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Cornell University

Associate Professor

Plant Breeding Symposium
Texas A&M University
2015

The Integration of Nutritional Genomics and High-Throughput Phenotyping: Progress and Prospects for Climate-Resilient High Provitamin A Maize

Michael Gore

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School of Integrative Plant Science

Cornell University

The global challenge

Population	**~7 Billion**
Less developed	**~5.8 Billion**
Undernutrition (caloric)	**~1 Billion (1 in 7)**
Malnutrition (micronutrients: Fe, Zn, I, Vitamin A)	**~2 Billion (1 in 3.5)**

10.9 million children under five die in developing countries each year. Malnutrition and hunger-related diseases cause 60 percent of these deaths. UNICEF, 2007

What can be done to reduce micronutrient deficiency for people in developing nations?

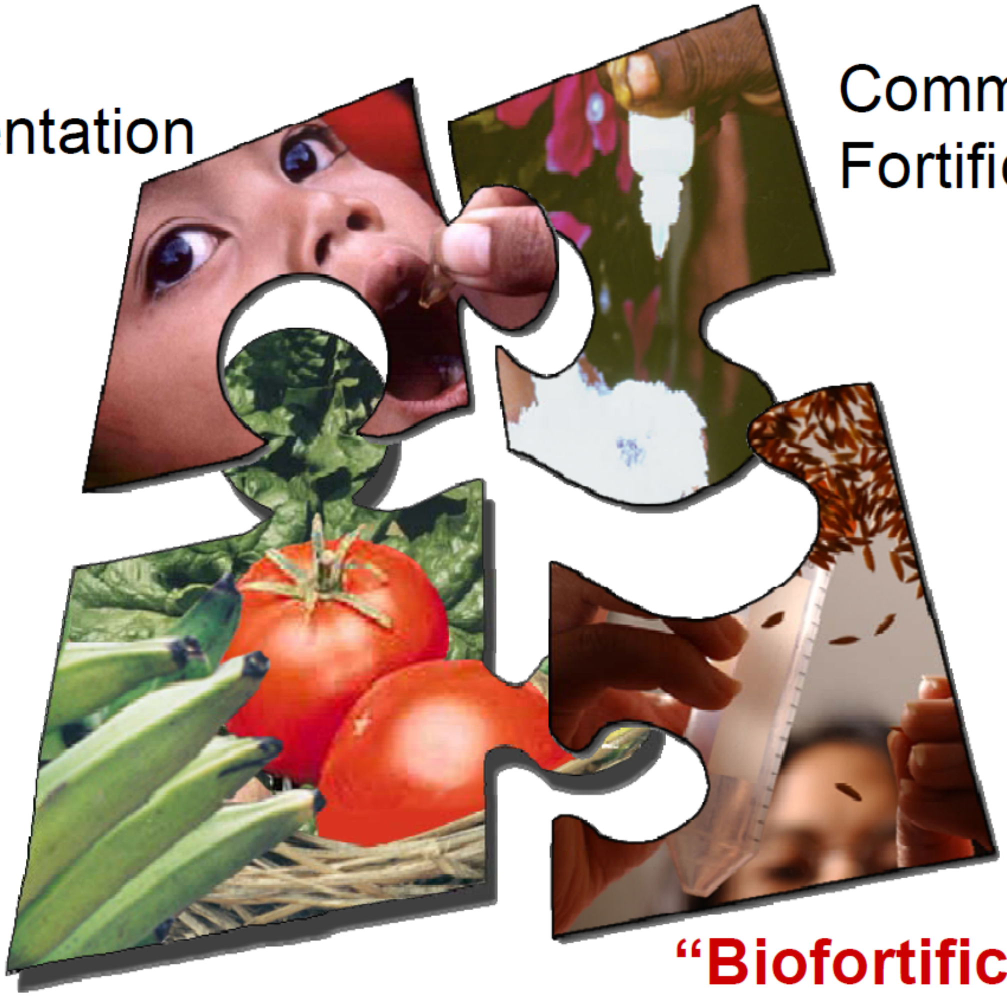


Image - Ross Welch

Biofortification

- Biofortification is focused on the rural poor and has the potential to be sustainable and cost-effective

Source: www. aboutharvest.com

- Identify genes associated with nutrient content in staple crops such as maize and cassava

- Increase nutritional value of locally adapted crop varieties by selecting on favorable alleles of these identified genes in breeding populations

Maize is a global staple crop

- Can account for more than 50% of total daily calories in African and Latin American countries with high nutritional deficiencies

Source: Torbert Rocheford

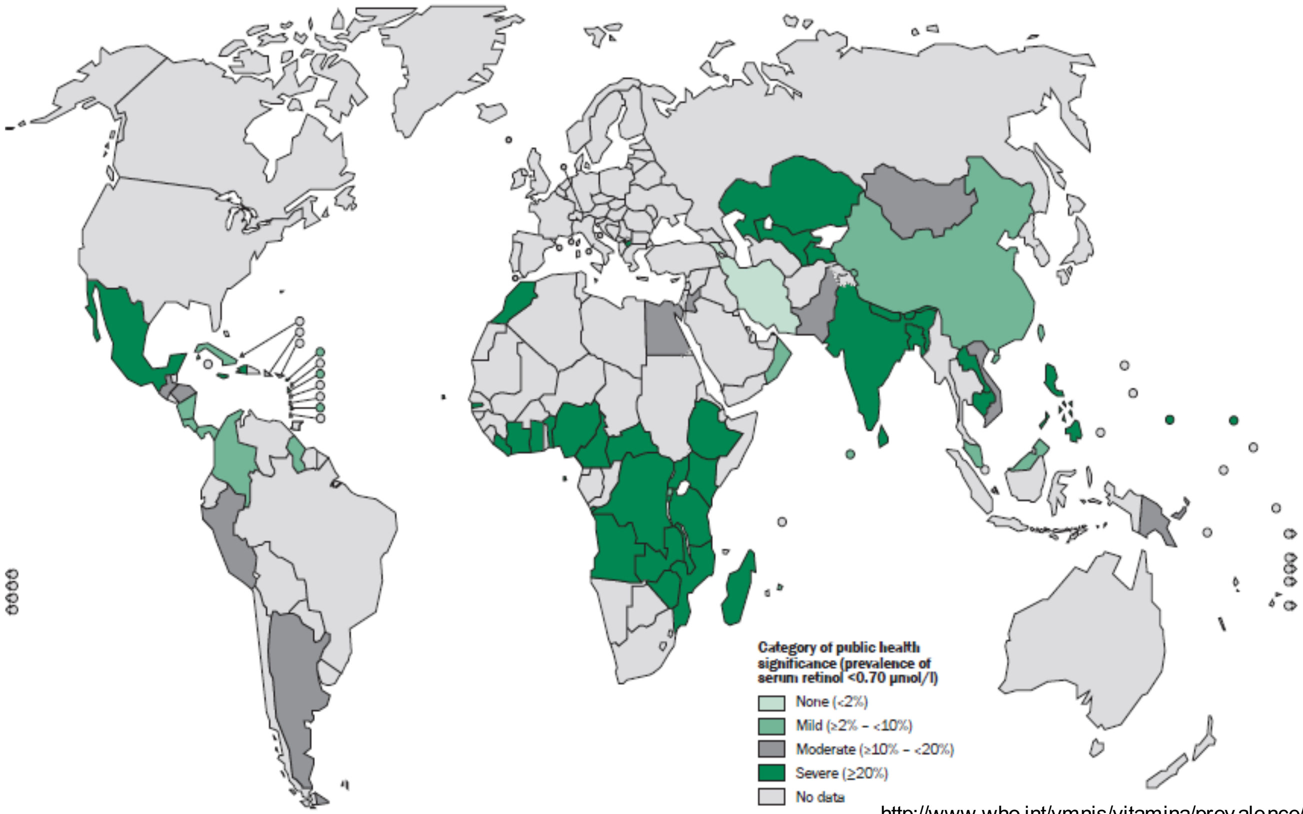
- In these countries, maize varieties typically do not provide grain with adequate daily levels of essential nutrients – Fe, Zn, vitamin A, vitamin E, B vitamins

- The tremendous genetic diversity of maize can be potentially harnessed by biofortification to develop nutrient-dense grain

Prevalence of vitamin A deficiency

- 7 million pregnant women and 127 million preschool-aged children are vitamin A deficient

Countries and areas with survey data: Preschool-age children

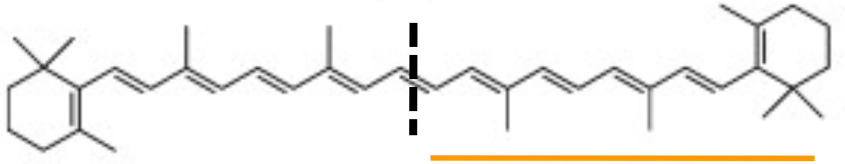


http://www.who.int/vmnis/vitamina/prevalence/vita_fig2a.pdf

- Vitamin A deficiency causes 600,000 early childhood deaths and blindness in 500,000 children each year

Provitamin A from endosperm of maize grain is converted to vitamin A in the body

Vitamin A



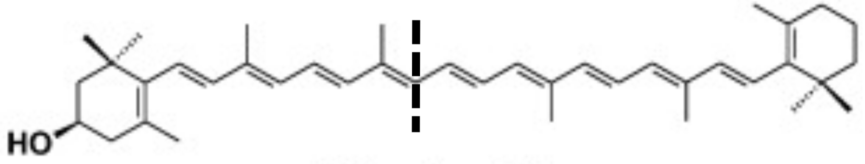
β-Carotene

Vitamin A (two retinyl groups)

Cleaved by *β,β-carotene 15,15'-monooxygenase* in intestine

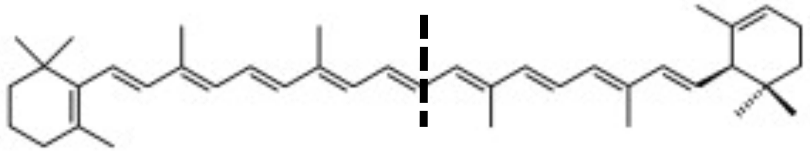
Lutein and zeaxanthin are non-provitamin A compounds but important for vision

Vitamin A



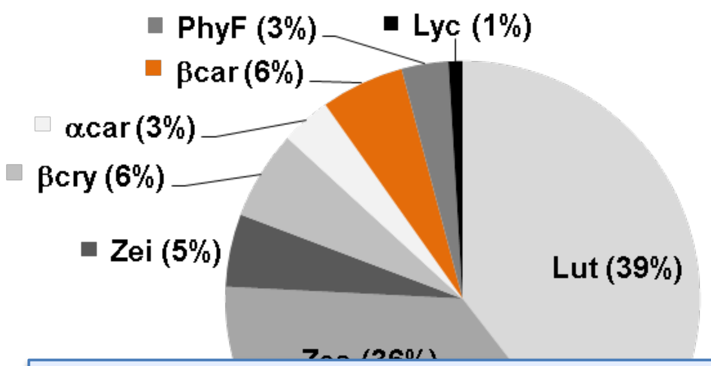
β-Cryptoxanthin

Vitamin A



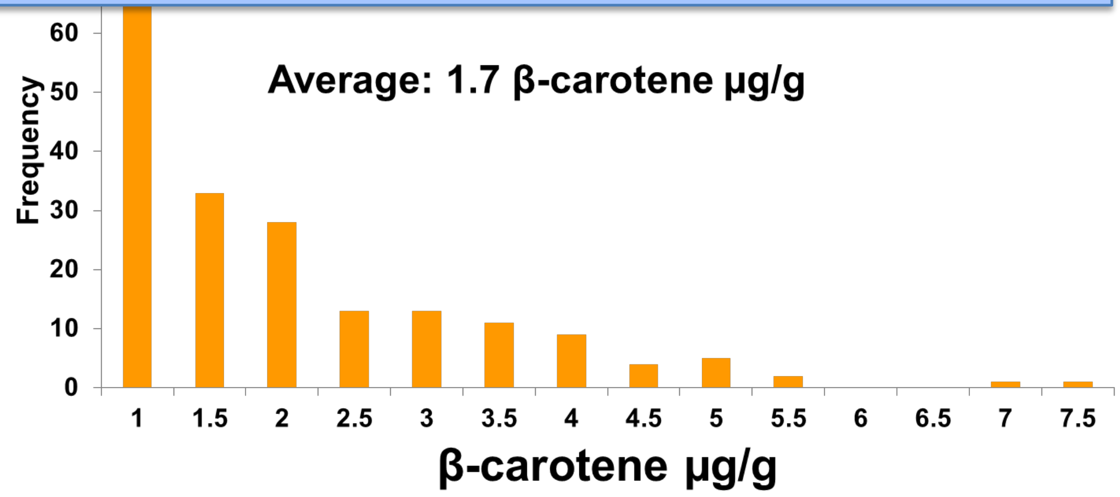
α-Carotene

Grain carotenoids in a diverse maize panel: gradient of light yellow to dark orange color



Highest ProVA Activity

Biofortification: HarvestPlus has an initial target of 17 µg/g β-carotene



Grain color and carotenoid content

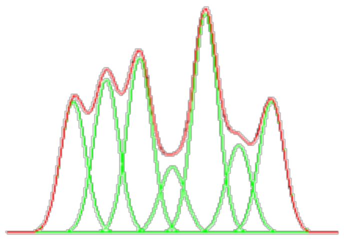
Total carotenoids
37.36 µg/g

Total carotenoids
8.48 µg/g

Harjes et al. 2008 Science

Nested Association Mapping panel-centered design to identify key genes and alleles

Phenotypic Data



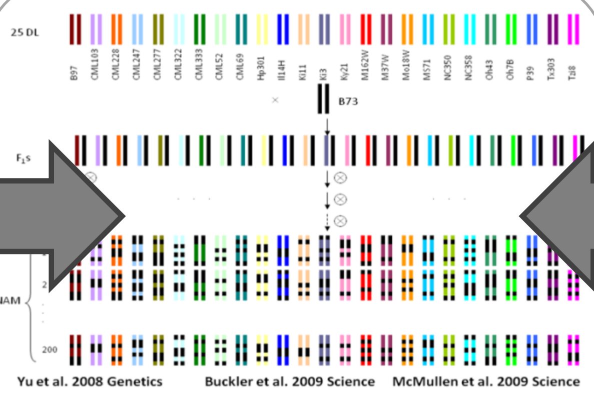
HPLC Quantification Mature Grain (2 yrs)

Carotenoids

Tocochromanols (vitamin E)

