Dissecting Complex Trait Variation with the *Drosophila* Synthetic Population Resource

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Motivation

Types of causative variant

Coding or regulatory ?

SNPs, simple InDels, CNVs ?

Mechanism

Molecular / cellular processes by which natural allelic variation leads to phenotypic change

Evolution

What forces maintain genetic variation ?

Mutation-Selection Balance

Variants unconditionally deleterious

Very rare (< 1% MAF)

Large effect

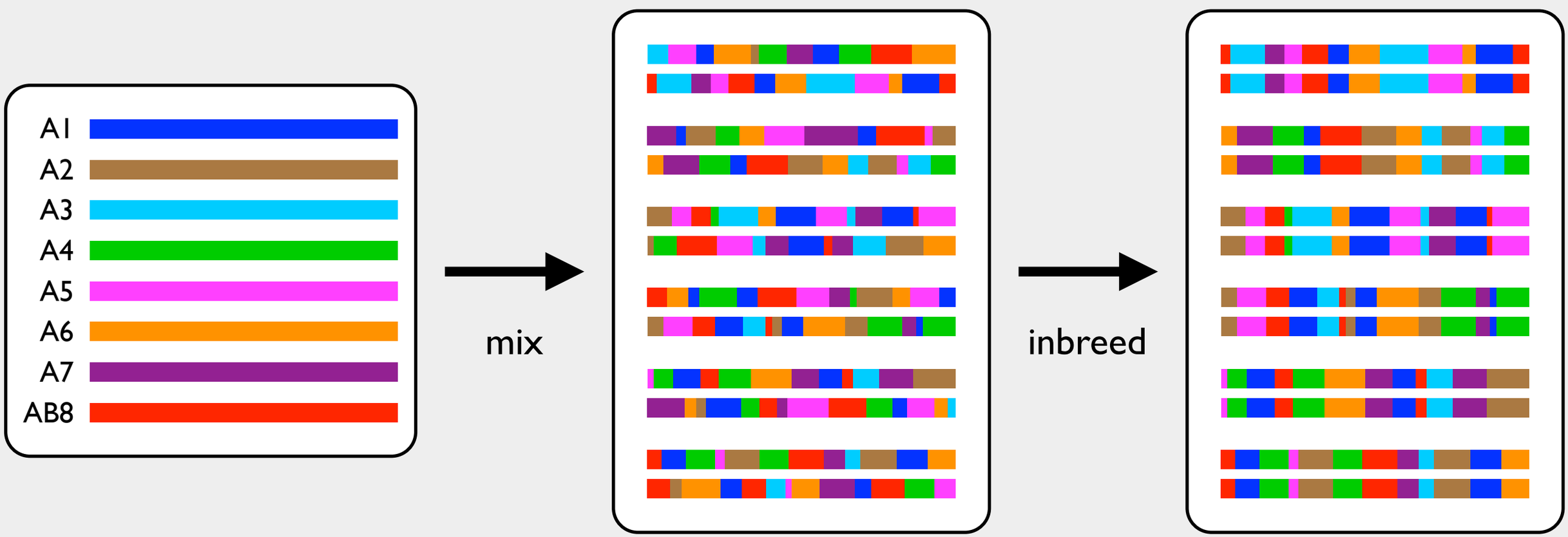
Balancing Selection

Variants maintained by selection

Intermediate-frequency

Subtle effect

Drosophila **S**ynthetic **P**opulation **R**esource



F_0

Founders

F_{50}

Synthetic recombinant population

F_{75}

RILs

King *et al.*, Genome Res. 2012

Empirical "Positive Control"

Overall activity of Alcohol Dehydrogenase (ADH) enzyme

Quantitative genetic variation of enzyme activities in natural populations of *Drosophila melanogaster*

(population genetics/modifier loci/regulatory elements/protein polymorphism)

C. C. LAURIE-AHLBERG†, G. MARONI‡, G. C. BEWLEY†, J. C. LUCCHESI‡, AND B. S. WEIR§

Quantitative analysis of RNA produced by Slow and Fast alleles of *Adh* in *Drosophila melanogaster*

(gene regulation/alcohol dehydrogenase/molecular evolution/polymorphism)

CATHY C. LAURIE* AND LYNN F. STAM*

Use of *in Vitro* Mutagenesis to Analyze the Molecular Basis of the Difference in *Adh* Expression Associated With the Allozyme Polymorphism in *Drosophila melanogaster*

Madhusudan Choudhary[1] and Cathy C. Laurie[2]

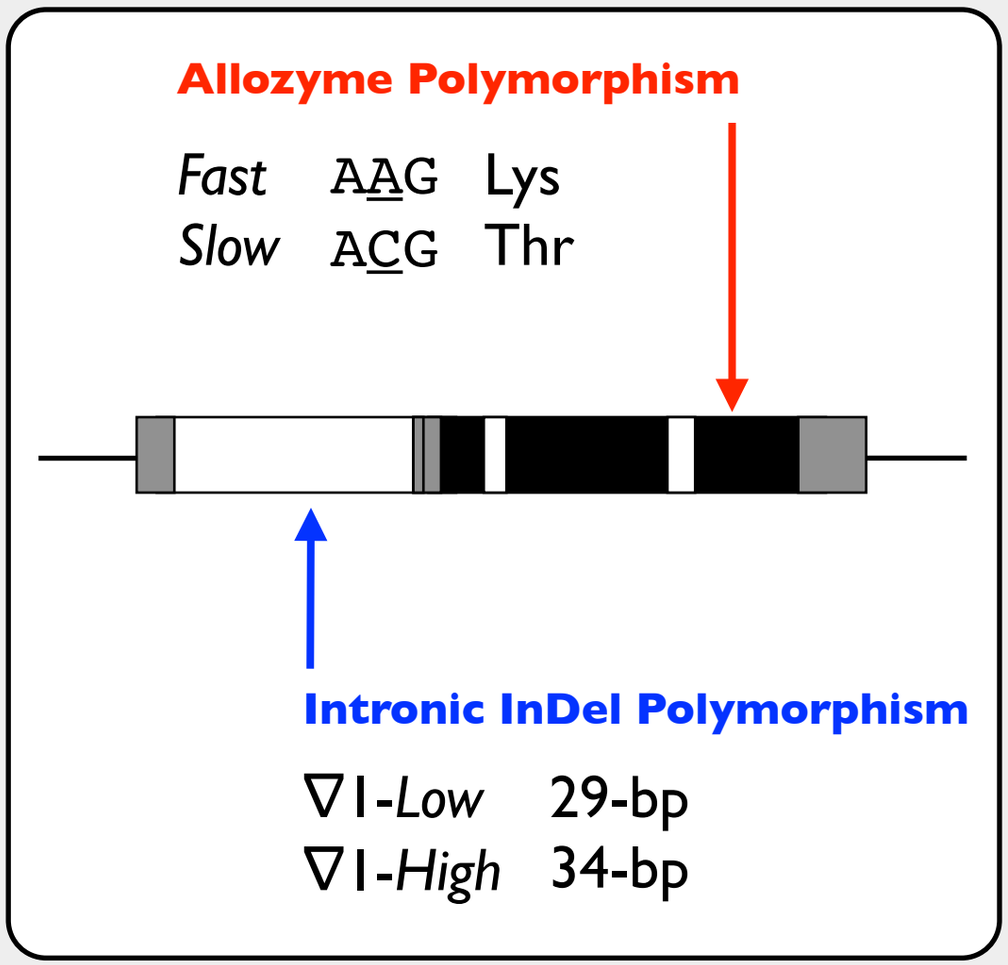
The Effect of an Intronic Polymorphism on Alcohol Dehydrogenase Expression in *Drosophila melanogaster*

