

Sawfly Resistance Breeding at CSU

Three More Years Down the Road



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Houston, We Have a Problem!

- 2008, 2009 – appearance of ‘Genou’ in Wyoming variety trials (1 bu/a higher yield than ‘Buckskin’)
- Fall 2009 – ‘Choteau’ spring wheat put into CSU crossing program (due to linked markers....)
- Summer 2011 – first documented sawfly damage in Colorado



Terri's Sweep Net



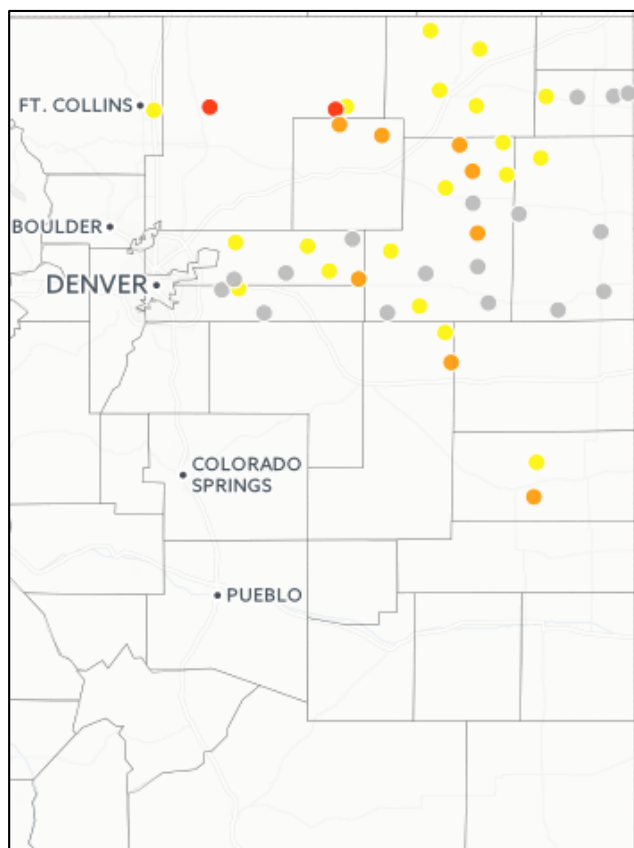
Ripper - worse



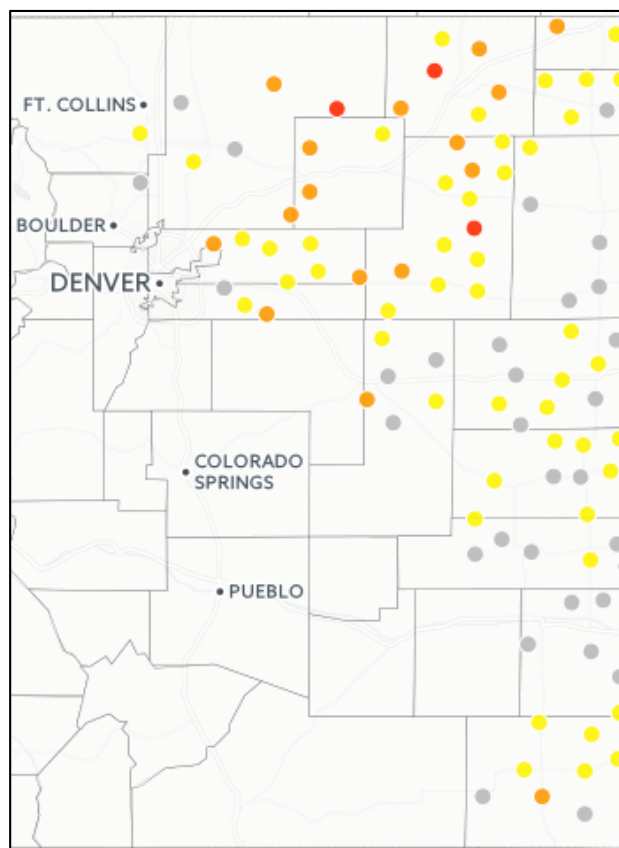
Hatcher - better

Wheat Stem Sawfly Surveys

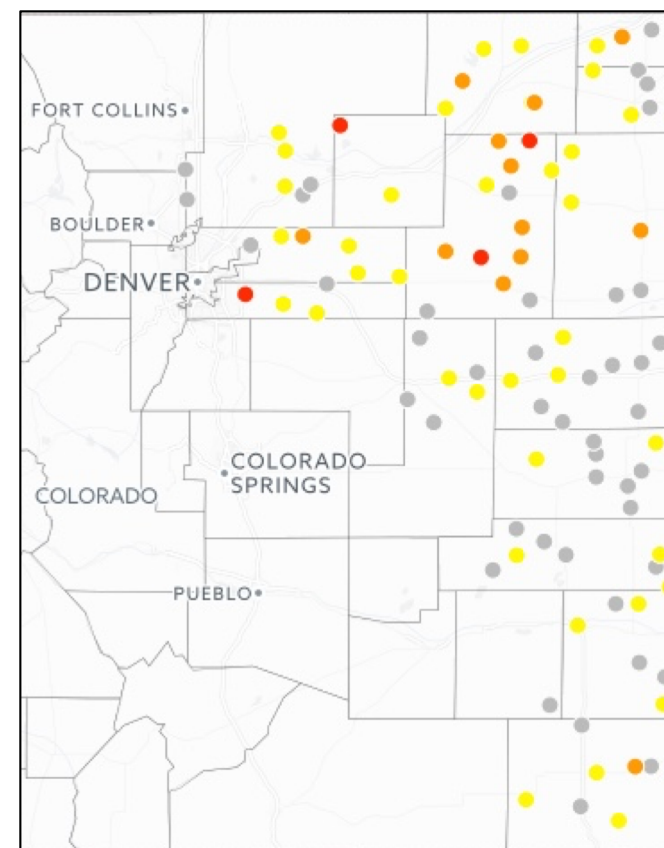
2012



2015



2017



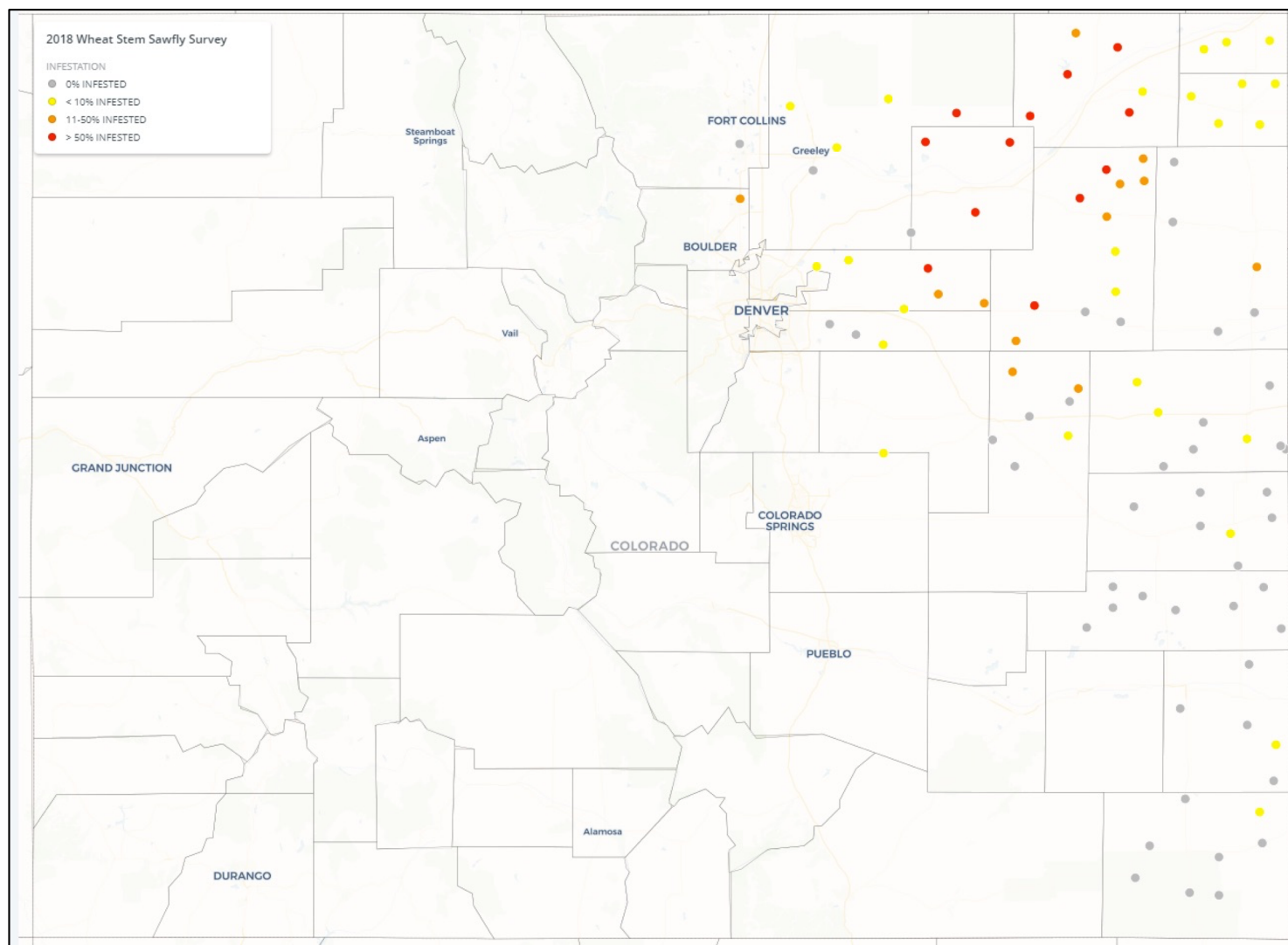
0% infestation

<10% infestation

11-50% infestation

>50% infestation

Wheat Stem Sawfly Survey – 2018



0% infestation

<10% infestation

11-50% infestation

>50% infestation

Variety Testing and Wheat Breeding

- Extension variety trials – Montana solid-stem lines vs *Byrd* winter wheat
 - *Judee* (2012) = 18 bu/a lower (33%)
 - *Bearpaw* (2013) = 8 bu/a lower (28%)
 - *Warhorse* (2014) = 13 bu/a lower (22%)
 - *Bearpaw* (2013-2015) = 12 bu/a lower (24%)
 - *Spur* (2015-2016) = 9 bu/a lower (12%)
 - *Loma* (2017) = 41 bu/a lower (41%)
 - Conclusion – cannot coast toward retirement!
- Breeding approaches
 - Doubled haploid breeding – accelerated line development, enrichment and selection using DNA markers
 - Selected bulk breeding – repeated phenotypic selection in segregating generations
