The University of Georgia

ESF

VirginiaTech
Invent the Future®

FHI American chestnut field trials

Jason Holliday, Carl Zipper, Brian Strahm
Department of Forest Resources and Environmental Conservation
Department of Crop and Soil Environmental Science
Virginia Tech

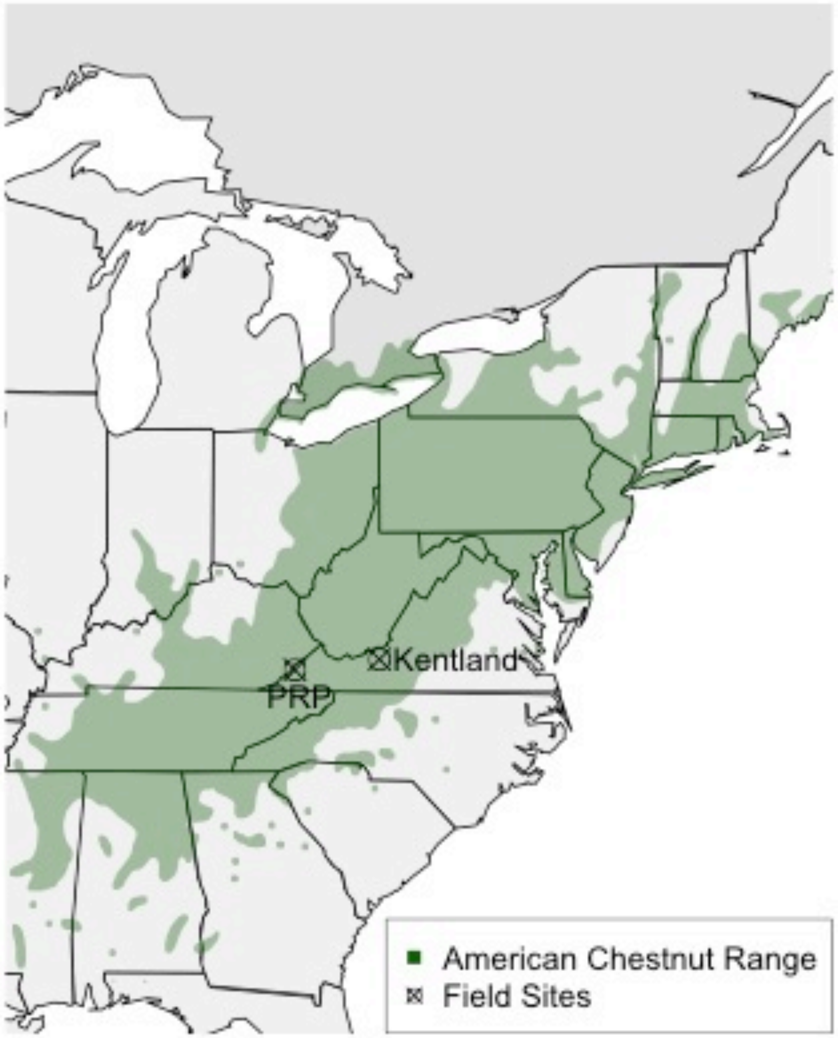
U.S. Endowment for Forestry and Communities

Forest Service
100 Years of caring for the land and serving people

Duke Energy®

Field trial locations

- Powell River Project
  - Active surface mine and site of reclamation research for past ~30 years
- Kentland Farm
  - 3200 acre VT research farm



Goals of field trials

- Evaluate resistance and adaptability of transgenics and cisgenics in common environments
- Determine appropriateness of these plants specifically for reclamation of mine lands
  - Presumably a very stressful environment for young trees, especially with respect to depauperate soils

Rationale for PRP

- Surface Mining Control and Reclamation Act (SMCRA) mandates reclamation but implementation has not been accompanied by widespread replacement of forests disturbed by mining
- Many active and abandoned mine sites comprise prime chestnut habitat (high elevation)
- Lack of competing vegetation

Rationale for Kentland 'Farm'

- Proximity to Virginia Tech allows frequent visit
- Upslope site similar to native chestnut niche
- Easy access for interested parties, but access controlled

Planting overview

- Powell River Project Coal Mine
  - Planted 96 trees June 13, 2013
    - Mix of SE and nut-derived cross-bred trees from UGA and TACF (2 families)
  - Planted 90 trees May 13 2014
    - 6 genetic constructs (with multiple events), plus some SE-derived non-transgenics (borders)
    - All trees from UGA
- Kentland Research Farm
  - Planted 360 trees, November 15, 2013
    - Mix of 27 genetic constructs (with multiple events) from UGA and SUNY, plus SE-derived pure American chestnut, Chinese chestnut, and empty vector transgenic controls
    - 1/3 trees from SUNY, 2/3 UGA

Candidate genes (CGs) that enhance blight resistance – transgenics

- Proof-of-concept for transgenic American chestnut
  - Oxalate oxidase (OxO) driven by 35S promoter (6 + events)
    - High level of enhancement (≥ Chinese chestnut) according to leaf assays

Intermediate level resistance

Susceptible

Candidate genes (CGs) that enhance blight resistance – cisgenics

- Three Chinese chestnut CGs appear to enhance blight resistance to intermediate levels (Chinese > I > American)
  - Laccase-like protein: located on QTL & differentially expression
    - flavanoid biosythesis, lignification
  - Proline-rich protein: located on QTL & differentially expression
    - cell wall modification (cross-linking)
  - Lipid transfer protein: Identified only by differential expression
    - plant defense (possible *Phytophthora* resistance)