~17,000 NAM runs

Maize Germplasm



Expression Profiling

Developing Kernel Libraries

NAM Founder Inbreds
Six dev. stages

Illumina Sequencing

~40,000 genes profiled

Genotypic Data

RNAseq
~1 million SNPs

+

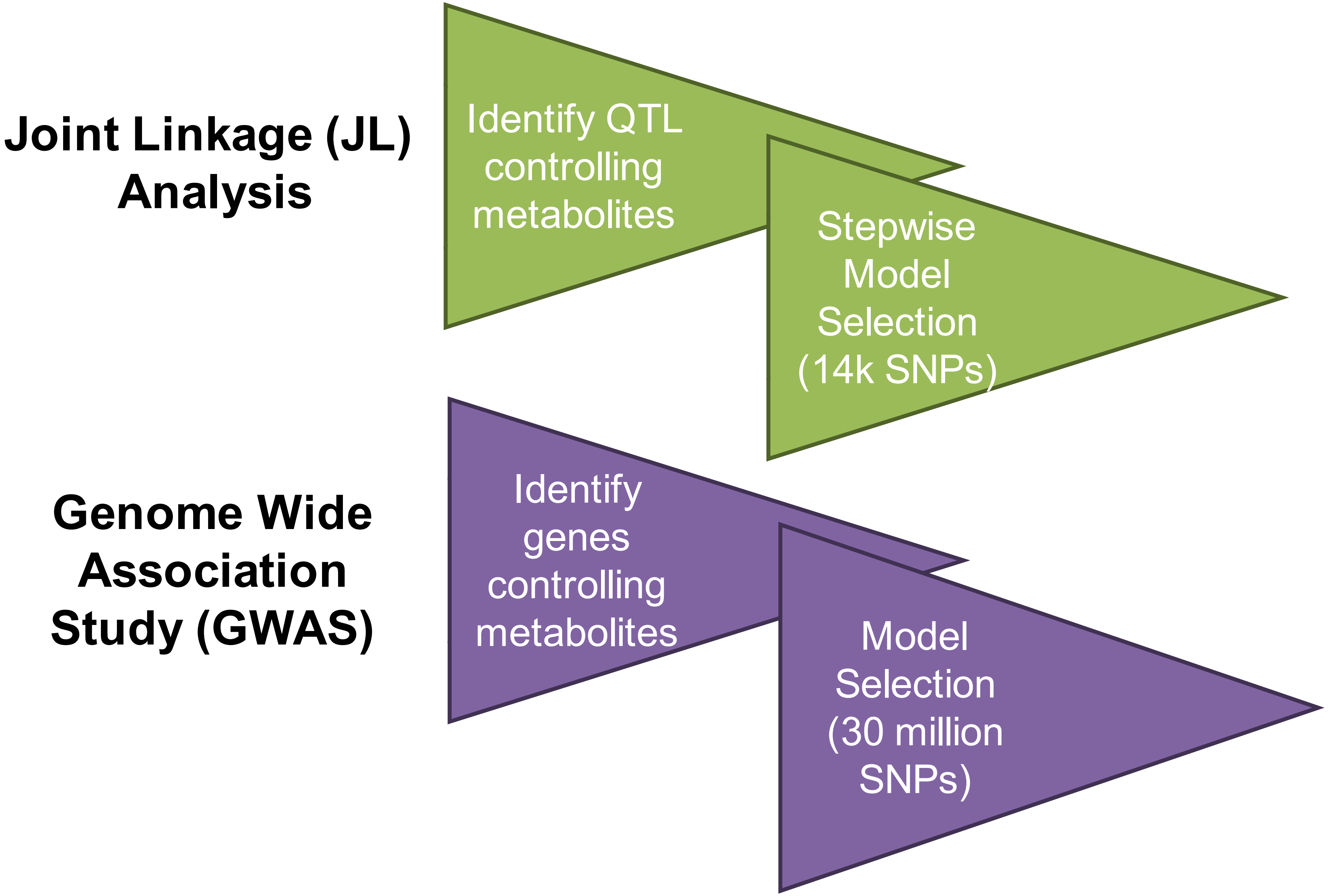
Hapmap v1
~1.6 million SNPs

HapMap v2
~26 million SNPs

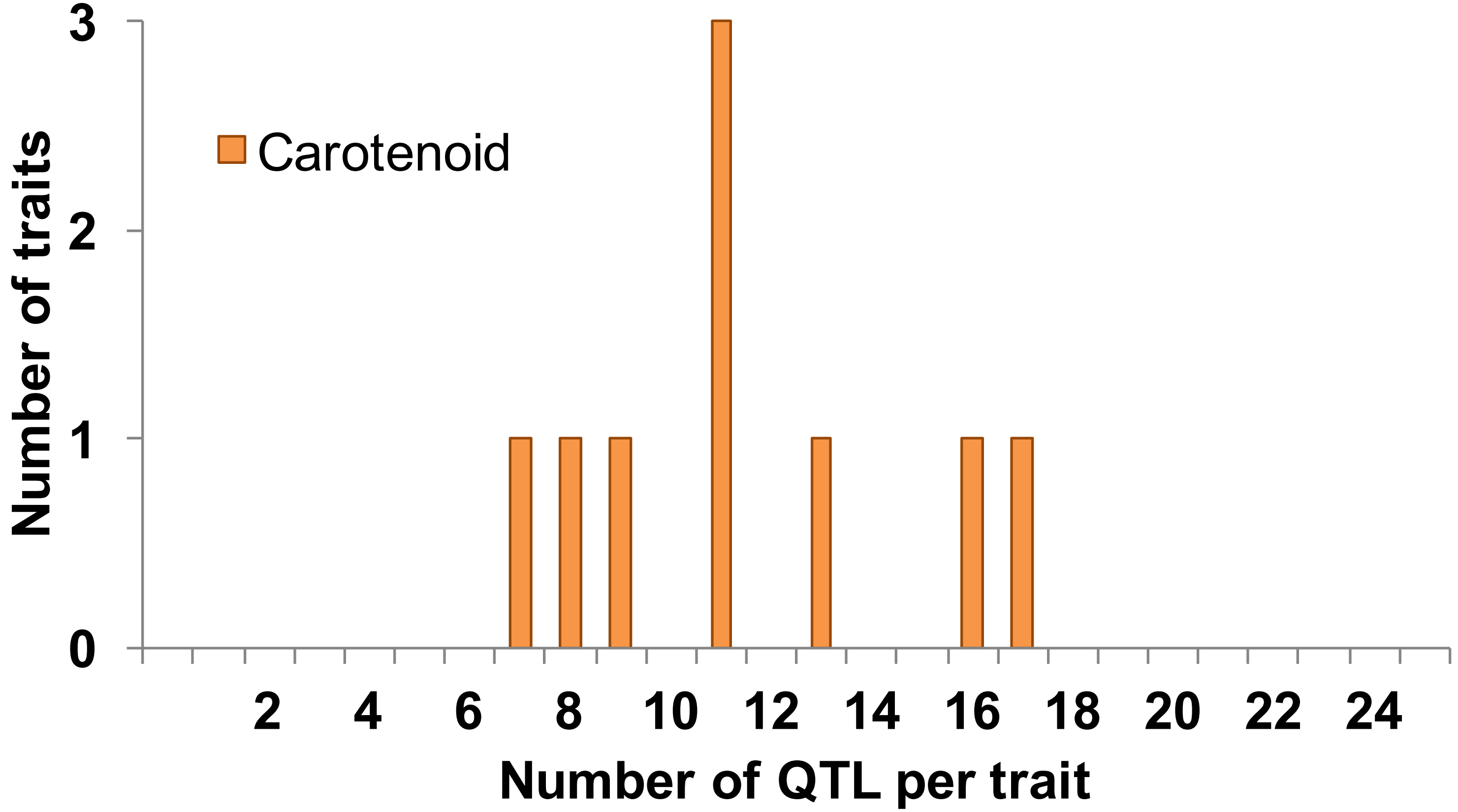
Alex Lipka

Cathy Kandianis

Statistical analysis of NAM panel

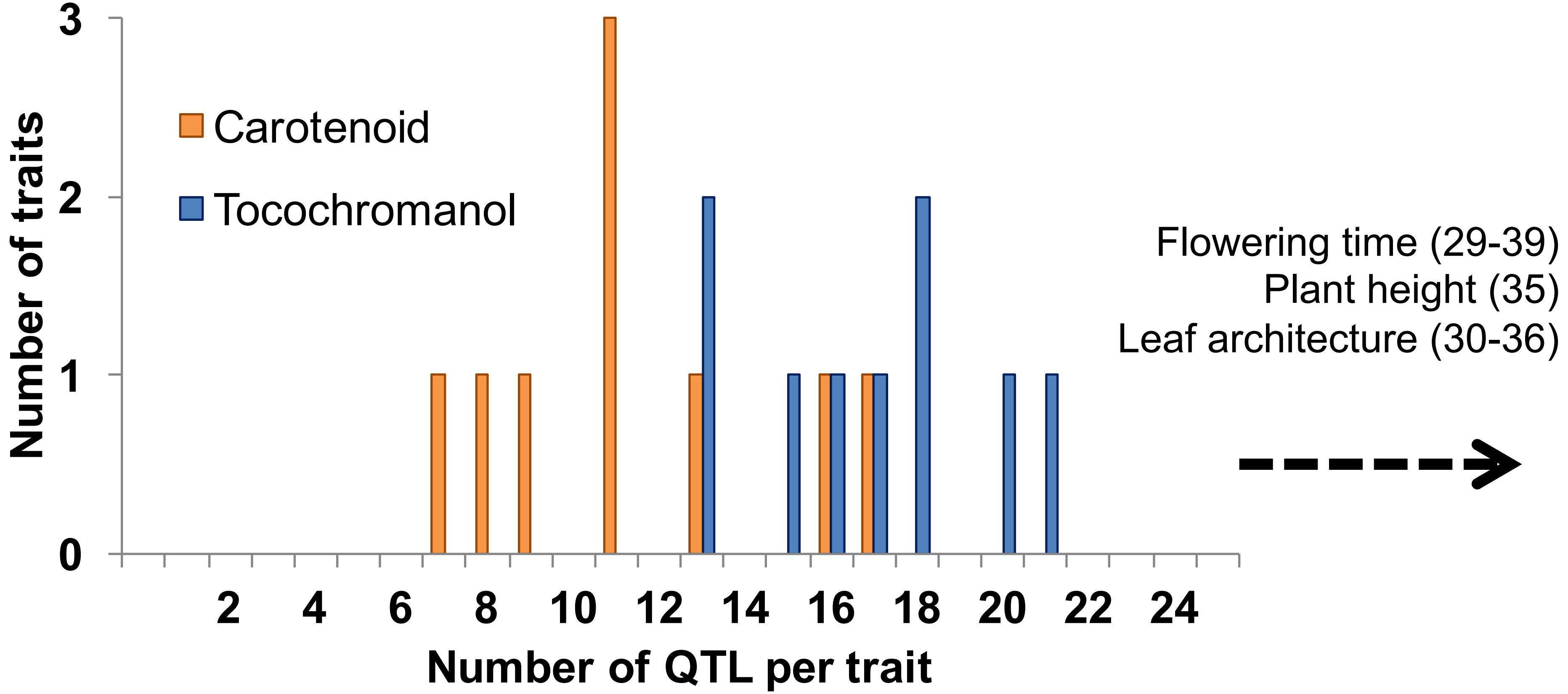


Identified 103 QTL for 8 carotenoids and total carotenoids in maize NAM grain



Heritability range of the 9 traits: 0.75-0.95

Fewer QTL identified for carotenoid traits relative to tocochromanol traits

