"So many genomes, so little time: the future of plant breeding"



David Francis (francis.77"at"osu.edu)



$\Delta G = k^* \sigma_{p}^* h^2$ Everything you need to know about breeding is in this equation

- The importance of genetic variation $(h^2 = \sigma_G / \sigma_P)$
- K, σ_p, h² are subject to disruptive technologies which may improve efficiency* of selection (or may just cost a lot of money).

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Larger populations improve measurement of σ_G

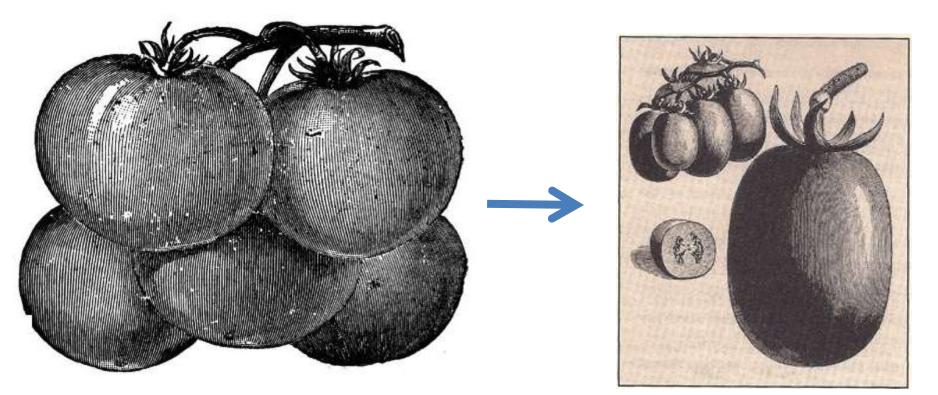
Efficiency

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"Those that fail to learn from history, are doomed to repeat it." **Winston Churchill**

"We learn from history that we learn nothing from history." George Bernard Shaw Plant breeding has always been more effective when coupled to new technology, whether that technology is production, harvesting, or breeding (e.g. development of mechanically harvested tomato)



S. pimpinellifolium, S. galapagense (plant form) S. pennellii, S. peruvianum, S. pimpinellifolium (resistance) as source of σ_{G}









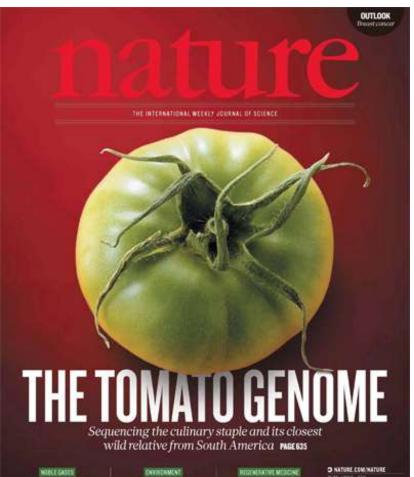
Many of the differences between market classes are due to genes from wild species **o**_G

Take home messages:

- NGS sequence data is providing a resource for allele identification, but...
 - Lack Reference (elite material)
 - Lack structure-function information
- Fastest way to evaluate new alleles is breeding
- CRISPR/CAS9 is the newest mutation breeding tool (loss of function will be more common)
- GS models show promise for prediction of performance
- Large populations and biological assessment Knowledge of traits and trait relationships

Since October 2014 We have ~500 tomato genomes in public databases: how can we use this information?

Tomato Genome Consortium - 2012 (Reference sequence)



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Resequencing projects 2014

Selected accessions Variant browner (fireFox) Unta Access Agrowment News Project Partners	ation Res	search	Publications	News & Calendar	About Wageningen UR	Work at	Phone book	Contact	
Selected accessions Two factors are essential for continued improvement of crop species by plant Variant browner (FireFox) breeding, tools to identify adequate genetic variation, and technology to efficiently (rejcombine useful ateles in new breeding lines. Material from wild relatives, ancestors and landraces held in germplasm collections of crop species contains ar underexploited wealth of genetic variation, and will therefore offer a useful gene pool to cope with existing and new breeding challenges.			1	50 Tomato Ge	enome ReSequer	ncing pro	oject		
Variant browner (FireFox) breeding, tools to identify adequate genetic variation, and technology to efficiently (re)combine useful ateles in new breeding lines. Material from wild relatives, ancestors and landraces held in germplasm collections of crop species contains ar underexploited wealth of genetic variation, and will therefore offer a useful gene pool to cope with existing and new breeding challenges.	ome :		-			**************************************	60.00		
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RESOURCE

Exploring genetic variation in the tomato (Solanum section Lycopersicon) clade by whole-genome sequencing



