

Elucidating the Genetic Architecture of Extremely Elevated Seed Stearic Acid in Soybean Using Genotyping-By-Sequencing

Crystal Heim¹, Jason D. Gillman²
¹University of Missouri-Columbia, Division of Plant Sciences
 crystal.buerke@gmail.com
²USDA-ARS, Midwest Area, Columbia, MO 65211

Introduction

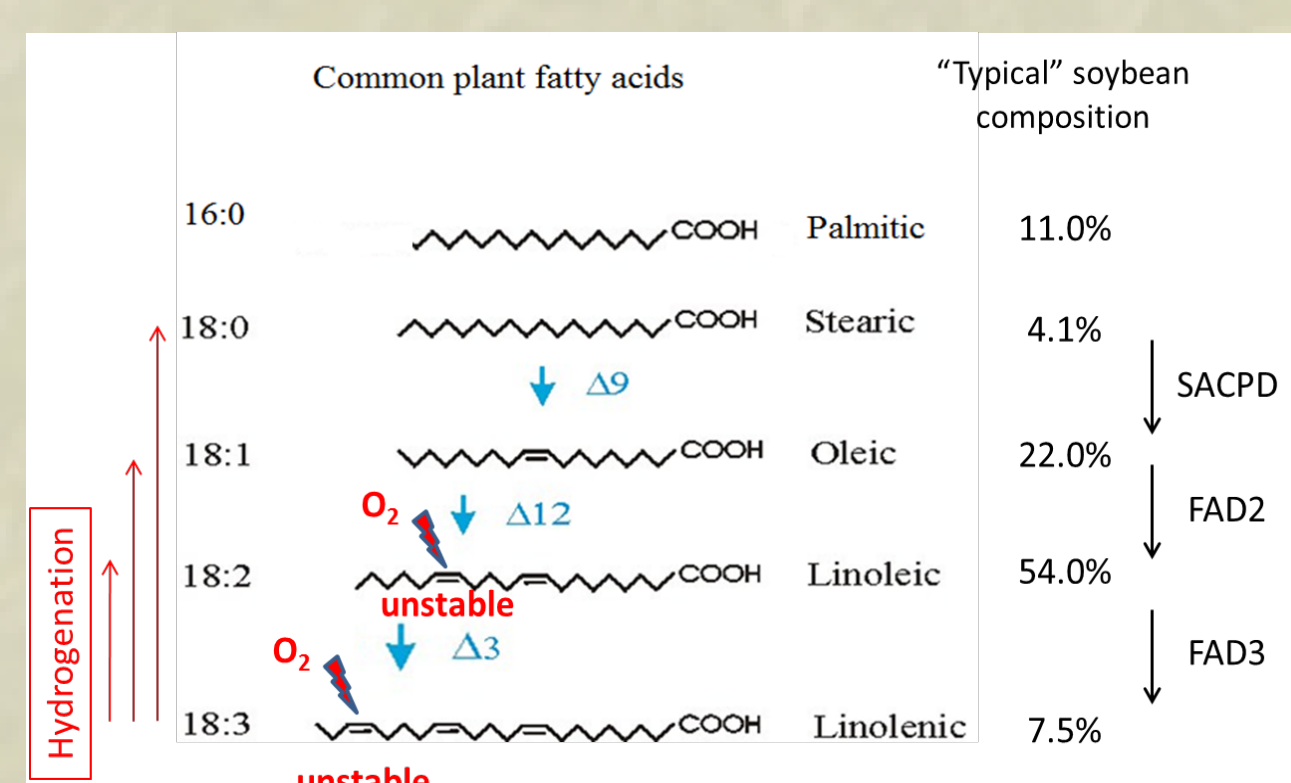
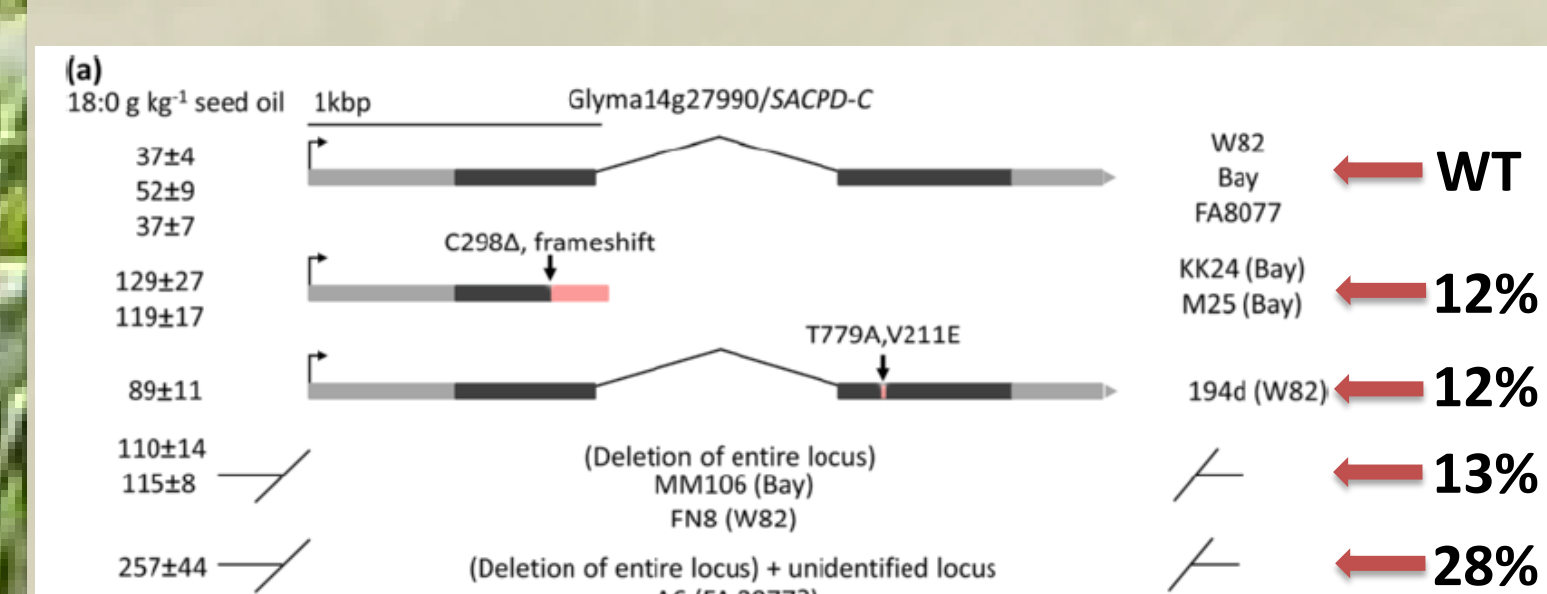
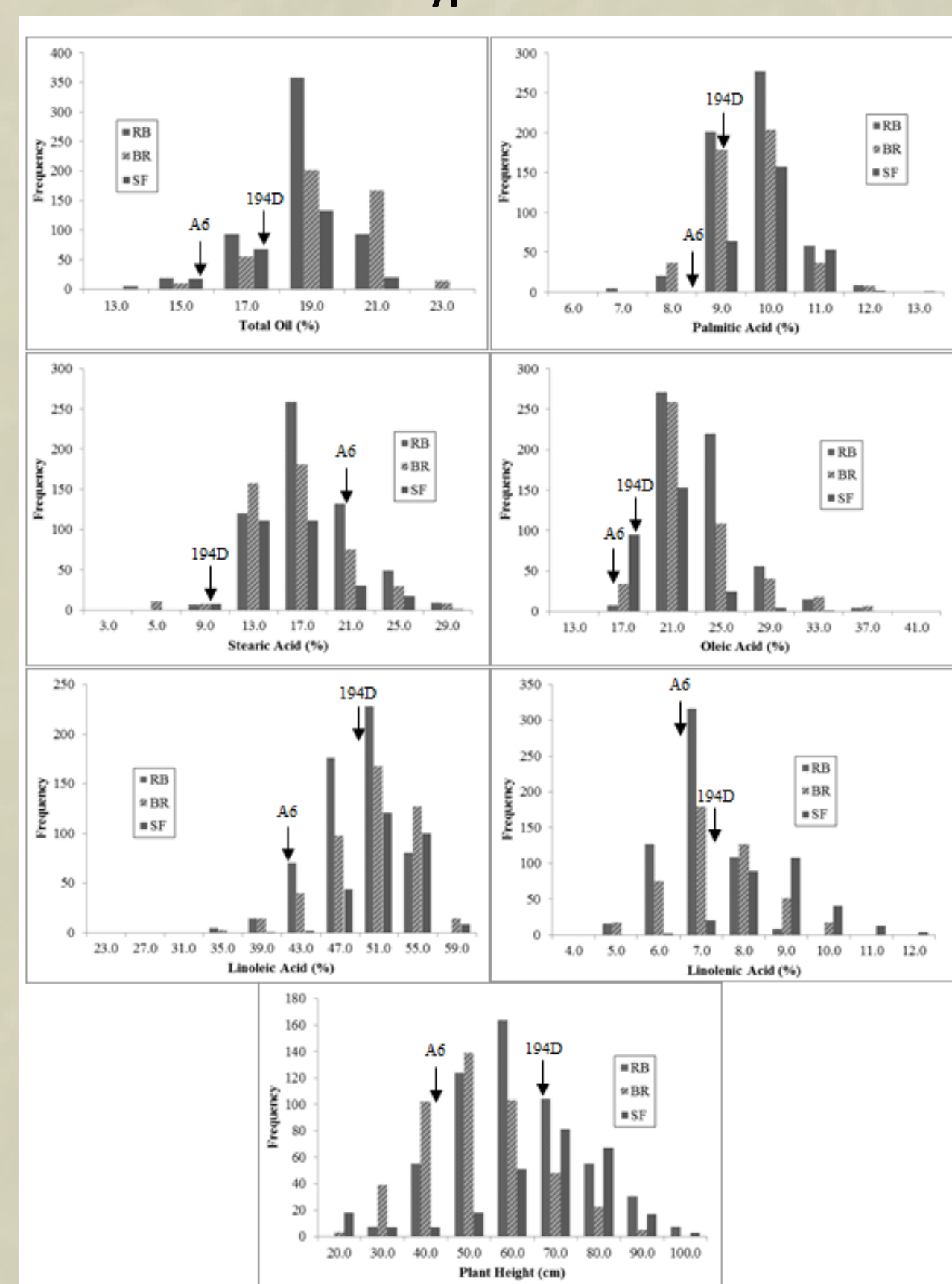