Cathy C. Laurie and Lynn F. Stam

Molecular Dissection of a Major Gene Effect on a Quantitative Trait: The Level of Alcohol Dehydrogenase Expression in *Drosophila melanogaster*

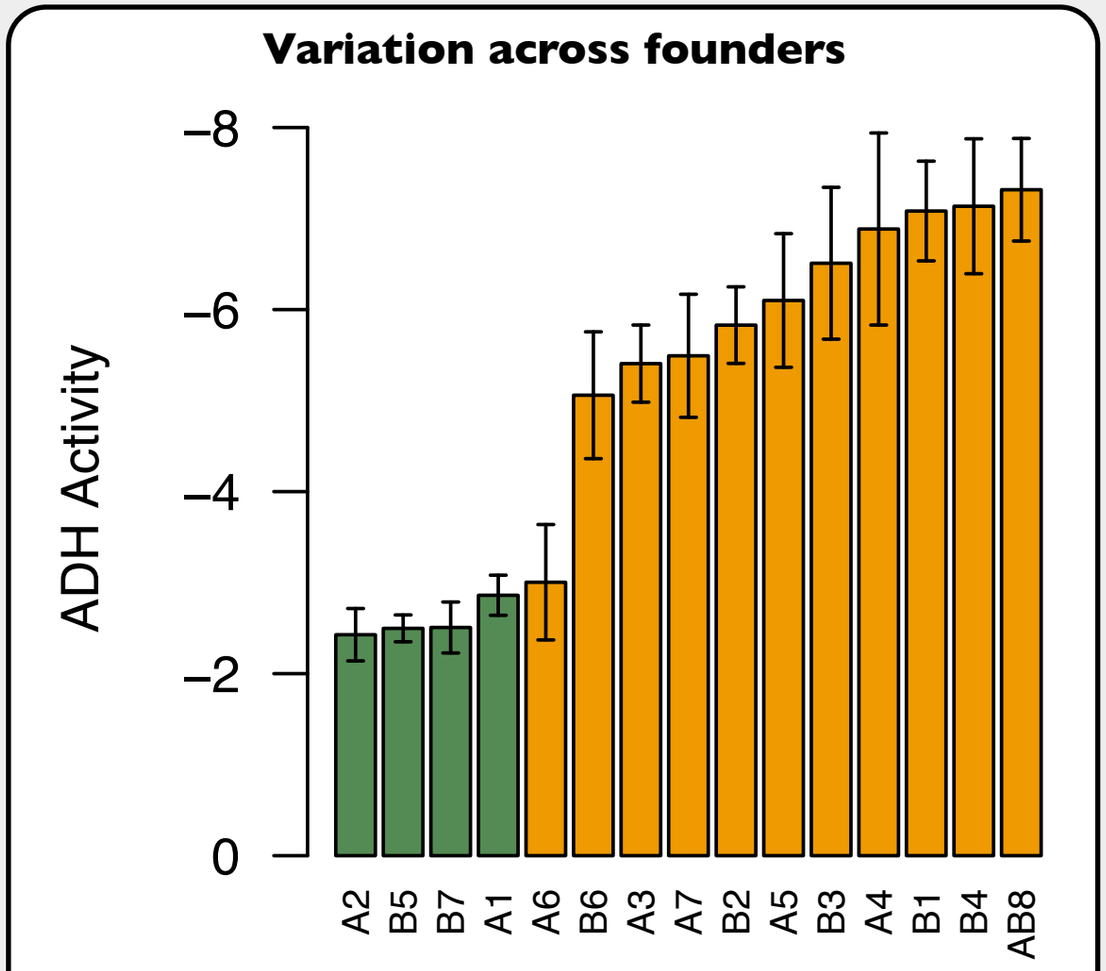
Lynn F. Stam and Cathy C. Laurie

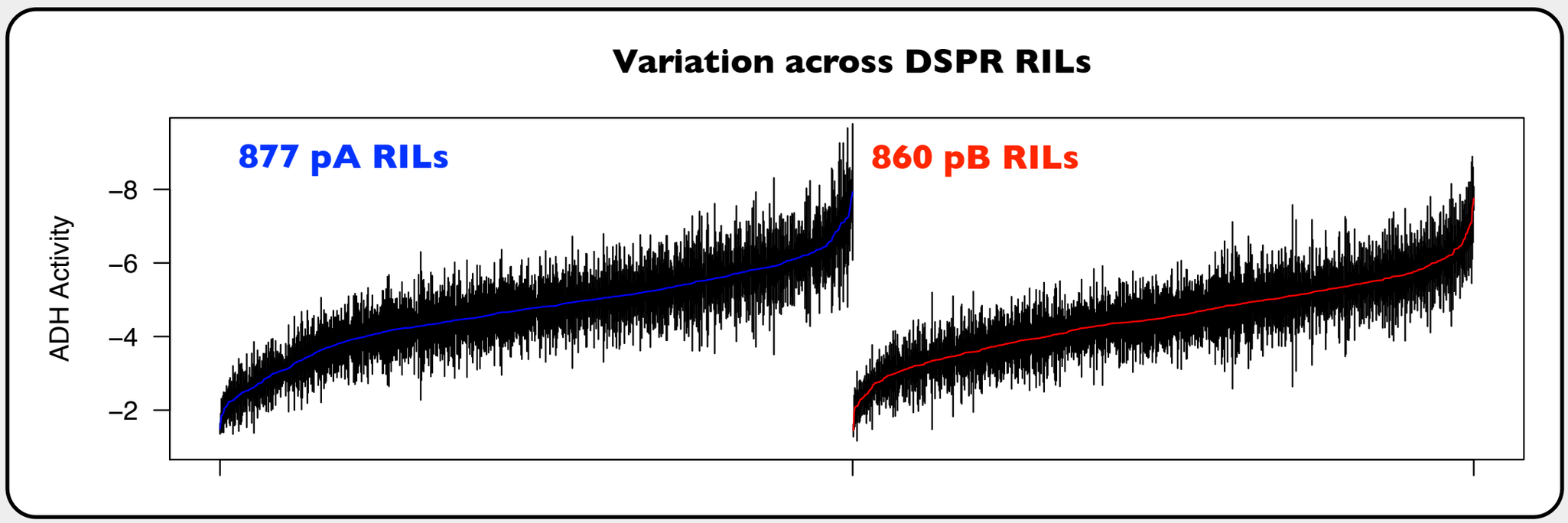
Classic studies on the quantitative genetics of ADH expression (Cathy Laurie)