アクセス 日本語要約にアクセスするためには、Nature Geneticsの構造が必要です(右参照)。

nature.com > Journal home > Table of Contents

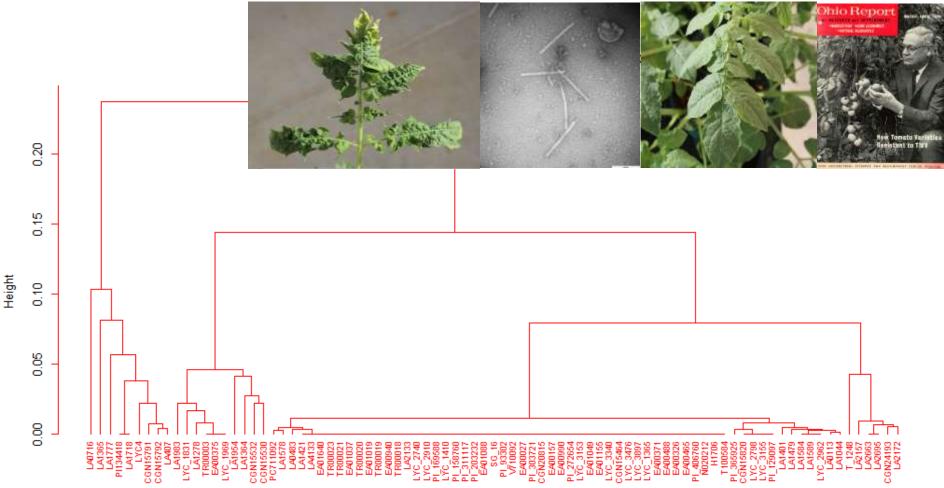
Article

Nature Genetics 46, 1220-1226 (1 November 2014) | doi:10.1038/ng.3117

Genomic analyses provide insights into the history of tomato breeding

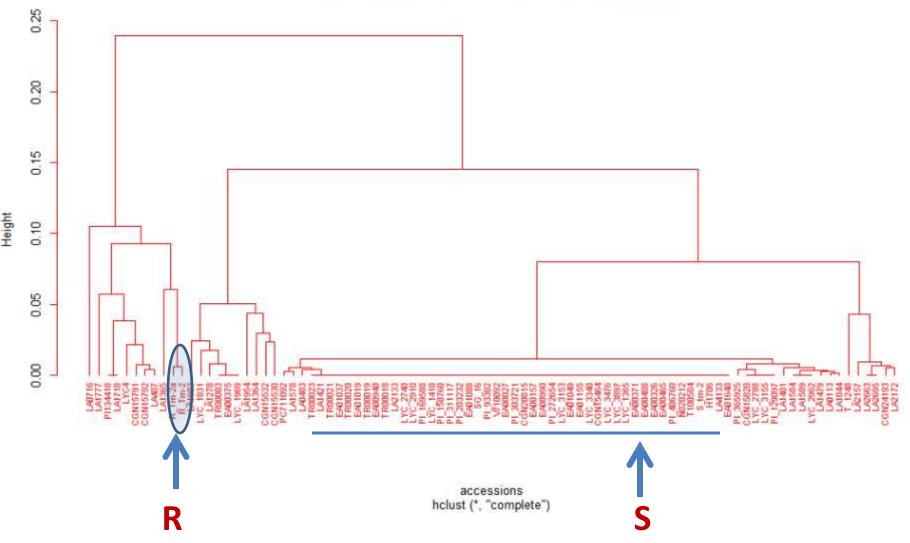
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Cluster of tm-2 alleles from of 85 unique genomes in "150 genome" project



accessions hclust (*, "complete")

Cluster of tm-2 alleles from of 85 unique genomes in "150 genome" project + 2 resistance alleles Tm-2 and Tm-2^a (let's sequence relevant germplasm...) Cluster of tomato accessions based on TM2 locus





- Evaluation of accessions for Tm2 DNA Sequence, virus symptoms, and antibody reactivity.
- Resistant phenotypes found on most nodes of accession tree
 Susceptible phenotypes found on most nodes of accession tree.
- Only predictability when we all ready know the allele.
- We lack sufficient structure-function information to predict new resistance alleles.

Efficient Gene Editing in Tomato in the First Generation Using the Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-Associated9 System¹

Christopher Brooks, Vladimir Nekrasov*, Zachary B. Lippman*, and Joyce Van Eck*

Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724 (C.B., Z.B.L.); Sainsbury Laboratory, Norwich Research Park, Norwich NR4 7UH, United Kingdom (V.N.); and Boyce Thompson Institute for Plant Science, Ithaca, New York 14853 (J.V.E.)

Creation of new genetic variation

- "Gene Editing" technologies
- 1) Zinc finger nucleases
- 2) Transcription activator-like nucleases (TALENs)
- 3) Clustered regularly interspaced short palindromic repeats
- (CRISPR)/CRISPR-associated 9(Cas9) endonuclease

1 and 2 are based on protein-DNA interactions, 3 is an RNA-guided DNA endonuclease system

To alter a target gene, supply Cas9 and the guide RNA Cas9 **Target DNA** VGG 20nt 5' **crRNA**

Easier to knock out (remove function) than to create a new function – there are exceptions to this rule

Image from: Horvath P, Barrangou R (2010). "CRISPR/Cas, the immune system of bacteria and archaea". Science **327** (5962): 167–170. tracrRNA

Andolfo et al. BMC Plant Biology 2014, 14:120 http://www.biomedcentral.com/1471-2229/14/120

How do we identify targets?

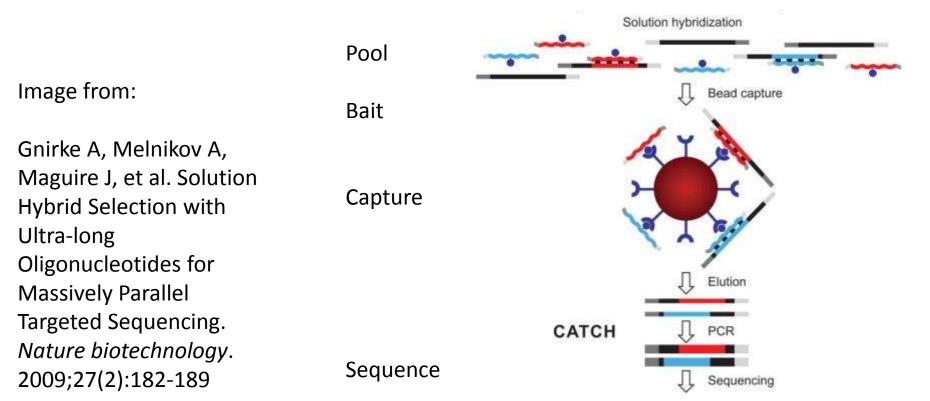
RESEARCH ARTICLE



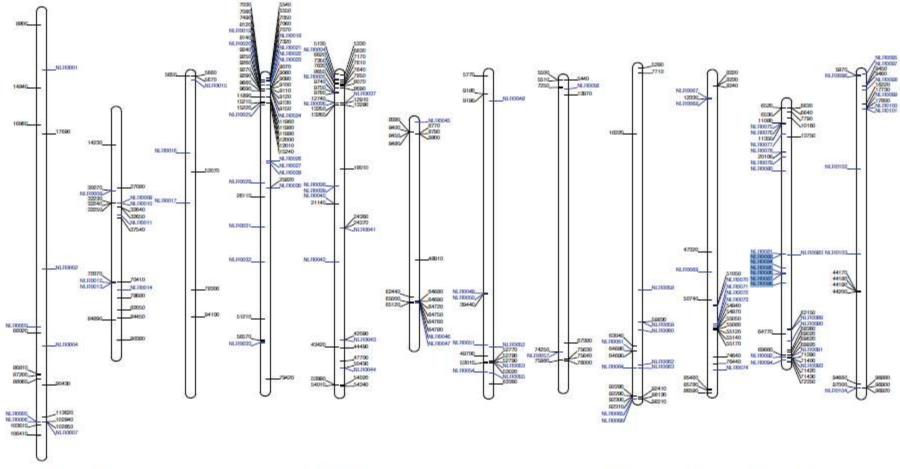
Open Access

Defining the full tomato NB-LRR resistance gene repertoire using genomic and cDNA RenSeq