 - Strategy – make crosses between elite lines and solid-stem donors
rapidly develop lines for evaluation under sawfly pressure
identify lines with potential for release, increase seed
recycle lines back into crossing program

Doubled Haploid (DH) Breeding



Make cross, grow F1
Pollinate with maize
Treat with hormones



Collect immature seeds
excise embryos
transfer to tissue culture



Regenerate haploid
plants in tissue culture



Vernalize, treat with
colchicine



Harvest DH seed,
increase

Doubled Haploid (DH) Breeding



DNA marker-assisted enrichment for stem solidness on chromosome 3BL

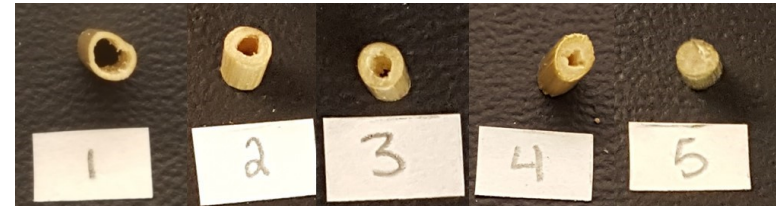
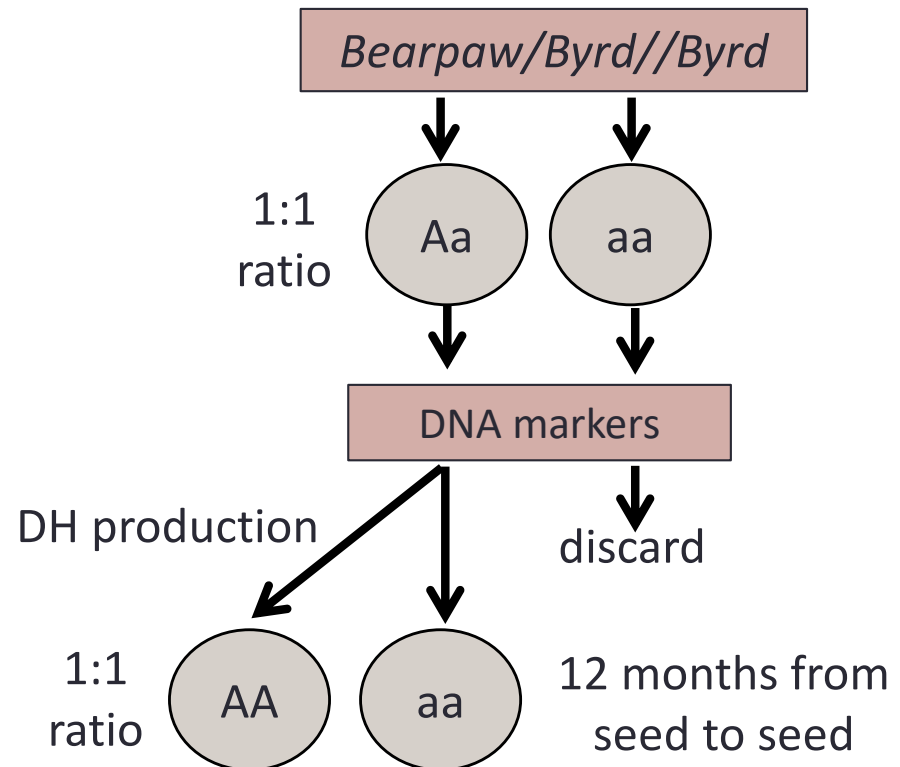


Image – Darren Cockrell, CSU



DH-Derived Semi-Solid Lines

- 264 DH lines generated, grown in field in 2015
 - Visual selection – 134 lines selected
 - Visually scored for solidness in the field at harvest, assayed for DNA markers associated with chromosome 3BL solidness gene
 - Selection history: 2016 - 102 lines, 2017 - 12 lines, 2018 - 4 lines

Entry	2016	2017		2018		Average				
	Yield WSS	Yield Non WSS	Yield WSS	Yield Non WSS	Yield WSS	Yield Non WSS	Yield WSS	Test Weight	Stem Cutting	Solidness
Byrd	57.7	71.9	56.8	53.1	69.8	60.9	61.4	57.3	5.8	6.6
Denali	60.9	62.2	54.8	54.4	65.7	59.2	60.5	57.5	5.6	6.3
CO15SFD092	59.3	64.8	56.6	54.9	68.6	59.7	61.5	57.3	2.4	13.2
CO15SFD107	60.0	64.9	53.9	54.3	67.5	59.7	60.5	58.2	2.3	12.7
Average	57.3	65.3	55.2	67.5	55.2	66.4	55.9	57.4		
Locations	2	9	2	8	2	17	6	23		