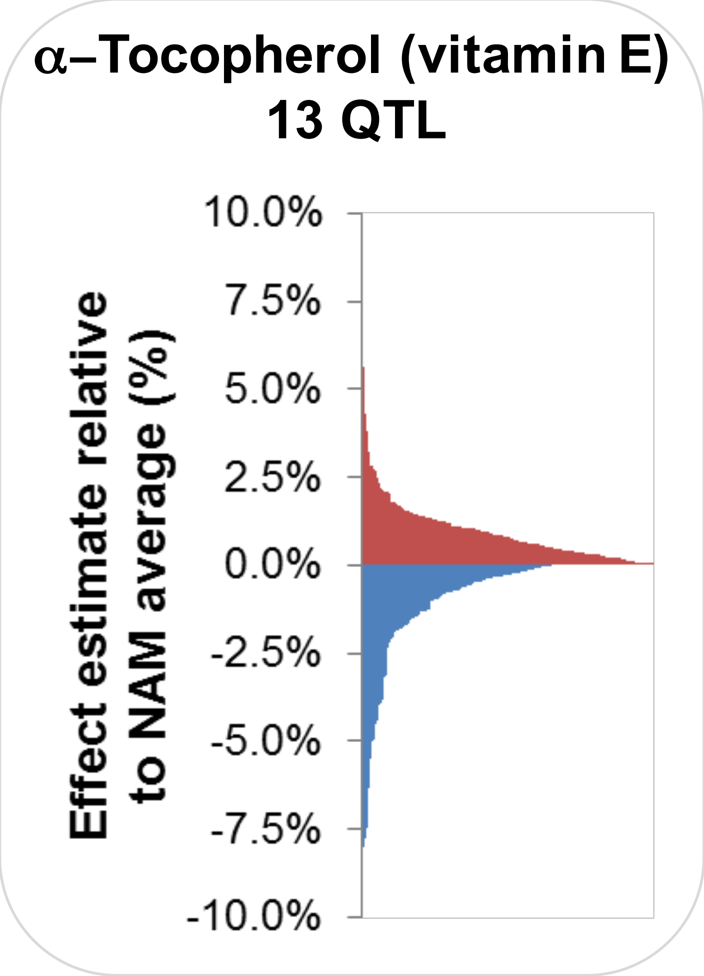


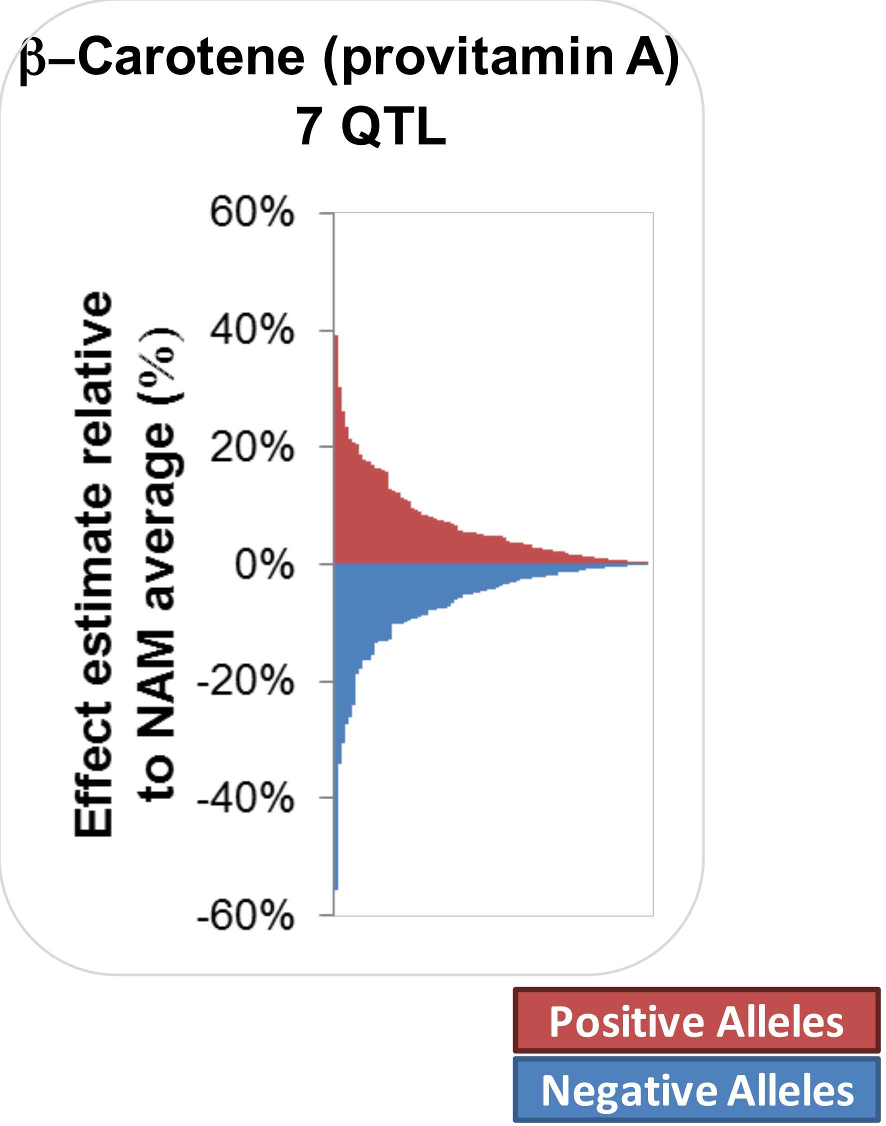
Carotenoids: Endosperm

Tocochromanols: Endosperm and Embryo

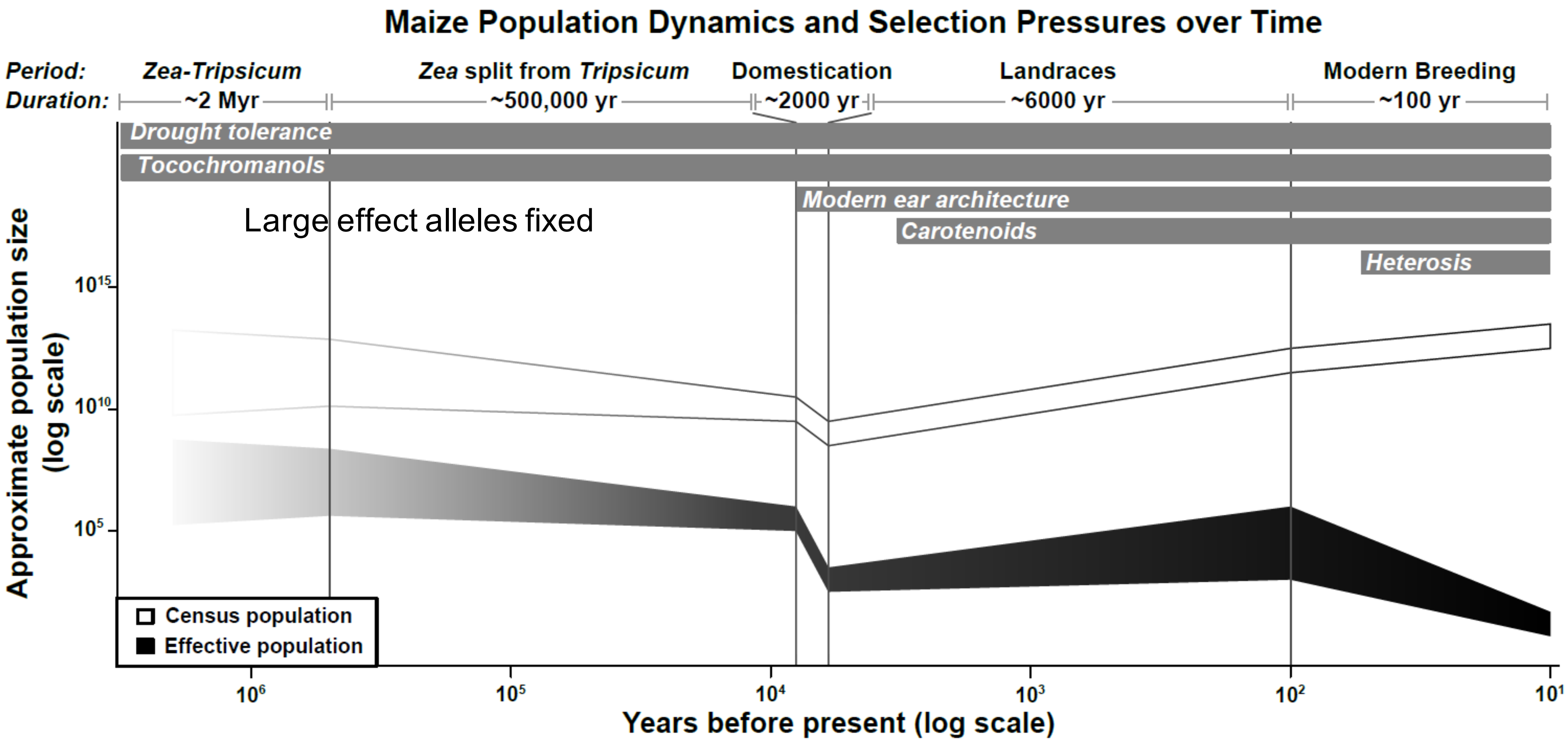
Heritability range of the 18 traits: 0.71-0.95

Carotenoid traits have larger relative QTL effects than tocochromanol traits





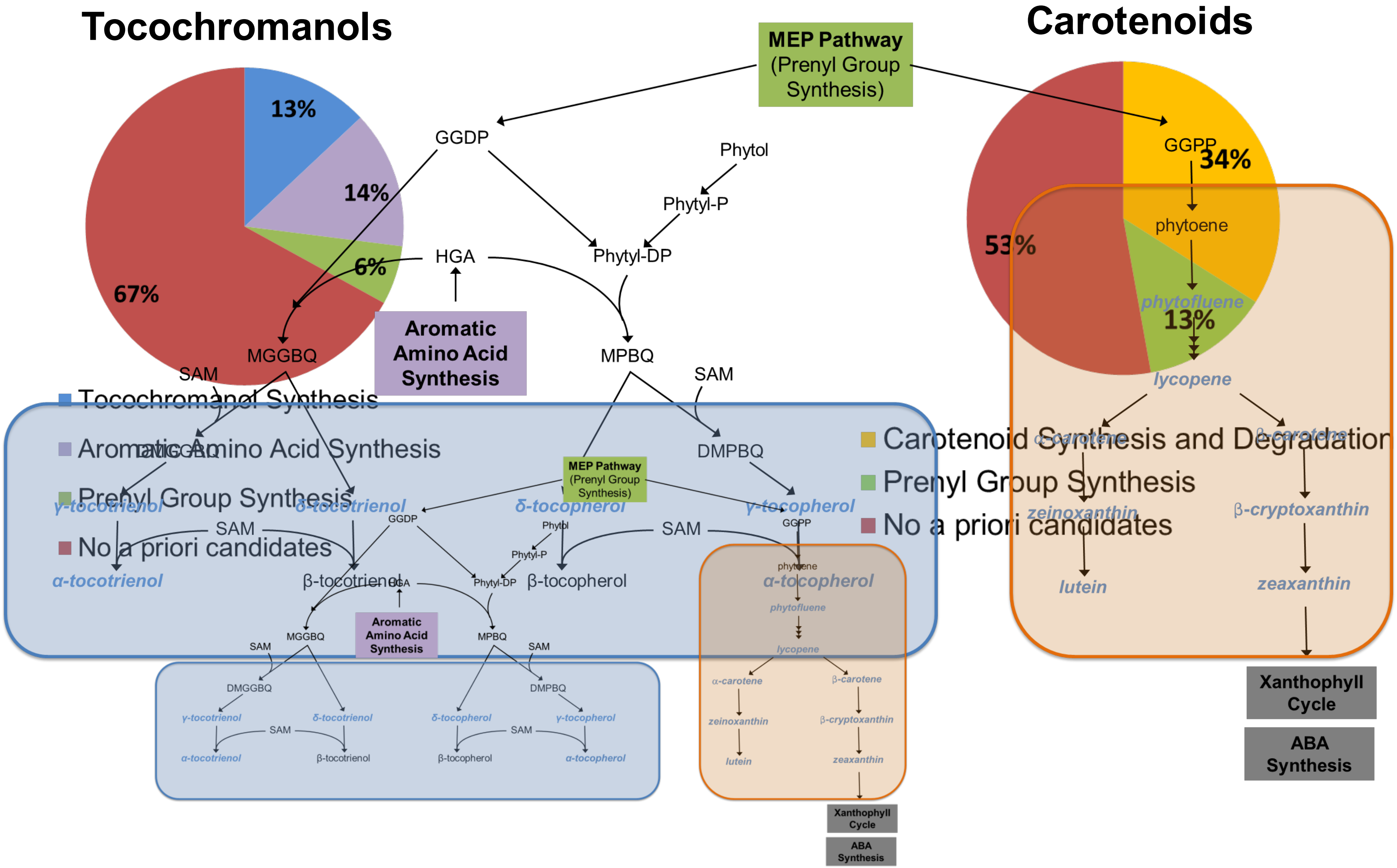
Grain endosperm carotenoid traits: fewer QTL and larger effect sizes because of shorter length of time for variation to accumulate



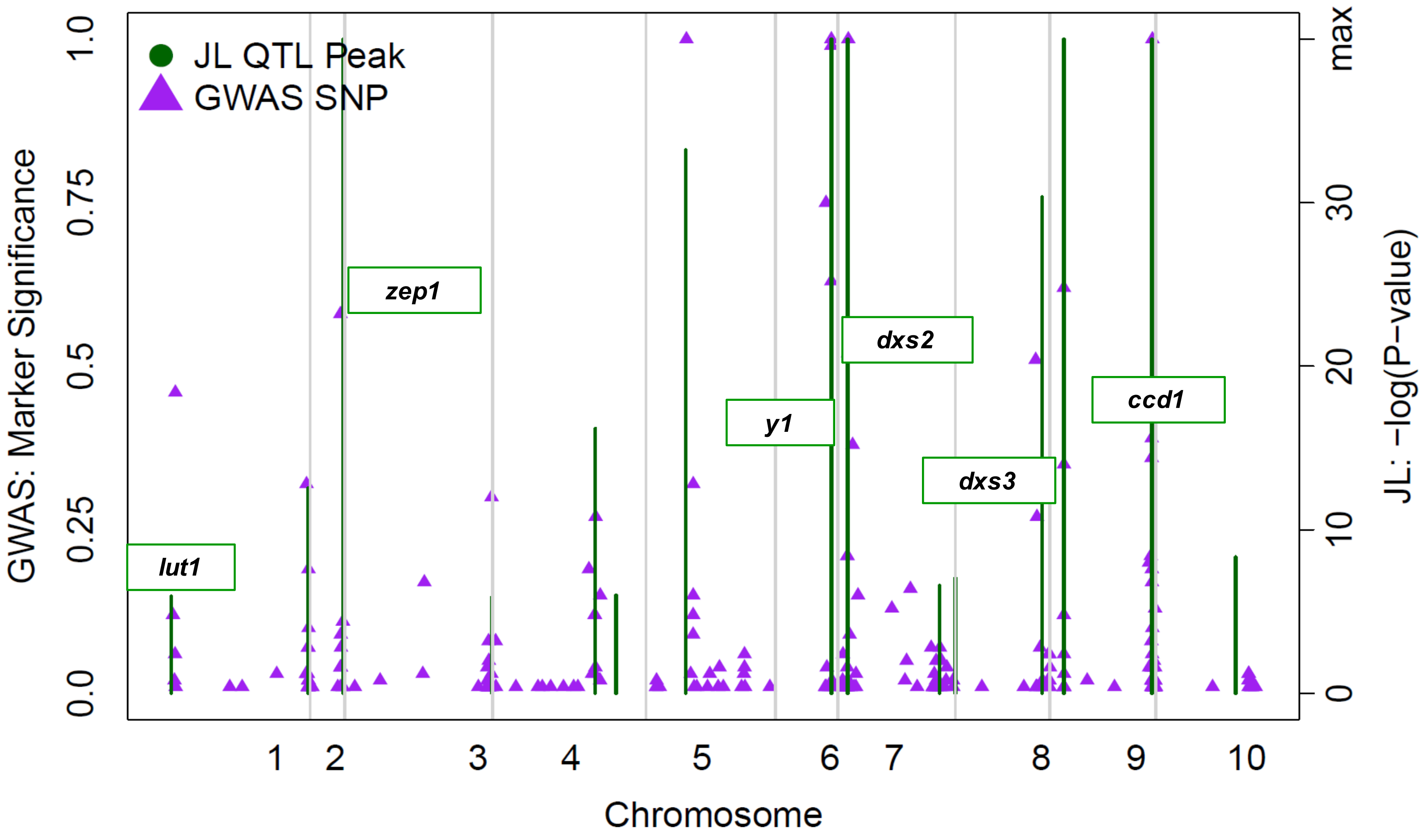
Grain endosperm carotenoid traits came under selection after domestication