Giuseppe Andolfo^{1,2}, Florian Jupe^{1*}, Kamil Witek¹, Graham J Etherington¹, Maria R Ercolano² and Jonathan D G Jones^{1*}



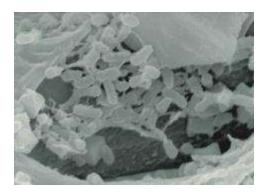
S. lycopersicum Heinz 1706



Chr1 Chr2 Chr3 Chr4 Chr5 Chr6 Chr7 Chr8 Chr9 Chr10 Chr11 Chr12

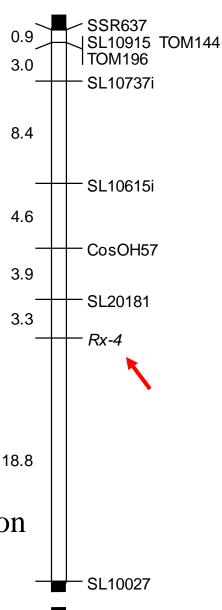
Figure 3 Chromosomal distribution of Heinz 1706 NB-LRR genes. The previously annotated NB-LRR genes [7] are shown in black and those discovered in this study are blue. Genes depicted to the left of the chromosome are on the forward strand and those on the right are on the reverse strand.

Example: T3 resistance from PI128216 IBC population



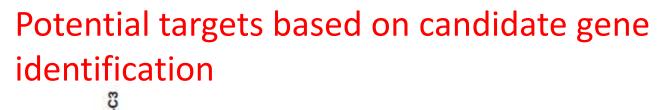
Robbins et al., 2009. Phytopathology

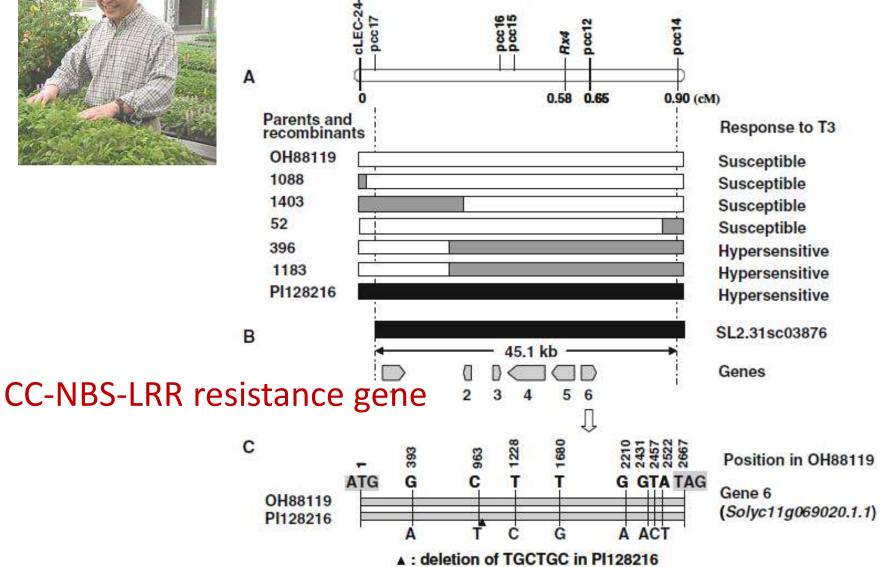
4 markers in 30 cM on chromosome 11

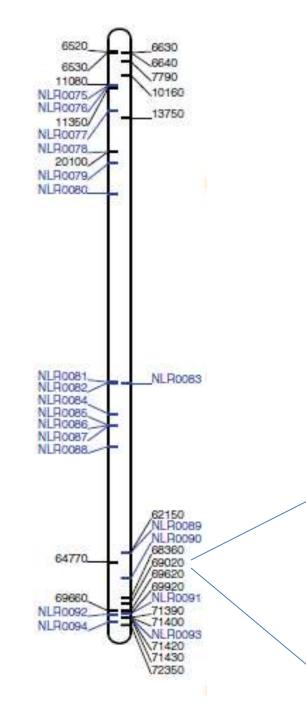


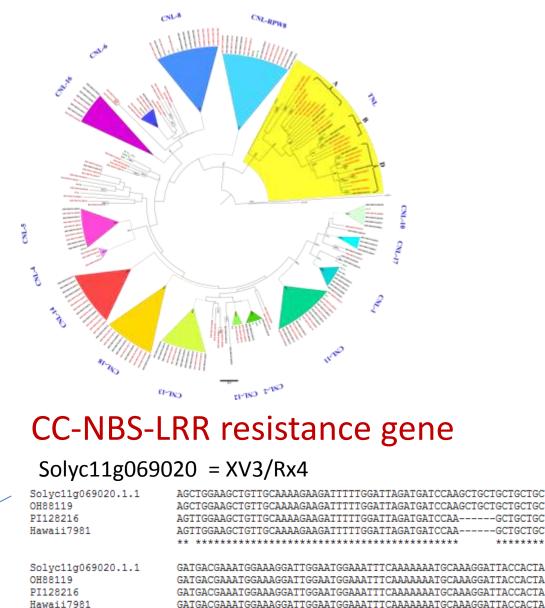












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OH88119

PI128216

Hawaii7981

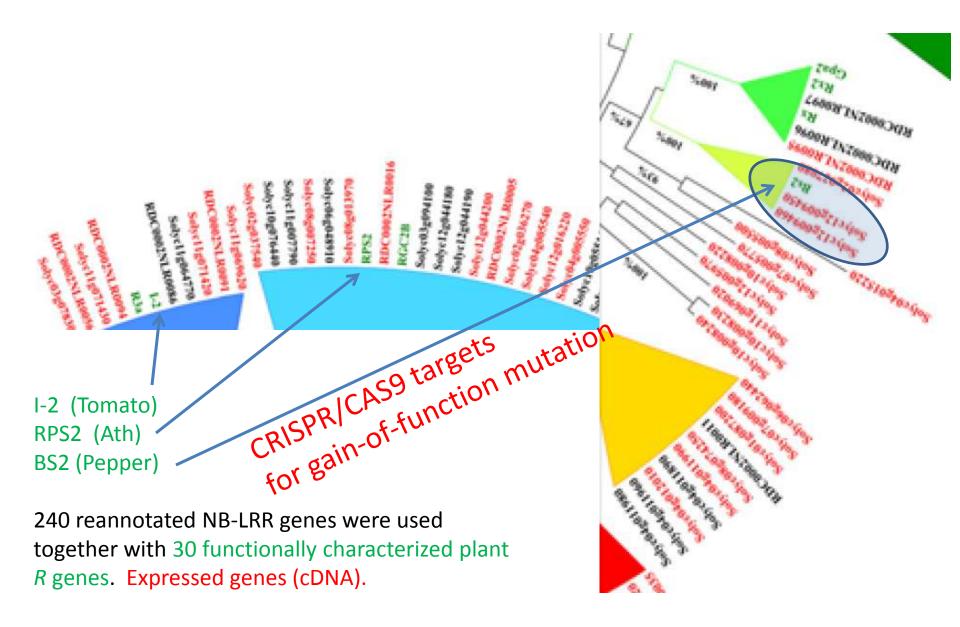
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-GCTGCTGC