ADH Activity Variation

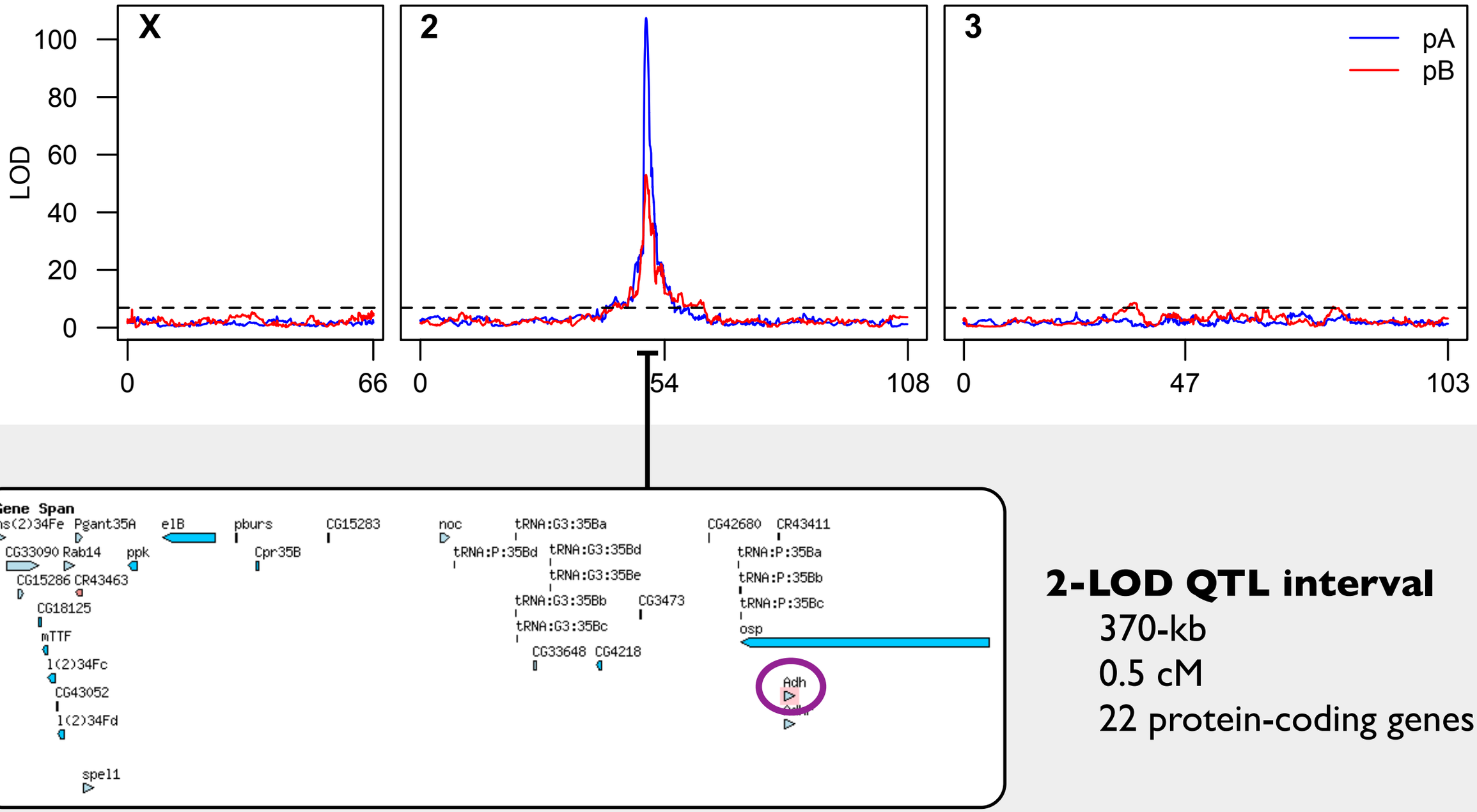
- ***Slow*** founders show consistently low ADH activity
- Variation among ***Fast*** founders - implies other loci involved
- Extensive phenotypic variation in DSPR





