

Searching for blight resistant American chestnuts

Linking genotype and phenotype to finish selection in an
American chestnut backcross seed orchards

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THE
AMERICAN
CHESTNUT
FOUNDATION®

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**Is this a blight
resistant tree?**



Photo by Eric Jenkins

**Can you tell
which of these
two trees is more
blight resistant?**



Photo by Eric Jenkins

Backcross challenge
Select the the 1% most
blight resistant trees





Design of BC_3F_2 seed orchards

- Plant each backcross line in 9 blocks
- 150 trees per line in each block
- 30,000 B_3F_2 s to screen for resistance per source
- 250 B_3F_2 selections per source



Initial phenotypic selection against blight susceptibility in seed orchards

Artificially inoculate stems at age two with *C. parasitica*



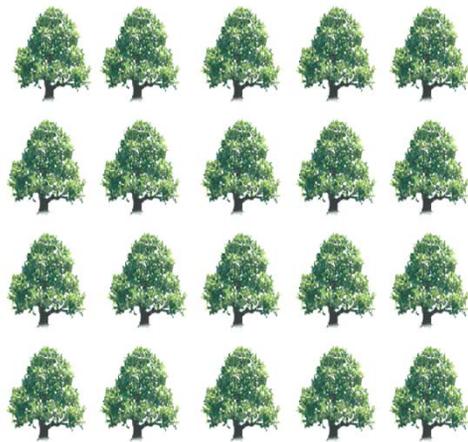
Progress on selection in seed orchards at TACF's Meadowview Research Farms

	Clapper	Graves
N trees planted	35,943	27,000
N trees remaining	4976	4250



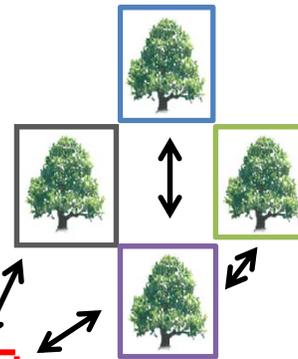
Progeny testing to make accurate selections for blight resistance

Unselected BC_3F_2 seed orchard

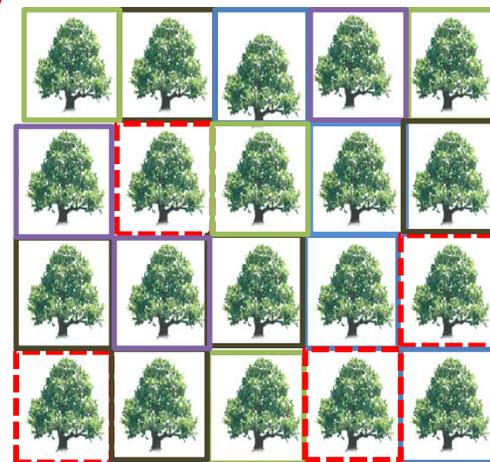
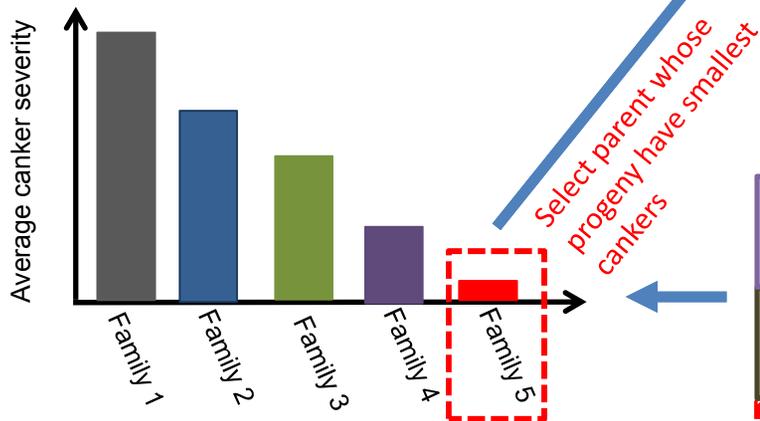


Artificially inoculate & cull susceptible

Partially selected seed orchard



Open-pollination among BC_3F_2 survivors



Artificially inoculate BC_3F_3 progeny from each selection candidate

Progeny testing alone is too slow to finish selection for blight resistance

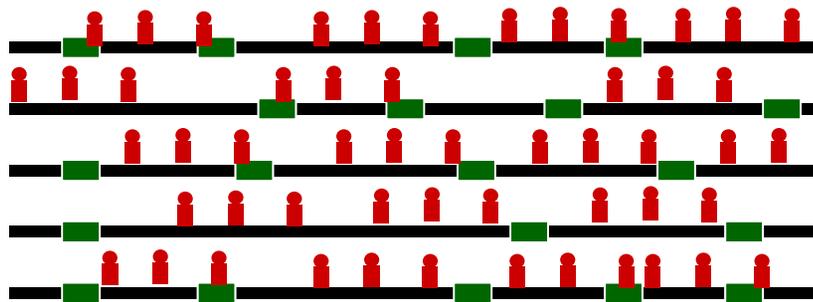
	Clapper	Graves
Number BC ₃ F ₂ trees remaining	5000	4200
N BC ₃ F ₂ parents progeny tested 2009-2017	300	300
N BC ₃ F ₂ to select	300	250