- 64 genes cloned from Chinese chestnut are in the process of being tested for enhancing blight and *Phytophthora* resistance

- Goal: identify at least three genes that confer resistance
  - pyramid (stacked) constructs to reach full blight resistance

Planting details

- Prior to planting, trees from each construct were assigned to blocks

- At planting, trees were in their block groups and randomly assigned to locations within the block

- Trees were each given two 21 g slow-release fertilizer tablets, straw matting was placed to help reduce water loss (June 2013 only), and a weed mat (1 m x 1 m) was secured around the base of each tree

Supplemental watering

- Frequency
  - 1-2 times per week for first few weeks after planting, then only as needed (not much needed for June 2013 and November 2013 plantings)
  - Visited site to check on trees more often

- Volume of water
  - Watered each tree by time to ensure even watering
    - 0.2-0.4 inches of "rain" per watering

Growth and health metrics

- Height (vertical and stem length), basal diameter, crown spread, form, stem count
  - Recorded immediately after planting and at end of growing season
- Tree health and other variables:
  - Leaf color, degree of leaf browning, degree of wilting, relative leaf density, die-back, tree form
  - Recorded monthly during growing season, 1st measurement right after planting

Powell River Project, June 2013 Cohort

Powell River Project June 2013 Planting

Clockwise from top left: laying down straw mats for moisture control, securing weed mats around all trees, providing supplemental water to trees

Powell River Project June 2013 Cohort

Survival (%) as of June 2014

Source	Nut or SE?	Variety	Block 1	Block 2	Block 3	Block 4	Mean
TACF	Nut	B3F3, Clapper	80.0	60.0	75.0	50.0	66.2
TACF	Nut	B3F3, Graves	33.3	83.3	83.3	83.3	70.8
UGA	Tissue	B3F3, Clapper	100	100	100	66.6	91.6
UGA	Tissue	B3F3, Graves	80.0	83.3	100	83.3	86.6
UGA	Tissue	76 x 5 OP (VDF)	90.0	80.0	100	100	92.5

- SE-derived trees are surviving better after first full year
  - Best performer is AC x CC x JC (no nut-derived comparison)
  - SE-derived Clapper and Graves families both had higher survival than nut-derived

June 2013 cohort cont'd

Growth/dieback from May 2013 to November 2013 (cm)

Source	Tree Variety	Block 1	Block 2	Block 3	Block 4	Mean
TACF	B3F3, Clapper	-21.6	-62.3	-5.1	-13.9	-25.7
TACF	B3F3, Graves	-8.8	2.5	-36.4	-35.3	-19.5
UGA	B3F3, Clapper	39.6	14.8	44.1	35.8	33.6
UGA	B3F3, Graves	7.5	36.9	-10.2	15.7	12.5
UGA	76 x 5 OP (VDF)	52.2	75.7	80.2	70.9	69.7

- Tissue-derived trees grew more (nut-derived trees on average died back)
  - Because they were tissue-derived or because they were bigger (or something else)?

Powell River Project June 2013 Cohort

Possible Blight Presence July 2014

Source	Nut/SE-derived	Tree Variety	# trees with cankers	# trees with yellowing/dying branches	% trees with possible blight that are dead/dying
TACF	Nut	B3F3, Clapper	1	2	33.3
TACF	Nut	B3F3, Graves	1	6	28.6
UGA	Tissue	B3F3, Clapper	1	5	0
UGA	Tissue	B3F3, Graves	1	3	0
UGA	Tissue	76 x 5 OP (VDF)	1	5	16.7

Powell River Project, May 2014 Cohort

May 2014 cohort
July 16, 2014

Powell River Project 2014 cohort survival (%) as of June 2014

Gene	Background	Block 1	Block 2	Block 3	Block 4	Borders	Mean	Mean number of trees per block
CAD	76-5xOP-2B	100	83.33	100	85.7	92.3	92.3	6.25
Prox	76-5xOP-2B	100	100	100	100	100	100	4.25
NPR1	RxT-22B	100	100	100	100	100	100	1.25
TL/TAGL	AW3-46B	0	100	0	N/A	33.3	33.3	0.75
Cyst1	WB484-3	N/A	0	100	N/A	50	50	0.5
ETF1	WB484-3	0	100	100	100	75	75	1
None	76-5 x OP (AC x CC x JC)	N/A	N/A	N/A	N/A	95	95	27
None	Nagle (AC)	N/A	N/A	N/A	N/A	100	100	5

- Low mean percent survival in some constructs due to low replication, too soon to draw conclusions