King *et al.*, Genome Res. 2012

ADH Enzyme Activity QTL

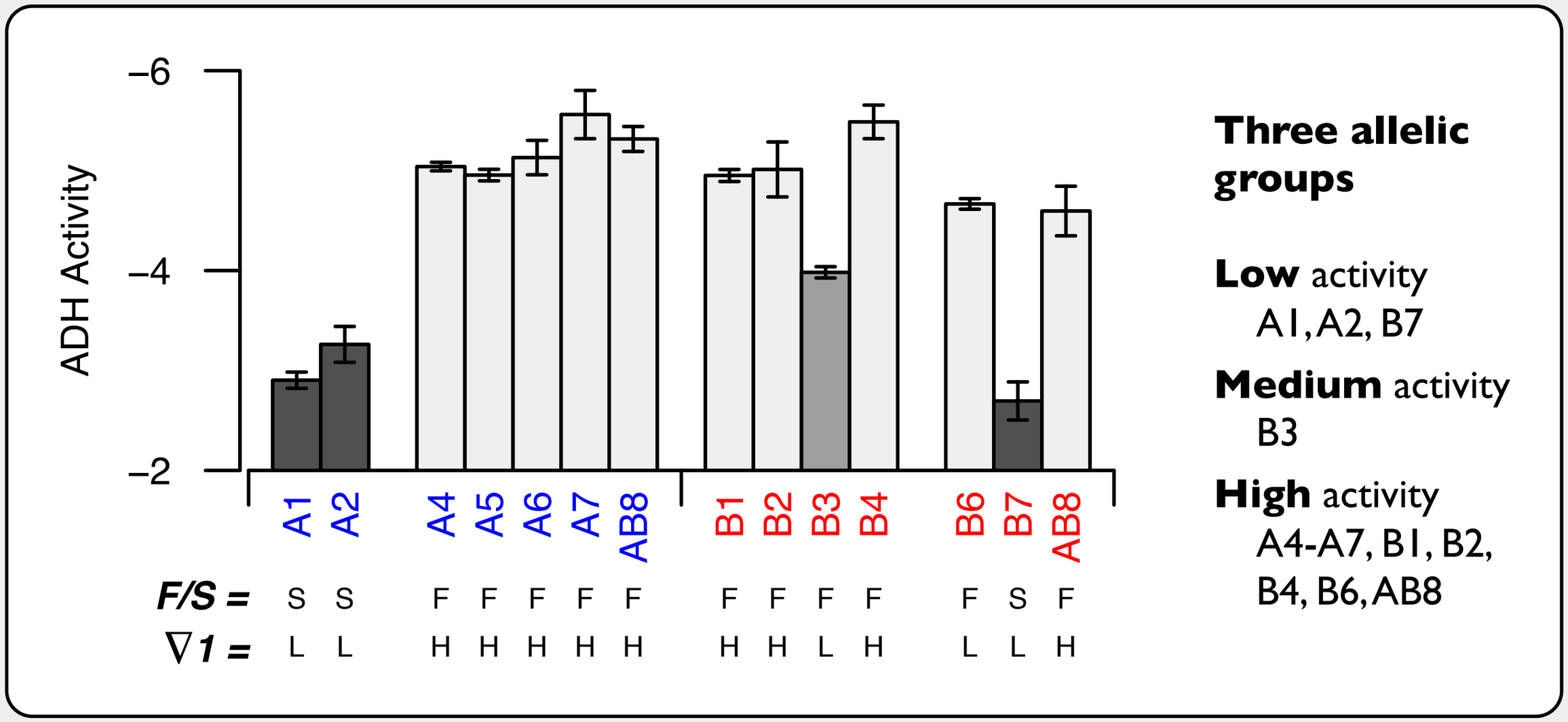


2-LOD QTL interval

- 370-kb
- 0.5 cM
- 22 protein-coding genes

King *et al.*, Genome Res. 2012

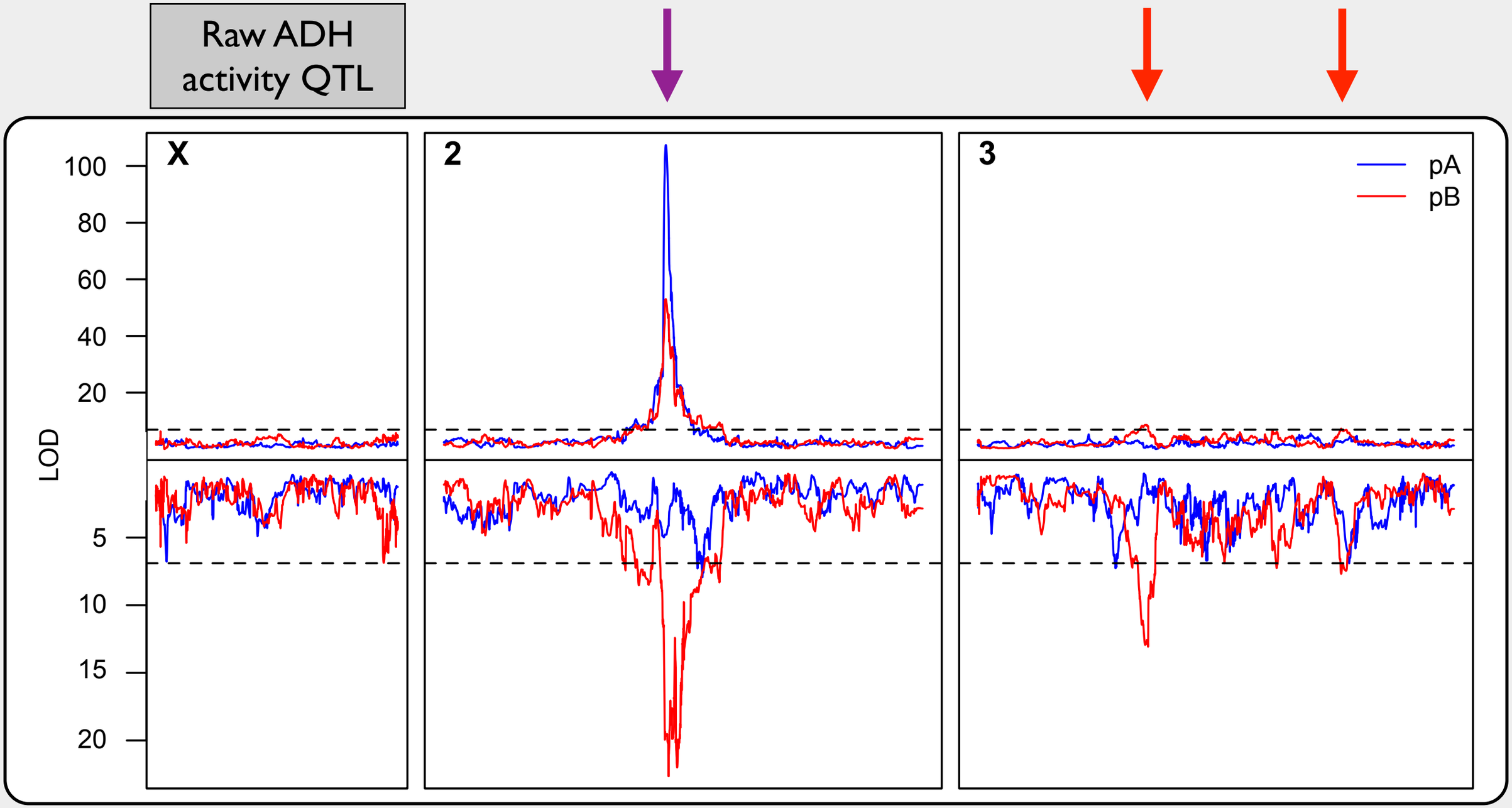
Adh QTL Phasing



Confirm effect of *Fast/Slow*, but other alleles at *Adh* are also involved

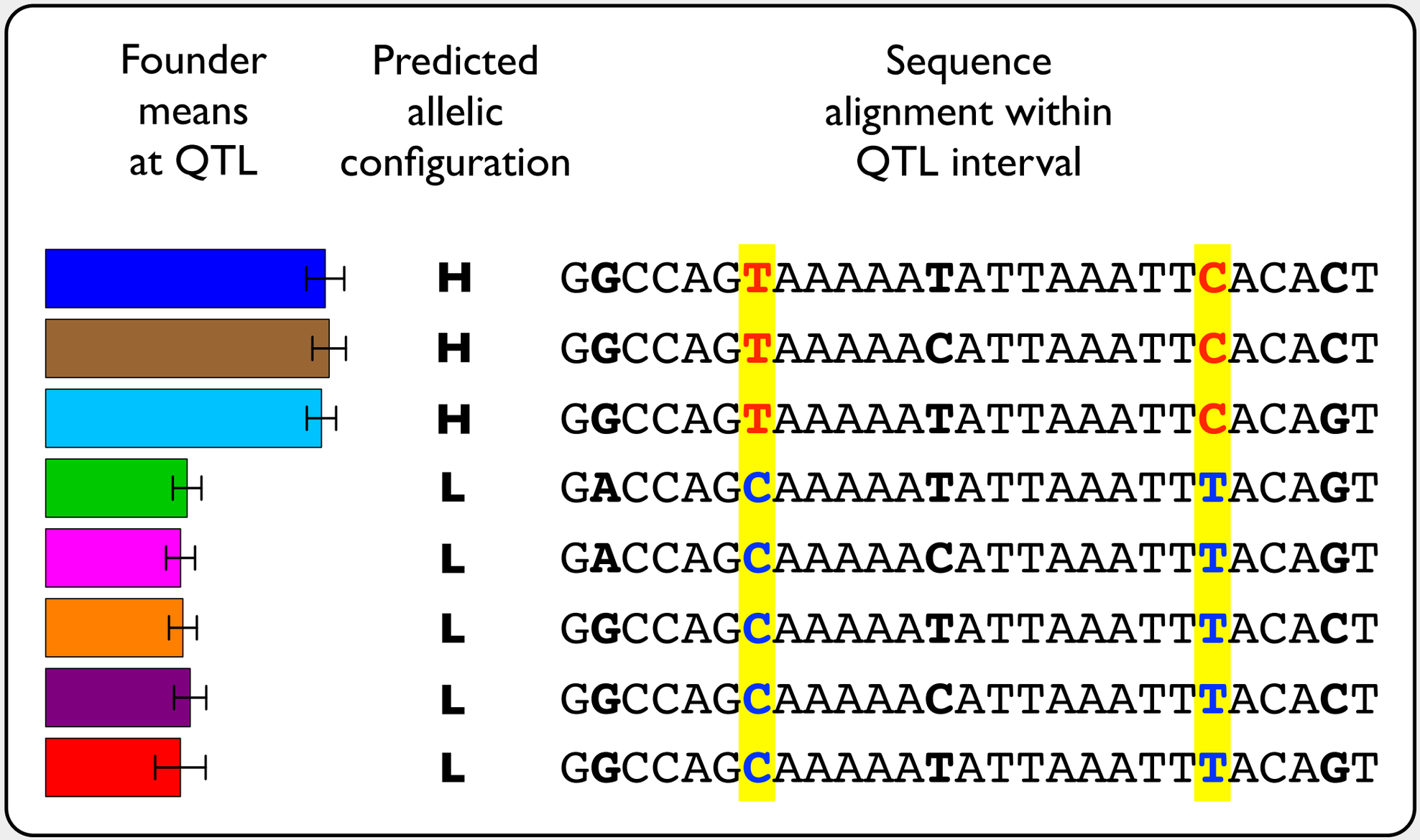