Modified from Wallace et al. 2013

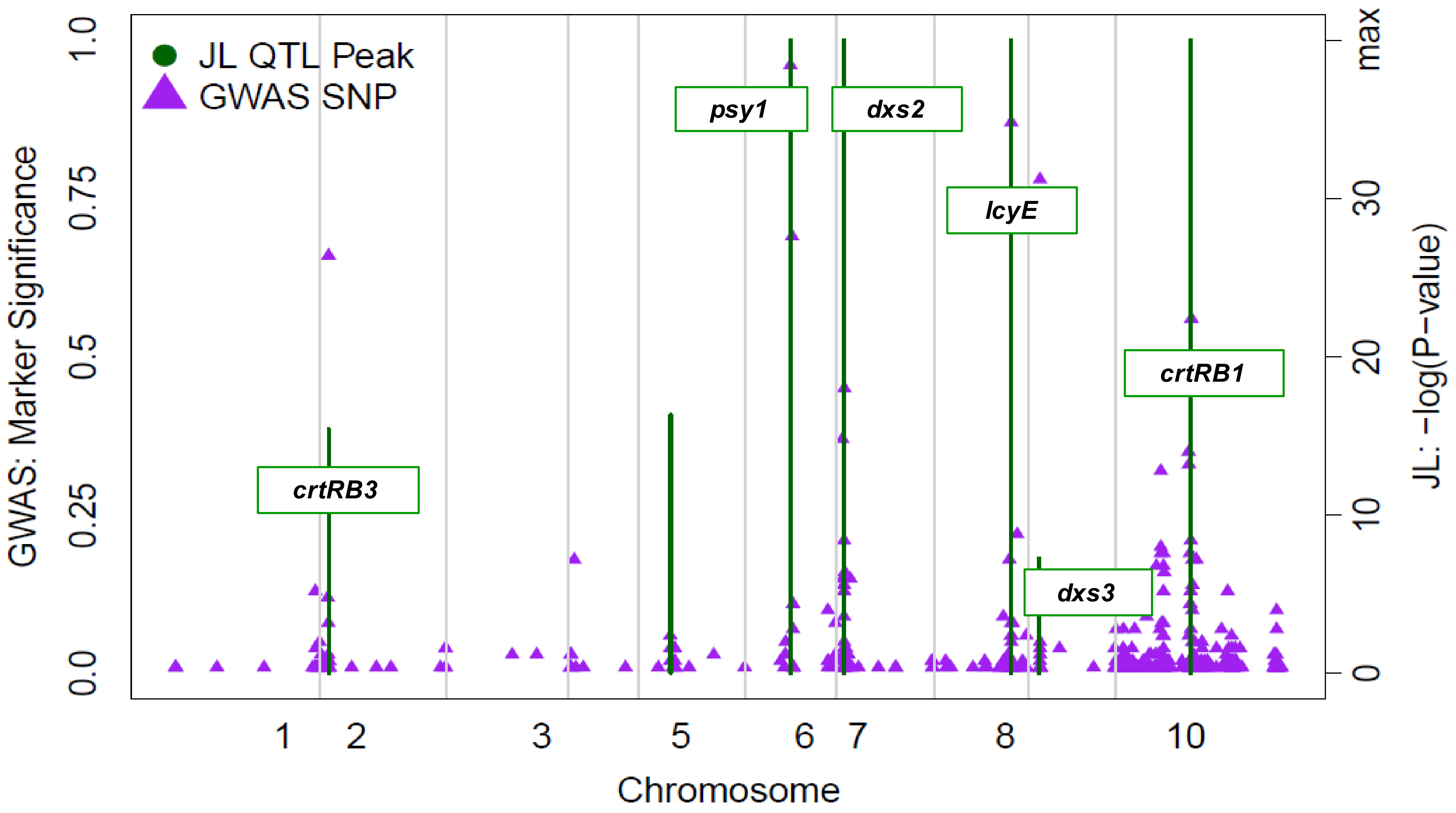
Intersection of candidate genes and QTL support intervals



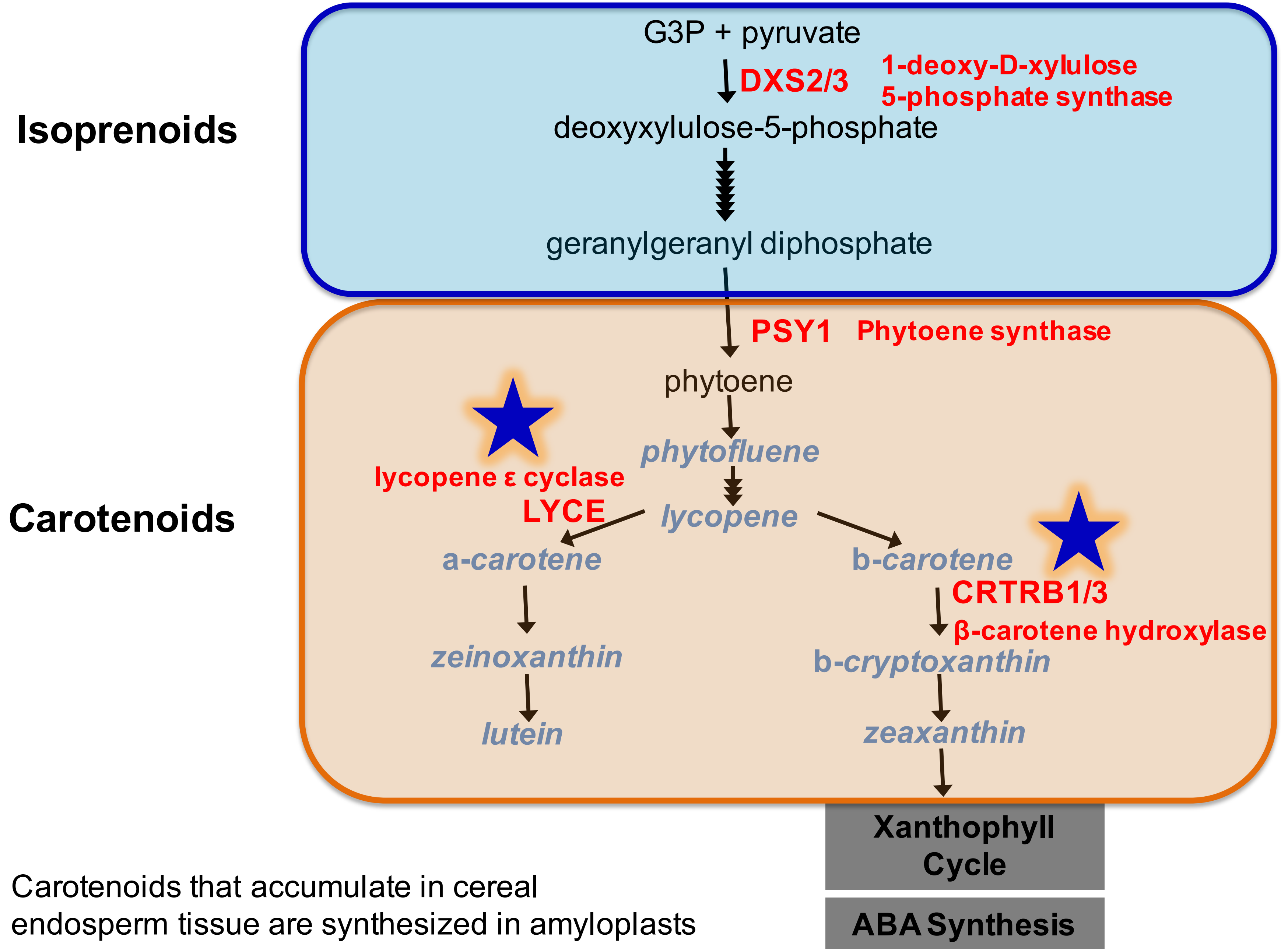
JL-GWAS: Resolving the 16 identified QTL associated with level of total carotenoids



JL-GWAS: Resolving the 7 identified QTL associated with level of beta-carotene

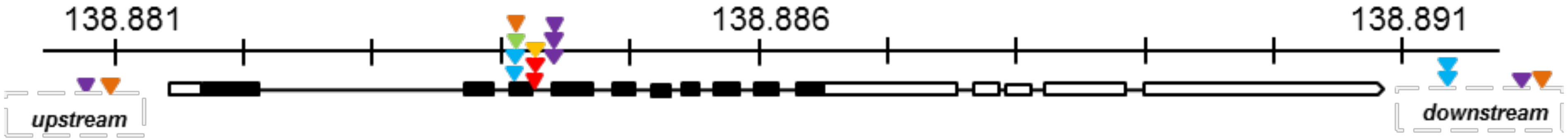


Pathway-level breeding for beta-carotene



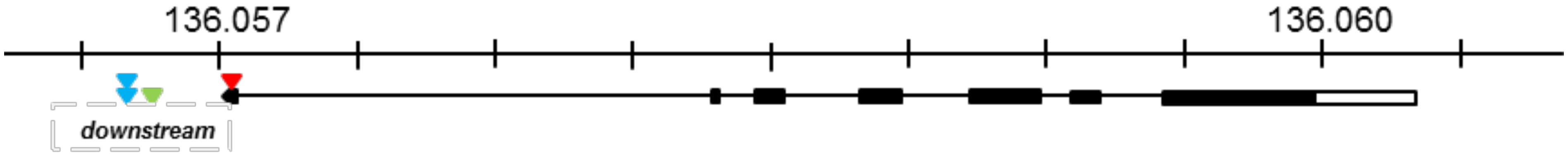
Carotenoids that accumulate in cereal endosperm tissue are synthesized in amyloplasts

GWAS signals proximal to candidate genes are within putative regulatory regions



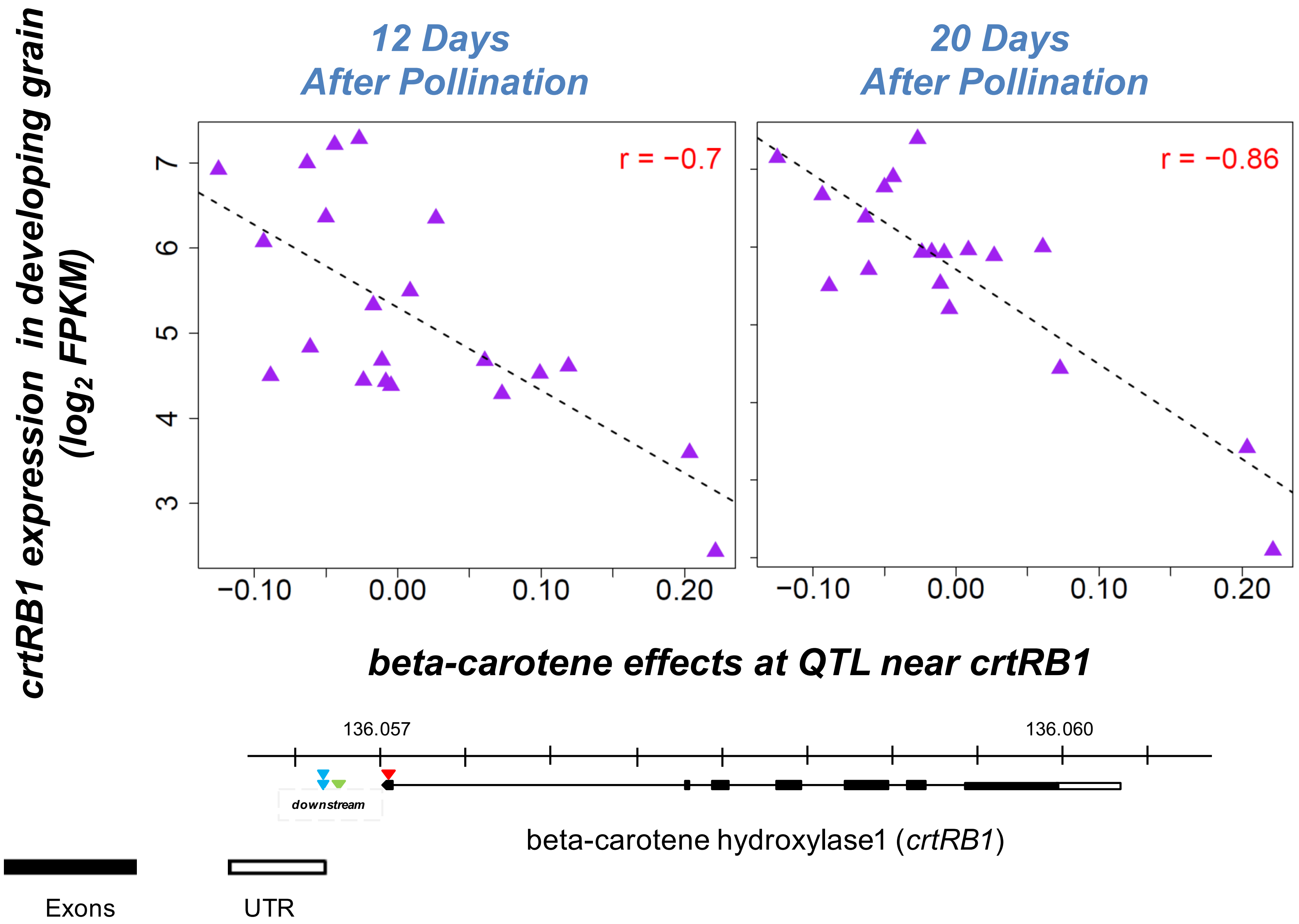
lycopene ε-cyclase (*lycE1*)

Does differential expression underlie the genetic basis of these loci?