N trees remaining after selection based on canker size >> N trees progeny tested



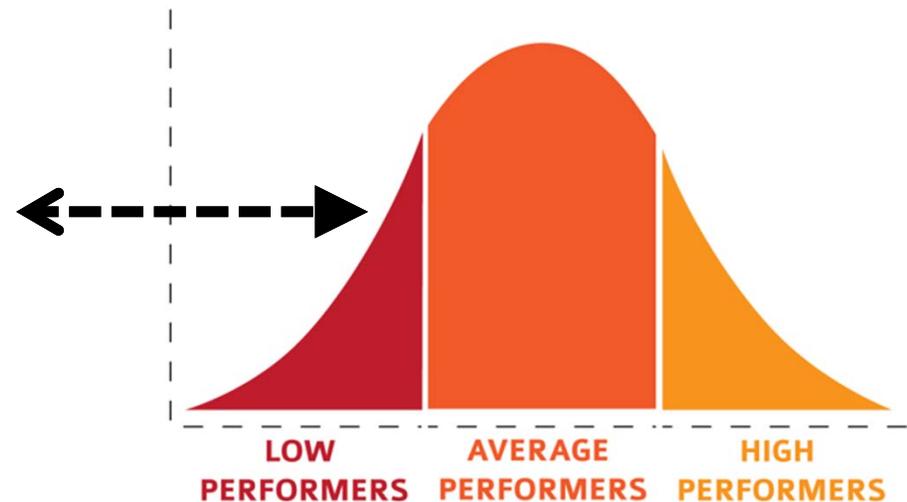
Genomic selection alleviates the need to screen progeny from ALL parent trees that remain after phenotypic selection