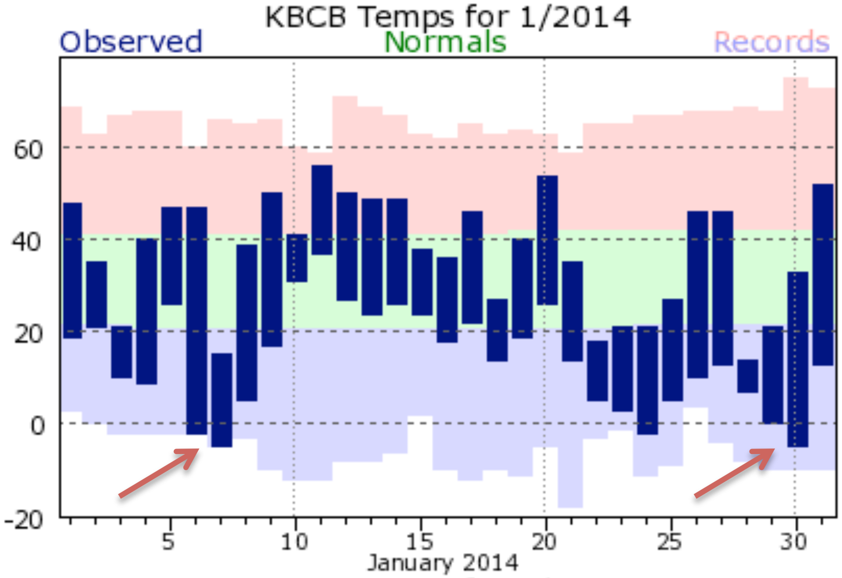
Plans for Powell River Project

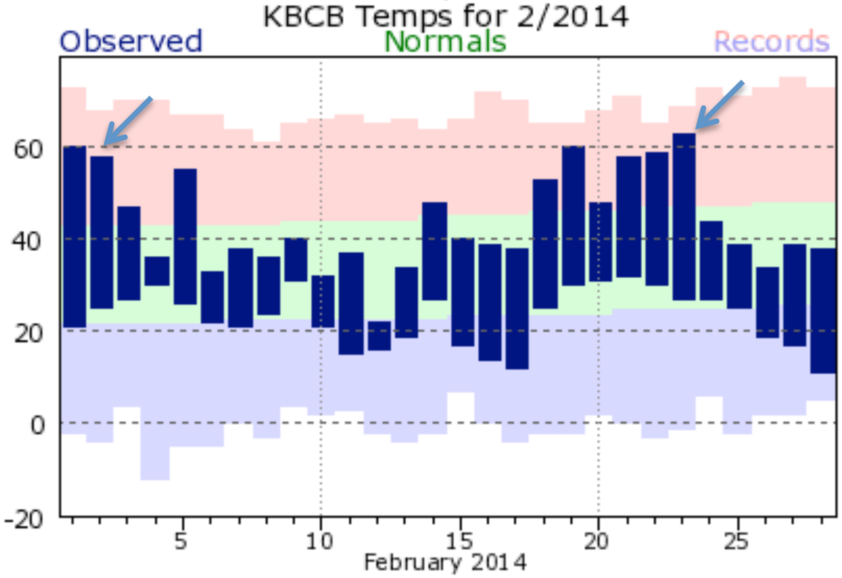
- Spring 2013 and 2014 plantings
  - Continue monthly measuring and monitoring until end of growing season
  - During winter, visit monthly to check on site, equipment, etc.
- Spring 2015 planting
  - Plant next batch of transgenics on mine site

Kentland Farm, Fall 2013 Cohort

Kentland Farm Fall 2013 cohort
June/July 2014

An unusual winter





ID/Source	Gene	Background	Block 1	Block 2	Block 3	Block 4	Block 5	Mean survival
Travis/SUNY	CC Laccase	AC	8.3	0	16.6	0	8.3	6.7
Darling/SUNY	oxalate oxidase	AC	0	0	20	0	9.09	5.8
Ellis/SUNY	none	AC	0	0	0	0	N/A	0
Qing/SUNY	none	CC	100	100	100	0	0	60
Empty vector control/ SUNY	none	AC	0	0	0	100	0	20
UGA	B-Gluc	WB484-3		50	100	50	33.3	58.3
UGA	CBS1	76-5xOP-2B	75	80	50	75	50	66
UGA	CBS1	WB484-3	0	N/A	N/A	N/A	N/A	0
UGA	GUSi	76-5xOP-2B	100	0	0	0	N/A	25
UGA	Lac	WB484-3	55.5	37.5	37.5	44.4	55.6	46.1
UGA	PRP	WB484-3	60	30	10	10	30	28
UGA	Thaum	RxT-22B	100	50	50	66.7	66.7	66.6
UGA	Thaum	WB484-3	25	25	0	33.3	33.3	23.3
UGA	YFPGUSi	RxT-29B	N/A	100	0	0	0	25
UGA	YFPGUSi	WB484-3	100	N/A	100	0	0	50
UGA	none	AC	100	0	N/A	0	100	50

- Overall low, but higher survival in trees from UGA
  - Fewer UGA trees leafed out during warm weather after Nov. planting
  - Larger trees more resilient to transplant stress, rodent nibbling

Kentland Farm Fall 2013 cohort
Early flowering May/June 2013

Early Flowering

- Flowering observed from 5-19-14 to 6-10-14
- Flowers removed, double-bagged, and disposed of

Gene	Background	Event	# trees	Total # flowers
CBS 1	76-5 x OP 2B	3	2	15
CBS 1	76-5 x OP 2B	7	3	10
CBS 1	76-5 x OP 2B	8	1	2

Kentland Issues and Solutions

1) Fall 2013 planting may have occurred too early

Issue: Trees (primarily from SUNY) leafed out during warm period immediately after planting, cold snap right afterward killed many of the trees

Solution: Delay planting, keep all trees in greenhouse until dormant

2) Heavy rodent damage

Issue: Heavy herbivory/damage from rabbits, voles, moles, etc.

- Possibly due to limited food due to a non-mast year and exacerbated by an **exceptionally harsh winter**

Solutions: Increased weed control before and after planting, added rodent guards to trees to minimize aboveground damage, more aggressive poison baiting

Plans for Kentland Farm

- Fall 2013 Planting
  - Continue monthly measuring and weekly monitoring of the site
- Fall 2014 Planting
  - Second cohort of transgenics
  - Minimize transplant stress
    - Ensure all trees are dormant before planting
  - Rodent control – minimizing tree mortality
    - More aggressive weed control pre-planting
    - Bait site more aggressively with poison
    - Install rodent guards, reconsider weed mats
- Innoculation toward end of project?