GCTGCTGC



CNL-RPW8



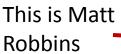
Yield Analysis and Prediction

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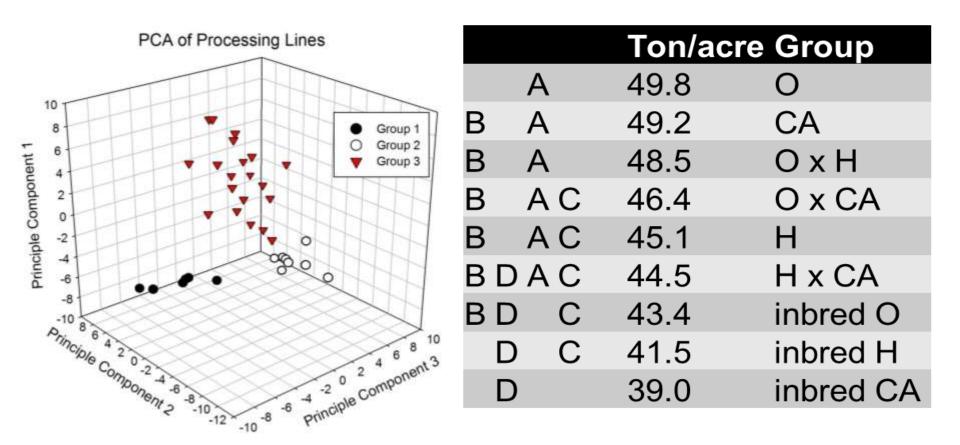
Why is David excited about GS and prediction?





Long-term Goal: Learn how to make a better hybrid

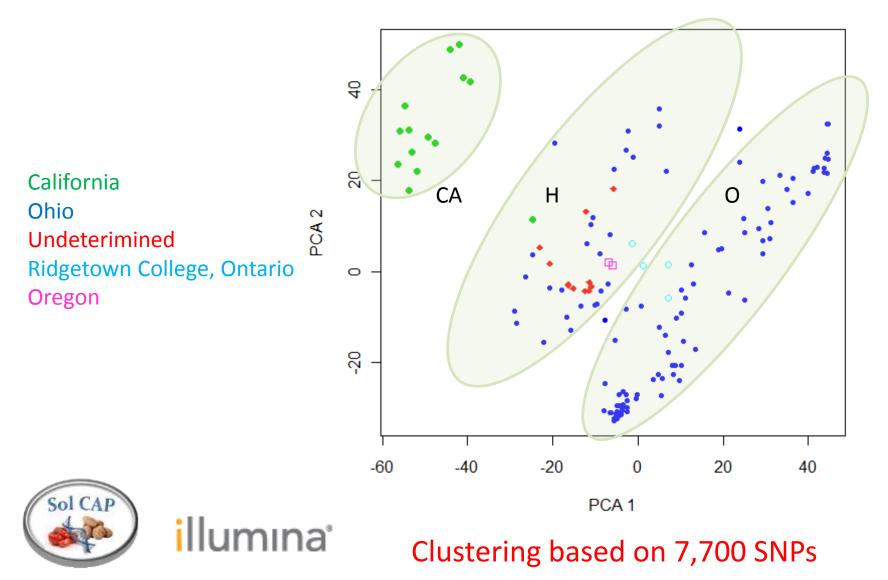
- Evaluate a partial diallele with 96 hybrids and parents (2 years, 2 locations, 2 reps/location RBC)
- Some evidence for hybrid vigor (hybrids preform ~5-10% better than parents)
- No evidence for heterotic groups



Best prediction of hybrid performance was parent performance (good parents make good hybrids)

Diversity in tomato populations (processing)

PC 1 vs PC 2



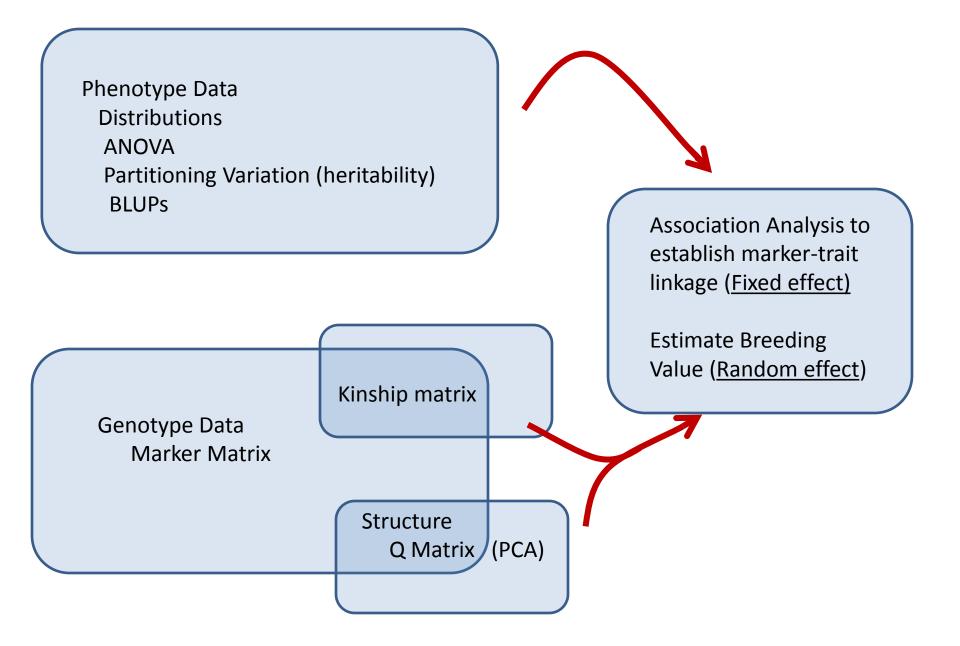
Genome Wide Selection (*Meuwissen T.H., Hayes B.J., Goddard M.E.* (2001) Prediction of total genetic value using genome-wide dense marker maps. *Genetics, 157, 1819–1829.*)