Variation in disease resistance in BC_3F_2 populations

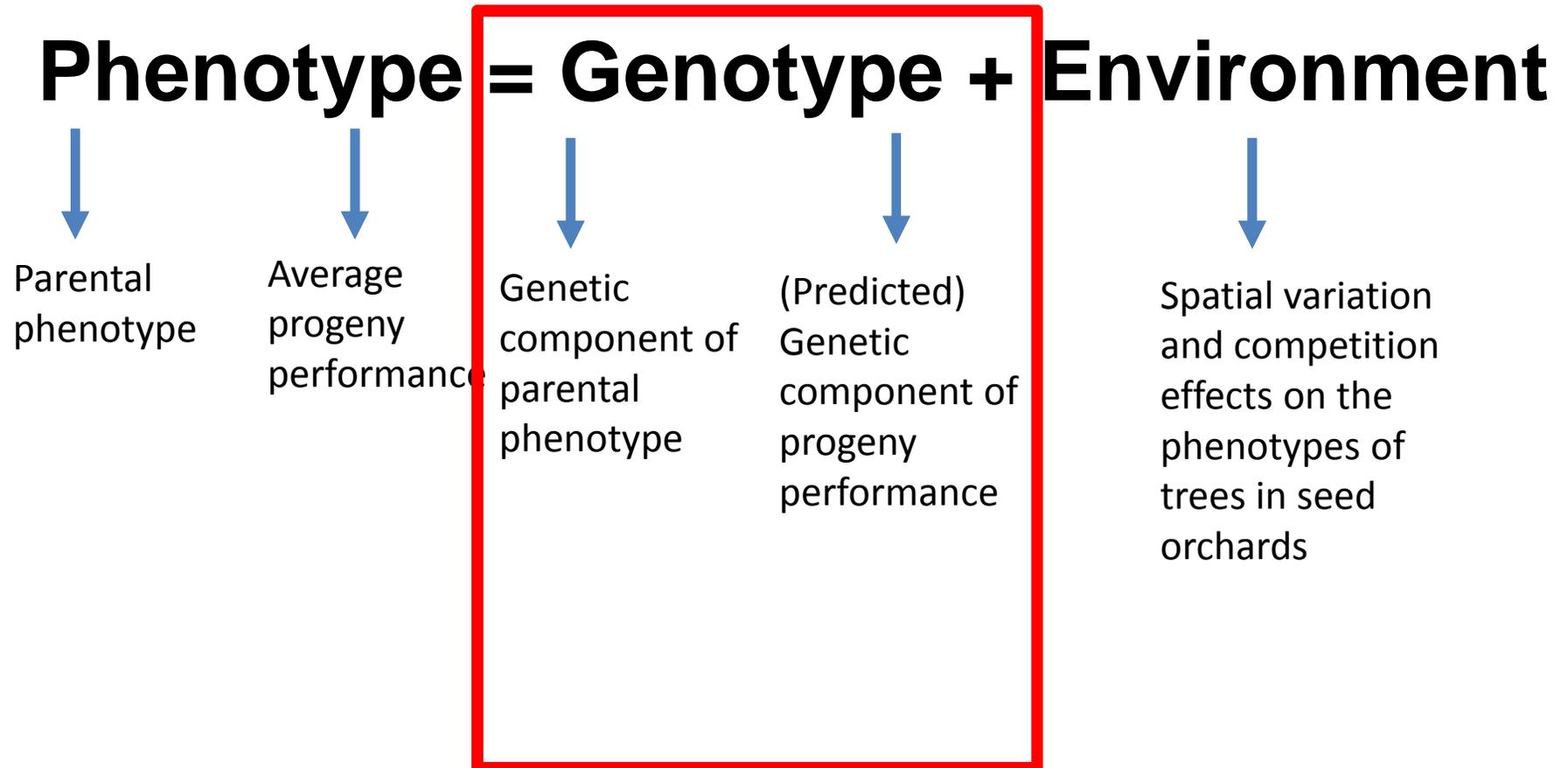


 DNA marker

 Gene involved in blight or PRR resistance



Final selections based on genetic component of parent phenotype + (genomic prediction of) progeny performance



Phenotyping parent trees in backcross seed orchards



Phenotyping by
Laura Barth & Eric Jenkins
Meadowview Research Farms

Phenotyping parents: Main stem alive/dead



Is the main inoculated stem alive or dead?

Phenotyping parents: Large cankers



Are there any cankers
larger than your palm?

Phenotyping parents: Exposed wood



Is wood showing through the blight infection?

Phenotyping parents: Sporulation



Is the blight fungus sporulating on the stem?

Phenotyping parents: Sunken/Swollen



Canker sunken, swollen, or neither?

Phenotyping parents: Callus rating



Extensive callus, some callus, no callus?

Phenotyping progeny: Severity rating and canker length



Rating 1
Minimal expansion beyond initial lesion



Rating 2
Some expansion



Rating 3
Large, sunken, sporulating

Canker length (cm)

Phenotyped 6 months after stems inoculated with weak and strong strains of *C. parasitica*

Genotyping-by-sequencing of parents

Genomic DNA

...TAACGAA**CCTTGGATCGTCATT**CGCATACTACTACGAAAATTGCATCATAA**ACCCGC**...

Restriction digestion

...TAACGAA  **CCTTGGATCGTCATT**CGCATACTACTACGAAAATTGCATCATAAA  **CCCGC**...