Semi-Solidness and Stem Cutting



CO15SFD107
Akron Colorado 2018

CO15SFD092
Akron Colorado 2018



Semi-Solidness and Stem Cutting

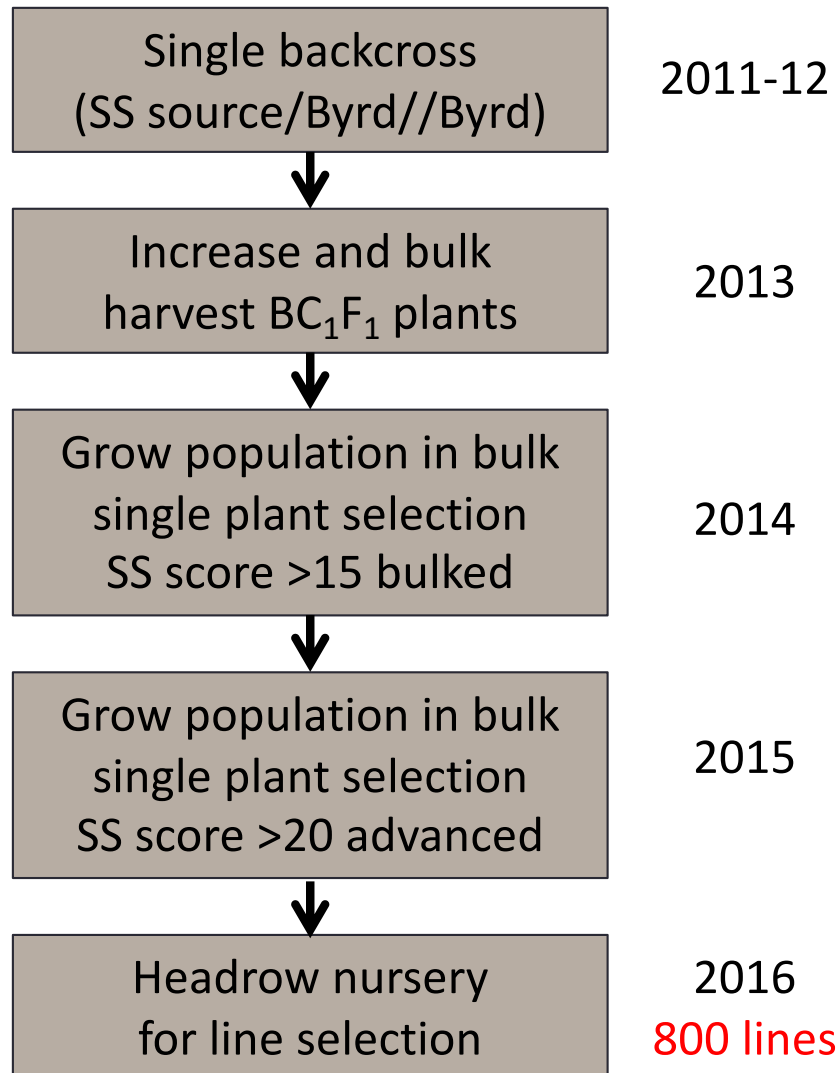


CO15SFD107
Akron Colorado 2018

CO15SFD107 (left) – cutting 20%
Canvas (right) – cutting 85%
New Raymer Colorado 2018



Selected Bulk Breeding



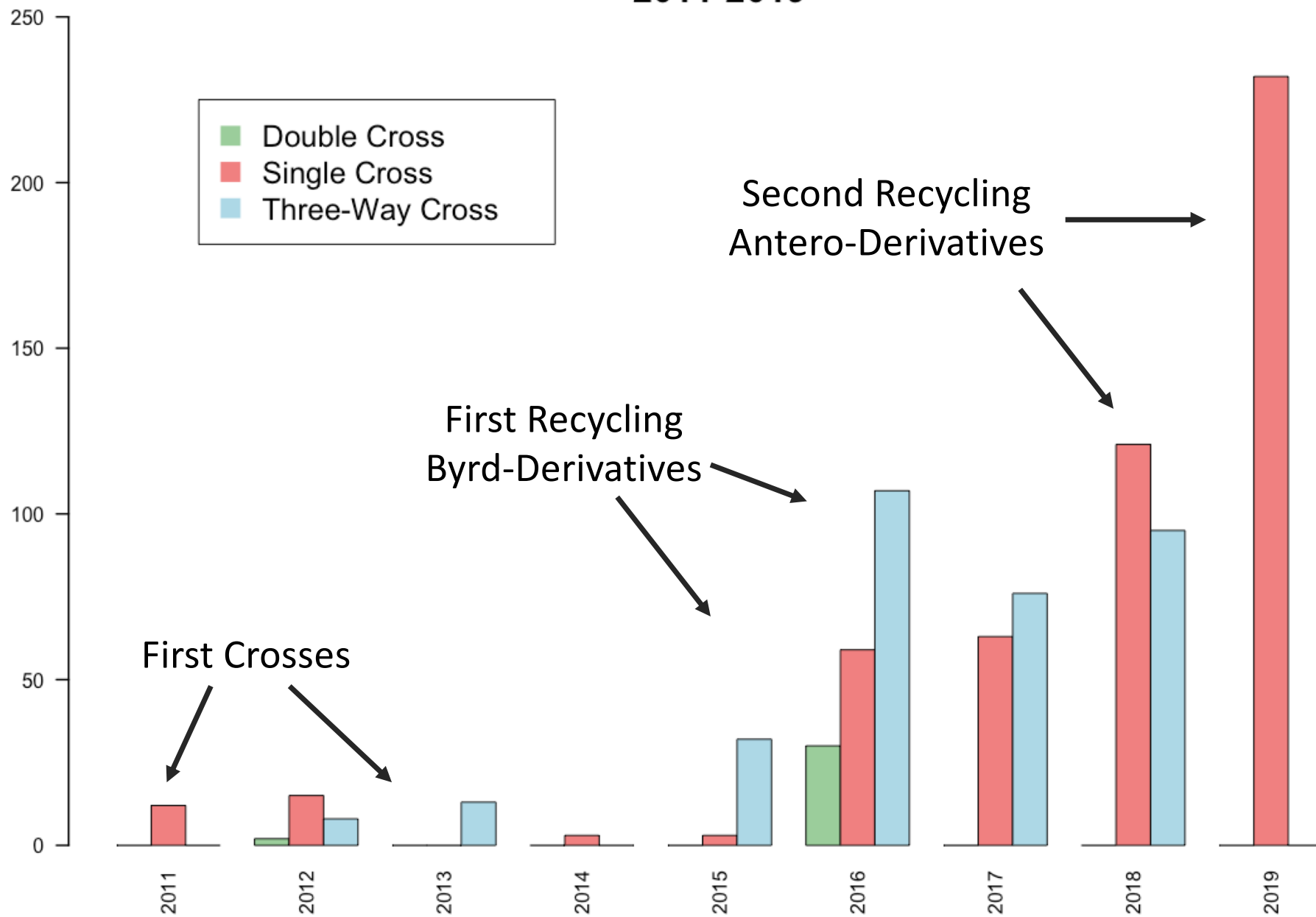
- Widely used for breeding for durable rust resistance in wheat
- Solid stem parents
 - Judee
 - Bearpaw
 - Warhorse
 - Spur
- Adapted parents
 - Byrd
 - Antero
 - Denali
 - New elite hard red, hard white lines