Figure 1. Soybean fatty acids: stability, typical composition

Materials and Methods

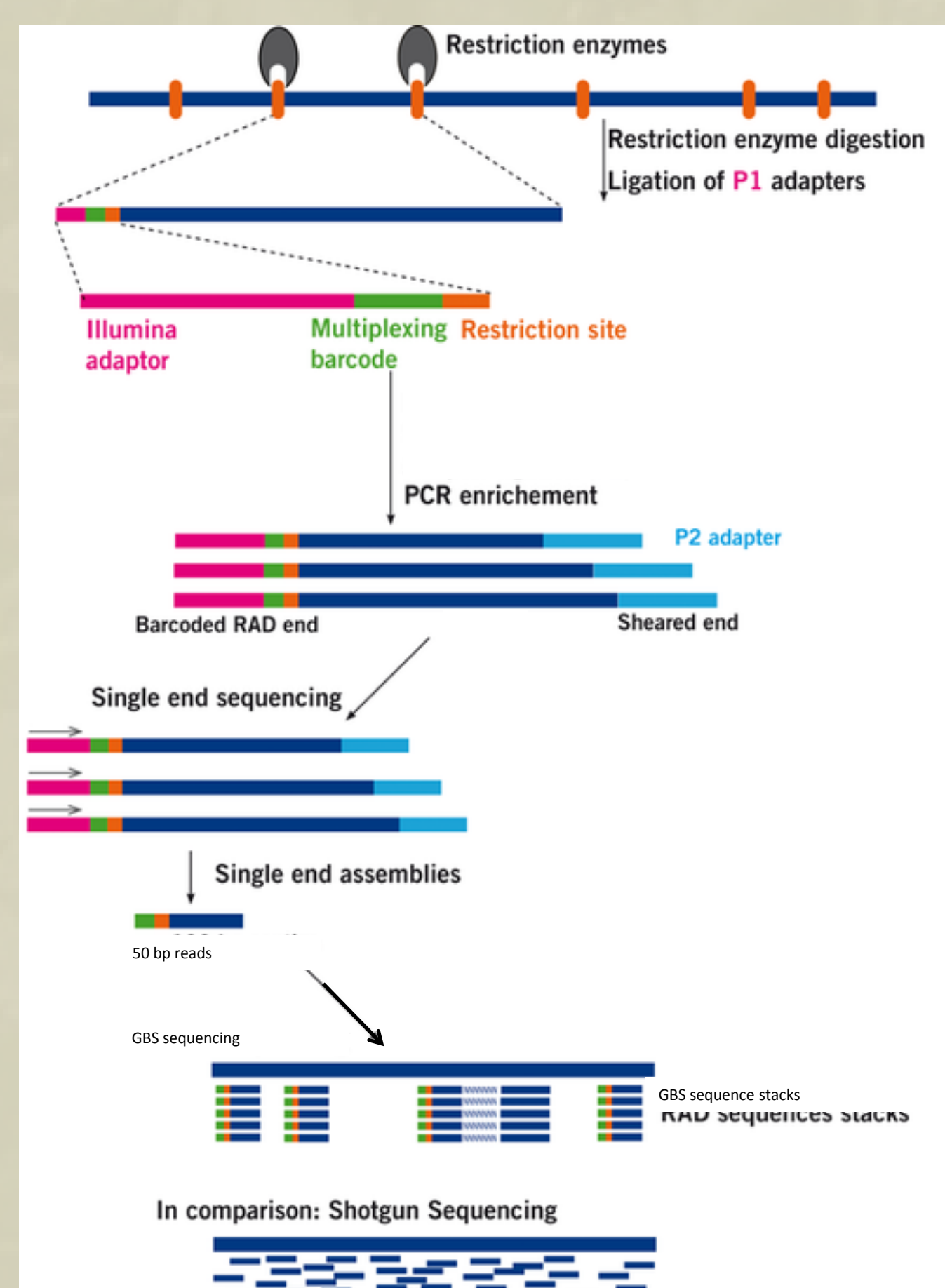