DNA Sequencing

CCTTGGATCGTCATTCGCATACTACTACGAAAATT
GGAACCTAGCA →

Template strand
Complementary strand

Alignment to reference genome and SNP detection

SNP SNP
↓ ↓
CCATCGAGCGTCCTT
CCTTGGAGCGTCATT



GBS by J. Holliday
and Q. Zhang, Vtech

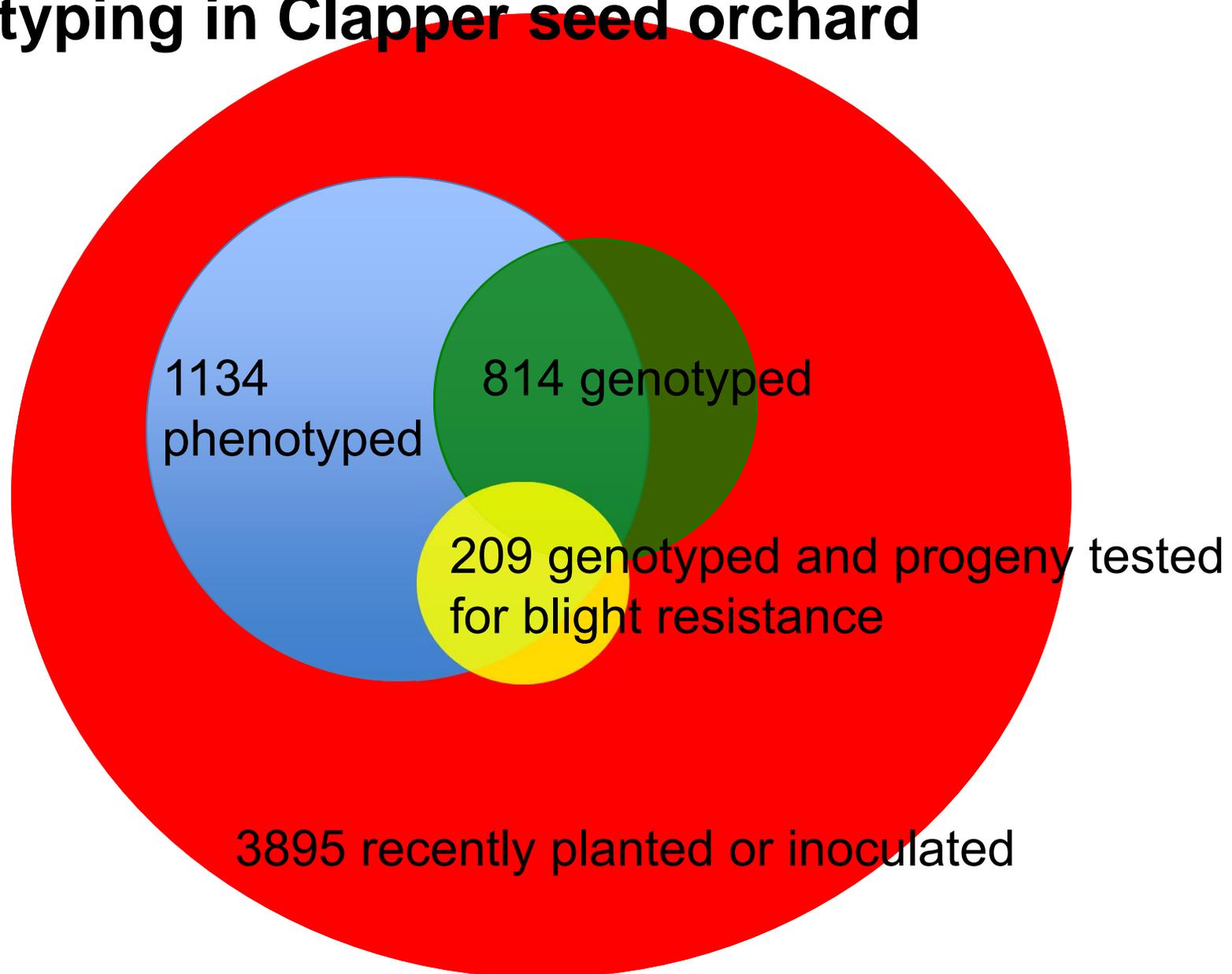
Individual A
Individual B

....TAACGAA**CCTTGGATCGTCATT**CGCATACTACTACGAAAATTGCATC.....