Selected Bulk-Derived Semi-Solid Lines

- About 800 headrow selections in field in 2016
 - Visual selection – 79 lines selected, scored for solidness at harvest
 - Trials at both Orchard and New Raymer in 2017
 - Selection history: 2018 - 5 lines; 2019 - 2 lines

Entry	2017	2018		Average			
	Yield WSS	Yield Non WSS	Yield WSS	Yield WSS	Test Weight	Stem Cutting	Solidness
Avery	54.2	71.8	55.7	55.0	57.3	5.5	6.2
Byrd	49.9	69.8	53.1	51.5	57.5	5.8	6.6
Denali	48.1	68.6	54.9	51.5	56.6	5.6	6.3
Snowmass	42.3	64.7	54.0	48.1	56.0	5.8	6.2
CO16SF065	50.8	68.7	55.6	53.2	57.5	2.6	15.7
CO16SF070	49.5	69.2	56.4	53.0	57.2	2.4	16.4
Average	46.8	68.6	54.5	50.7	57.0		
Locations	2	8	2	4	12		

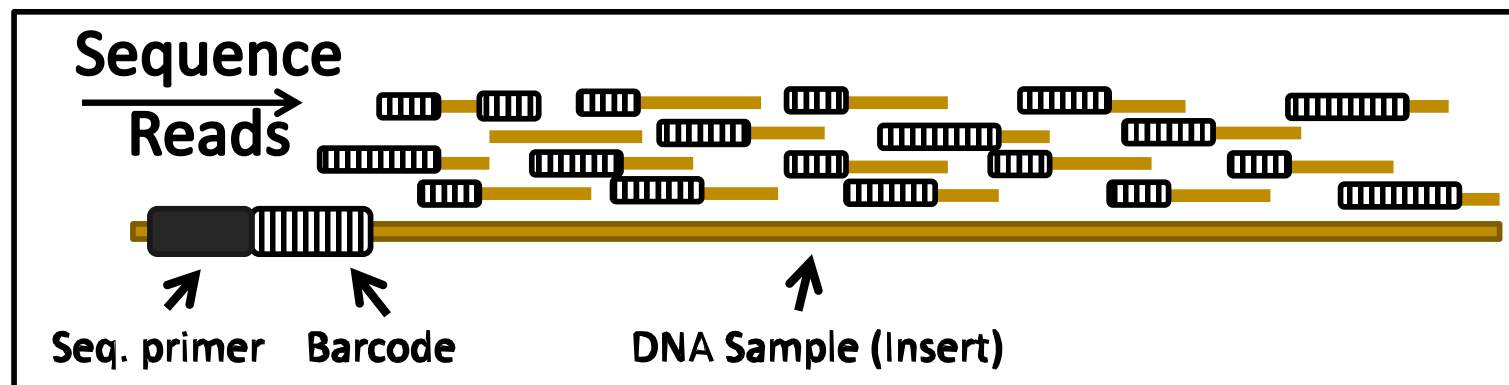
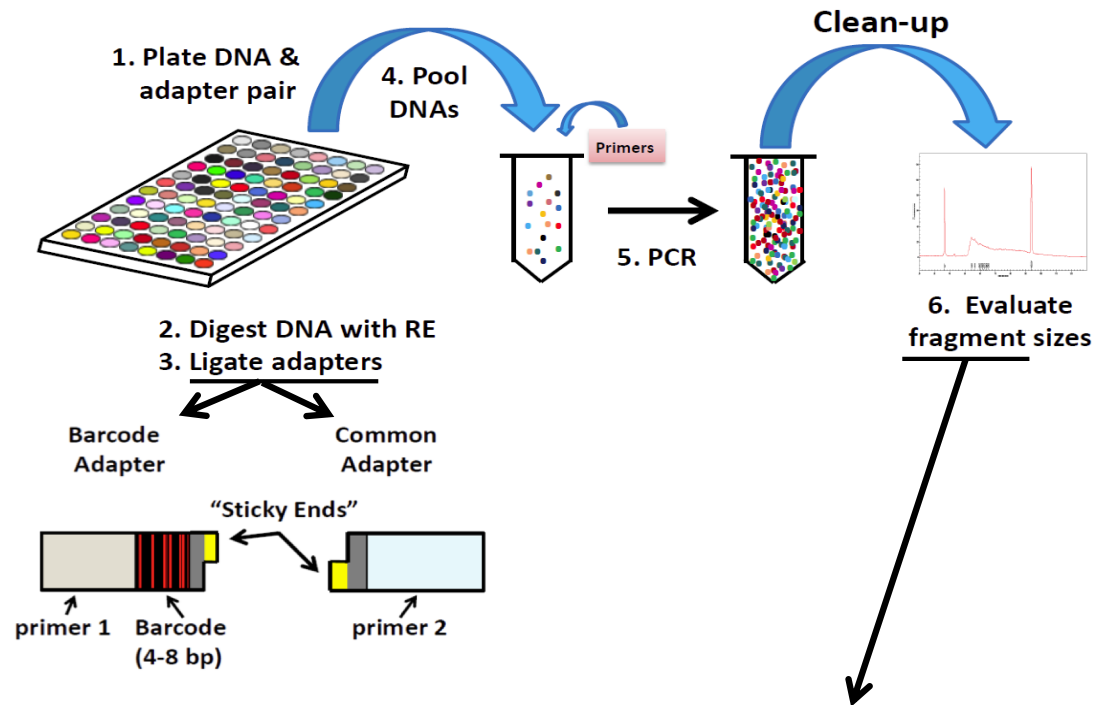
Number of Breeding Program Crosses 2011-2019



Genotyping by Sequencing (GBS)

Elshire et al., PLoS One 6(5): e19379 (2011)

Poland and Rife, Plant Genome 5:92–102 (2012)



Evolution of GBS

IWGSC Survey Sequences (v1)
+ 90K SNP array
“Pseudo-Reference”
96-plex, Illumina HiSeq 2500



IWGSC Survey Sequences
(v2 and v3)
Chromosome assembly
192-plex, Illumina HiSeq 2500



IWGSC Whole Genome Assembly
(v0.4)
Illumina + PopSeq
384-plex, Illumina HiSeq 4000