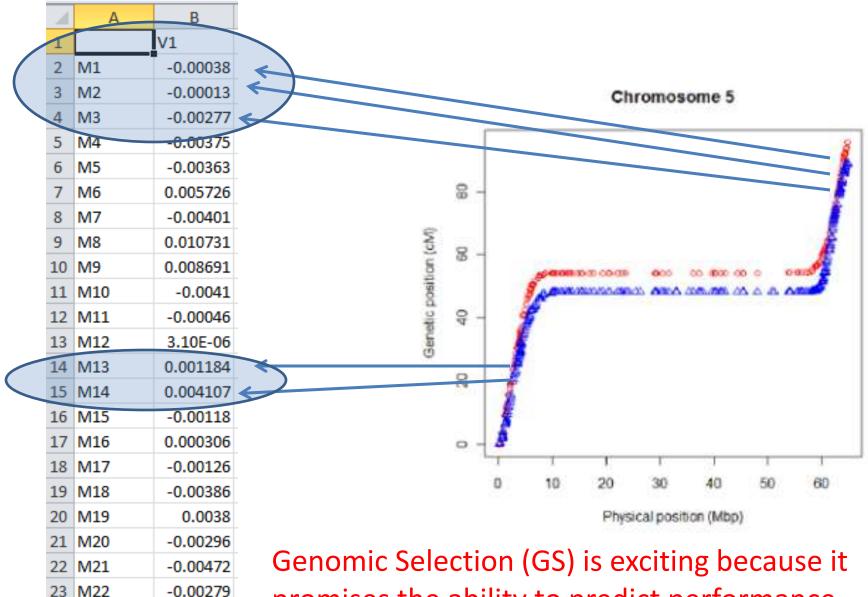
- Detection of sequence variation is no longer limiting
- Promise of predictive models

$\gamma = \mu + \ddot{X} \beta + \in$

 γ is the vector of phenotypic values for n individuals $\ddot{\mathbf{X}}$ is the n x K marker matrix for K markers $\boldsymbol{\beta}$ is the estimated breeding value associated with each marker

Goal: Predict the performance of an individual based on genotype



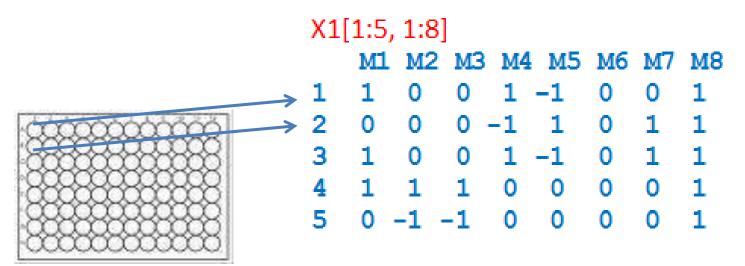


24 M23

0.002641

promises the ability to predict performance based on sequence variation (C/T).

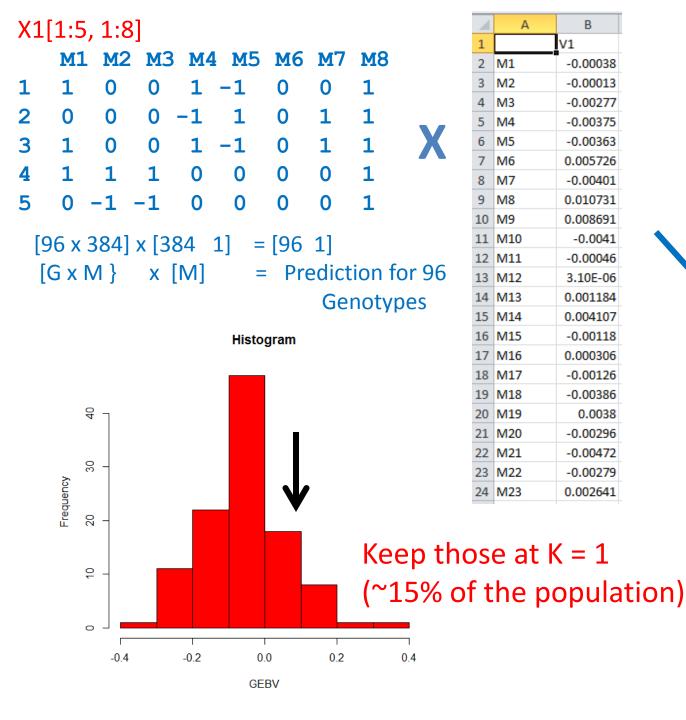
How prediction models work:



DNA extracted and allele specific assays conducted to determine genotypes.

The result is a matrix of marker data coded based on allele dose.

Selections made based on "model" (developed from previous experience with performance and sequence information).



	А	В
1		x
2	14	-0.36451
3	17	-0.2875
4	104	-0.28532
5	15	-0.27402
6	76	-0.27182
7	96	-0.27018
8	8	-0.26794
9	9	-0.25418
10	49	-0.24986
11	92	-0.20341
12	23	-0.20324
13	11	-0.20033
14	34	-0.19169
15	66	-0.18804
16	39	-0.18763
17	65	-0.17746
18	16	-0.16764
19	28	-0.16176
20	89	-0.16098
21	100	-0.14011
22	4	-0.13922
23	69	-0.138
24	63	-0.13526
25	35	-0.13342
26	48	-0.12348

В

-0.00038

-0.00013

-0.00277

-0.00375

-0.00363

0.005726

-0.00401

0.010731

0.008691

-0.0041

-0.00046

3.10E-06

0.001184

0.004107

-0.00118

0.000306

-0.00126

-0.00386

-0.00296

-0.00472

-0.00279

0.002641

0.0038

٧1

SolCAP Collection 140 inbred lines evaluated in CA and OH (2 year, 2 locations RBC design)