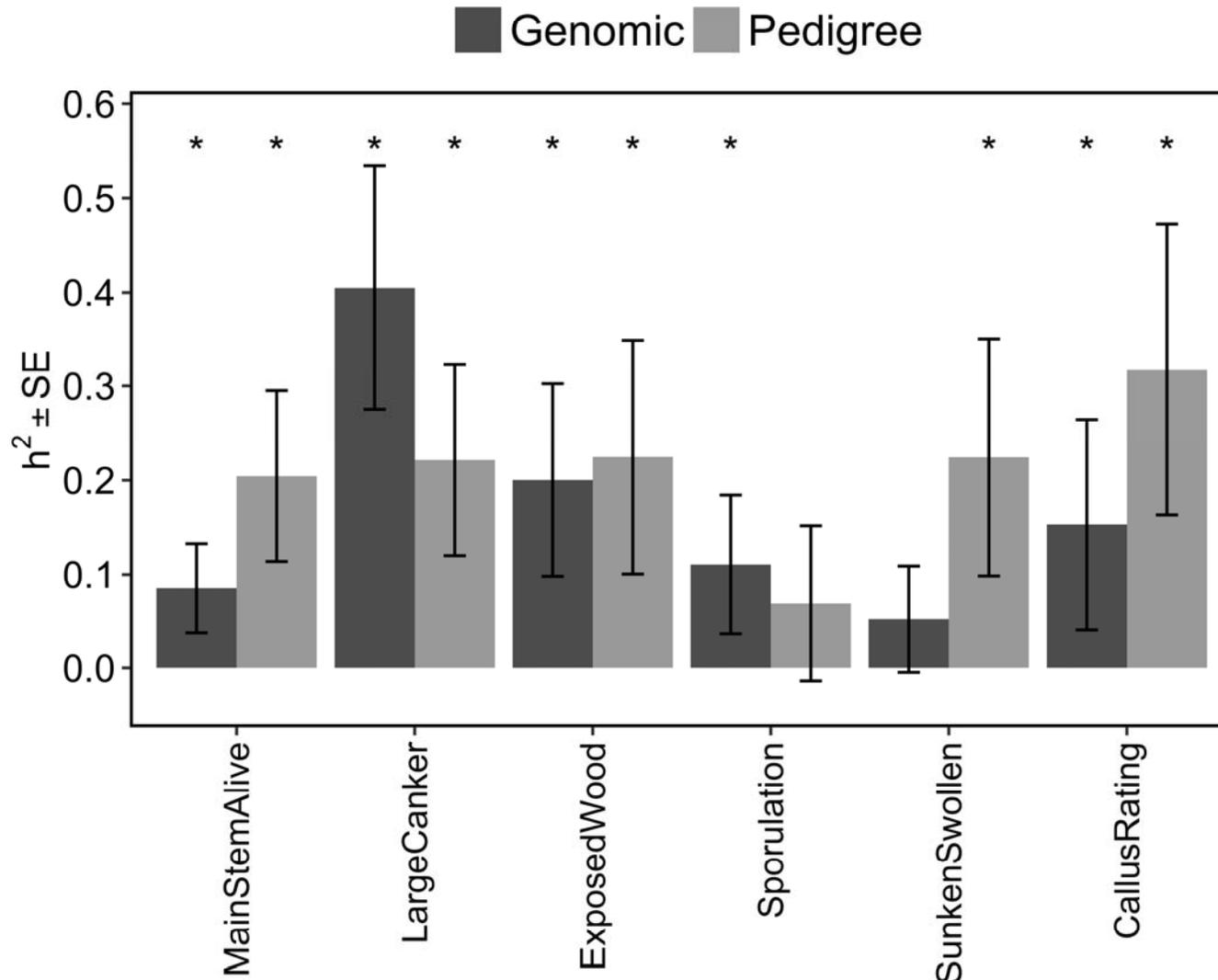
Reference genome

71504 SNPs, 1230 BC₃F₂ trees genotyped to date

Progress on phenotyping, progeny testing, and genotyping in Clapper seed orchard



Blight phenotypes of parent trees are heritable and also have a large environmental component