Additional ADH QTL

Raw ADH activity QTL



Control for *Adh* haplotypes defined by *F*/*S* and ∇1

QTL to Causative Variants



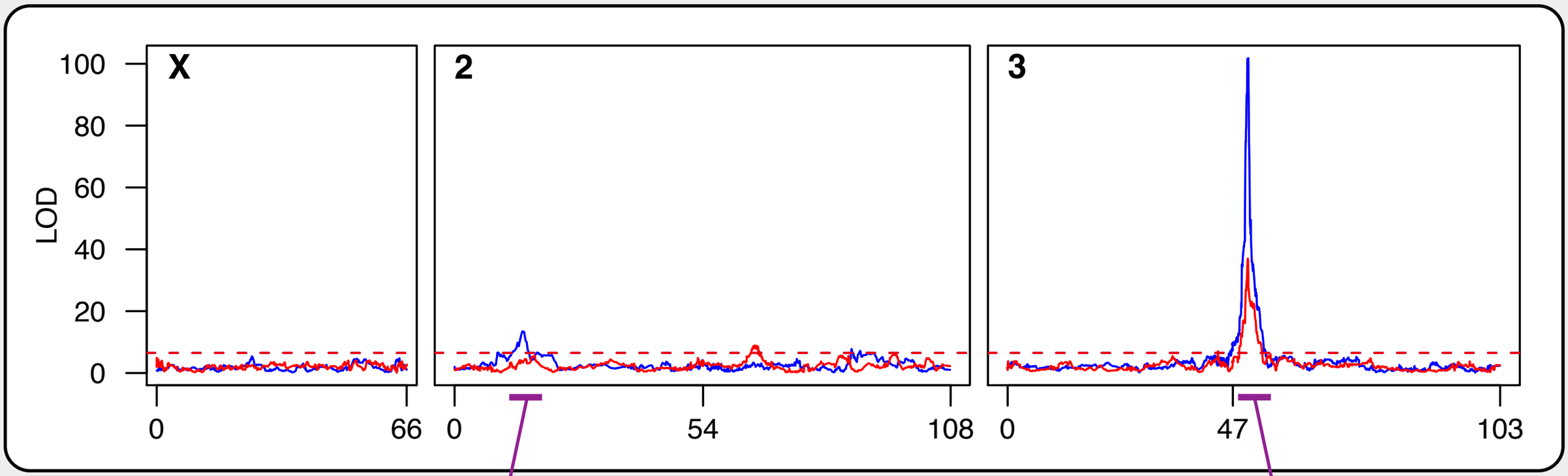
Few “in-phase” Polymorphisms

	SNP	nsSNP	InDel
Q1	169	3	48
QR2	6	0	8
QR3	102	25	89
QR4	12	1	12
QR6	537	233	490
QR7	2	6	11
QR8	37	2	33

Q1
Includes known *F/S Adh* variant

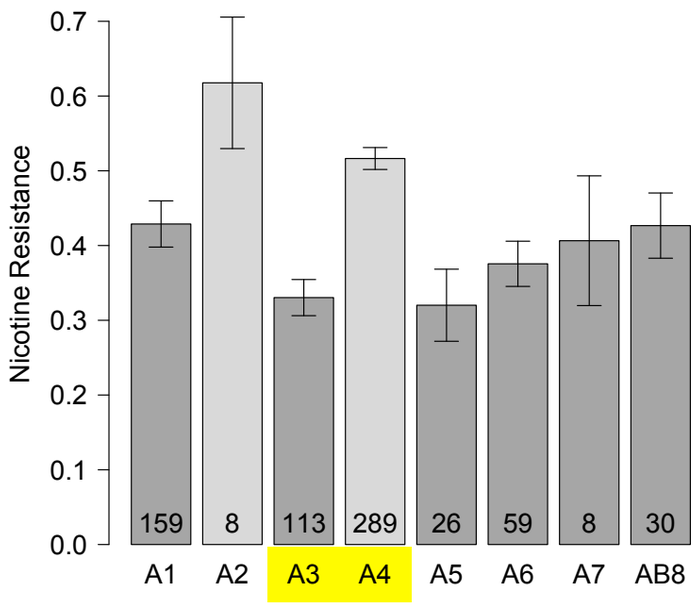