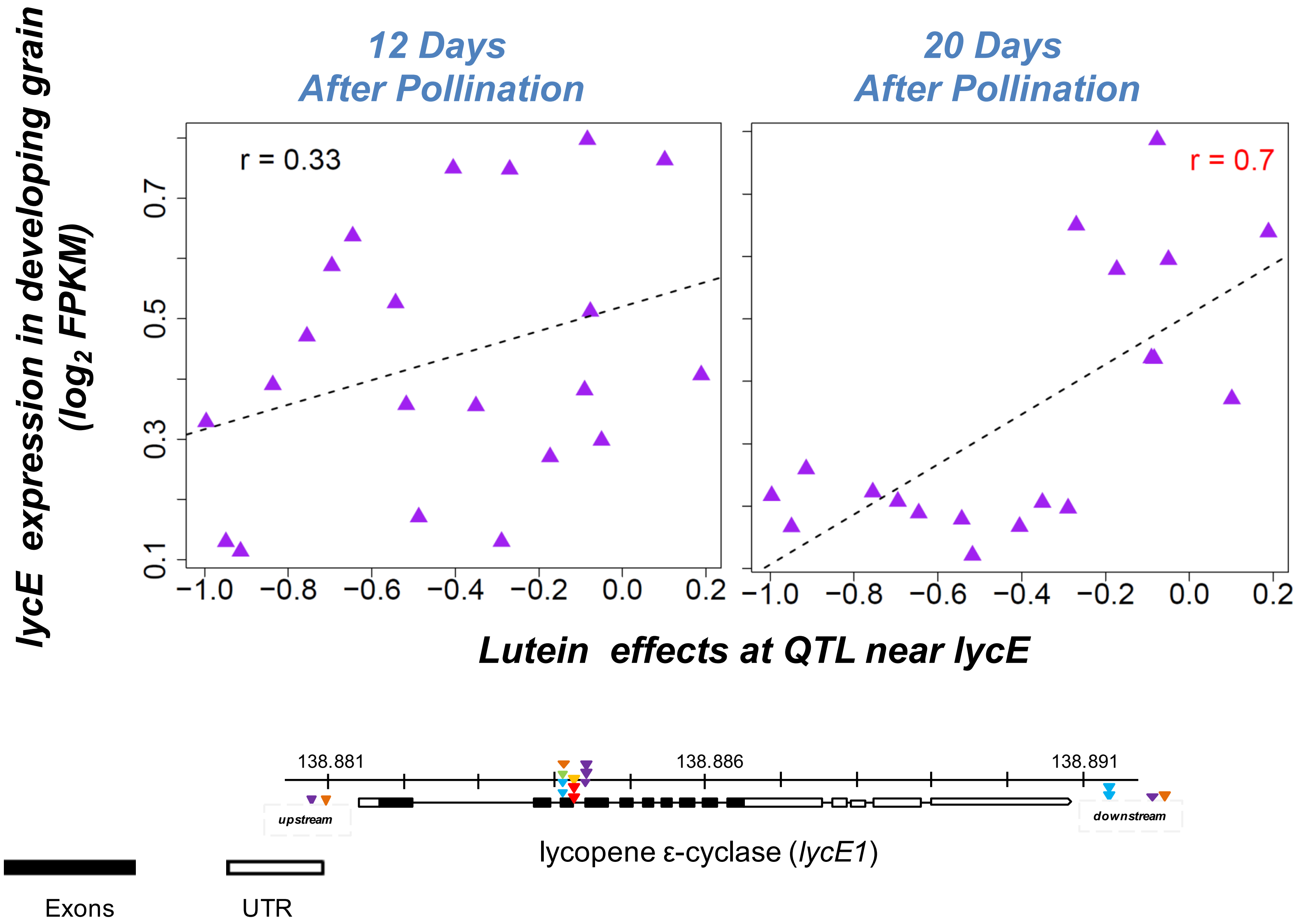


β-carotene hydroxylase (*crtRB1*)

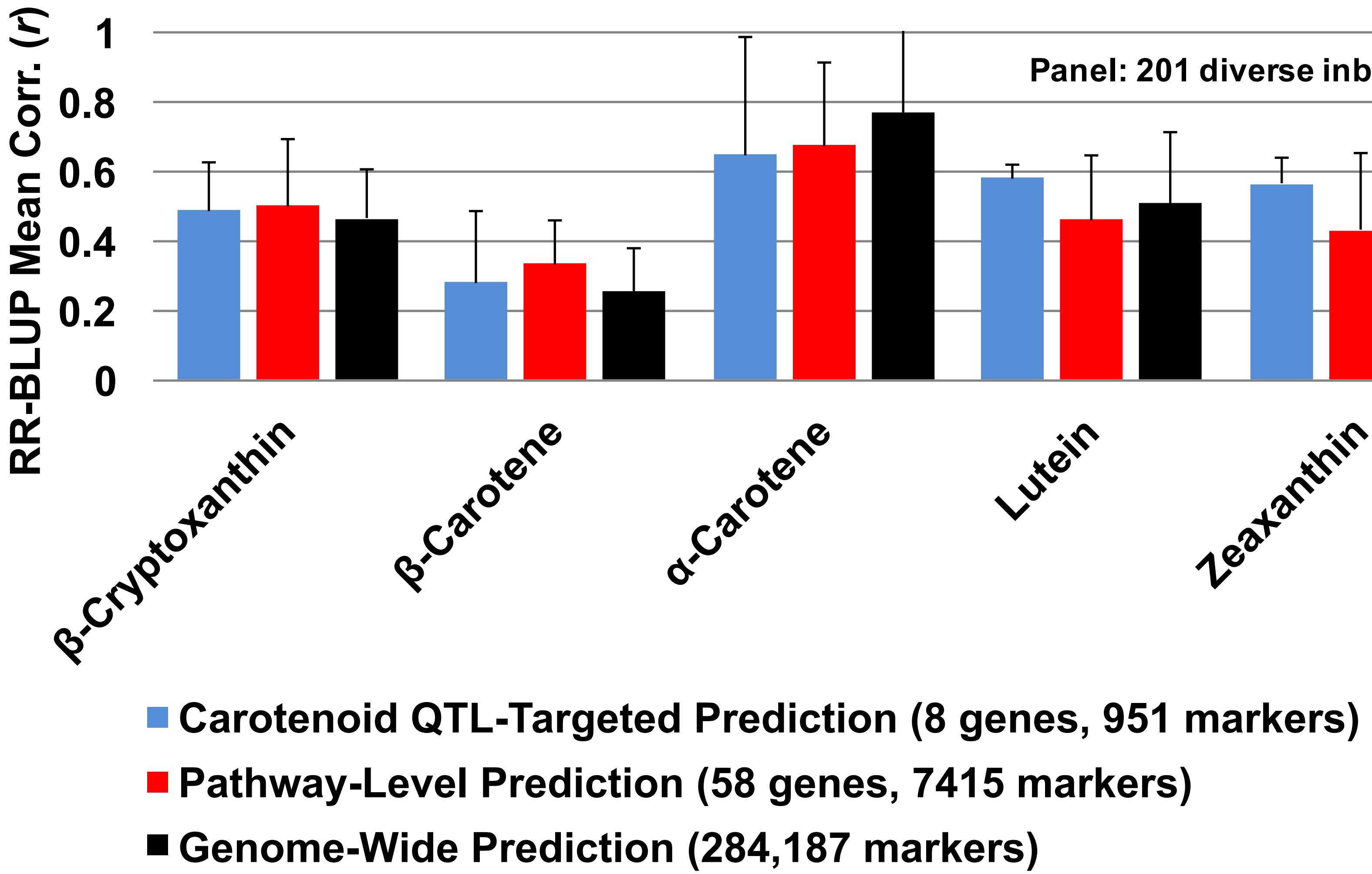
Consistent correlation: *crtRB1* expression, beta-carotene QTL effects



Stage-specific correlation: *lycE* expression, lutein QTL effects



Markers within ± 250kb of 8 QTL associated with carotenoid levels in prior linkage studies are as predictive as genome-wide markers



Owens, Lipka et al., 2014 Genetics

Standardized mean corr. resulting from the fivefold cross-validation and S.D.

Christine Diepenbrock

We Collaborate with

HarvestPlus
Better Crops • Better Nutrition

Our research results flow to CIMMYT, IITA in Africa, successfully using MAS for *lycE* and *crtRB1* In Zambia for high provitamin A maize

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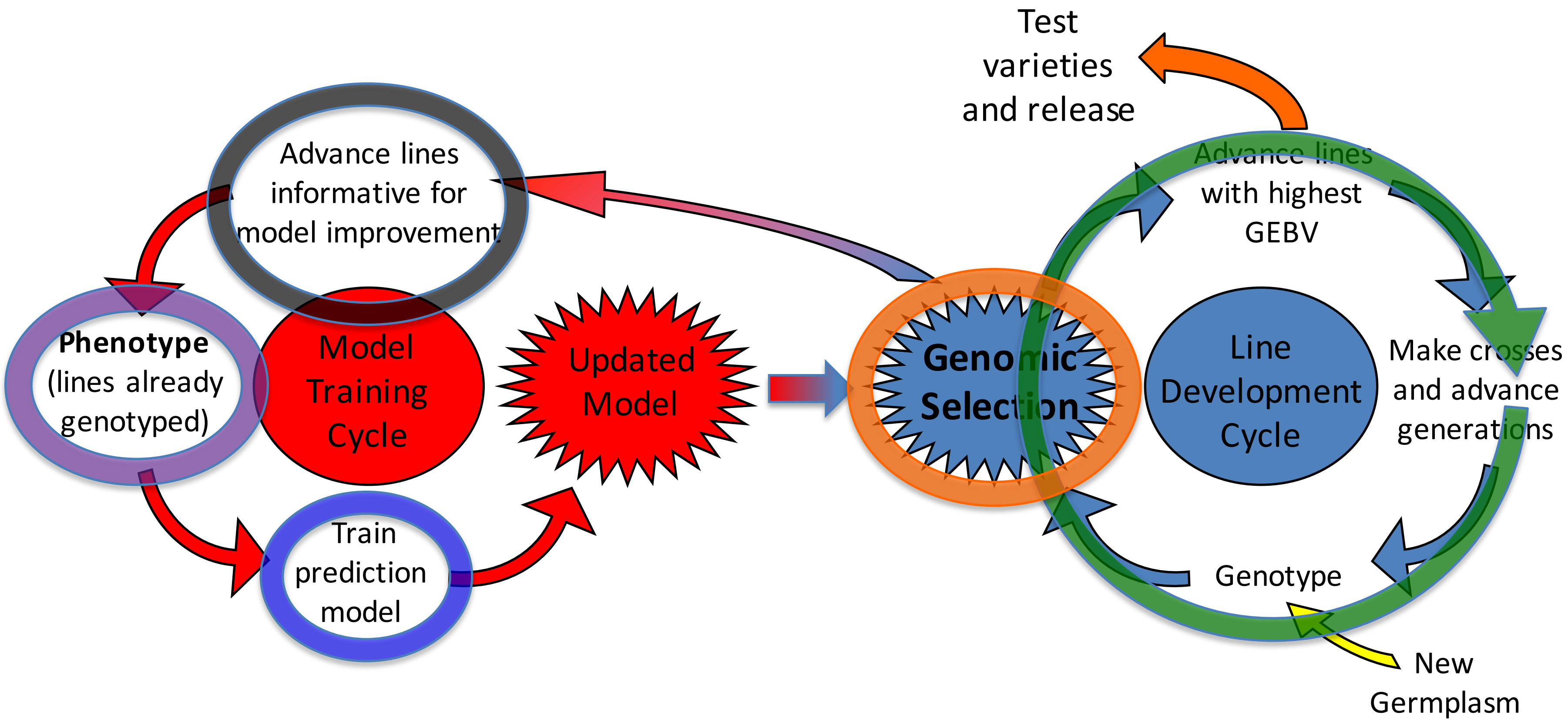
CGIAR

Sustainable maize production is threatened by increasingly variable weather patterns and diminishing fresh water resources

https://www.flickr.com/photos/cimmyt/5190627819/

Tanzanian farmer with drought-affected maize

Genomic selection: faster development of nutrient-dense, stress tolerant maize varieties



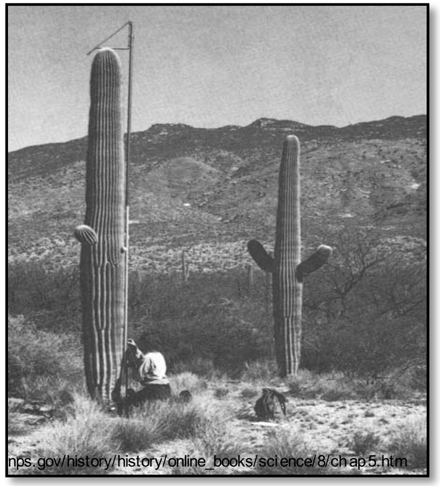
Research needs: **train prediction models**, **accelerate recombination**, **manage the population**, **identify informative lines**, and **improved phenotyping capacity**.