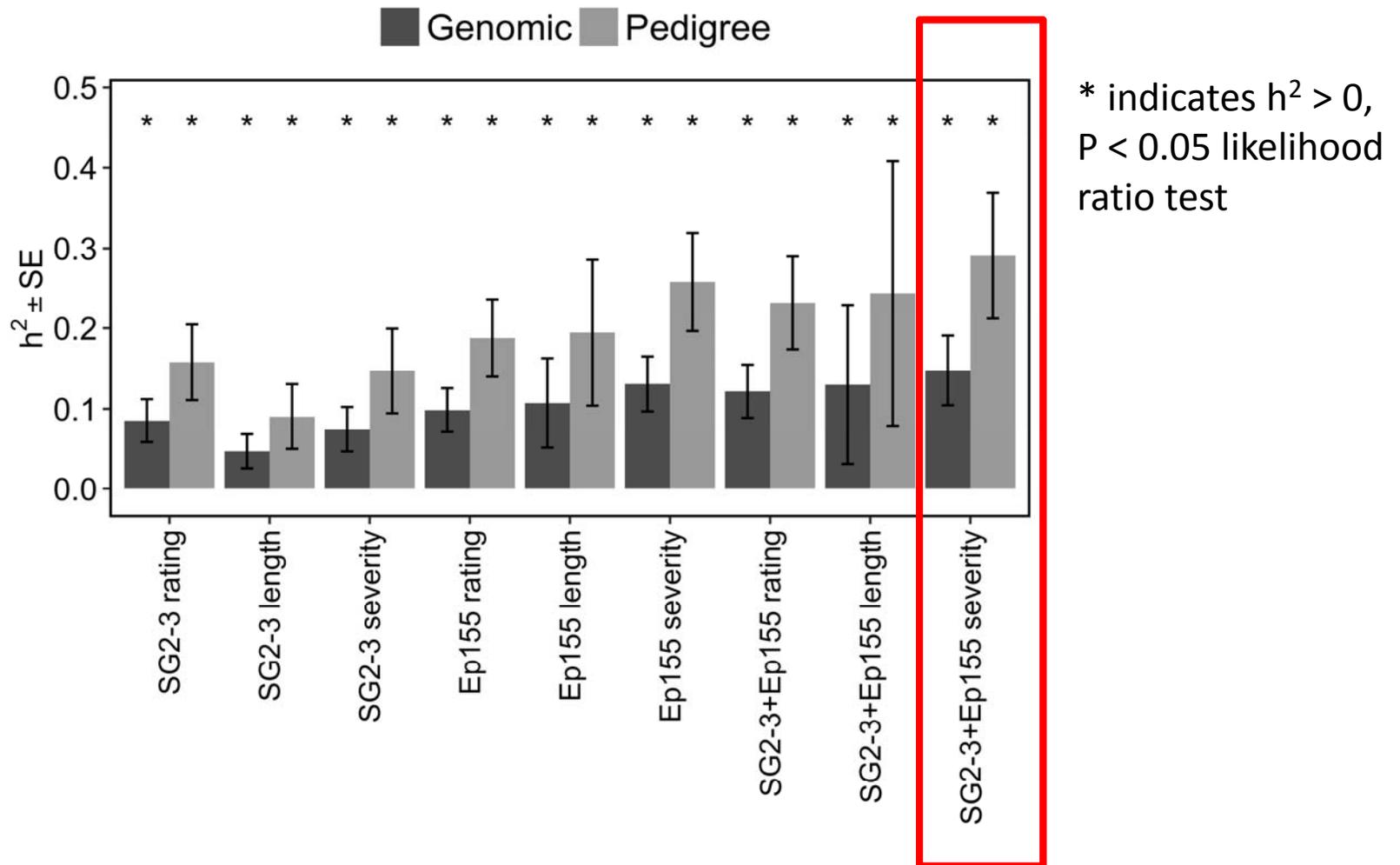


1134 trees
phenotyped for
main stem
alive/dead

645 trees w/ live
main stem
phenotyped for
additional traits

* indicates $h^2 > 0$,
 $P < 0.05$ likelihood
ratio test

Among progeny, some of the variation in canker severity inherited from parents, but most of variation is “environmental”



Cross-validation of genomic prediction of progeny performance: subdivide and repeat

Create model using 9/10th of training population

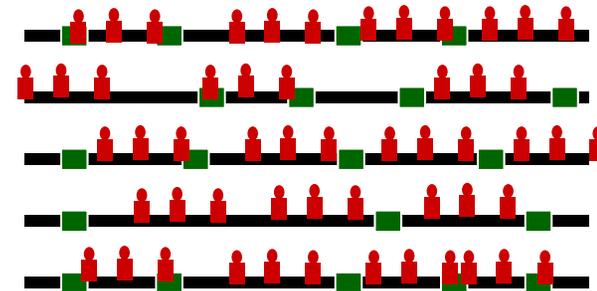


$$= \mu + X\beta$$

Block and year effects

$$+ \begin{bmatrix} -0.1 \\ +0.5 \\ +0.9 \end{bmatrix}$$

Estimated marker effects



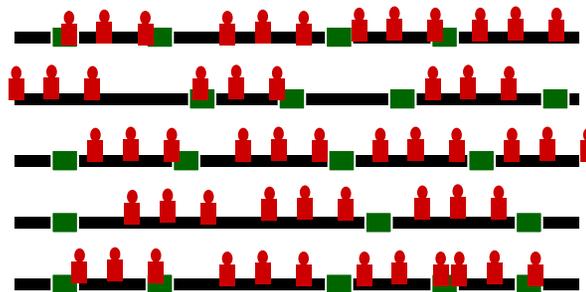
Genome-wide markers

Progeny canker severity

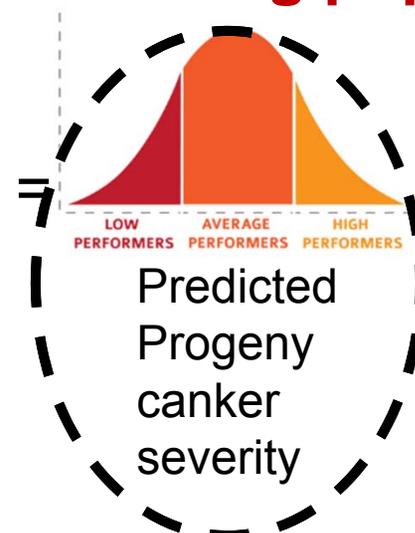
Then predict resistance using 1/10th of training population