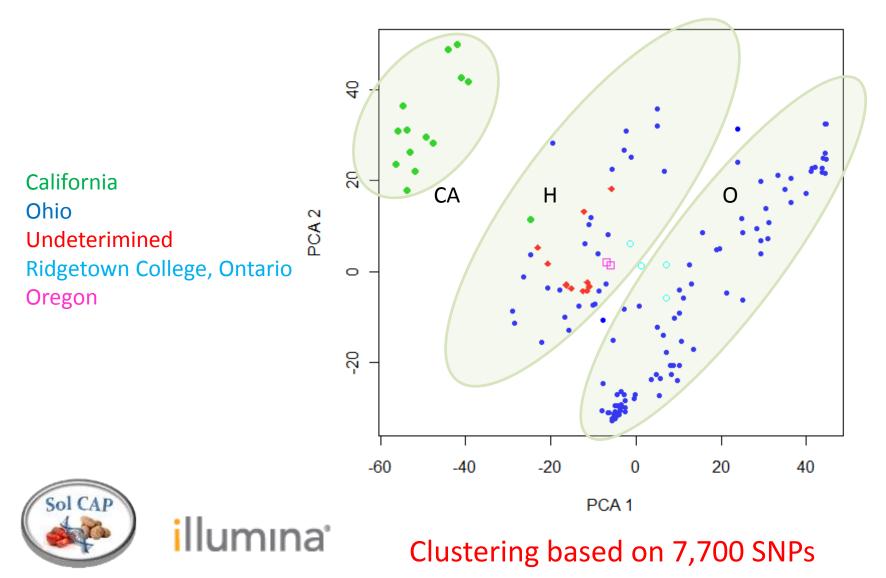
288 Nested RIL progeny (OH2641 x OH987034; OH7814 x OH987034; OH2641 x OH981136) (O x H; O x H, O x CA)

Augmented Experimental design (2 year, 2 locations)



Populations (SolCAP) and (OxH; OxH, OxCA)

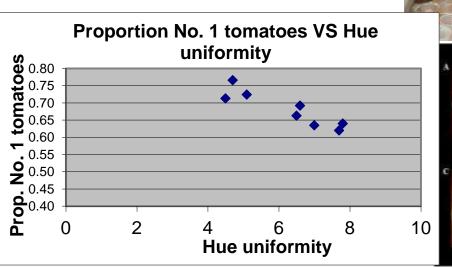
PC 1 vs PC 2



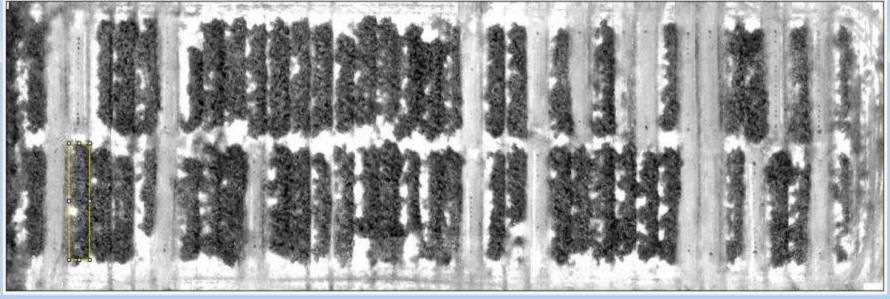
Traits: Total traits measured: 52 Yield, digital phenotyping and chemical meas.

Reduced to 22 most informative (h², PCA and other methods)

- Yield (total and marketable)
- Color and Color uniformity
- BRIX
- pH
- Vitamin C
- Fruit Size and Shape







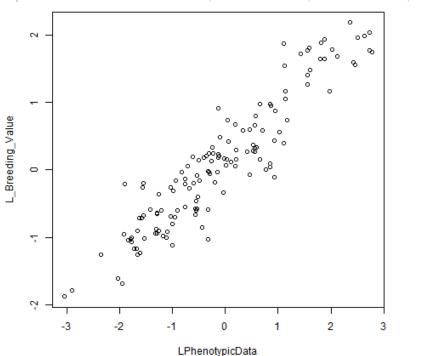


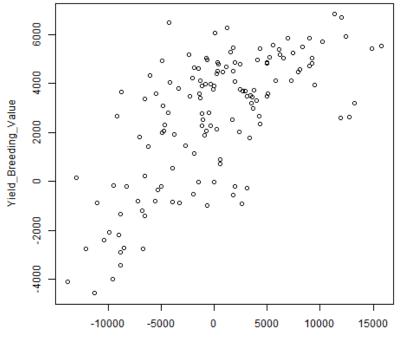


Predicting inbred-line performance from inbred line data Data from SolCAP data (140 varieties, 7,700 markers)

Trait	P_value	R ²
Color_Unifromity	3.99E-69	0.895
L	3.98E-61	0.863
hue	8.97E-52	0.812
Fruit Size	1.15E-30	0.618
Yield	6.11E-20	0.454
Brix	1.77E-66	0.885



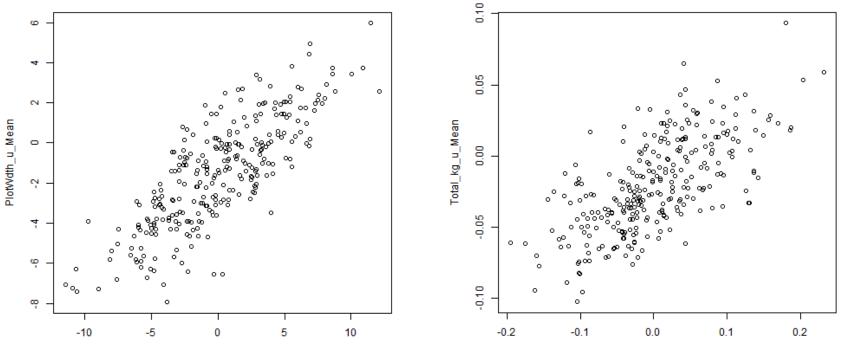




YieldPhenotypicData

Predicting inbred-line performance from inbred line data Results from nested RIL (280 x 384 markers)

Trait	P_value	R ²	
PlotWdth	1.75E-64	0.639	
Total_kg	1.29E-38	0.449	← Yield
Total_Frt	6.27E-33	0.396	
Ripe_kg	1.48E-16	0.212	
Ripe_Frt	1.64E-35	0.421	
Frt Size	7.02E-68	0.658	← Fruit Size



PlotWdthPhenoInfo

Total_kgPhenoInfo

Predict hybrid performance from inbred data

Training Populations (Genotype and Phenotype) SolCAP (inbreds) Nested RIL (inbreds)

Hybrids

Predict genotype from inbred data Predict performance using GW model(s) developed for inbreds

Compare prediction with actual performance

ļ!	M1	M2	M3	M4	M5
Inbred 1	1	1	0	0	0
Inbred2	1	0	1	1	0
Inbred3	0	1	0	1	1
F1 1x2	1	0.5	0.5	0.5	0
F1 1x3	0.5	1	0	0.5	0.5
F1 2x3	0.5	0.5	0.5	1	0.5
ł	-lybrid ge	enotype	matrix		
(actimated from inbrod gapatypes)					