Modified from Heffner, E.L. et al. 2009. Crop Sci. 49: 1–12

Contrasting evolutionary rates between genotyping and phenotyping platforms

1997 – Capillary DNA sequencer

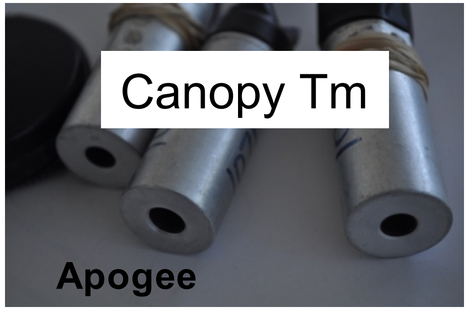
1974 – Steel measuring tape



2015 – Single molecule DNA sequencer

2015 – Barcoded measuring tape

HTP: Proximal sensors, platform, and vehicle

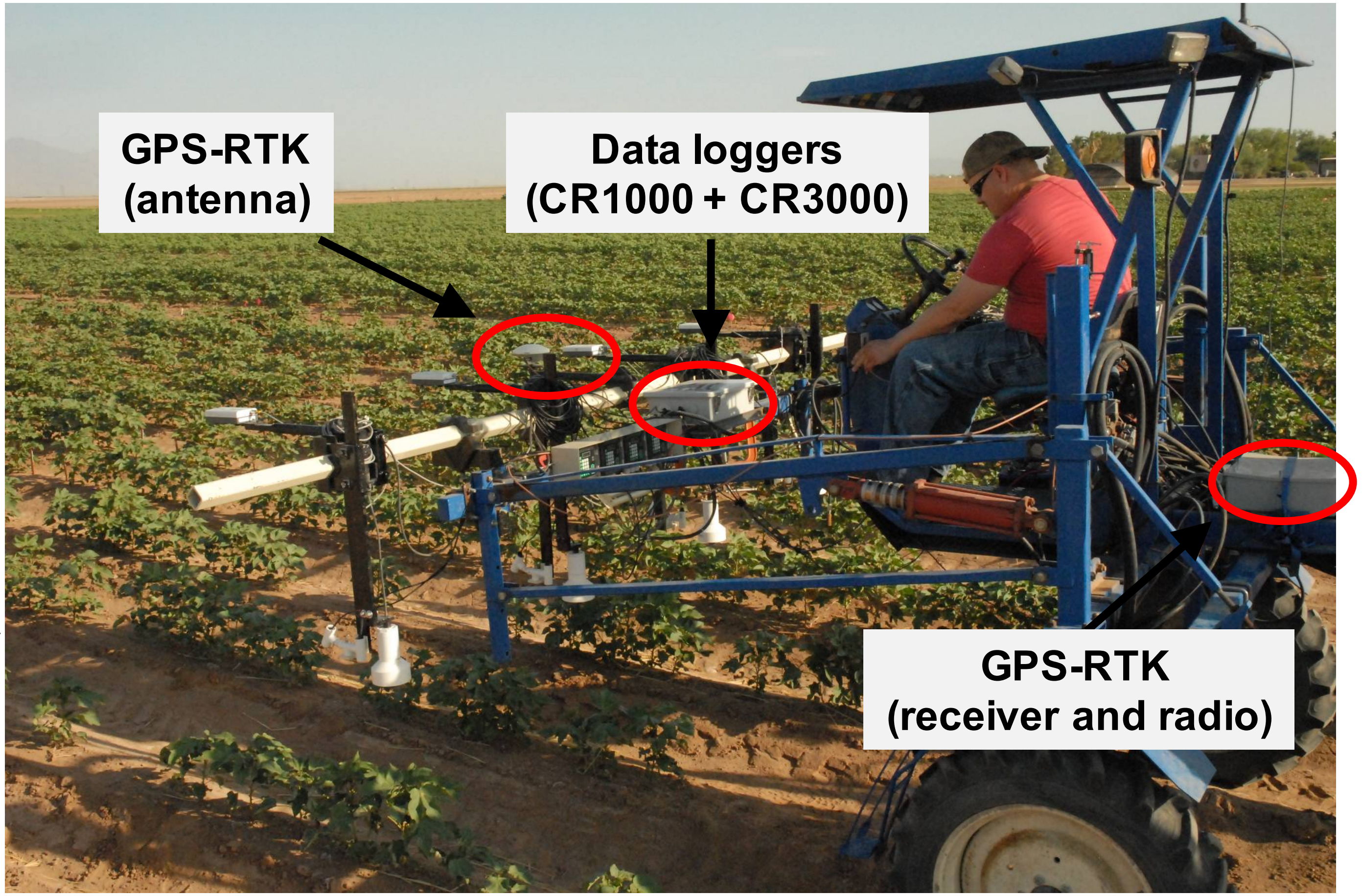


Infrared thermometer

Pulsar db 3-m

Ultrasonic Transducer

Multi-spectral crop canopy sensor

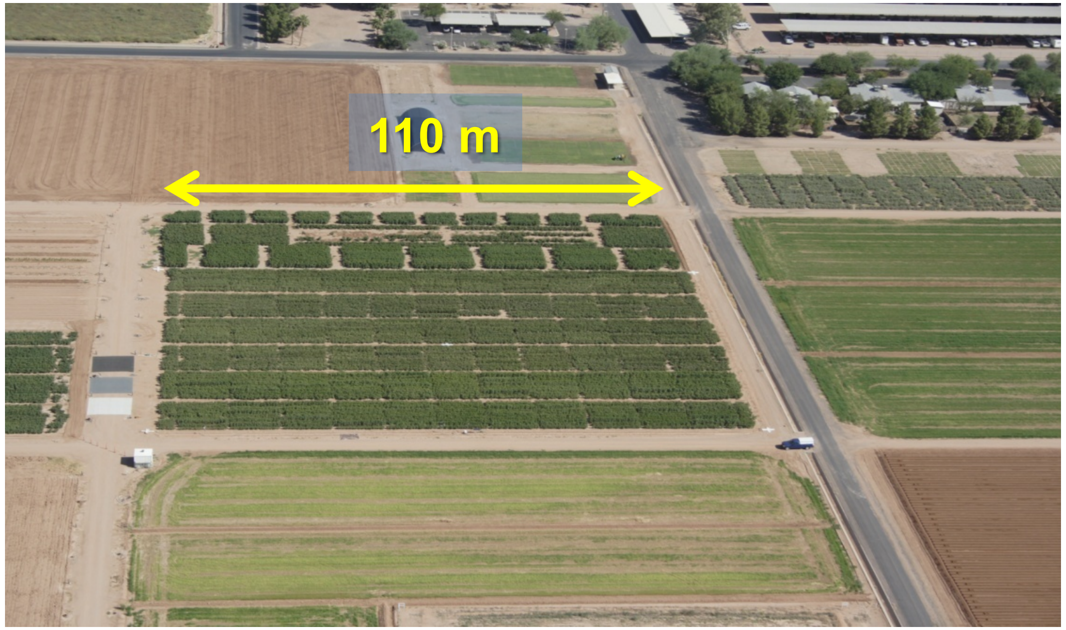


High-clearance tractor
Average speed of 2.82 km/h
1 data point/meter (1 Hz)

Duke Pauli

Andrade-Sanchez, P., Gore, M. A. et al. 2014 Functional Plant Biology

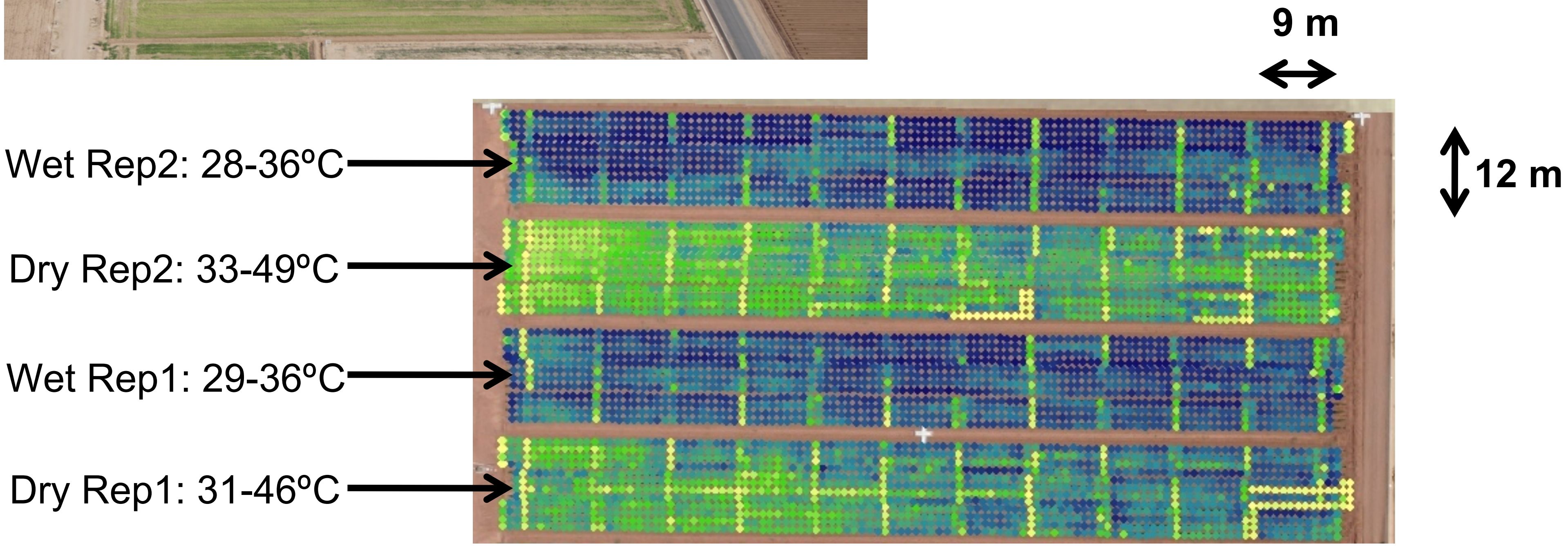
HTP: Canopy temperature



Central Arizona: clear skies, very limited rain, high temperatures

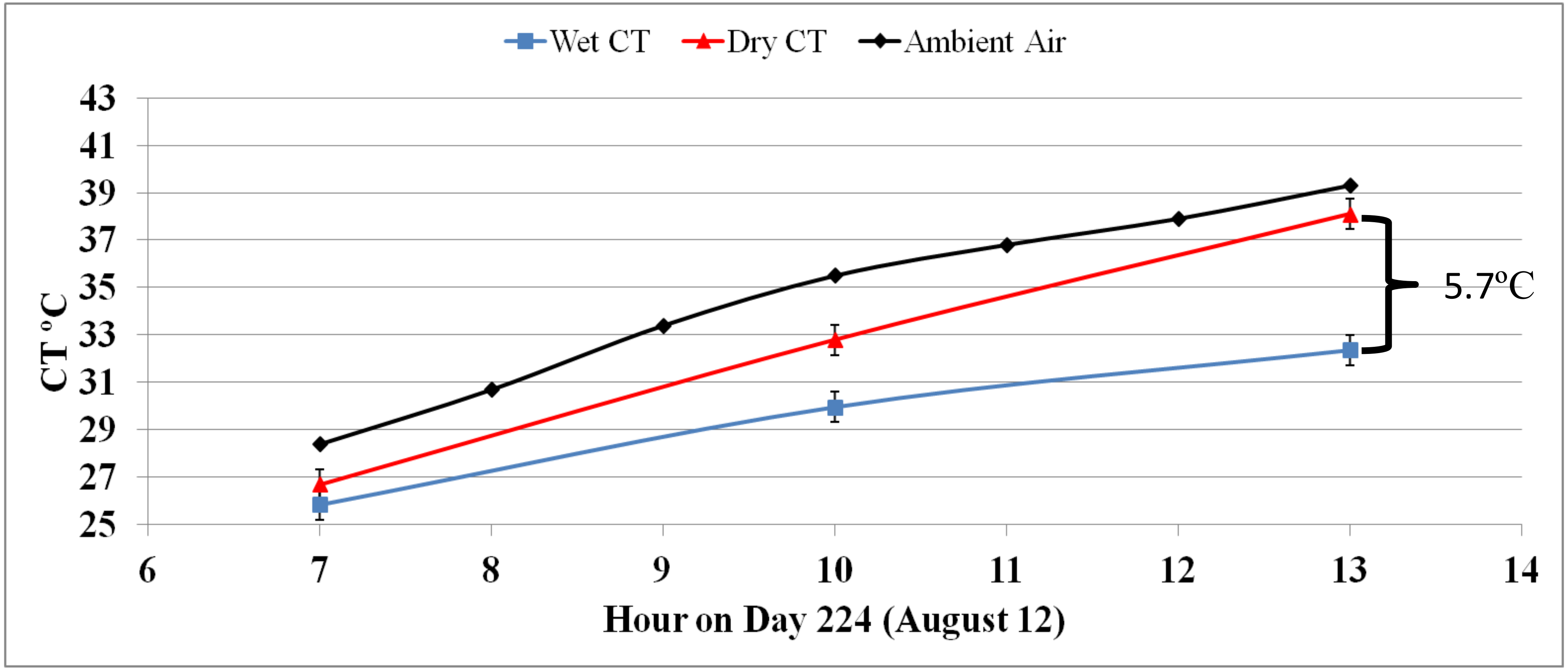
TM-1×NM24016 population: 94 RILs (*Gossypium hirsutum;* Upland cotton)

Treatments: 100 and 50% ET (2 reps) by drip irrigation



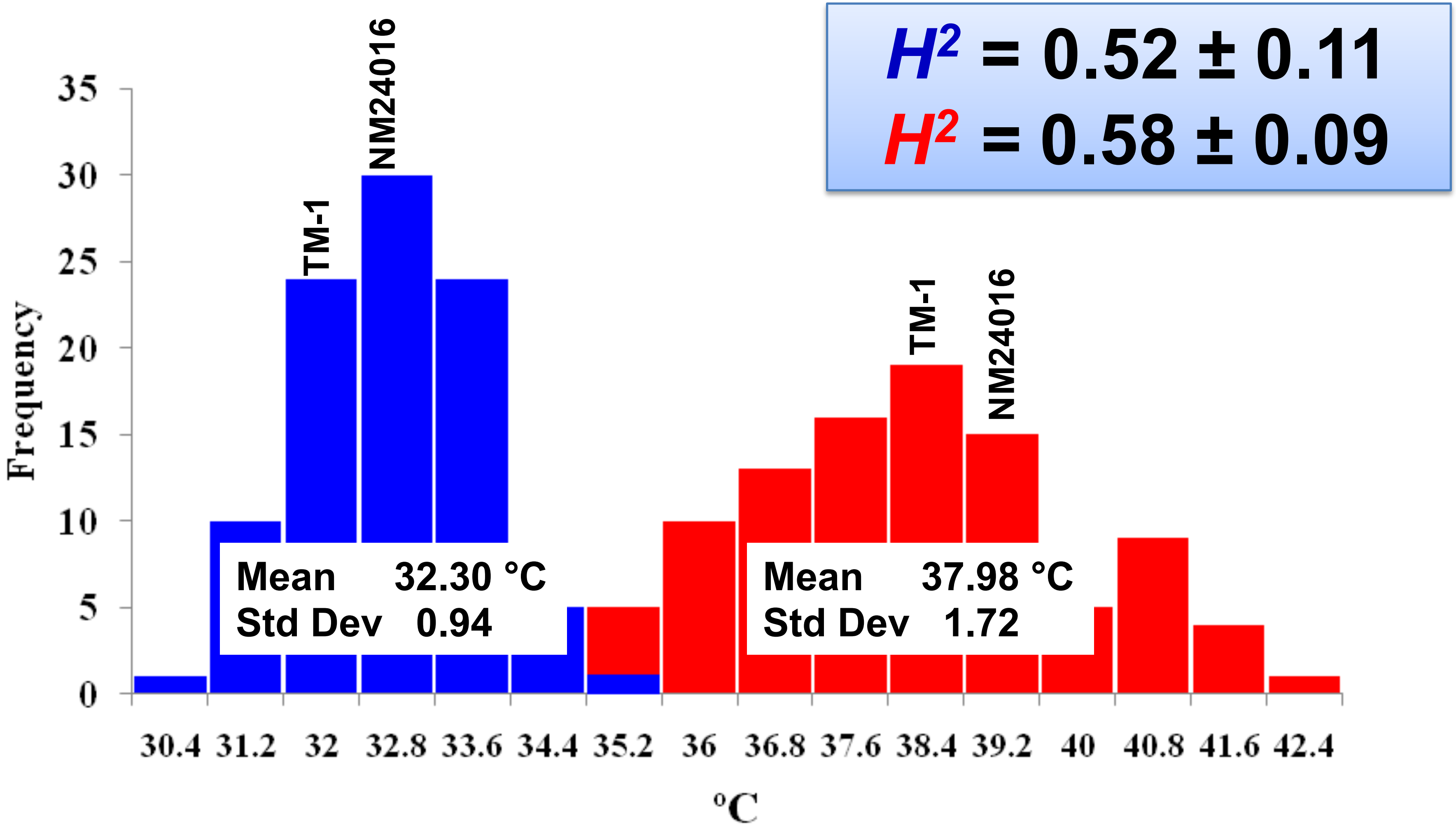
1pm MST on day 224 (12-Aug)