Looking ahead

- Current FHI funding ends in about 1.5 years
- Extending the life of these trials would provide valuable data on long-term resistance, but regulatory compliance is costly
- Deregulation of these trees would allow us to continue the trials indefinitely
  - More on that issue from Bill and Adam later

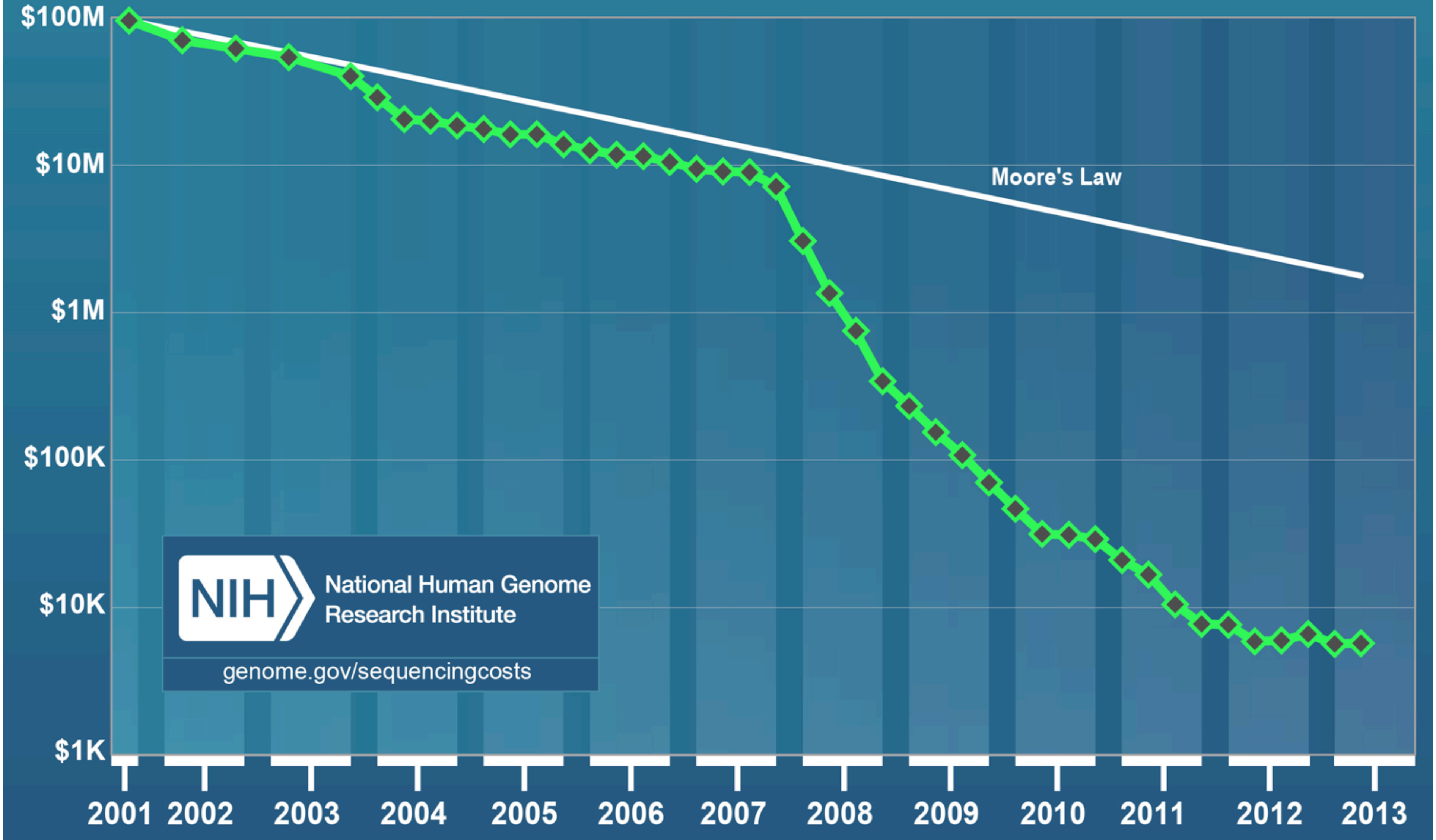
Use genomics to accelerate backcross breeding

Jason Holliday, Dana Nelson (USFS), Fred Hebard (TACF)

Genomic selection

- Introduction of Chinese chestnut resistance alleles and subsequent backcrossing an alternative to trans/cisgenics
  - Greater social acceptability?
- The goal: maximize resistance alleles while minimizing Chinese chestnut alleles
- Next-gen sequencing coupled with multi-locus models can advance both of these objectives