(estimated from inbred genotypes)

Μ

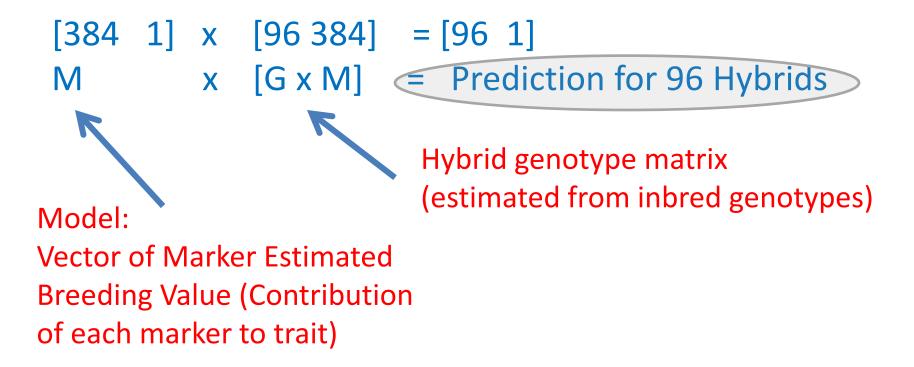
 $[384 \ 1] \ x \ [96 \ 384] = [96 \ 1]$ x [G x M]

Model:

Vector of Marker Estimated **Breeding Value (Contribution** of each marker to trait)

	А	В
1		V1
2	M1	-0.00038
3	M2	-0.00013
4	M3	-0.00277
5	M4	-0.00375
6	M5	-0.00363
7	M6	0.005726
8	M7	-0.00401
9	M8	0.010731
10	M9	0.008691
11	M10	-0.0041
12	M11	-0.00046
13	M12	3.10E-06
14	M13	0.001184
15	M14	0.004107
16	M15	-0.00118
17	M16	0.000306
18	M17	-0.00126
19	M18	-0.00386
20	M19	0.0038
21	M20	-0.00296
22	M21	-0.00472
23	M22	-0.00279
24	M23	0.002641
	-	

	M1	M2	M3	M4	M5
Inbred 1	1	1	0	0	0
Inbred2	1	0	1	1	0
Inbred3	0	1	0	1	1
F1 1x2	1	0.5	0.5	0.5	0
F1 1x3	0.5	1	0	0.5	0.5
F1 2x3	0.5	0.5	0.5	1	0.5



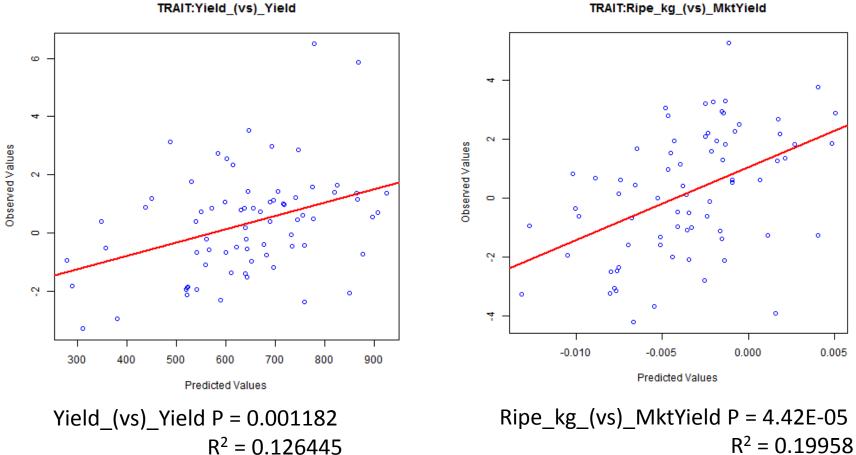


How does predicted performance relate to actual performance?

Predicting Hybrid Performance from inbred-line data

Model from SolCAP (n = 140 K = 7,700)

from RIL (n = 280 K = 384)

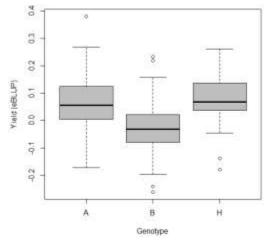


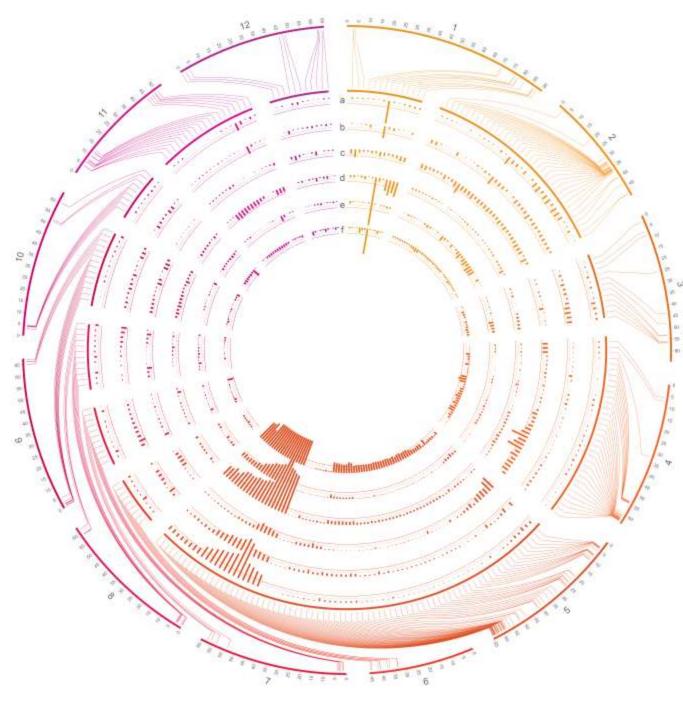
TRAIT:Ripe kg (vs) MktYield

Association analysis

a = Yield as BLUP b = Yield normalized c = fruit weight d = fruit number e = vine size

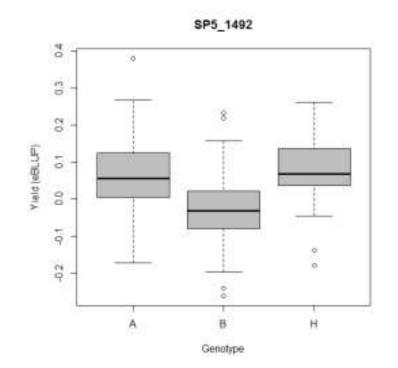
SP5_1492





Currently: Testing to see if additive models can be improved by incorporating non-additive effects