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

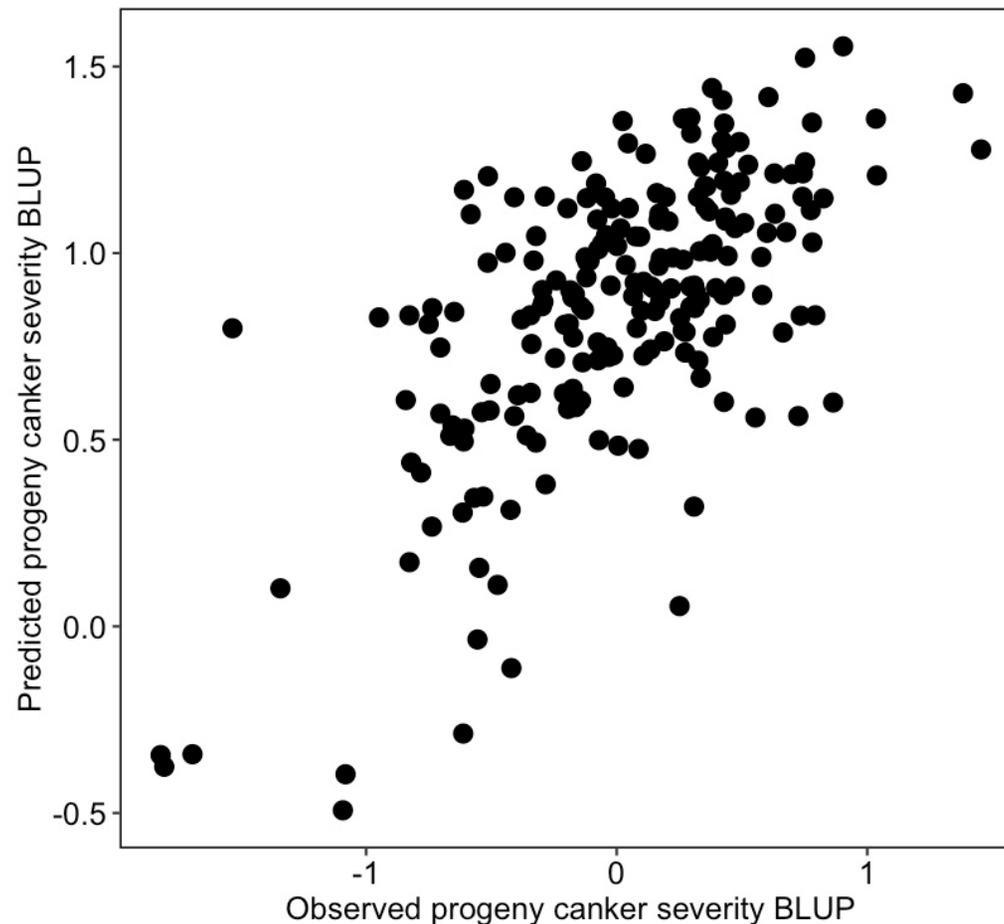
Marker effects



Marker genotypes

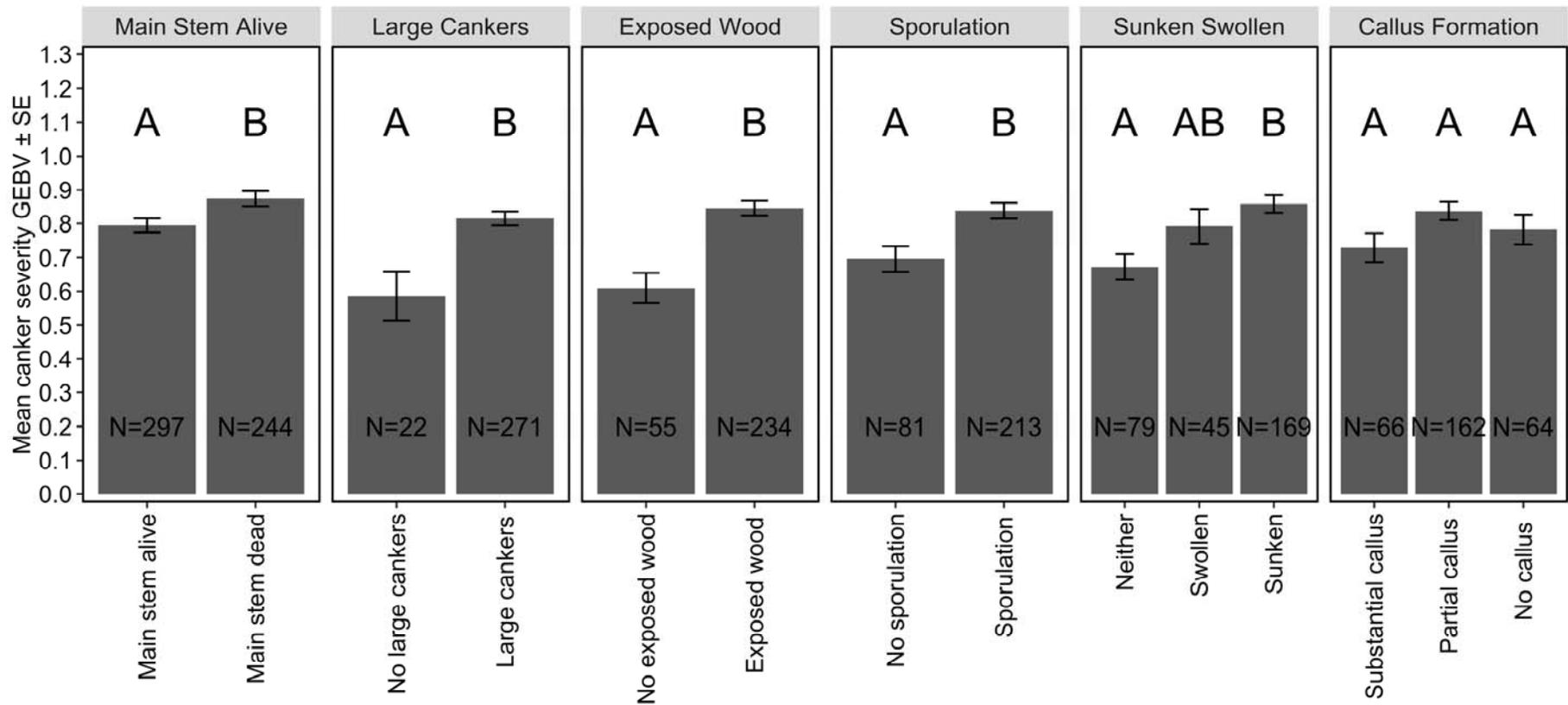


Cross-validation results: genomic prediction correlated with observed average canker severity of progeny