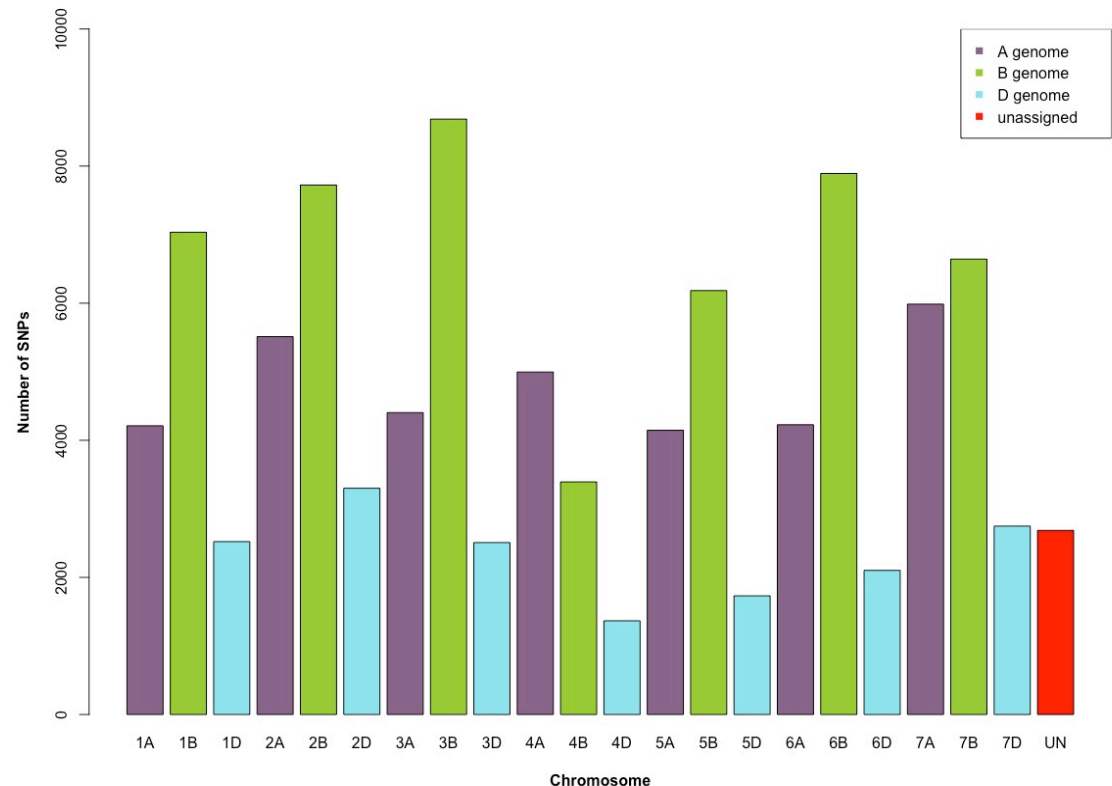


IWGSC RefSeq
(v1.0)
Chinese Spring reference
384-plex, Illumina HiSeq 4000

2012 – 22K SNPs, 384 individuals
\$19 per sample, 48% missing data



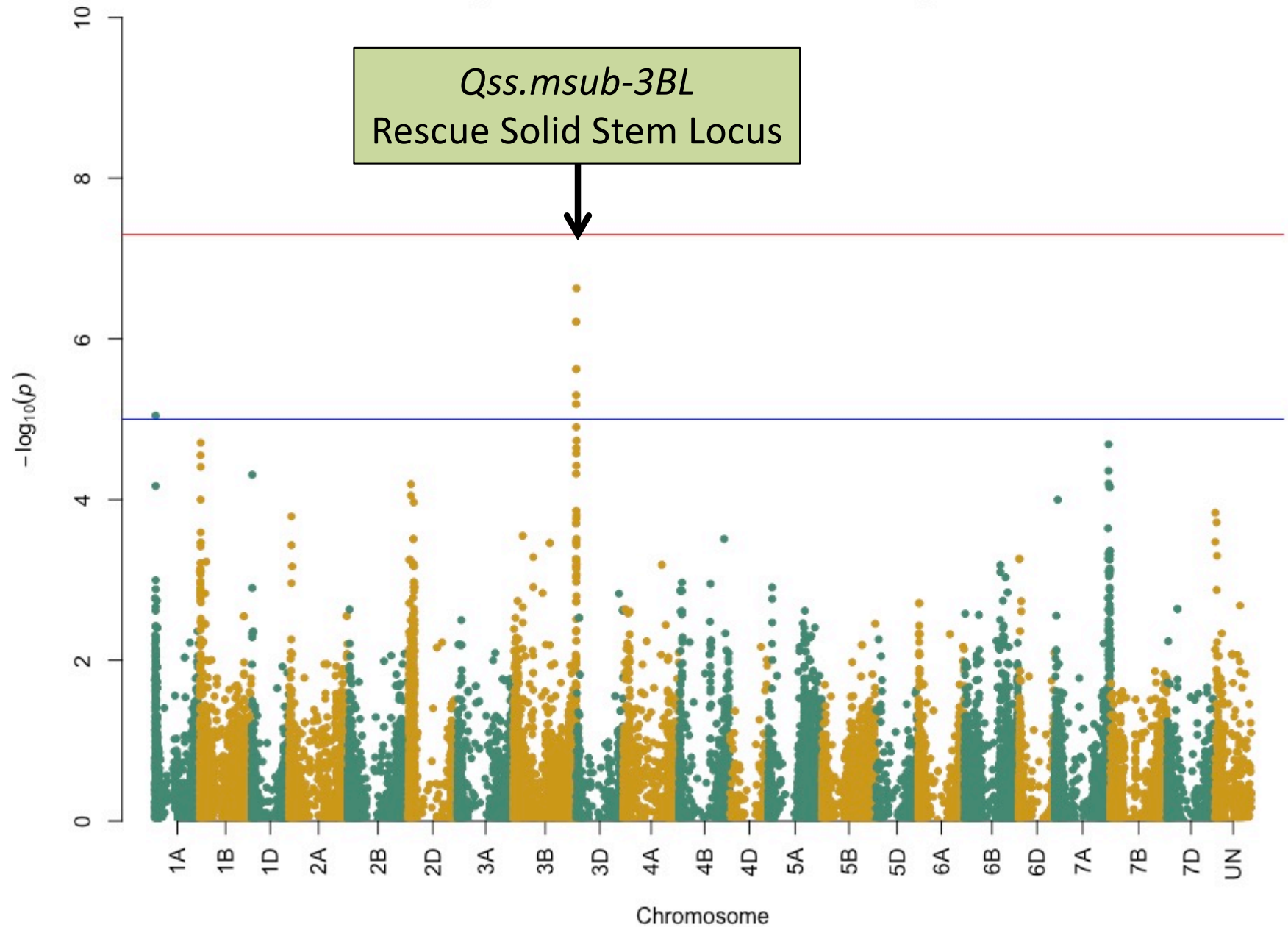
2019 – 100K+ SNPs, 20K individuals
\$8 per sample, 31% missing data



