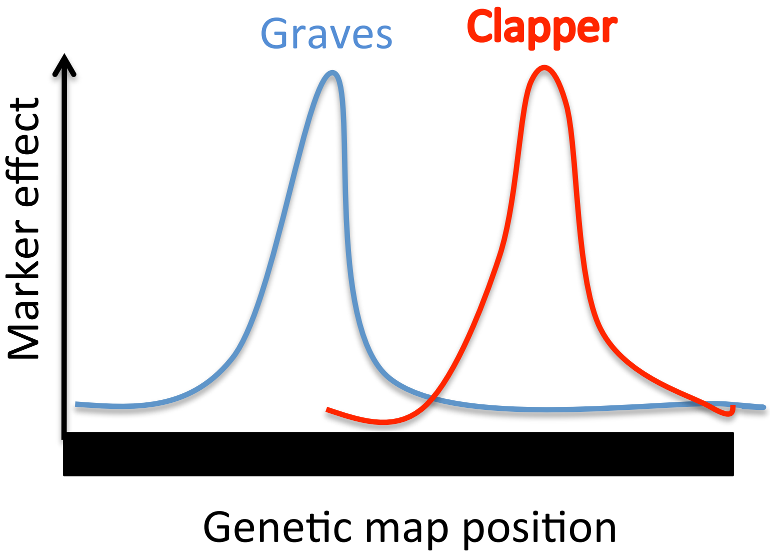


Predicted pathogen resistance

# 2. Determine if there are unique and shared blight resistance loci in Clapper and Graves?



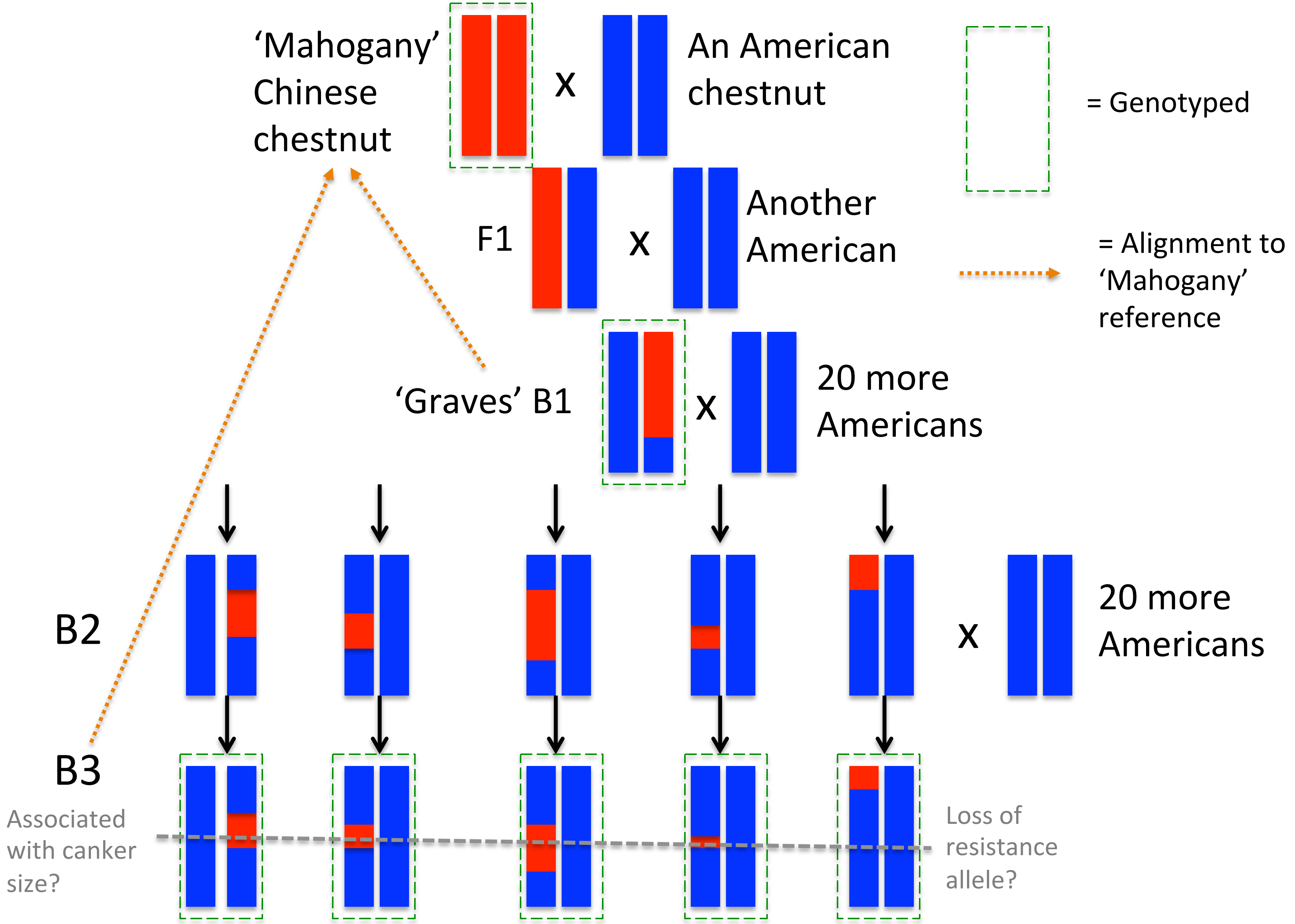
Same genes



Different genes

Would crossing Clapper and Graves increase the number of resistance alleles in the progeny?

# 3 . Trace inheritance of Chinese chestnut chromosomal segments in American chestnut backcross hybrids



'Mahogany' Chinese chestnut

x

An American chestnut

F1

x

Another American

'Graves' B1

x

20 more Americans

B2

x

20 more Americans

B3

Associated with canker size?

Loss of resistance allele?

⬚ = Genotyped

⇢ = Alignment to 'Mahogany' reference

THE
AMERICAN
CHESTNUT
FOUNDATION®

Fred Hebard, Laura Georgi, and Jeff Donahue

Virginia Tech
1872

Jason Holliday & Mihir Mandal

# Thanks!

Forest Health Initiative
Advancing Forest Health through Biotechnology