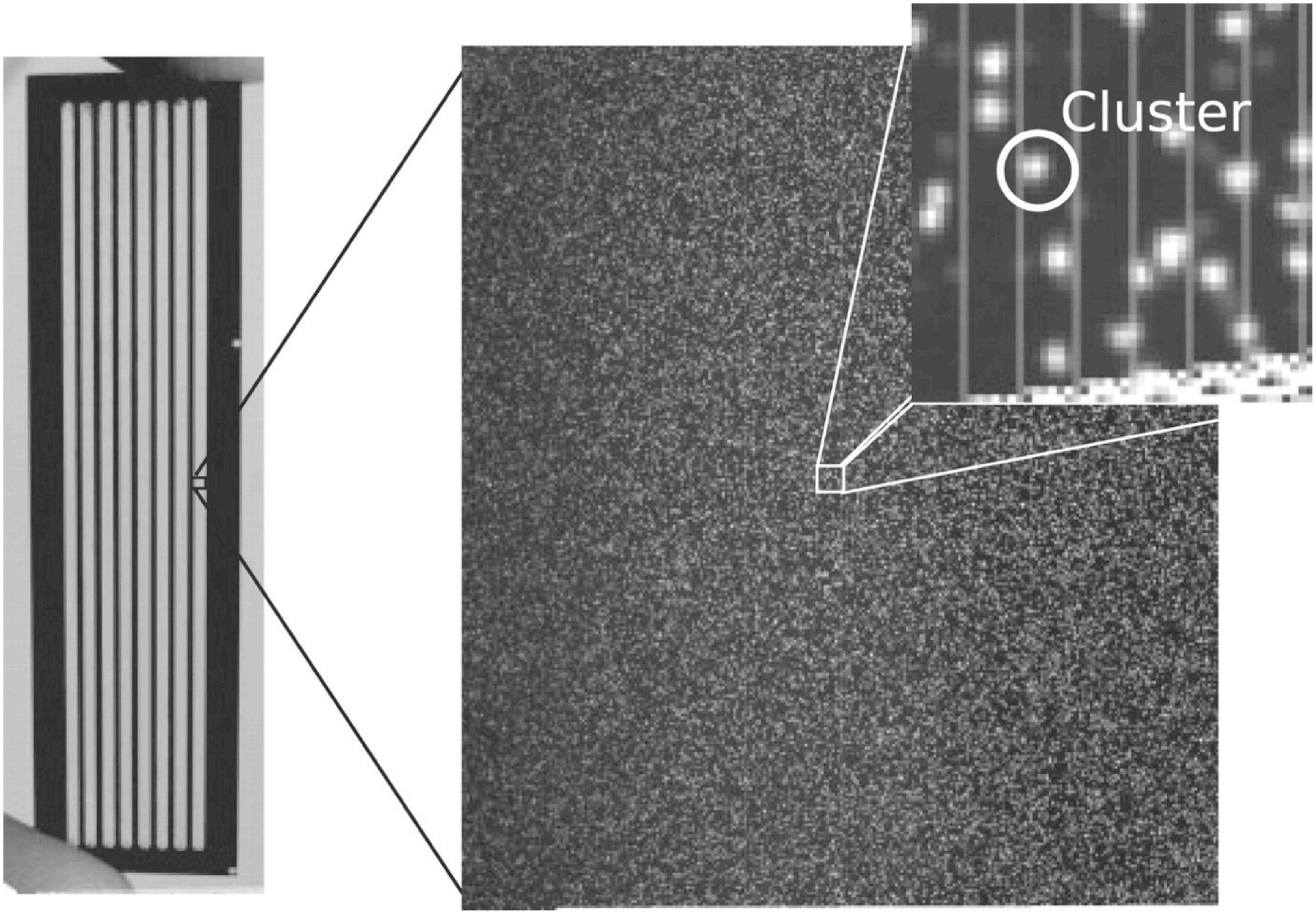
Cost per Genome



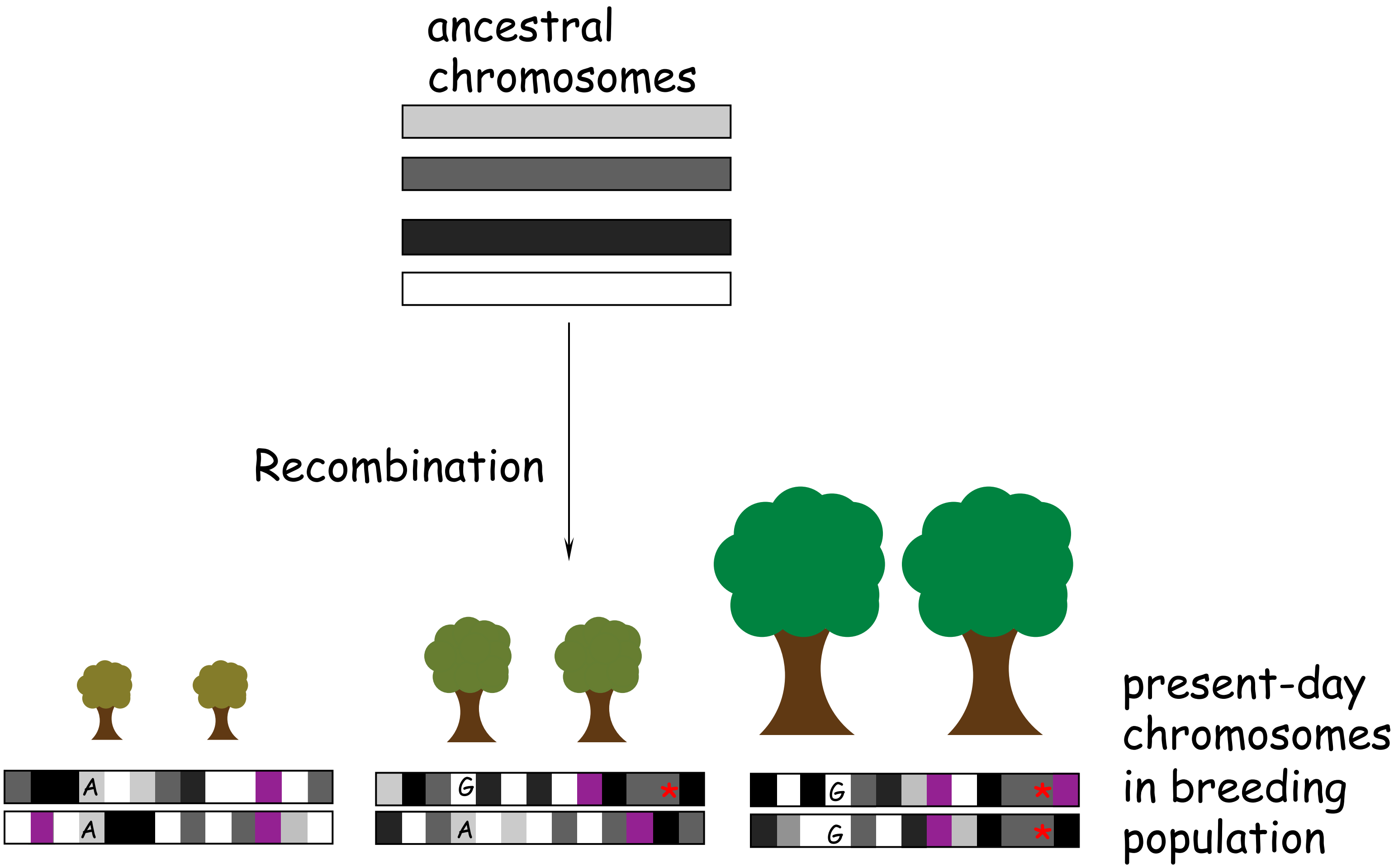
National Human Genome Research Institute