Phenotypic distributions

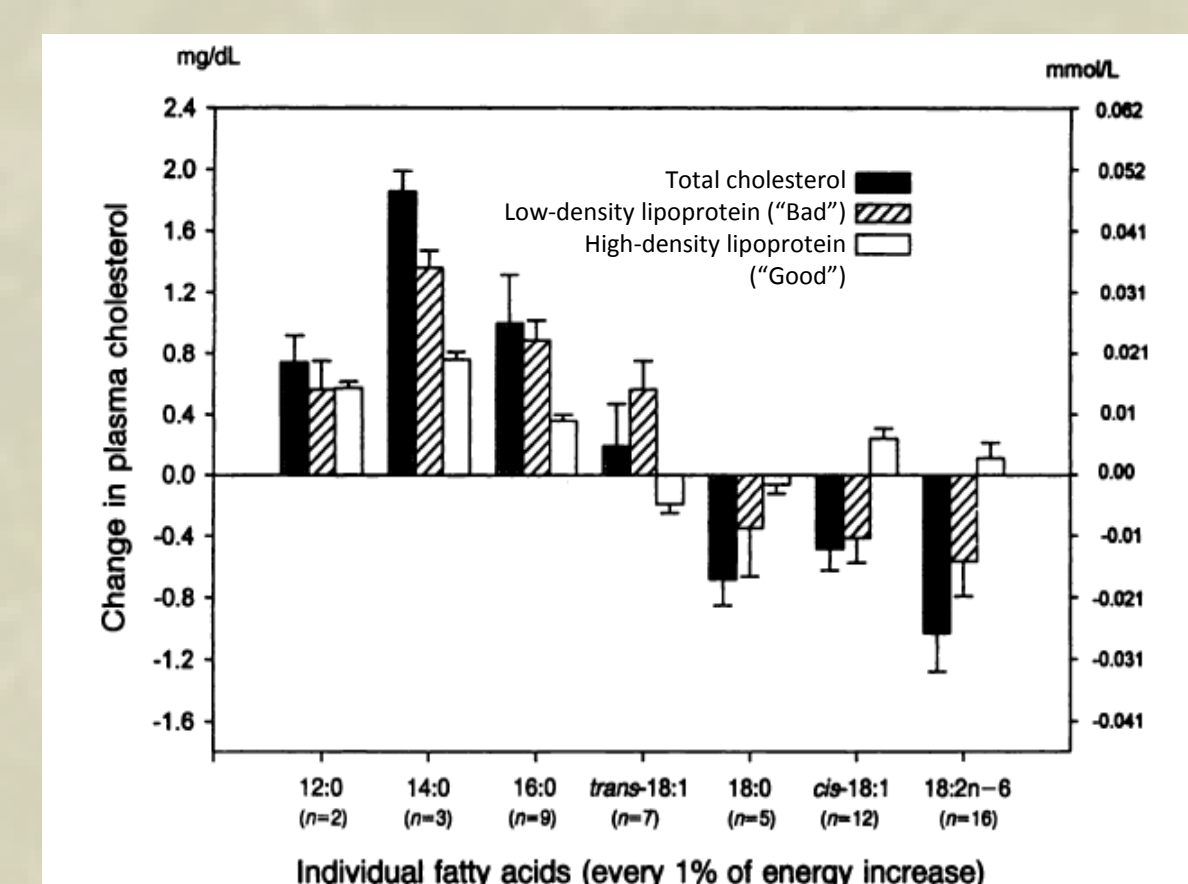
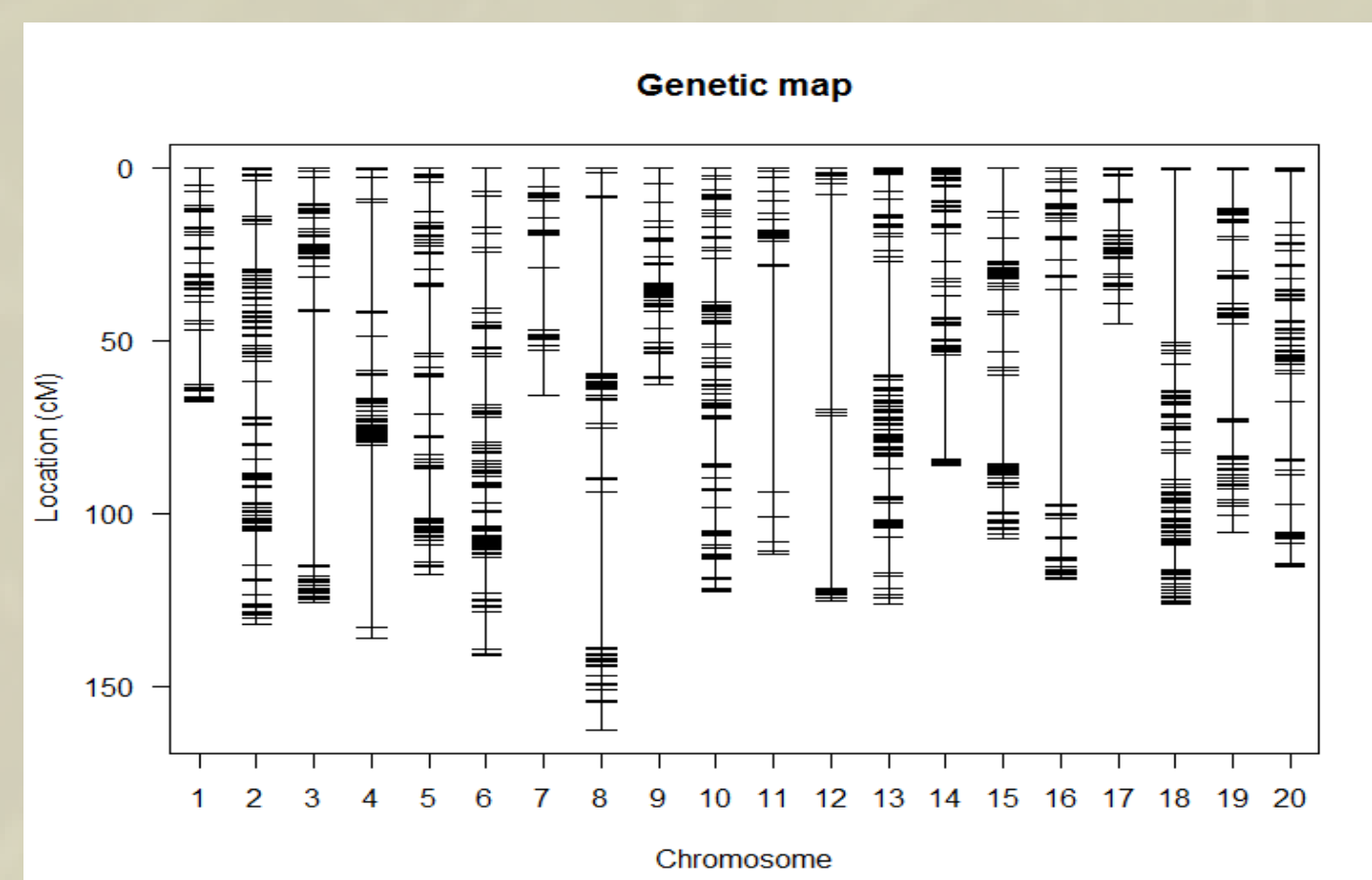