QR2
Includes frameshift mutation in CG7377 and intronic deletion in CG6024

Nicotine Resistance QTL



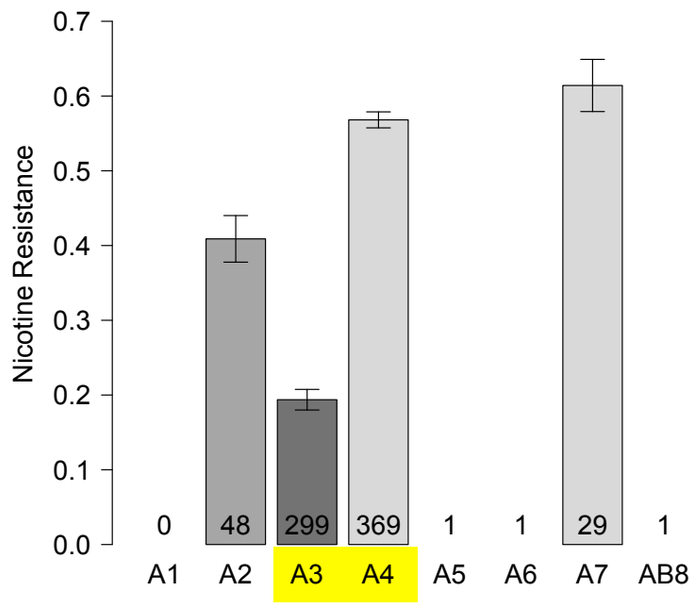
Biallelic QTL
14.4% variation

220-kb
0.84 cM
27 genes



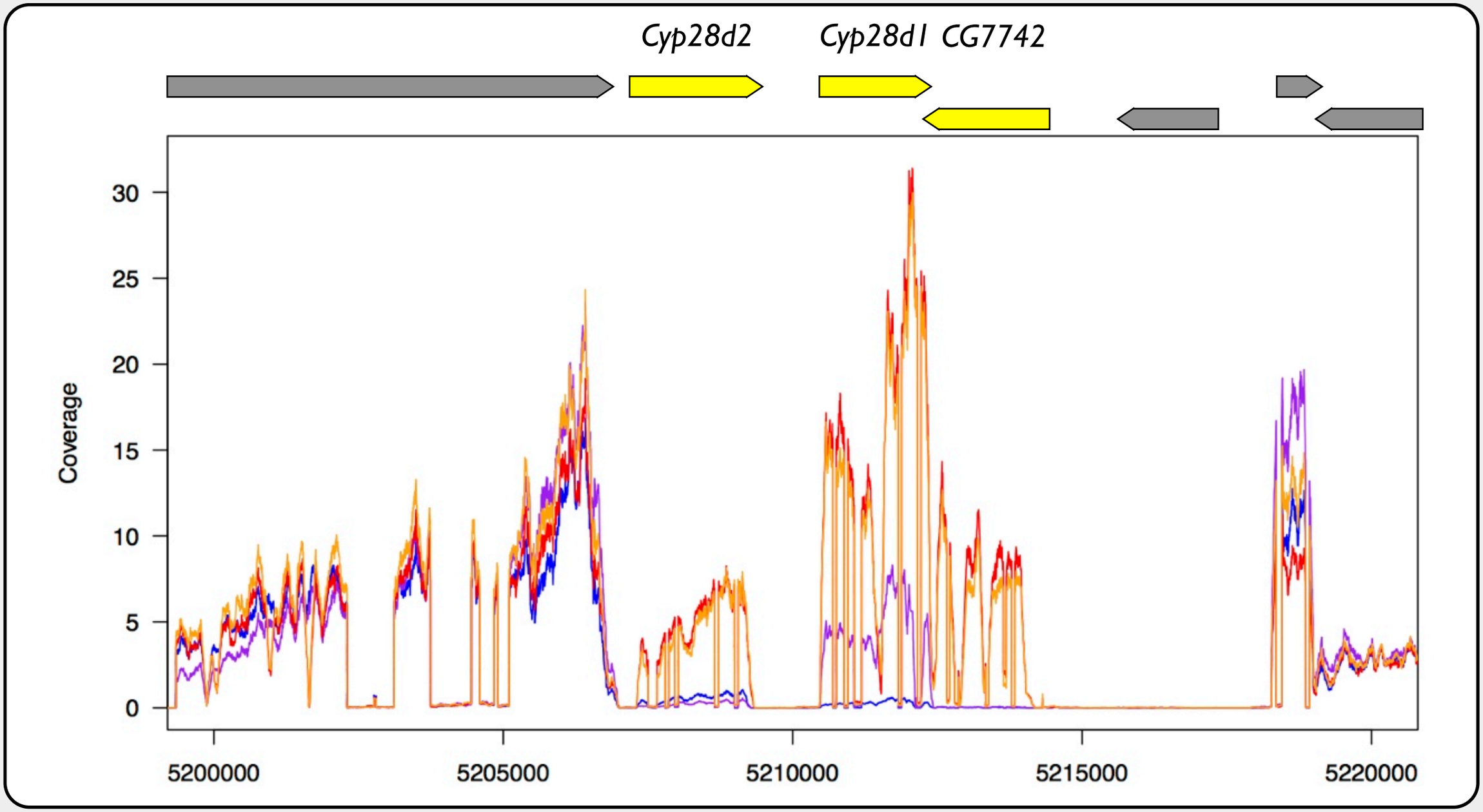
Multiallelic QTL
51.5% variation

190-kb
0.21 cM
32 genes



Marriage *et al.*, in preparation

Expression Candidates (2L)