genome.gov/sequencingcosts

Genotyping-by-sequencing



Genomic Selection – the concept



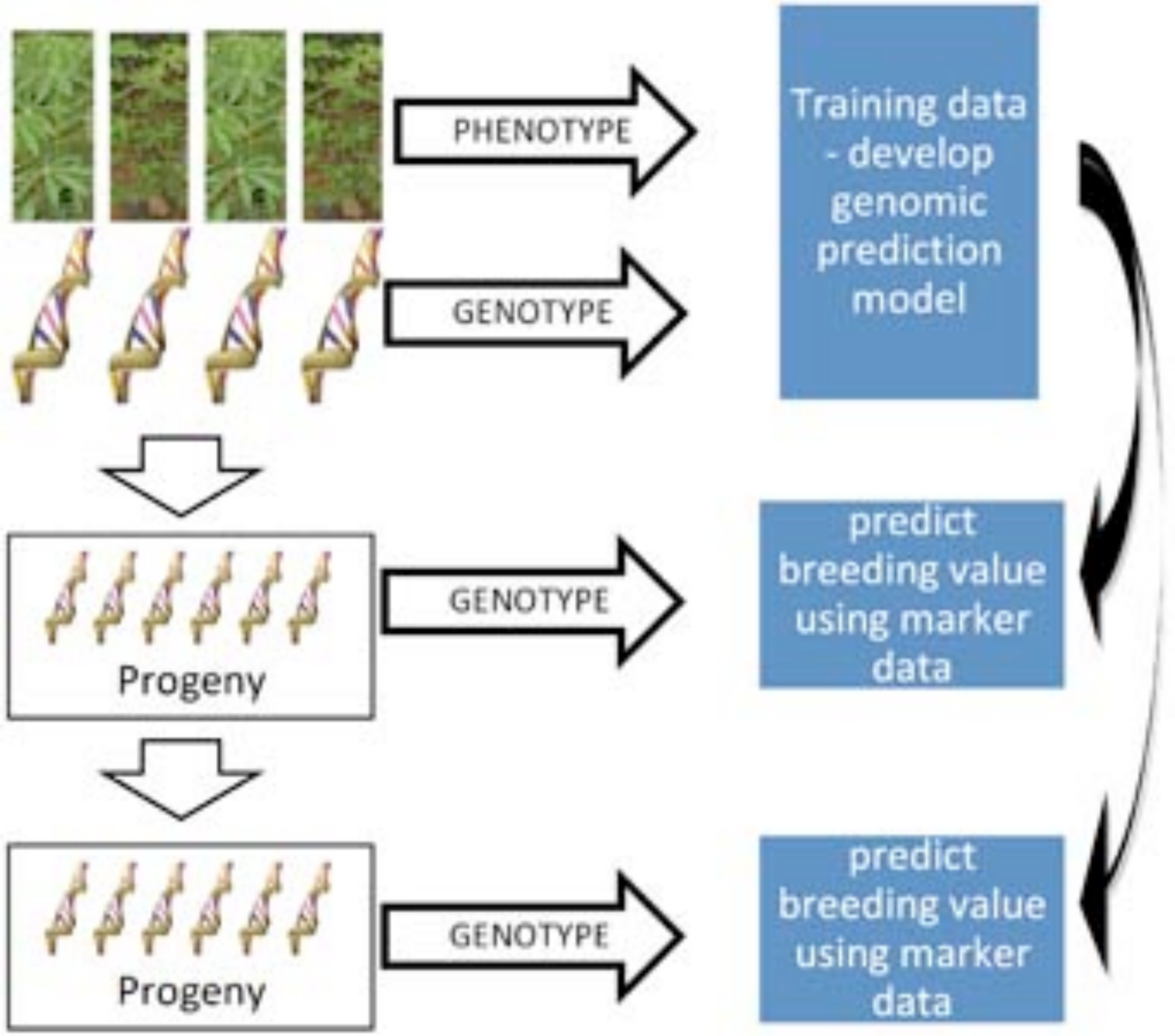