Supplemental Figure 3 Frequency distributions of soybean seed traits

GBS genotyping



27,672 SNPs were identified, with an average SNP coverage of 10.98-fold. SNPs were filtered to <20% missing data, allele frequency between 0.2 and 0.8, bringing the SNP count down to 5,423. Chi-square test removed severely distorted markers (at F_{4.5}), resulting in a final count of 2,977



Individual fatty acids and their effects on cholesterol levels

Stearic Acid (C18:0)

- Unsaturated fatty acids, such as linolenic (C18:3) and linoleic (C18:2), are oxidatively unstable, and tend to go rancid rapidly
 - These fatty acids must be hydrogenated to increase stability (Figure 1)
- The hydrogenation process results in the formation of trans fatty acids, which are tightly linked with heart problems in humans and "not generally regarded as safe" by the USDA
- Stearic acid (C18:0) is a long chain fully hydrogenated fatty acid that has been shown to have no negative effect on LDL levels (unlike other saturated fatty acids)
- Soybean oil with elevated saturated fatty acid content is desirable for solid fat baking applications, and could potentially eliminate the need for expensive solid fats like cocoa butter and heart-unhealthy fats such as palm oil

Table 1. Fatty acid composition of A6 and its parent FA 8077.

Line	Fatty acid					
	Palmitic	Stearic	Oleic	Linoleic	Linolenic	Arachidic
A6	8.0	28.1	19.8	35.5	6.6	2.0
FA 8077	8.4	4.4	42.8	36.7	7.6	< 1.0

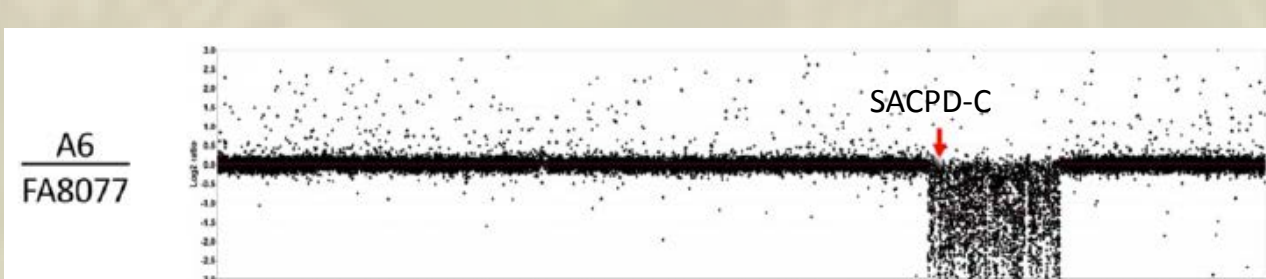
Mutant lines: A6 and 194D

A6 is a mutant characterized by several large deletions, including ~1/8 chromosome deletion on Gm14 predicted to encompass 30 genes from the Glyma 1.0 high confidence gene set, including SACP-D-C.