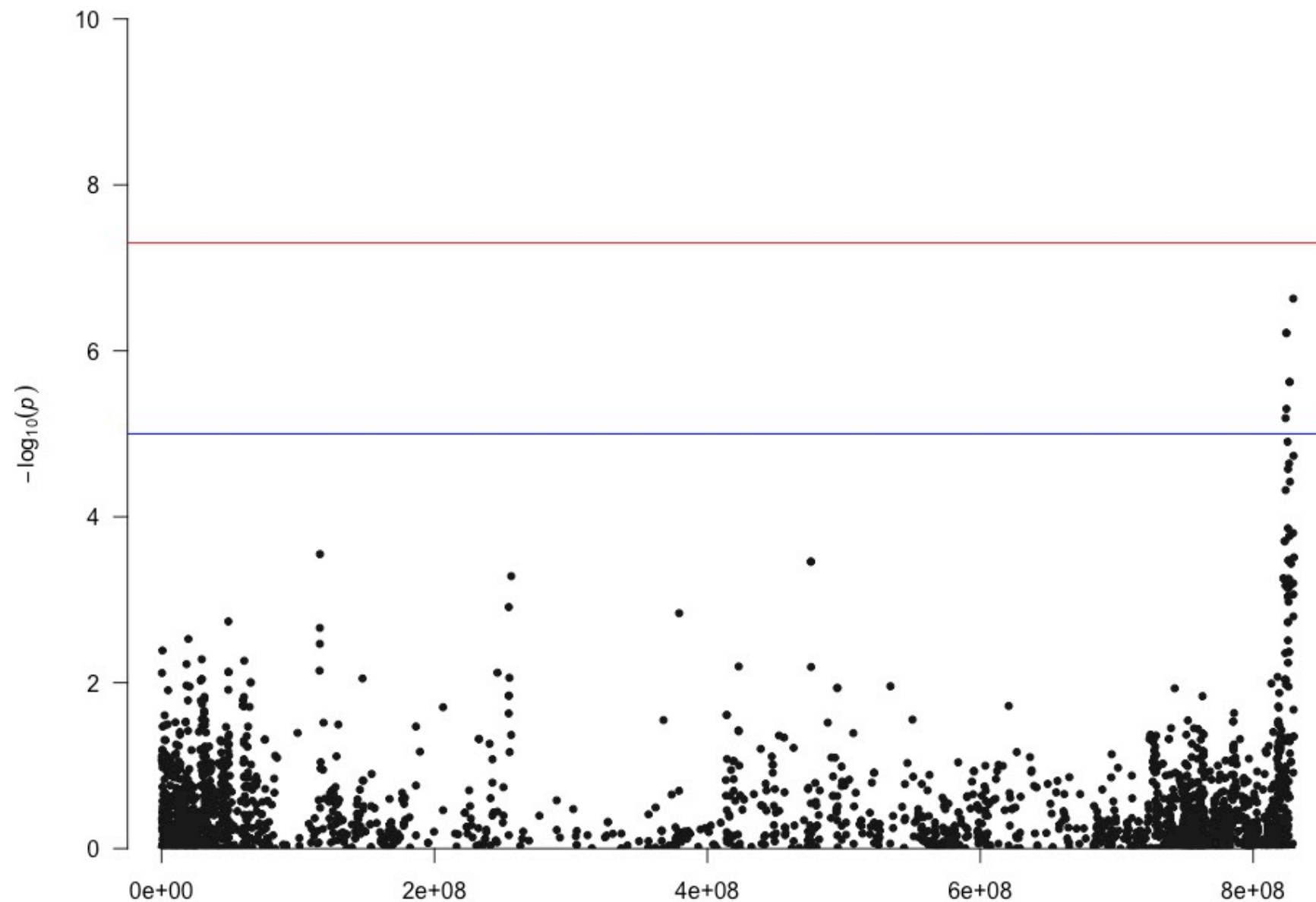
Genomics-Assisted Breeding

- High-throughput DNA markers
 - Genotyping-by-sequencing (GBS) – one-step discovery of single nucleotide polymorphisms (SNPs) across the genome
 - Inexpensive, high density, uniform distribution
 - Application leveraged across multiple breeding objectives
- Plant phenotypes
 - Grain yield
 - End-use quality – dough mixing properties, loaf volume
 - Disease resistance – stripe rust, wheat streak mosaic virus
 - Response to sawfly infestation
- Breeding applications
 - Genome wide association study (GWAS) – gene and haplotype discovery
 - Genome wide selection (GS) – breeding value estimation
- Dataset
 - New Raymer, Orchard field trials (2014-2018)
 - 459 different entries (solid-stem and hollow-stem)
 - Mixed linear model – grain yield, test weight, stem cutting

Genome Wide Association - WSS Cutting Score
(459 individuals x 29996 markers)

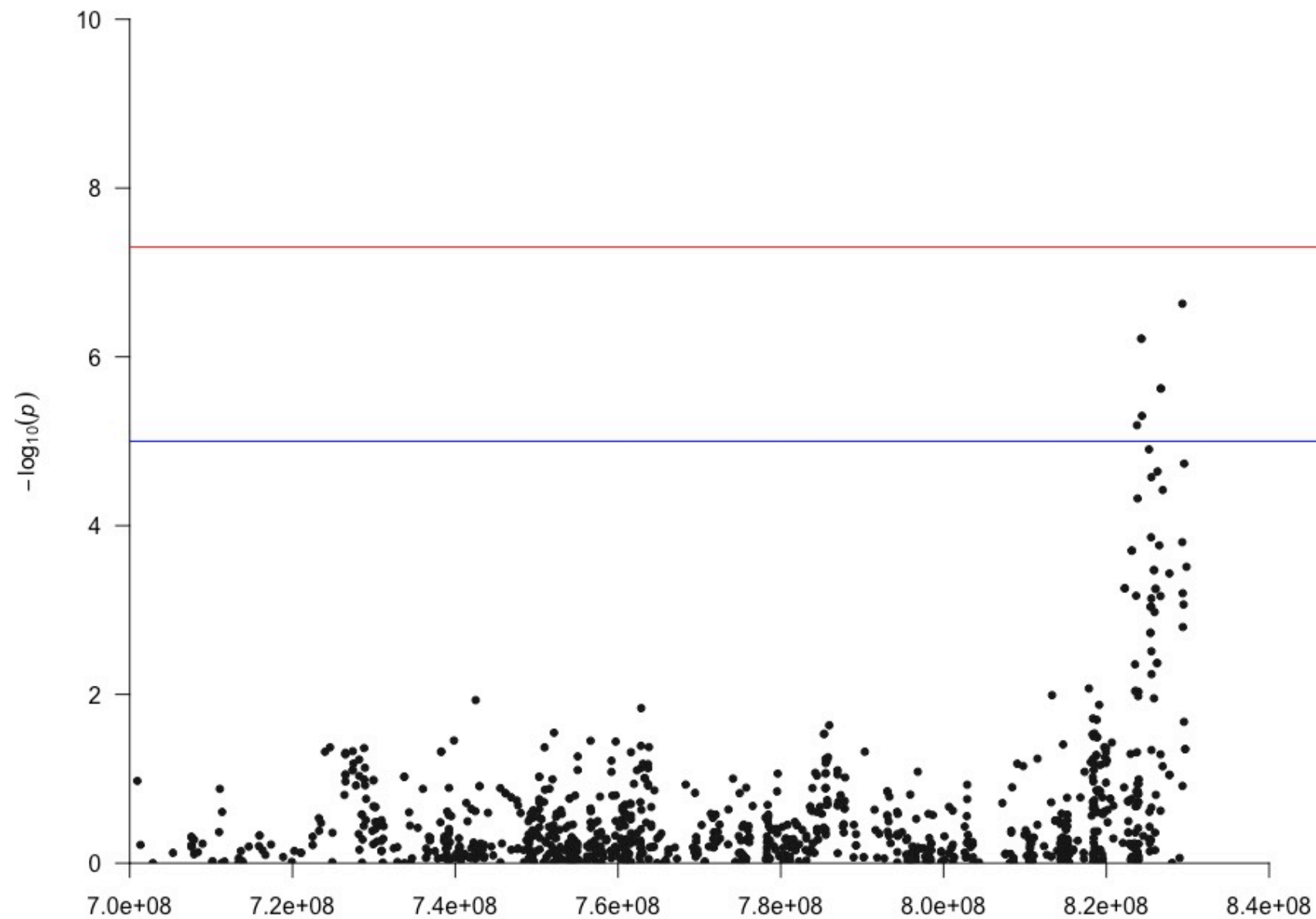


Genome Wide Association - WSS Cutting Score
(459 individuals x 29996 markers)



Chromosome 3BL Position

Genome Wide Association - WSS Cutting Score
(459 individuals x 29996 markers)



Chromosome 3BL Position

Chromosome 3BL Haplotypes – 459 individuals (subset)