Significant time-by-treatment interaction for canopy temperature



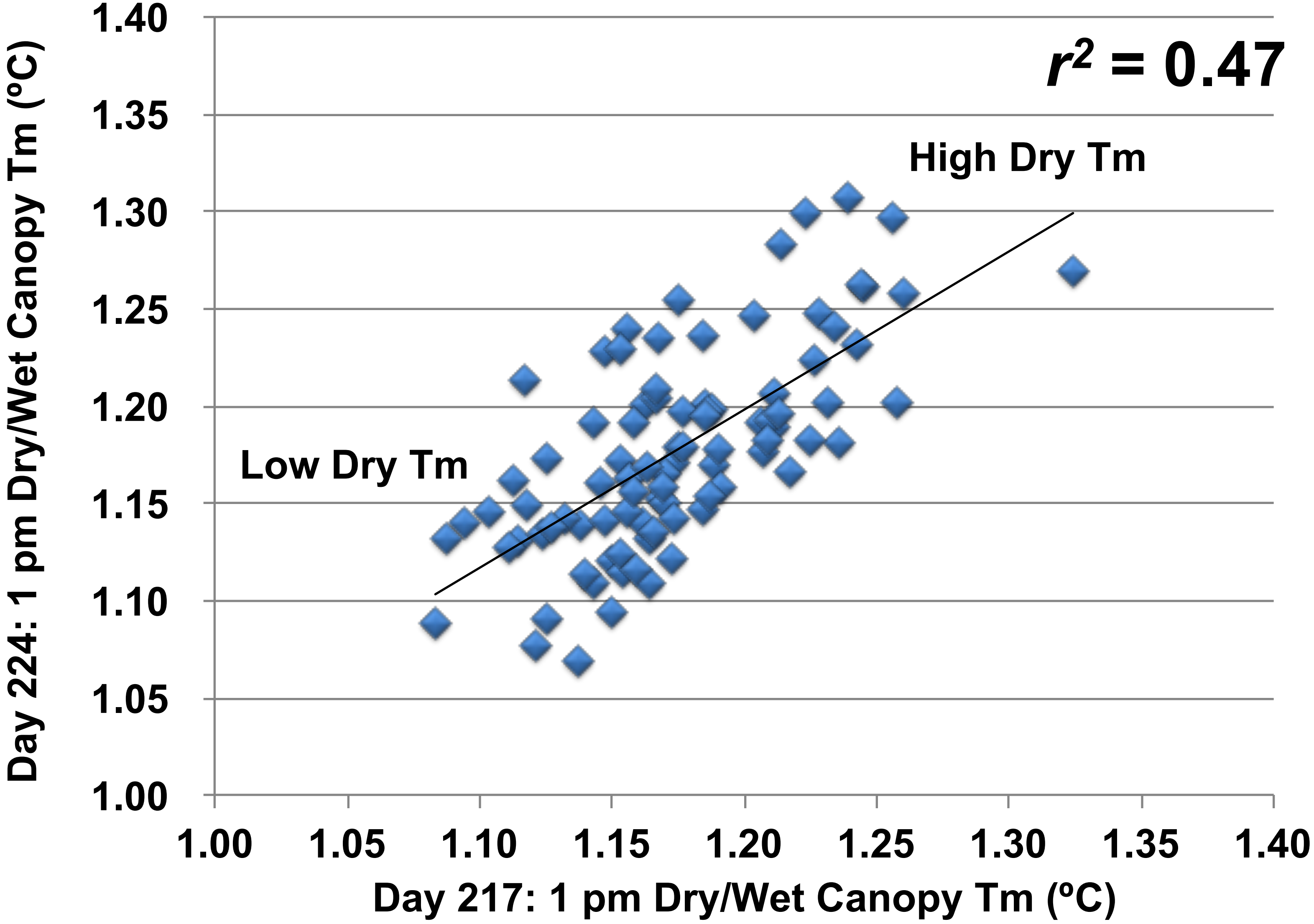
Treatment	$P<0.05$
Time	$P<0.0001$
Treatment*Time	$P<0.0001$

Phenotypic variation: Canopy Tm



Wet and Dry Plots at 1 pm on Day 224 (Aug 12)

Repeatability of Dry/Wet Canopy Tm



GBS-QTL Analysis: Dry/Wet Canopy Tm

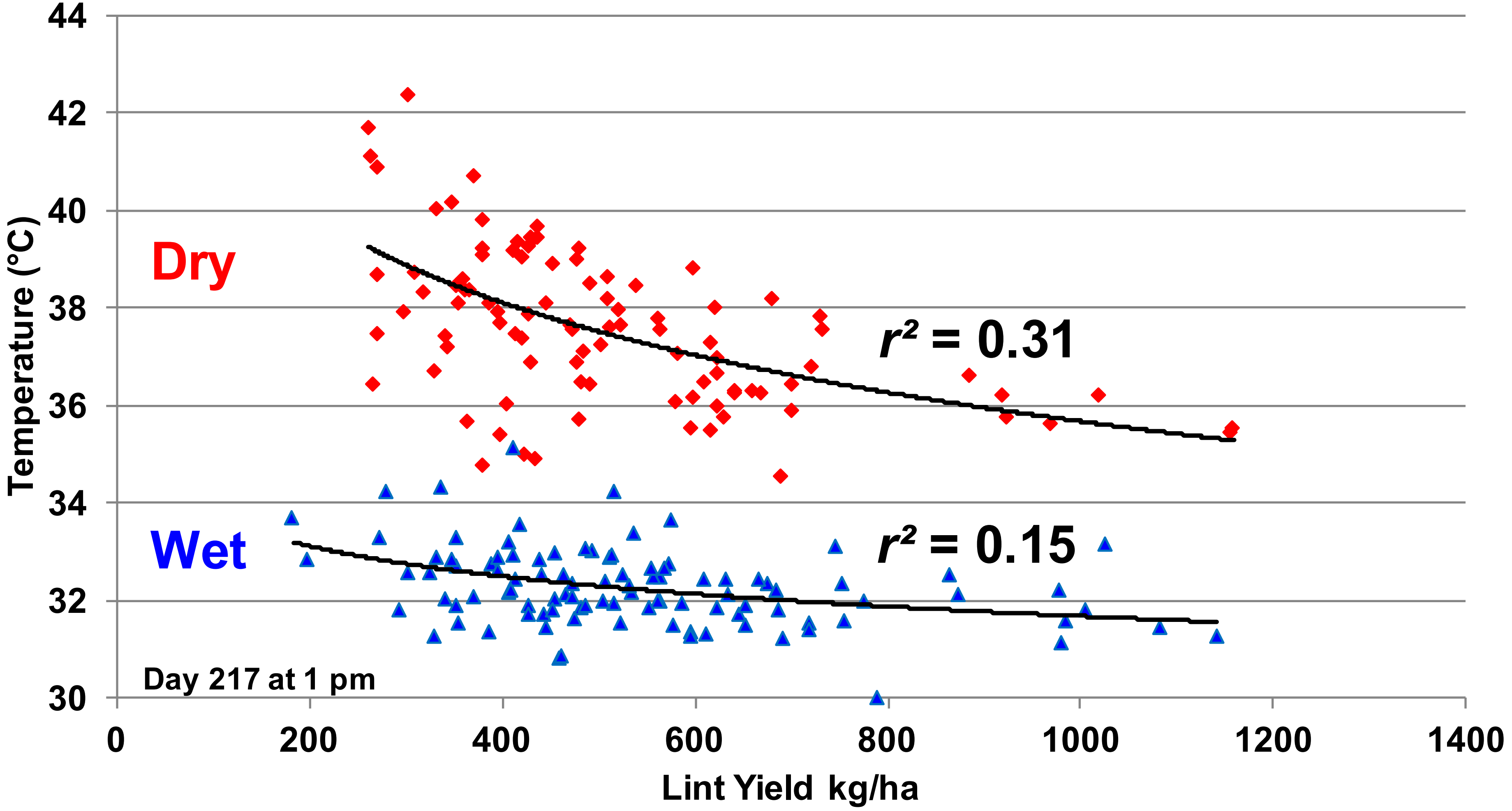
Chr_LG_cM	c10_38_5	c12_51_18	c17_68_14	c20_83_18
Day 217 7 am	-0.006	0.003	-0.005	-0.001
Day 217 1 pm	**-0.019**	0.008	**-0.016**	**0.015**
Day 224 7 am	-0.004	0.005	-0.004	0.002
Day 224 10 am	-0.011	0.010	-0.009	0.009
Day 224 1 pm	**-0.020**	**0.018**	-0.010	**0.020**

Negative – decrease °C **Positive** – increase °C

All statistically significant allelic effects are indicted by a black rectangle border

Considered identical QTL if support intervals overlapped

Increased leaf transpiration rate under drought stress contributes to higher lint yield



Higher leaf transpiration provides more of an adaptive advantage in terms of yield when grown under drought

Small unmanned aircraft systems: potential to combine the throughput of aerial imaging with the precision of a ground-based system

Margaret Krause

Duke Pauli

James Clohessy

Nicholas Kaczmar

Plant phenotyping: hand-held for breeders

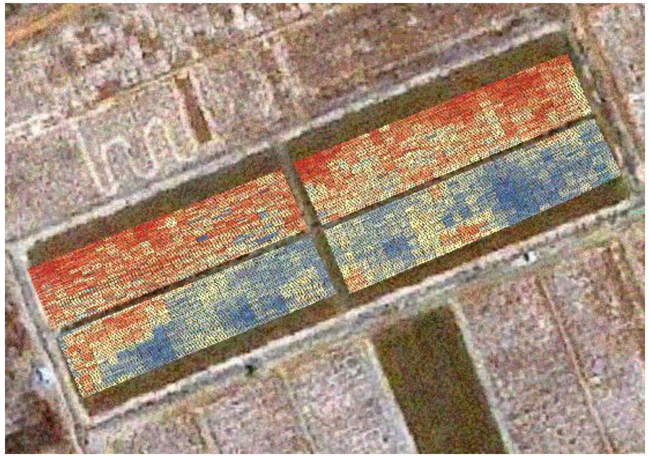
- Sensors: temperature, height, infrared imaging
- Hardware: intelligent real time processing
- Flexible: functional with or without RTK-GPS

Component	**Prototype Price**
Raspberry Pi Model B+, ARM1176, 512MB RAM	$35
5MP Camera IR filter removed	$25
IR Temperature Sensor MLX90614	$16
IR Range Sensor GP2Y0A21	$13
High Resolution Sonar MB1023	$30
Battery & Solar Panel 5000mAh capacity, 1W charging	$28
3 Axis Accelerometer ADXL335	$6
MicroSD Storage 16GB	$9
Additional Hardware LCD screen, buttons, case, etc	$30
Total Prototype Cost	**$192**

James Clohessy

Combined drought and heat stress trial at CIMMYT-Harare in Zimbabwe

Skywalker UAS



- Hybrids generated from CIMMYT/HarvestPlus breeding material
- Field-based, high-throughput phenotyping of physiological responses
- GWAS and GP for carotenoid, agronomic, and physiological traits

Christine Diepenbrock

Additional Research Projects

Accelerated Development of Commercial Hydrotreated Renewable Jet Fuel from Redesigned Oil Seed Feedstock Supply Chains
Funding Source: USDA-NIFA-DOE BRDI
Investigators: H. Colvin; M. Gore; C. McMahan; M. Jenks; J. Dyer; A. Landis; M. Fraley

Elodie Gazave

Securing the Future of Natural Rubber – An American Tire and Bioenergy Platform from Guayule
Funding Source: USDA-NIFA-DOE BRDI
Investigators: T. Isbell; M. Gore; M. Jenks; J. Dyer; D. Long; D. Archer; S. Frey; D. Galloway; T. Tomlinson

Dan Ilut

Accelerating Oat Breeding for Nutritional Quality: beta-glucans, lipids, and antioxidants
Funding Source: PepsiCo
Investigators: M. Sorrells; M. Gore; J.-L. Jannink; O. Hoekenga

James Clohessy

USDA United States Department of Agriculture National Institute of Food and Agriculture
U.S. DEPARTMENT OF ENERGY
PEPSICO

Additional Research Projects

Elucidating the genetic basis and relationship of root post-harvest physiological deterioration tolerance and carotenoid levels in West African cassava germplasm
Funding Source: BMGF PEARL
Investigators: N. Ndubuisi and M. Gore


Njoku Damian Ndubuisi

Relevance of gender in trait preferences of cassava small-scale farmers in Uganda
Funding Sources: BMGF, NextGen Cassava and CGIAR RTB
Investigators: P. Iragaba; M. Gore; H. Tufan; R. Bezner-Kerr; NaCRRI


Paula Iragaba

Breeding methods and germplasm for improved nutritional quality of sweet corn
Funding Sources: Hatch NIFA and Cornell Startup Funds
Investigators: M. Gore, M. Smith


Billie Guilliatt


Matt Baseggio

Genetic diversity of common bean landraces in US Southwest
Funding Source: Cornell Startup Funds
Investigators: M. Gore and Native Seeds Exchange