Correlation predicted v. observed = 0.67

Blight phenotypes of mother trees correlates with genomic predictions of progeny average canker severity



Selection index for blight resistance

Parent main stem survival

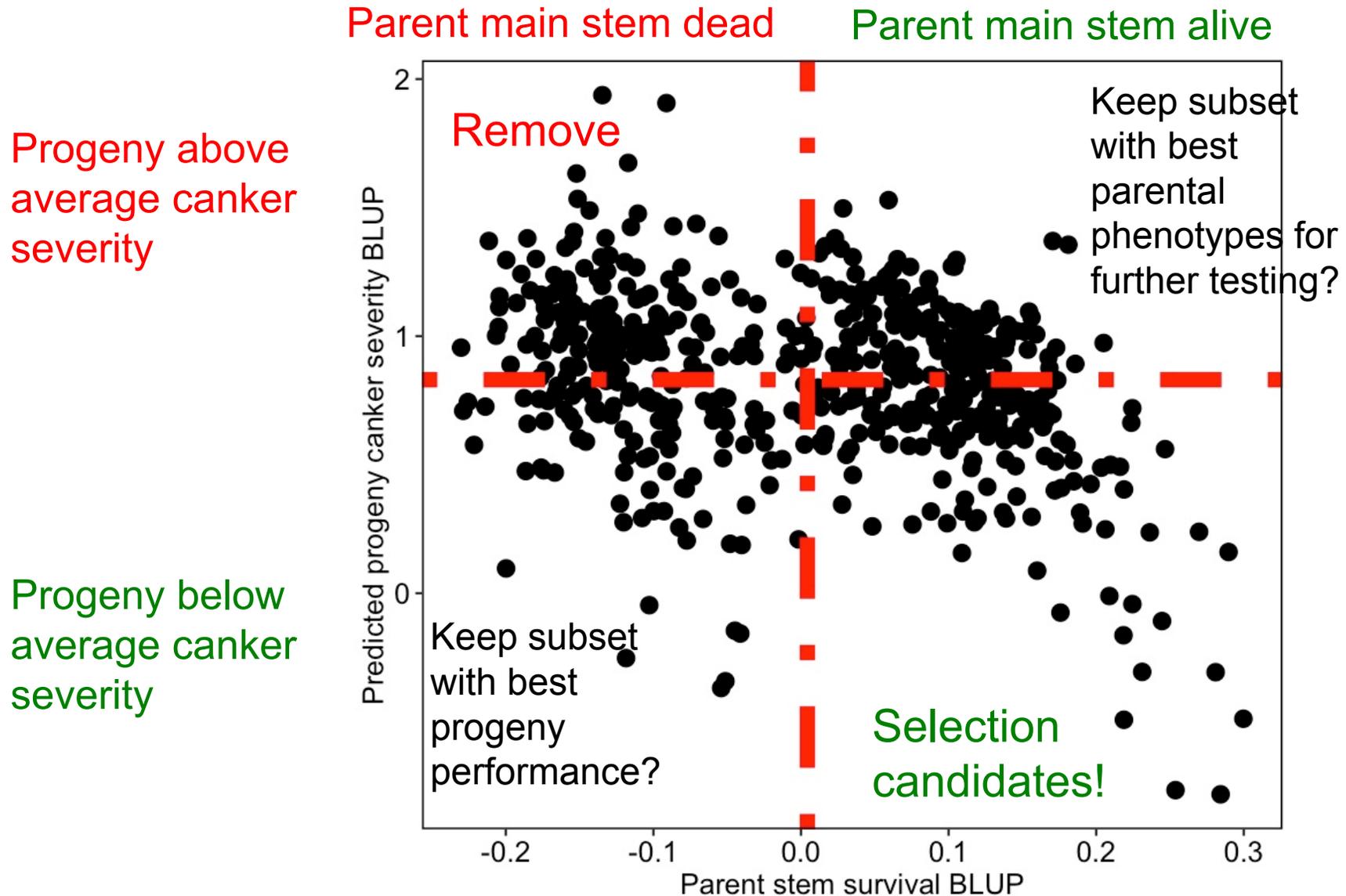
+

Parent canker traits on main stem

+

Progeny (predicted) canker severity

Selection for genetic component of parent phenotype and predicted progeny performance



Conclusions



Susceptible
American
chestnut

Examples of selections with good phenotype and good progeny performance

Funding

Colcom Foundation American chestnut reference genome



United States Department of Agriculture
National Institute of Food and Agriculture

Development of genomic selection models

**Anonymous
donor**

Additional genotyping and
phenotyping for genomic,
prediction