3BL
QTL

Entry	Cutting	S3B_823763468	S3B_824302584	S3B_824302608	S3B_824374886	S3B_826696909	S3B_826696912	S3B_829341613
CO15R770	3.0	C	A	G	T	C	G	G
CO12D158	3.1	C	A	G	T	C	G	G
CO16D068W	3.2	C	A	G	T	C	G	G
CO15R317	3.2	C	A	G	T	C	G	G
Snowmass 2.0	3.8	C	A	G	T	C	G	G
Hatcher	4.2	C	A	G	T	C	G	G
Canvas	5.3	C	A	G	T	C	G	G
Breck	5.4	C	A	G	T	C	G	G
Antero	5.7	C	A	G	T	C	G	G
Langin	5.9	C	A	G	T	C	G	G
Avery	6.0	C	A	G	T	C	G	G
Byrd	6.3	C	A	G	T	C	G	G
Snowmass	6.3	C	A	G	T	C	G	G
Denali	6.4	C	A	G	T	C	G	G
CO15SFD107	2.4	A	C	A	C	T	A	A
CO15SFD032	2.6	A	C	A	C	T	A	A
CO15SFD024	2.9	A	C	A	C	T	A	A
Bearpaw	3.0	A	C	A	C	T	A	A
CO15SFD062	3.1	A	C	A	C	T	A	A
CO16SF029	3.3	A	C	A	C	T	A	A
CO16SFD014	4.5	A	C	A	C	T	A	A
CO16SFD016	4.9	A	C	A	C	T	A	A
CO16SFD004	5.3	A	C	A	C	T	A	A
CO16SFD008	6.0	A	C	A	C	T	A	A
CO16SF078	2.4	A	C	A	C	T	A	A
CO16SF070	2.9	A	C	A	C	T	A	A
CO16SF065	3.1	A	C	A	C	T	A	A
CO16SF049	4.4	A	C	A	C	T	A	A
CO16SF076	4.5	A	C	A	C	T	A	A
CO16SF071	4.5	A	C	A	C	T	A	A
CO15SFD092	2.8	C	C	A	C	T	A	A
CO11D444	4.6	C	C	A	C	T	A	A
CO14R466	3.7	C	A	G	T	T	A	A
CO15D098R	4.0	C	A	G	T	T	A	G
TAM 114	4.8	C	A	G	T	T	A	G

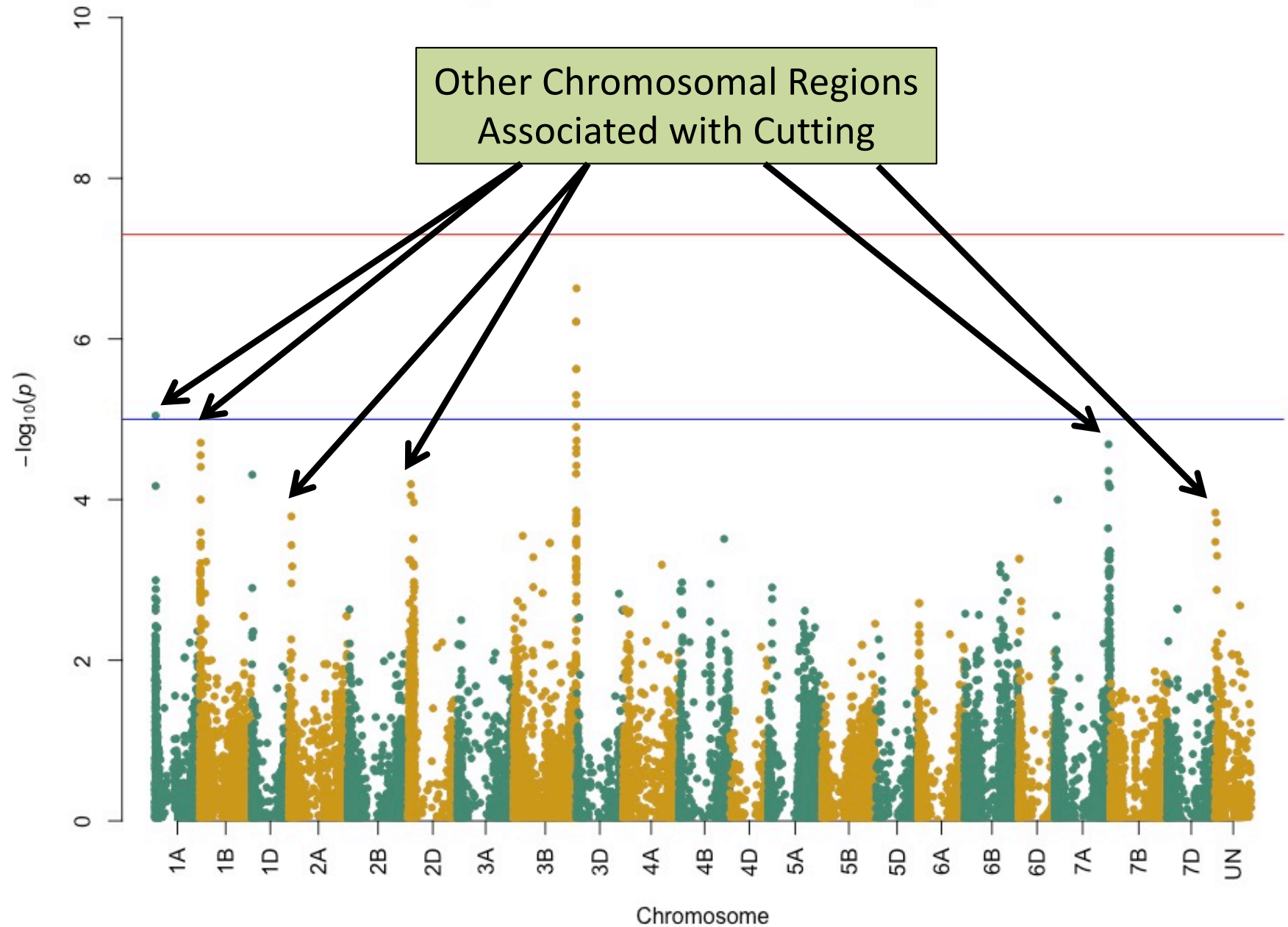
NA

Bearpaw

Judee

misc

Genome Wide Association - WSS Cutting Score
(459 individuals x 29996 markers)