ancestral
chromosomes

Recombination

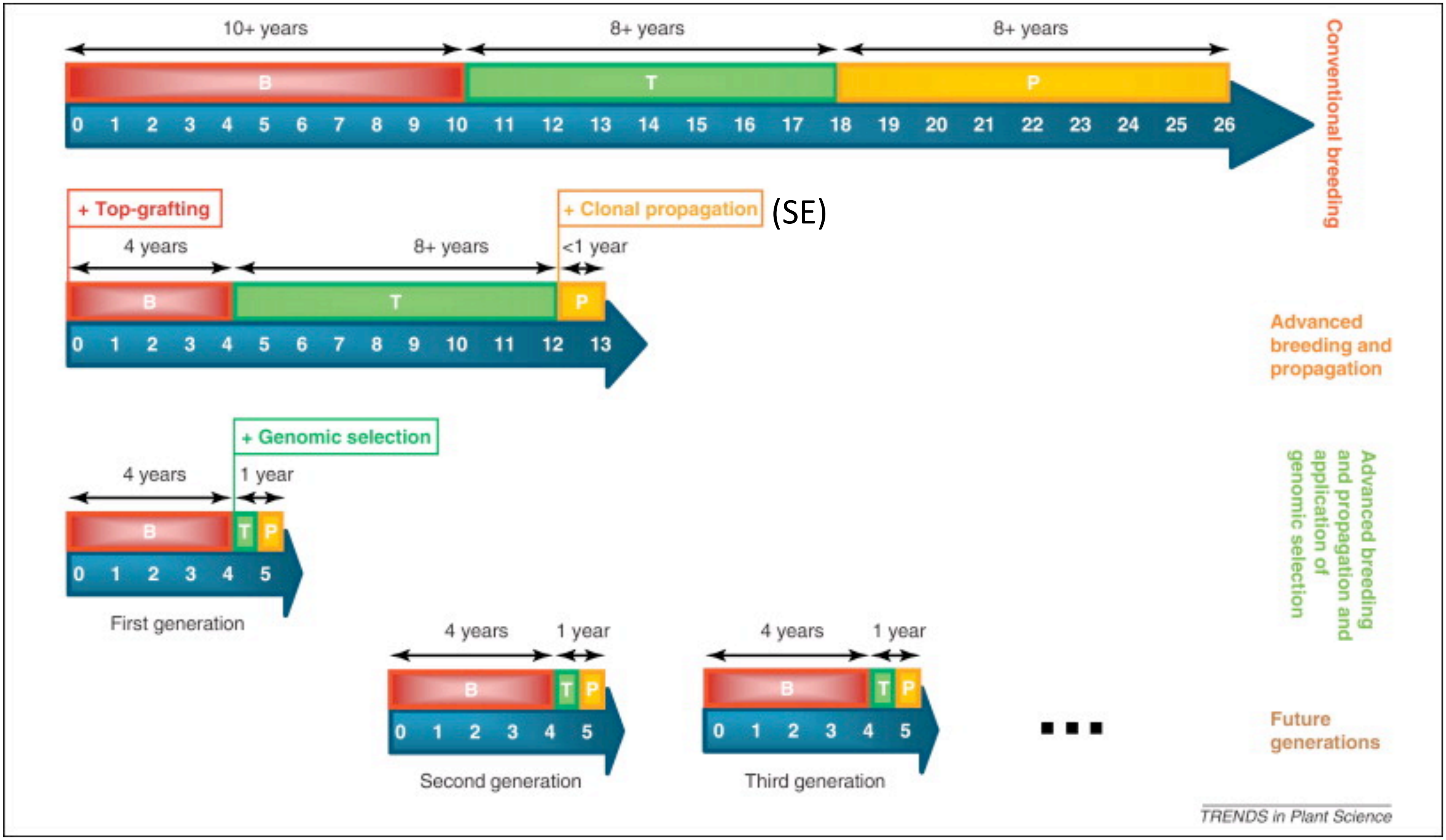
present-day
chromosomes
in breeding
population