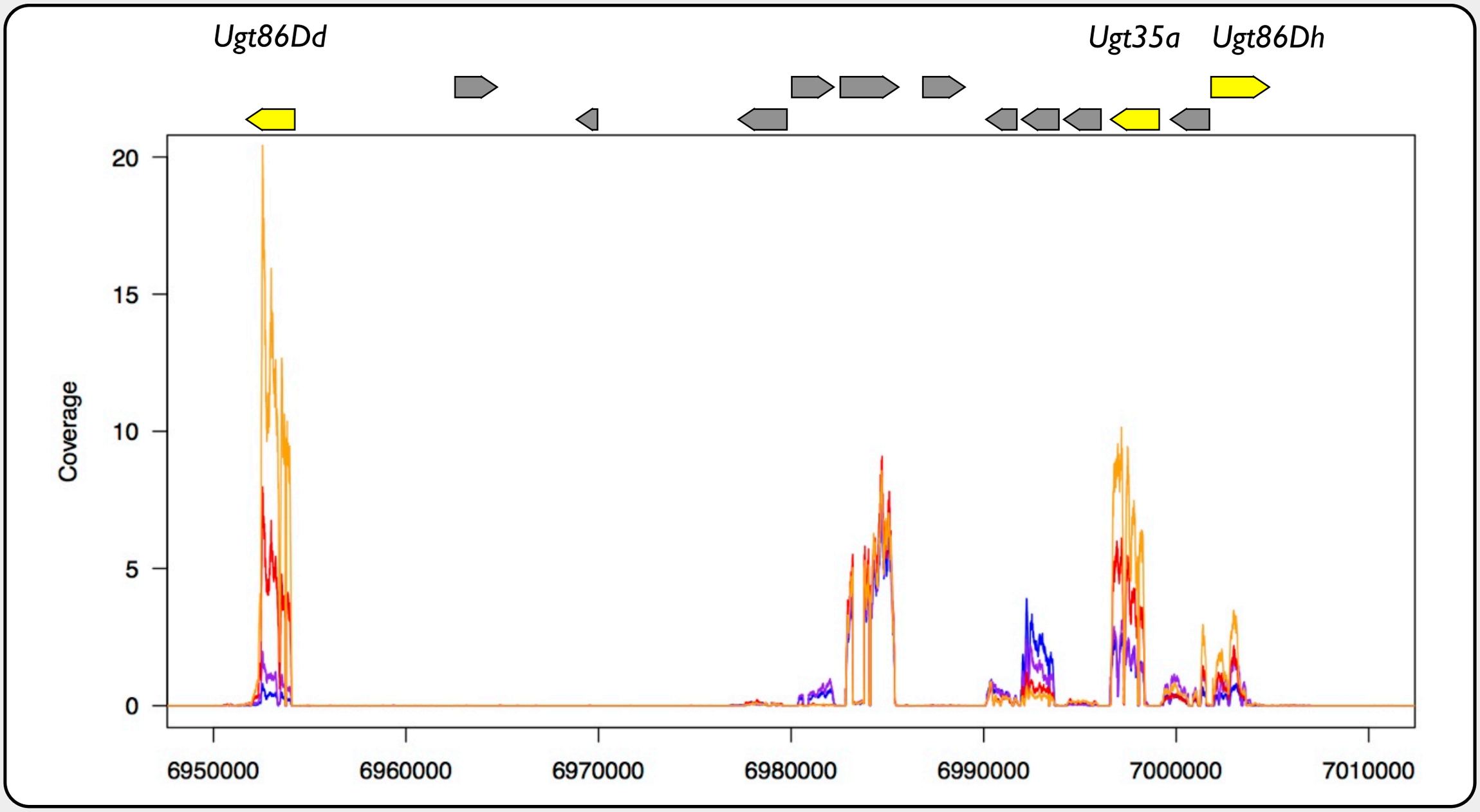
A3 (regular)

A4 (regular)

A3 (nicotine)

A4 (nicotine)

Expression Candidates (3R)