A6 exhibits poor germination and severe field defects such as reduced height and yield

194D is an EMS-induced mutant with moderate levels of stearic acid (~12%) due to a point mutation (V211E) in SACP-D-C (Figure 4)

Previous work by Zhang et al (2008) and first demonstrated that the elevated stearic acid trait in soybean is due to loss of function mutations affecting a stearyl-acyl carrier protein-desaturase gene, SACP-D-C,



Comparative Genome Hybridization of A6 and its progenitor line

GBS/QTL mapping

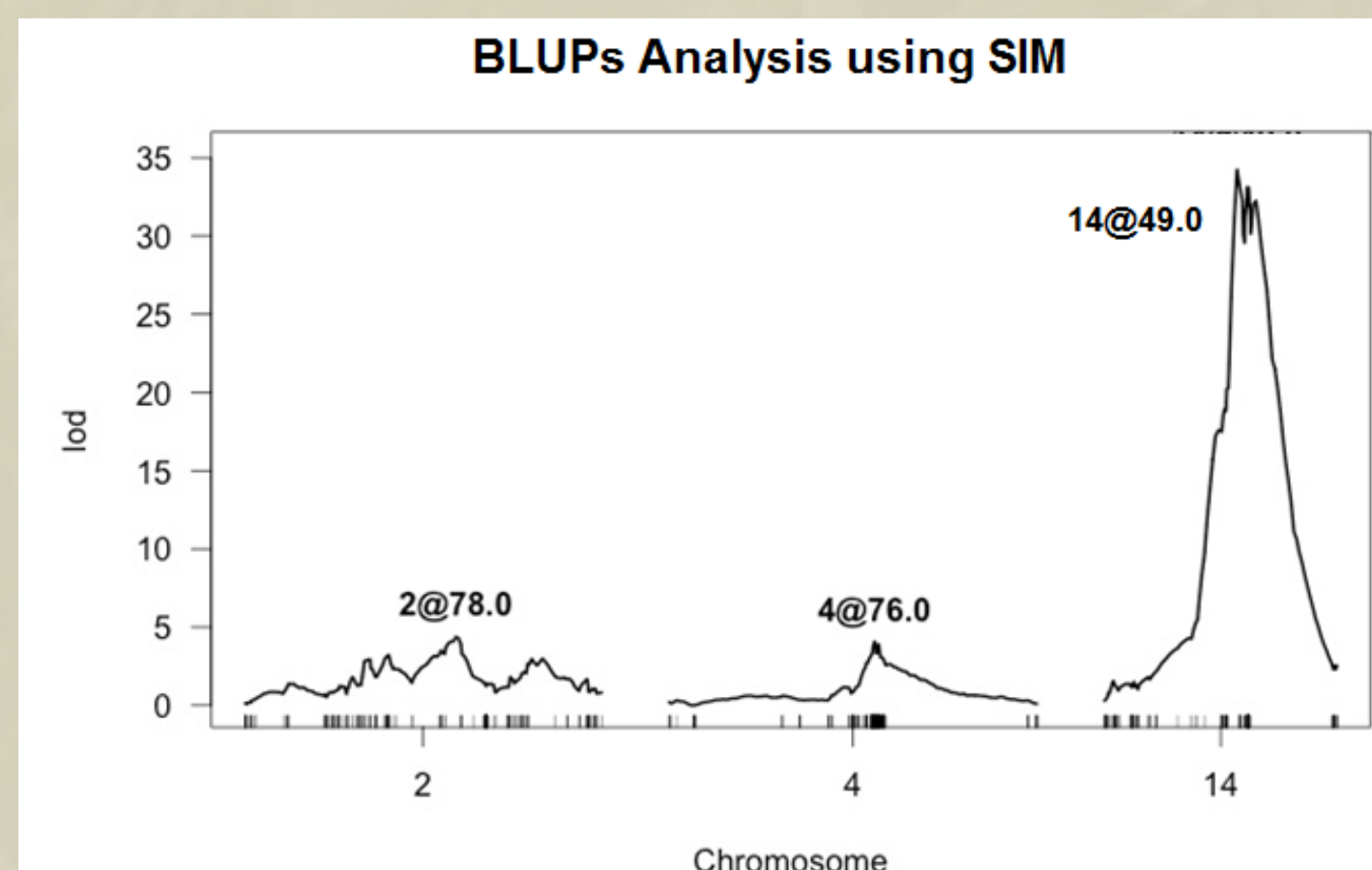
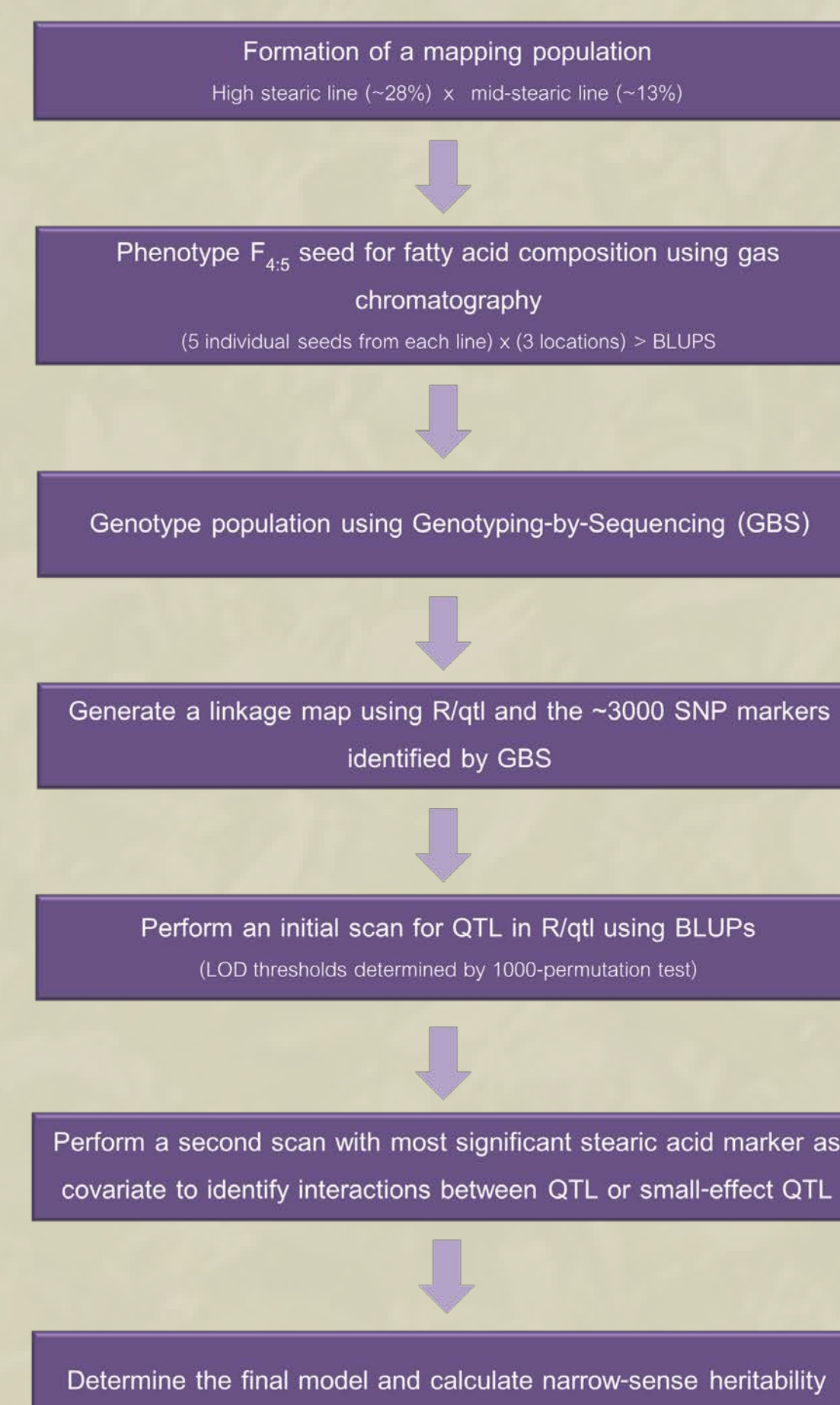