	А	В
1		V1
2	M1	-0.00038
3	M2	-0.00013
4	M3	-0.00277
5	M4	-0.00375
6	M5	-0.00363
7	M6	0.005726
8	M7	-0.00401
9	M8	0.010731
10	M9	0.008691
11	M10	-0.0041
12	M11	-0.00046
13	M12	3.10E-06
14	M13	0.001184
15	M14	0.004107
16	M15	-0.00118
17	M16	0.000306
18	M17	-0.00126
19	M18	-0.00386
20	M19	0.0038
21	M20	-0.00296
22	M21	-0.00472
23	M22	-0.00279
24	M23	0.002641



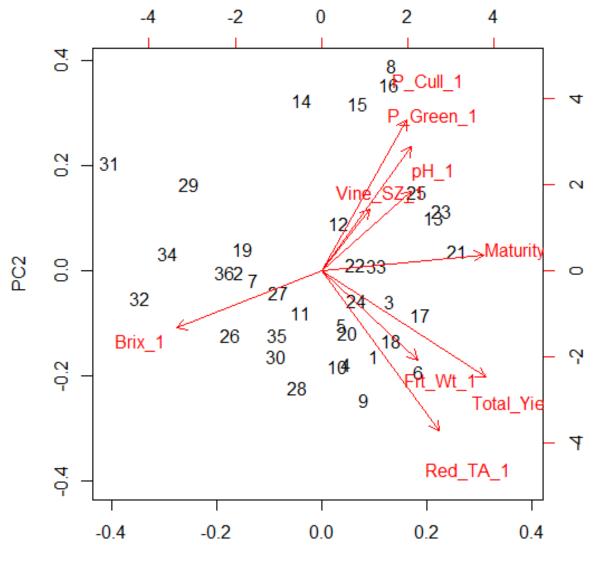
= better prediction?

Ability to predict performance based on genetics is an exciting development, but "beyond mountains there are mountains".

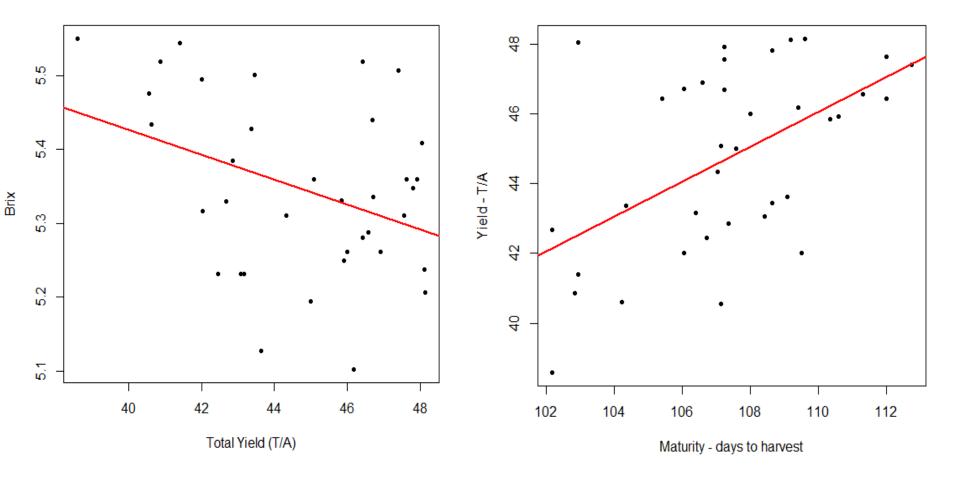
- What are we selecting for $(\gamma) \gamma = \mu + \ddot{X} \beta + \in ?$
- Yield, Brix, Color, Viscosity, Resistance (n = 5-17)
- GWS for multiple traits requires that we have robust Multi Trait Indices (MTI) for selection



Trait weights



PC1



<u>Trait</u>	Weight		
Yield	+2		
Color	-1		
Brix	0		
etc			

Traits: What can historical data tell us about trait models?

California PTAB

- 20 years
- 585 varieties
- 8.8 million of loads

Farm Advisor Variety Trials

- 10 years
- 173 varieties

Traits Measured

- Yield
- Brix
- рН
- Color
- Defects
- Number of loads produced
- Can we predict performance in market with traits value?



Model	P-value	Percent	Stand.
WIOUEI	I -value	accuracy	dev.
MTI ₂ ^V	0.001	35	9
yield	0.03	29	9
Yield xBrix	0.534	23	12
random	-	14	7

V = Multi-Trait Index weighting Yield, BRIX, Color and limited use.
 Evaluated based on ability to predict market success (% load and longevity) in varieties from PTAB and UCCE year data set.

Conclusions: Response to challenges can be faster 1) Genetic resources remain a foundation; 2) Sequence data in public data bases allows rapid evaluation of new alleles for function, the best way to do this is still through breeding (Eka's talk); 3) Whole genome models have predictive capability for individual performance and hybrid performance; prior knowledge of QTL position helps (Debora's talk); 4) Population size is more important than marker number; 5) Knowledge of selection targets will be key to success.

Thank you for your time.

Questions?







Some up coming meetings



SolGenomics: From Advances to Applications



September 12 – 16, 2016 • Davis California USA

Shameless advertisement:



Plant Breeding Summer Workshops

Session 1: Field Design and Analysis

May 23-27 2016

Introduction to R Statistical Software Linear models of phenotype, heritability, & gain under selection Randomized Complete Block Designs Augmented and lattice designs for trials with many entries Best Linear Unbiased Predictors (BLUPs) Partitioning Variation and estimating heritability Genotype by environment interactions Selection indices Field Tour

Session 2: Marker Assisted Selection

June 6-10 2016

Introduction to R for marker-trait analysis Molecular marker platforms and scoring Basic marker-trait models Populations for breeding and mapping Linkage analysis and Linkage disequilibrium (LD) Genetic Variation in Populations Selecting for Recombination Background Genome Selection Population sizes required for selection Field Tour

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Session 3: Genome Wide Analysis June 27-July 1 2016

Introduction to R packages rrBLUP, GAPIT, BLR Random effects models The structure of breeding populations Association Mapping Genome wide selection (GWS) Incorporating genomic selection in a breeding program Selection for multiple traits



All 3 = HCS 8825

Individual workshops as HCS 8806 (Methods)

Reminder: Support germplasm center(s) http://tgrc.ucdavis.edu/Donate/

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Silvia Francis,

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