Blanket entire genome with markers and use these to predict phenotypes

Genomic Selection – the process



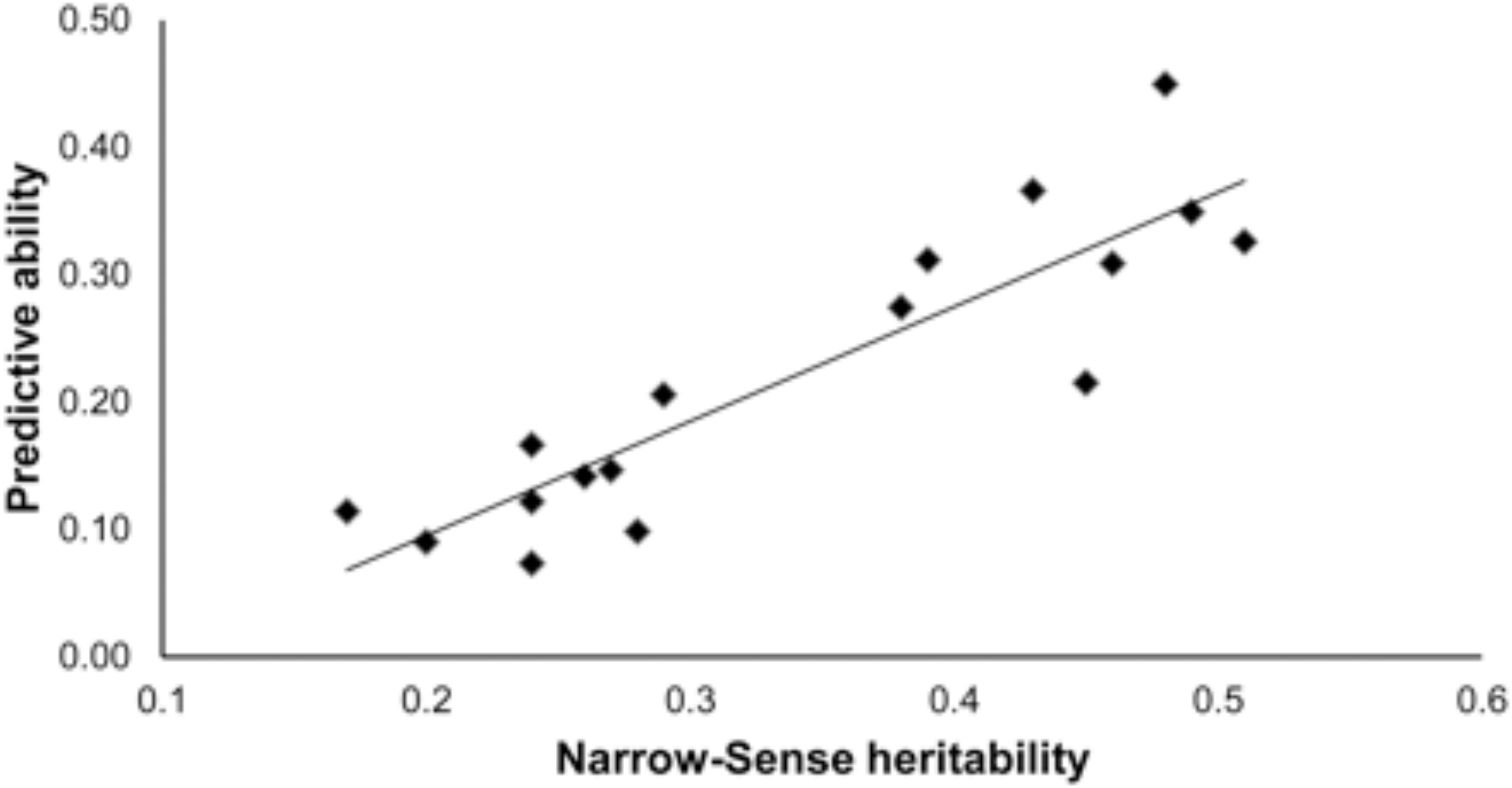
Genomic Selection – the advantages



B = Breeding T = Testing P = Propagation

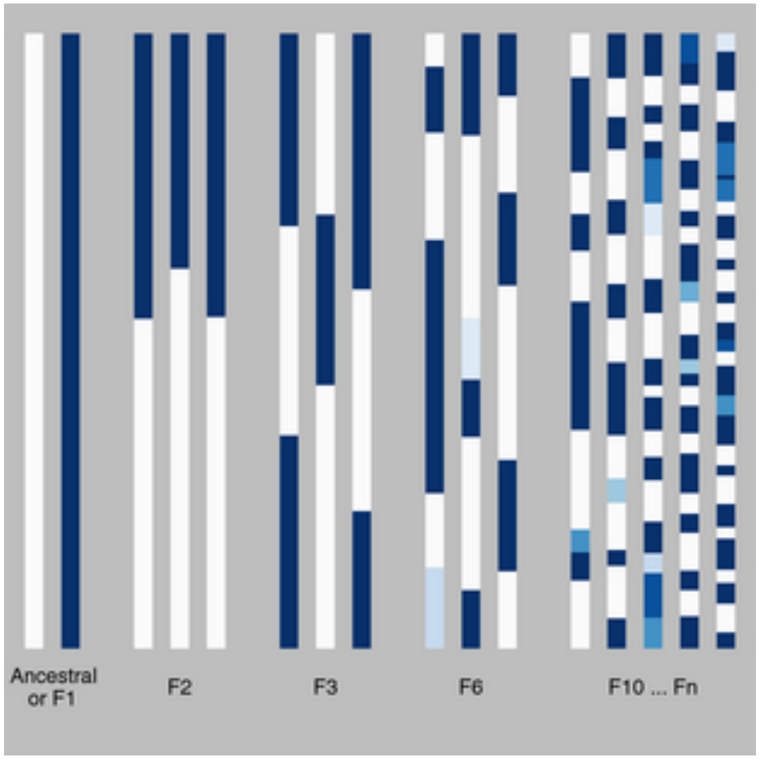
Harfouche et al 2012

Success of GS in trees



Tracking resistance alleles *and* Chinese chestnut background

- A critical goal of backcross breeding is to maximize blight resistance while minimizing extraneous Chinese chestnut chromosomal segments
- Phenotypic selection can do a good job at the former but it is difficult to quantitatively assess the latter
- A unique advantage of GS in the case of chestnut breeding is the ability to identify the **rare** offspring that harbor resistance alleles but little other Chinese chestnut background



Gompert and Buerkle 2013

Plans – Genomic selection

- Two-enzyme system: common cutter and rare cutter
- Select ~500 phenotyped progeny from TACF backcross breeding program
- Sequence in 96-plex format
- Train model on a subset of the samples (~80%) and validate with the rest
    - Various models will be tested

Acknowledgements

- Duke Energy, USFS, US Endowment for Forestry and Communities – Funding
- FHI scientific advisory committee – hours upon hours of unpaid support
- The American Chestnut Foundation – Access to invaluable plant material and expertise
- The Institute for Forest Biotechnology – Coordination and regulatory process
- Sara Koropchok (Virginia Tech) – field trial manager

U.S. Endowment
for Forestry and Communities

Forest Service
100 Years of caring for the land and serving people

Duke
Energy®