Di Wu

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David Fang

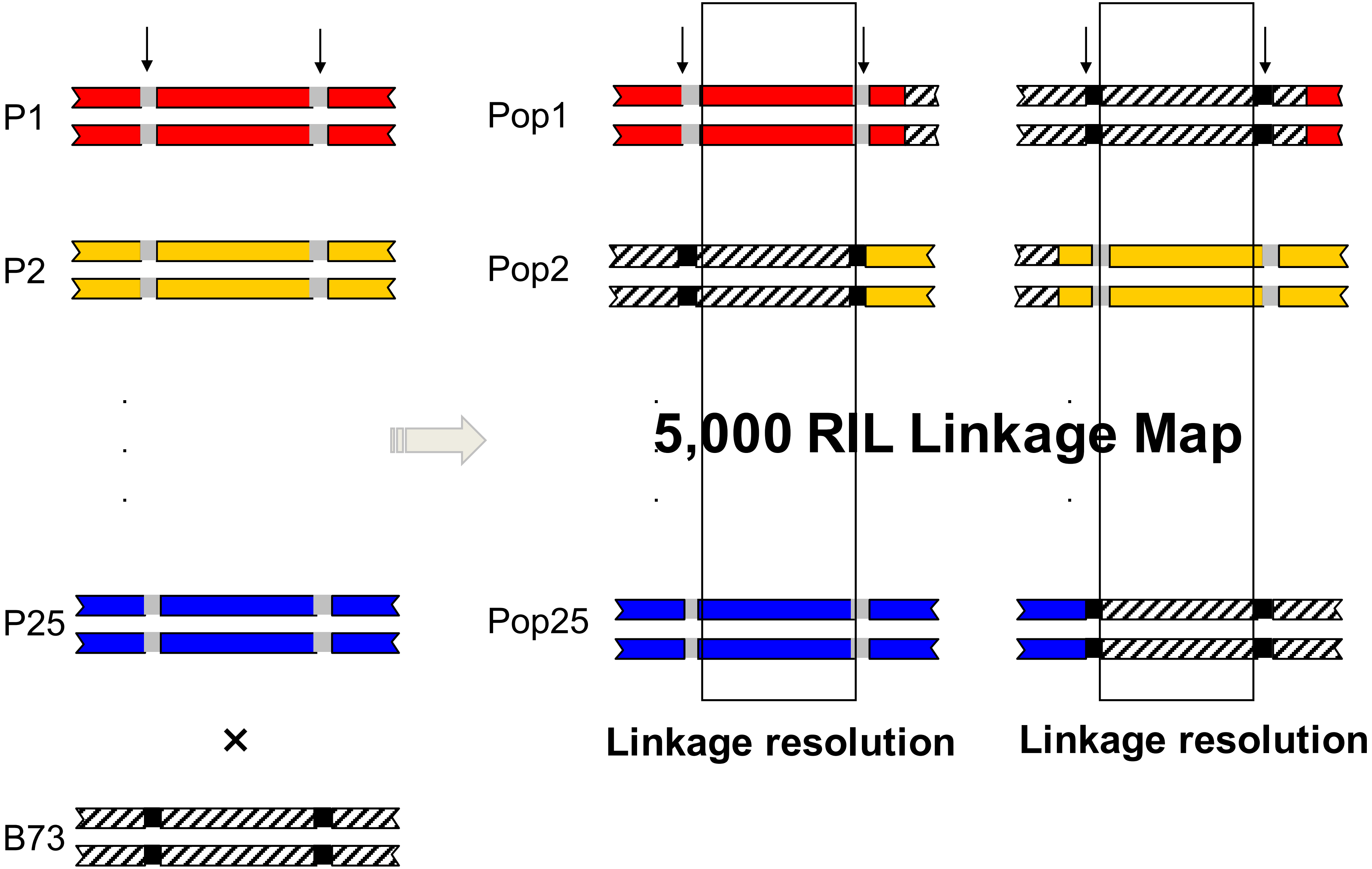
COTTON INCORPORATED

USDA

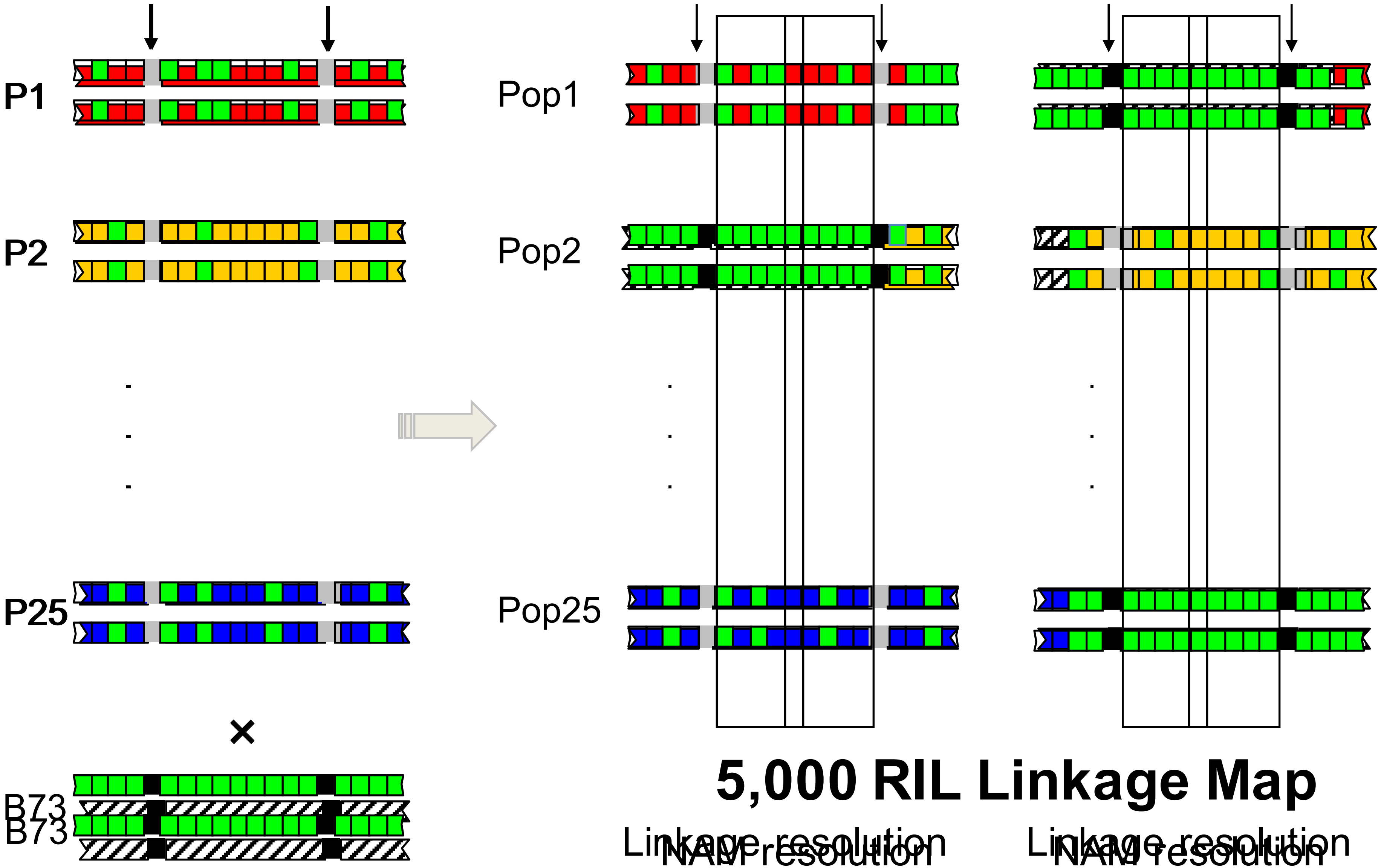
National Science Foundation
WHERE DISCOVERIES BEGIN

PGRP Award# 1238187

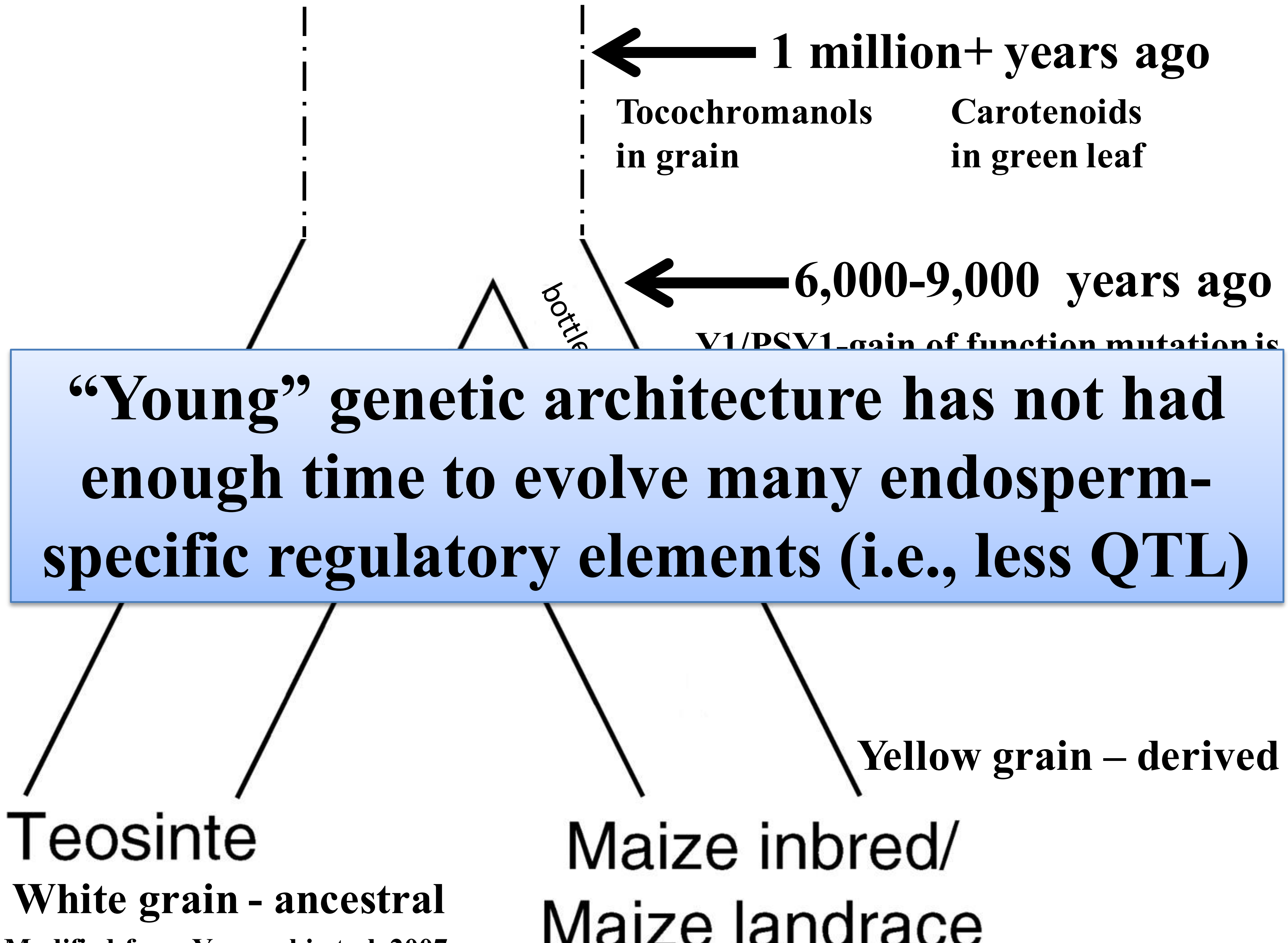
The 5,000 RILs are genotyped with 14k GBS SNP markers for NAM joint linkage



Whole-genome resequencing of parents and impute 30M SNPs onto recombination blocks

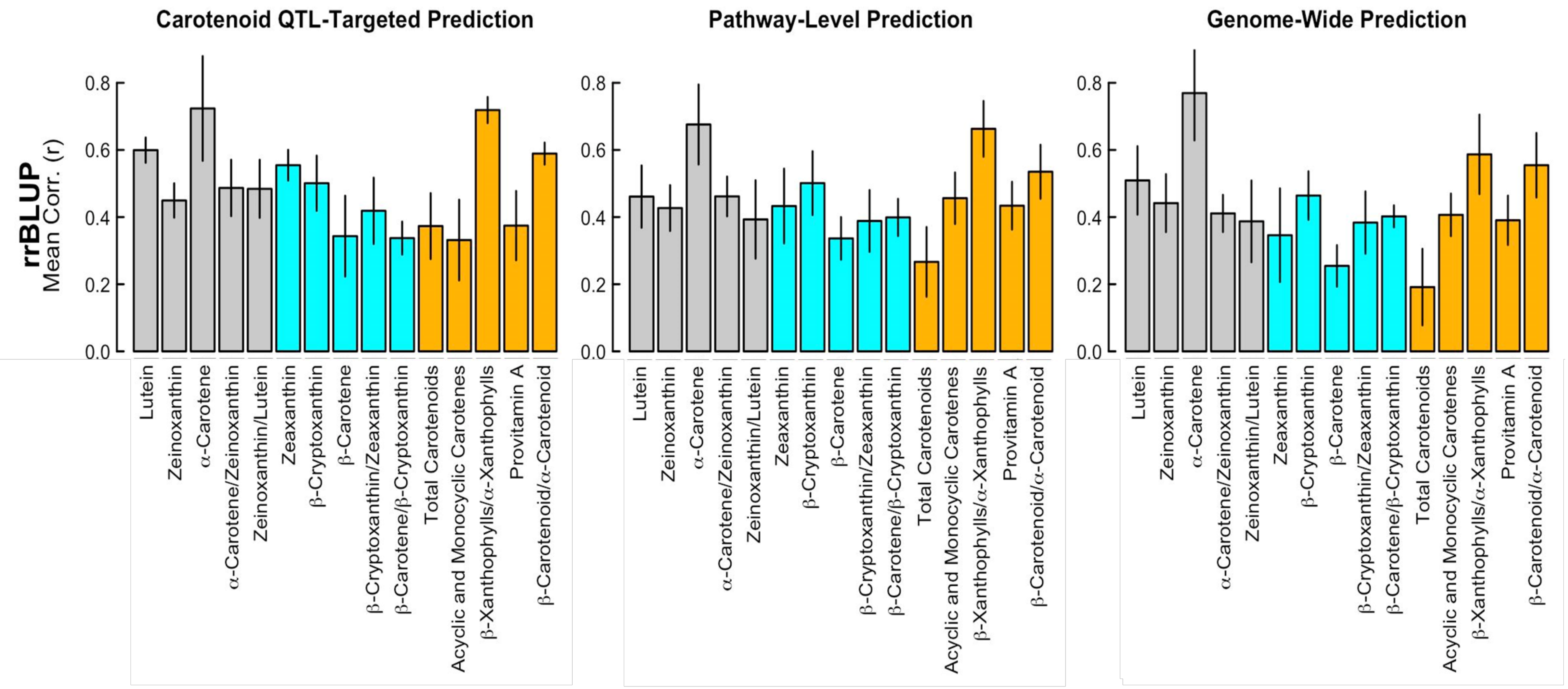


5,000 RIL Linkage Map



Modified from Yamasaki et al. 2007

Markers at candidate genes associated with *a priori* QTL predict carotenoid traits as well as genome-wide marker set



Owens et al., 2014, in press

Genomic prediction models: diverse maize panel

Harvest of Orange Maize for Vitamin A Nutrition Efficacy Trial

Zambia


Africa Acceptance – using orange to overcome preference For white grain and concerns about yellow grain. Orange also associated with more total carotenoids, more flux into pathway. Thus, more that can be modulated to provitamin A.

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Biology, Texas A&M University

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MEPS: Molecular & Environmental Plant Sciences

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Soil & Crop Sciences, Texas A&M University

Graduate and Professional Studies, Texas A&M University

Plant Breeding Symposium, Texas A&M University, 2015