Figure 1 QTL results for the seed stearic acid trait using BLUPs and the SIM method.

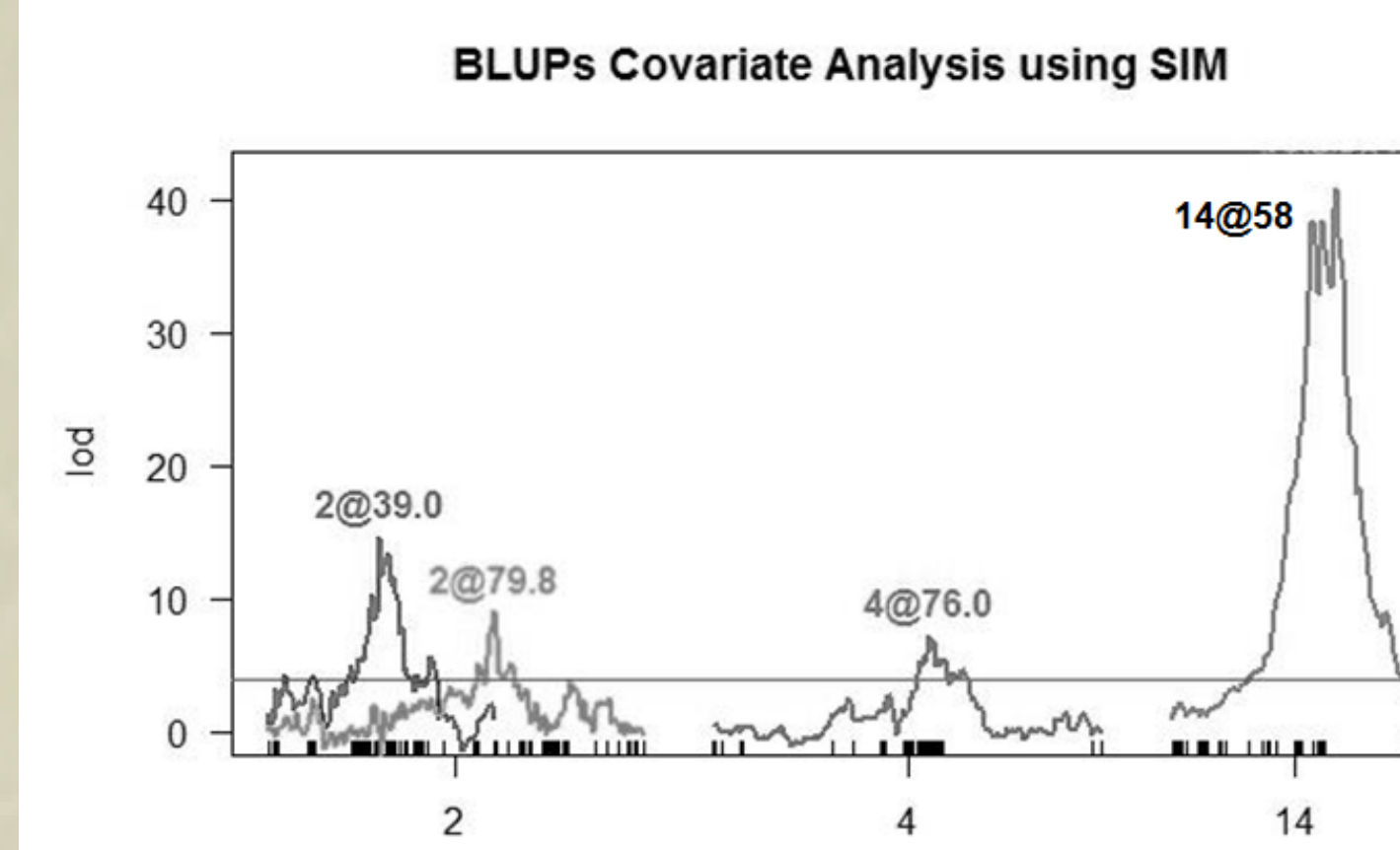


Table 5 Summary of QTL results for 7 traits in soybean A6x194D cross

Trait	QTL	Chr	SNP marker nucleotide	Position (cM)	1.5 Interval	LOD	R ² (%)	QTL effect ^a
Palmitic Acid	q-2p	2	Gm2-7138451	44.58	38-128	5.55	10.49	+0.36%
	q-2-2a	2	Gm2-1420665	2.45	0-8	6.70	13.06	+0.44%
	q-14p	14	Gm14-12506615	44.91	42-58	6.89	13.26	+0.44%
	q-2.1a	2	Gm2-5946912	39	38-42	14.63	13.75	-1.16%
	q-2.2a	2	Gm2-1552379	79.79	78-80	9.05	7.86	-0.52%
Stearic Acid	q-4a	4	Gm4-1831293	75.99	73-78	7.23	6.12	+1.24%
	q-14a	14	Gm14-4220409	58	57-59	40.84	56.76	-5.00%
	q-4a	4	Gm4-1946976	76.80	74-80	6.57	12.17	+1.37%
	q-14a	14	Gm14-3229452	51	49-54	13.7	20.95	+2.2%
Oleic Acid	q-4a	4	Gm4-32351007	78.88	73-84	6.74	14.75	-2.31%
	q-14a	14	Gm14-34918500	54	45-63	4.48	9.49	+2.02%
Linoleic Acid	q-2a	2	Gm2-1552379	79.79	78-81	7.05	10.4	+0.33%
	q-4a	4	Gm4-2732180	78.12	76-84	7.04	10.39	-0.21%
Plant Height	q-8a	8	Gm8-44116750	150	148-152	6.93	10.2	-0.15%
	q-10a	10	Gm10-65310798	89.68	81-96	8.13	12.16	-0.42%
	q-13a	13	Gm13-4141355	118.19	108-125	6.48	9.48	-0.17%
	q-14a	14	Gm14-32372635	51.27	44-53	5.52	7.97	-0.25%
	q-10b	10	Gm10-44893959	90	88-94	9.70	20.3	-4.54 cm
	q-19p	19	Gm19-4939820	100.55	91-104	5.2	10.42	+0.3 cm
	q-9a	9	Gm9-6801513	16	15-17	6.85	14.43	+0.28%
	q-14a	14	Gm14-4220409	62	49-68	9.43	20.58	+0.71%