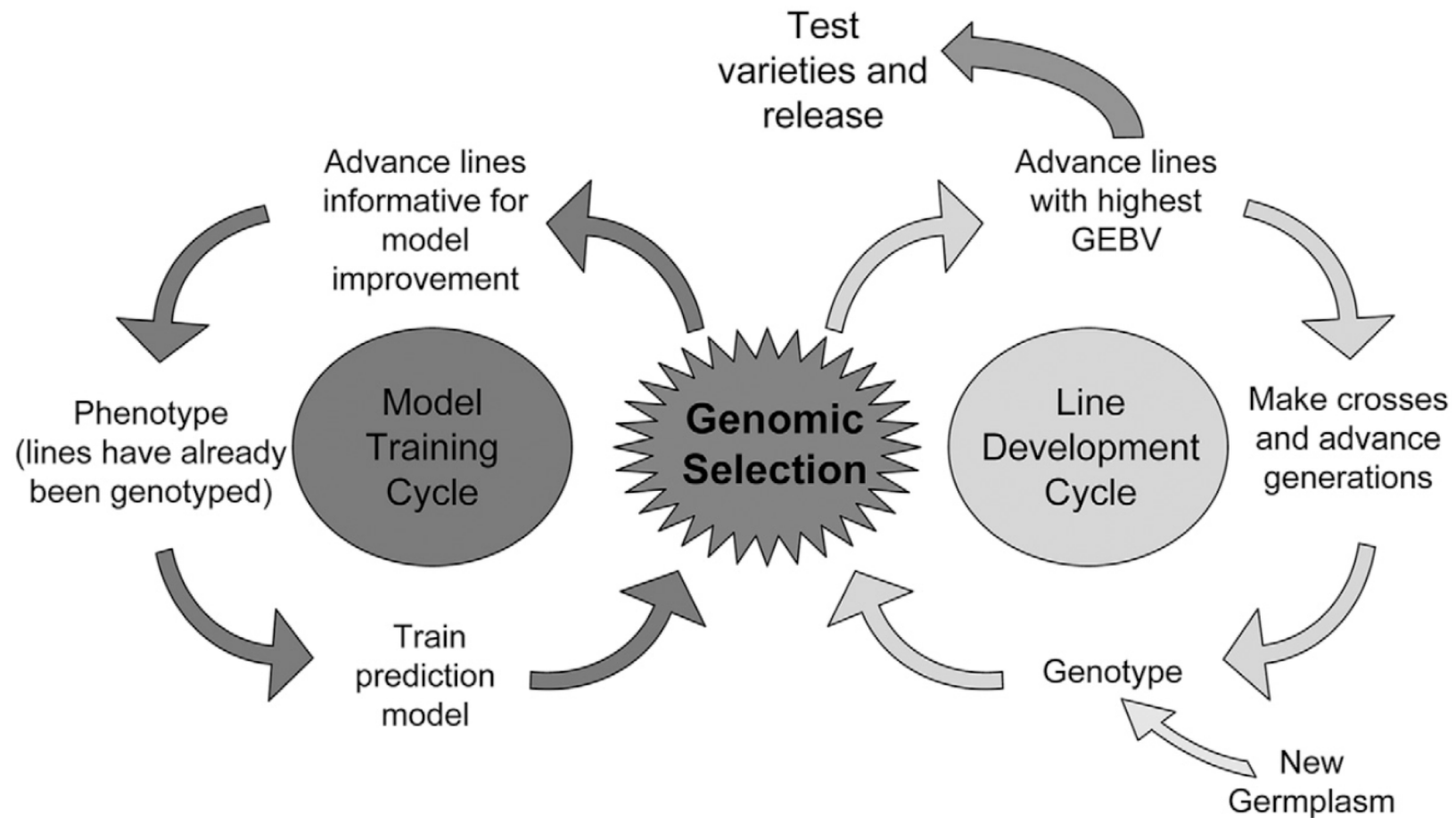
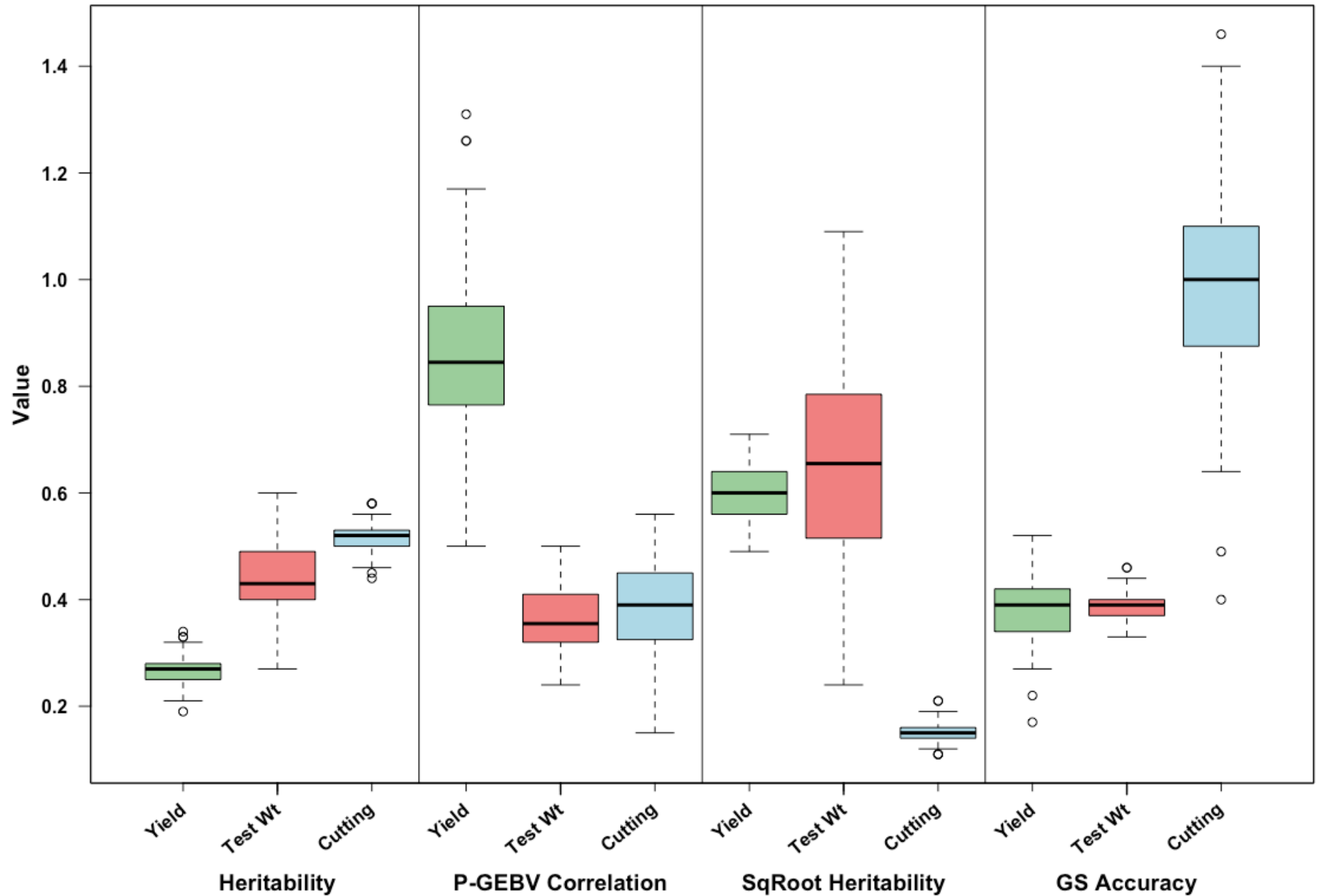


Figure 2. Flow diagram of a genomic selection breeding program. Breeding cycle time is shortened by removing phenotypic evaluation of lines before selection as parents for the next cycle. Model training and line development cycle length will be crop and breeding program specific. (GEBV = genomic estimated breeding value.)

Elliot L. Heffner, Mark E. Sorrells, and Jean-Luc Jannink
Genomic Selection for Crop Improvement
Crop Science 49:1-12 (2009)

GS Prediction Accuracy and Trait Heritability

Five-fold cross validation (n=100 iterations)



Closing Thoughts

- Deployment of adapted semi-solid and solid cultivars
 - Adoption of initial cultivars – what level of yield drag is acceptable?
 - Integration with sawfly population "forecasting"?
 - Is semi-solidness adequate under heavy infestation?
- Resistance sources – diversification, durability, effectiveness, stacking
 - *Bearpaw, Judee (Rescue)* – *Qss.msub-3BL*
 - *Conan* – early expression of solidness
 - *Sr2* – adult plant stem rust resistance
 - *PI 166471* (Turkish landrace) – three new QTLs on chromosome 1B
 - Non solid-stem based resistance – non-preference? antibiosis?
- Applied wheat cultivar development
 - Genomics-assisted breeding
 - Stripe rust resistance (etc.) – Byrd derivatives as base
 - Hard red and hard white varieties (Ardent-CWRF Premium Program)
 - Herbicide tolerance – *Clearfield**, *CoAXium™*

Acknowledgements



Colorado Wheat
Administrative Committee



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Darren Cockrell

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Questions?