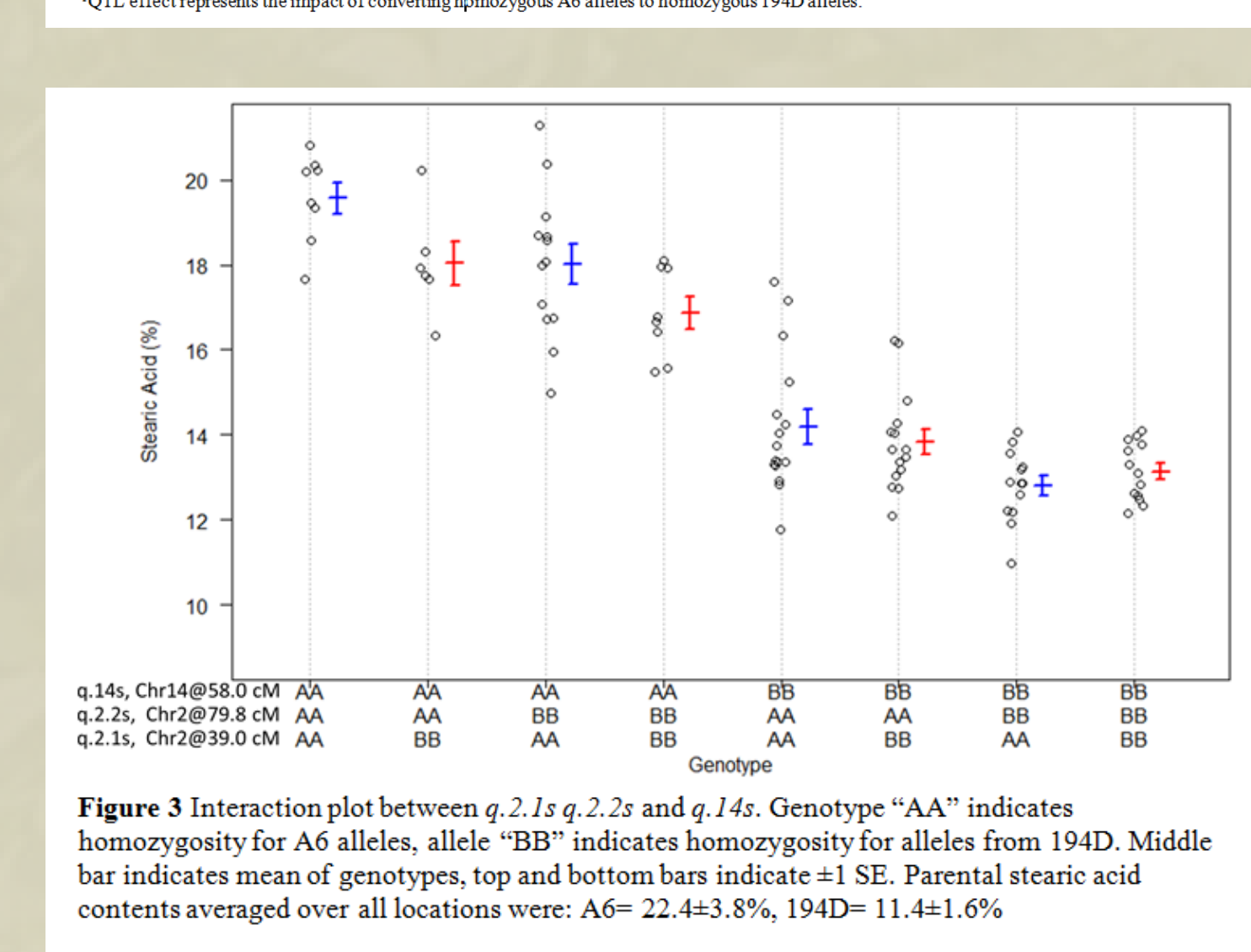
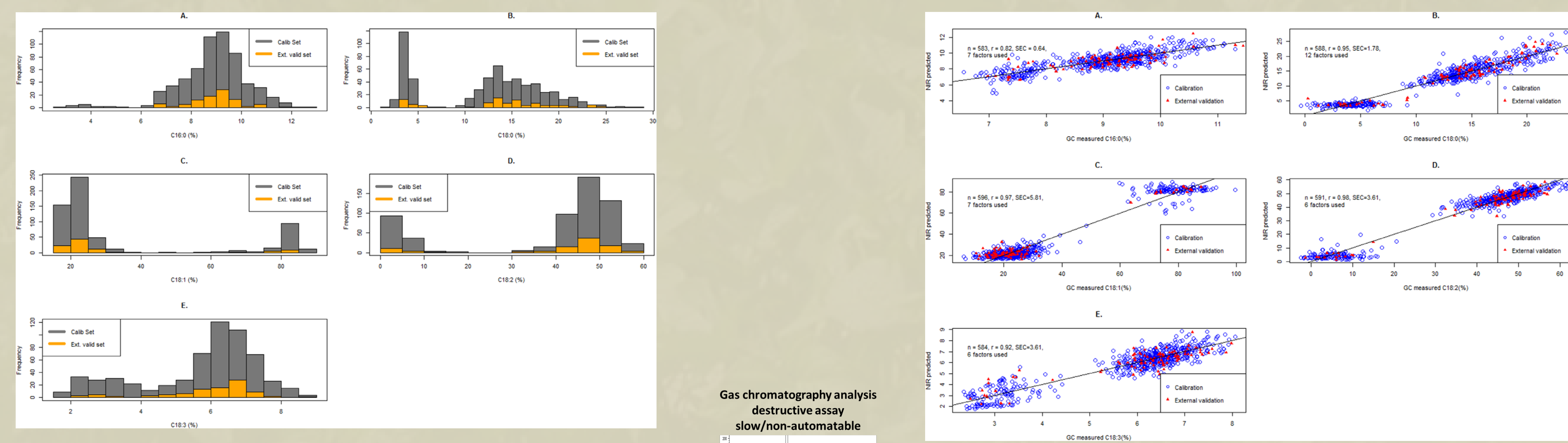
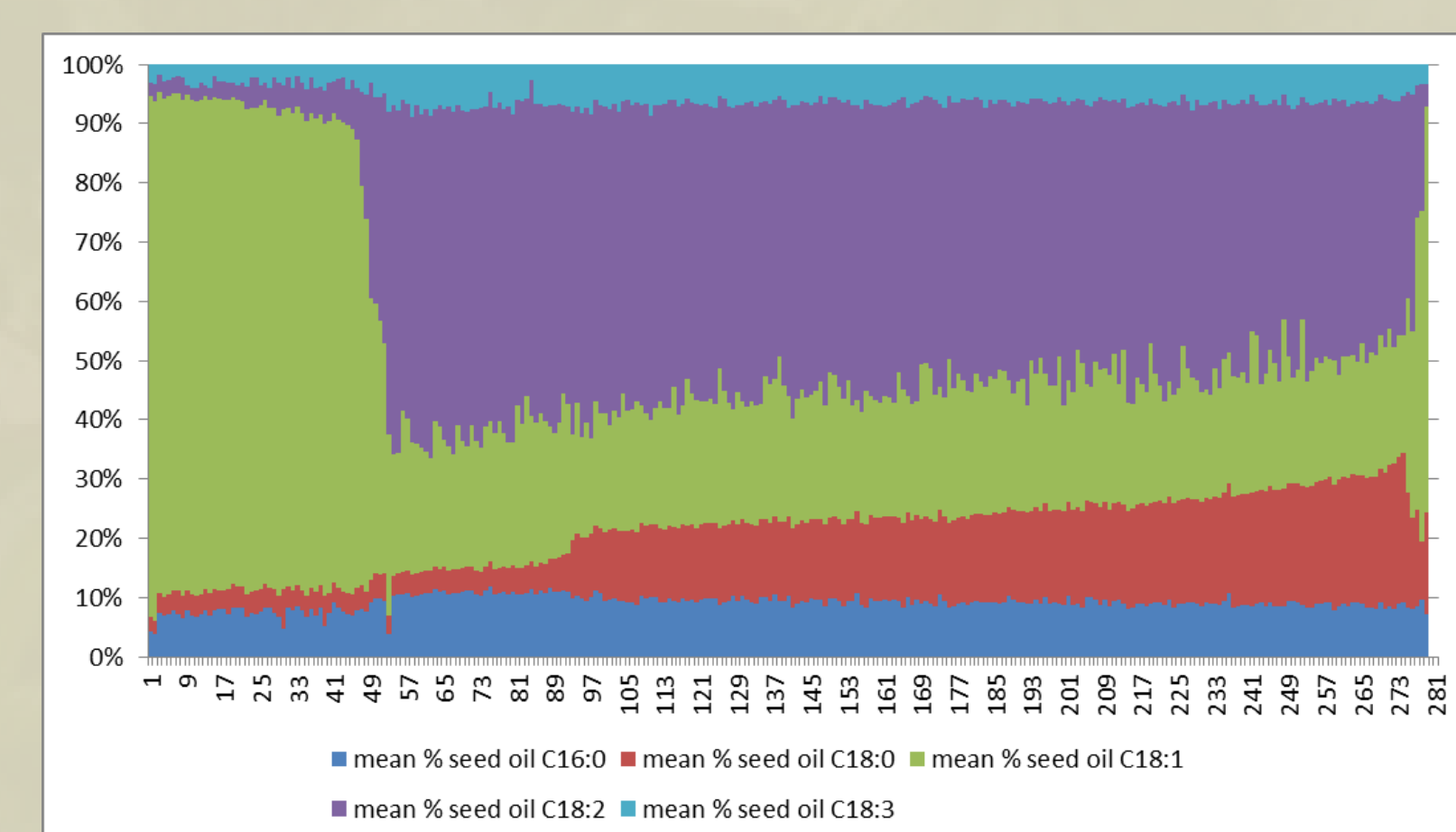


Figure 3 Interaction plot between q.2.1a q.2.2a and q.14a. Genotype "AA" indicates homozygosity for A6 alleles, allele "BB" indicates homozygosity for alleles from 194D. Middle bar indicates mean of genotypes, top and bottom bars indicate ±1 SE. Parental stearic acid contents averaged over all locations were: A6= 22.4±3.8%, 194D= 11.4±1.6%

Fatty acid NIRS calibrations



Phenotypic distribution in NIRS calibration data set



Gas chromatography analysis
destructive assay
slow/non-automatable

NIRS model accurately predicts oil composition from spectral data

Near Infrared Reflectance
non-destructive
rapid/automatable

Correlation coefficients >0.9 for 4 soybean fatty acids

Instrument: monochromator model FOSS 6500 using the transport quarter cup (dimension 97mm x 55mm).

Software: UnScrambler® software 6.11

A Karn, CB Heim, S. Flint-Garcia, K Bilyeu, JD Gillman "Development of rigorous fatty acid near-infrared spectroscopy quantitation methods in support of soybean oil improvement." *JAOCS*, in press (September 2016)

Future Plans

High stearic line A6 and selected high stearic (>20%, A6 x 194D) progeny have recently been resequenced to identify causative alleles.

2016 Multi-location phenotype stability study just completed.

Eventual germplasm release (>20% C18:0).



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References

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