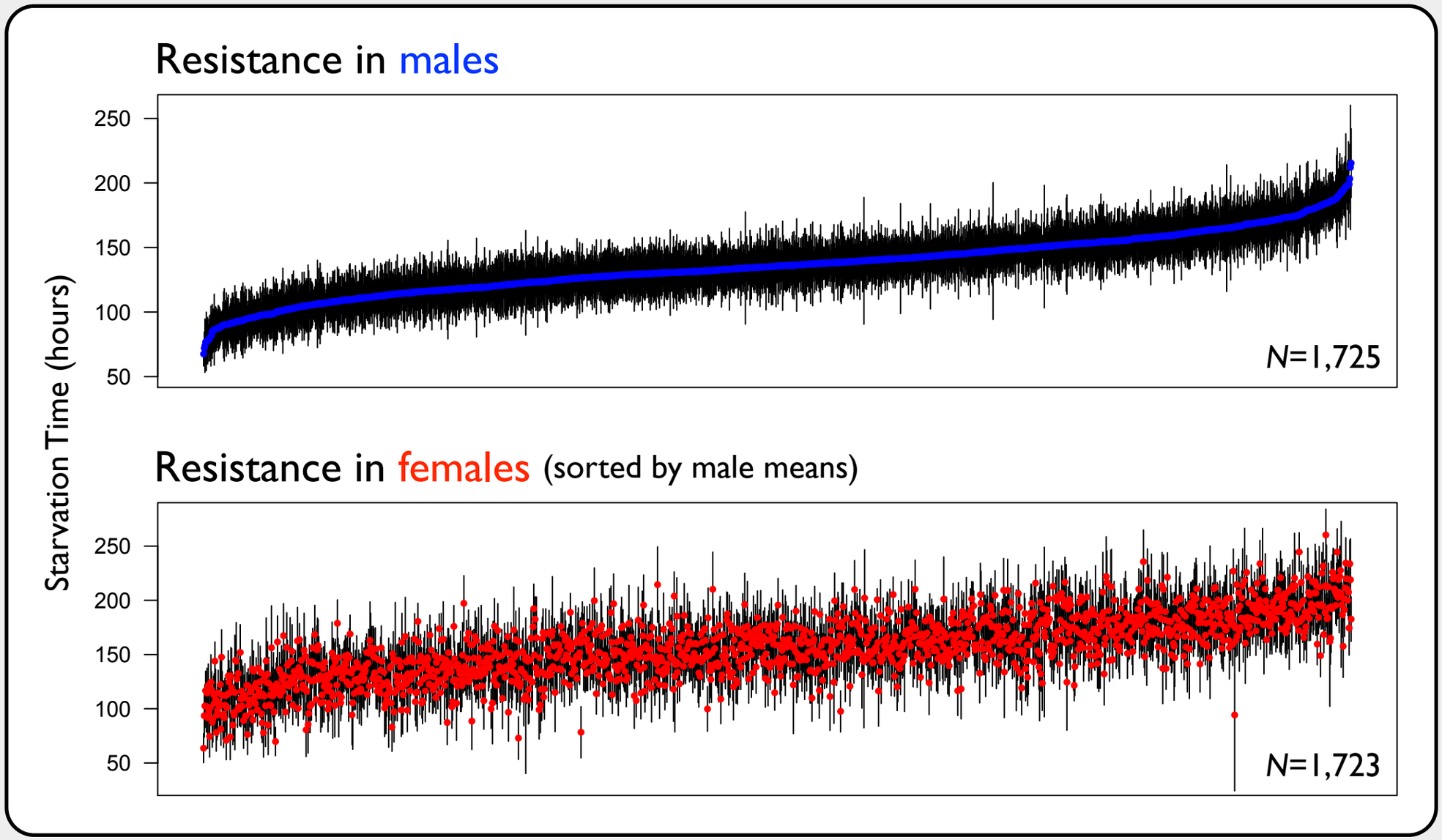
A3 (regular)

A4 (regular)

A3 (nicotine)

A4 (nicotine)

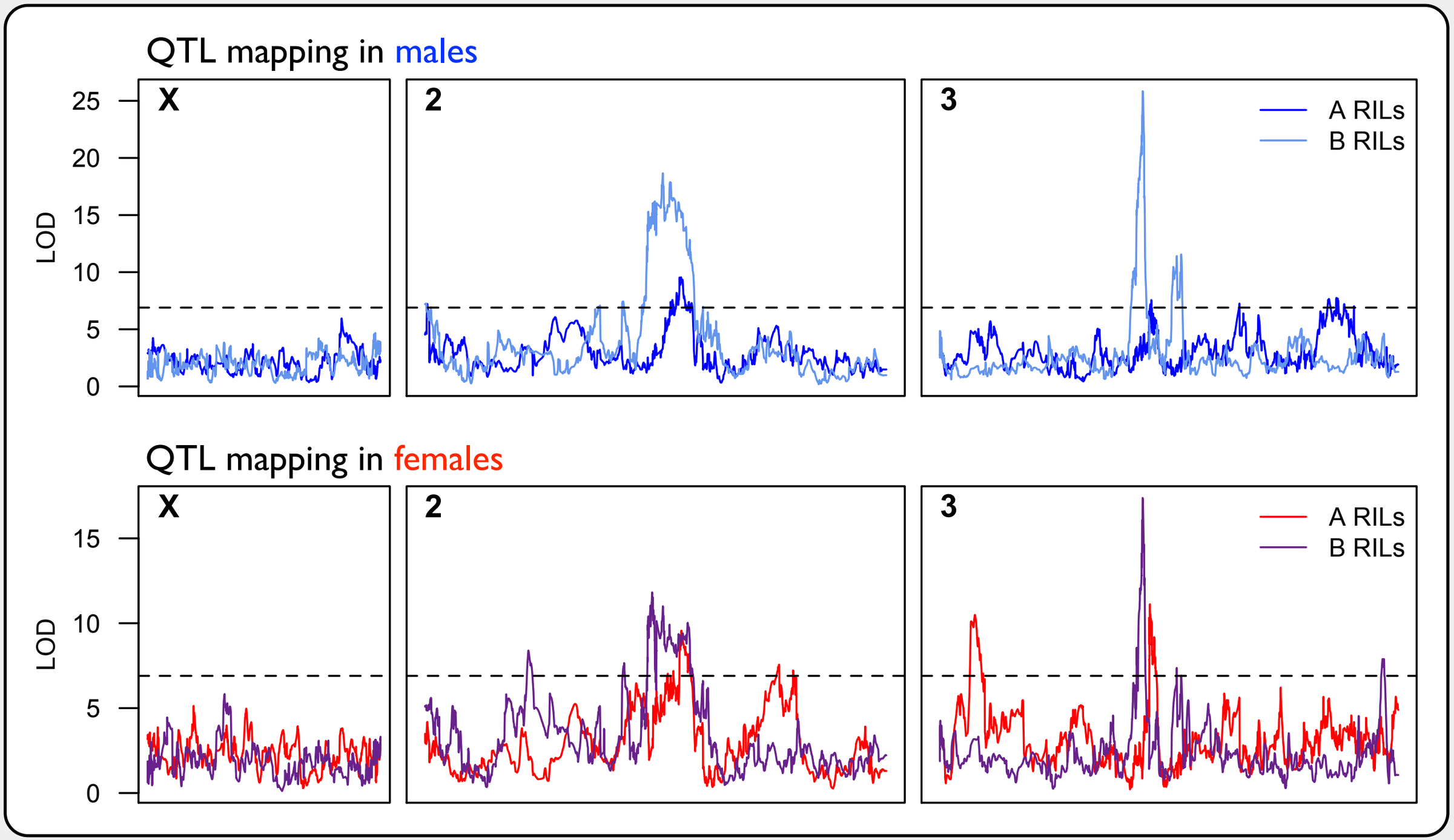
Starvation Resistance in DSPR



- Heritability, H^2 = 52-55%

McNeil *et al.*, in preparation

Numerous Starvation QTL

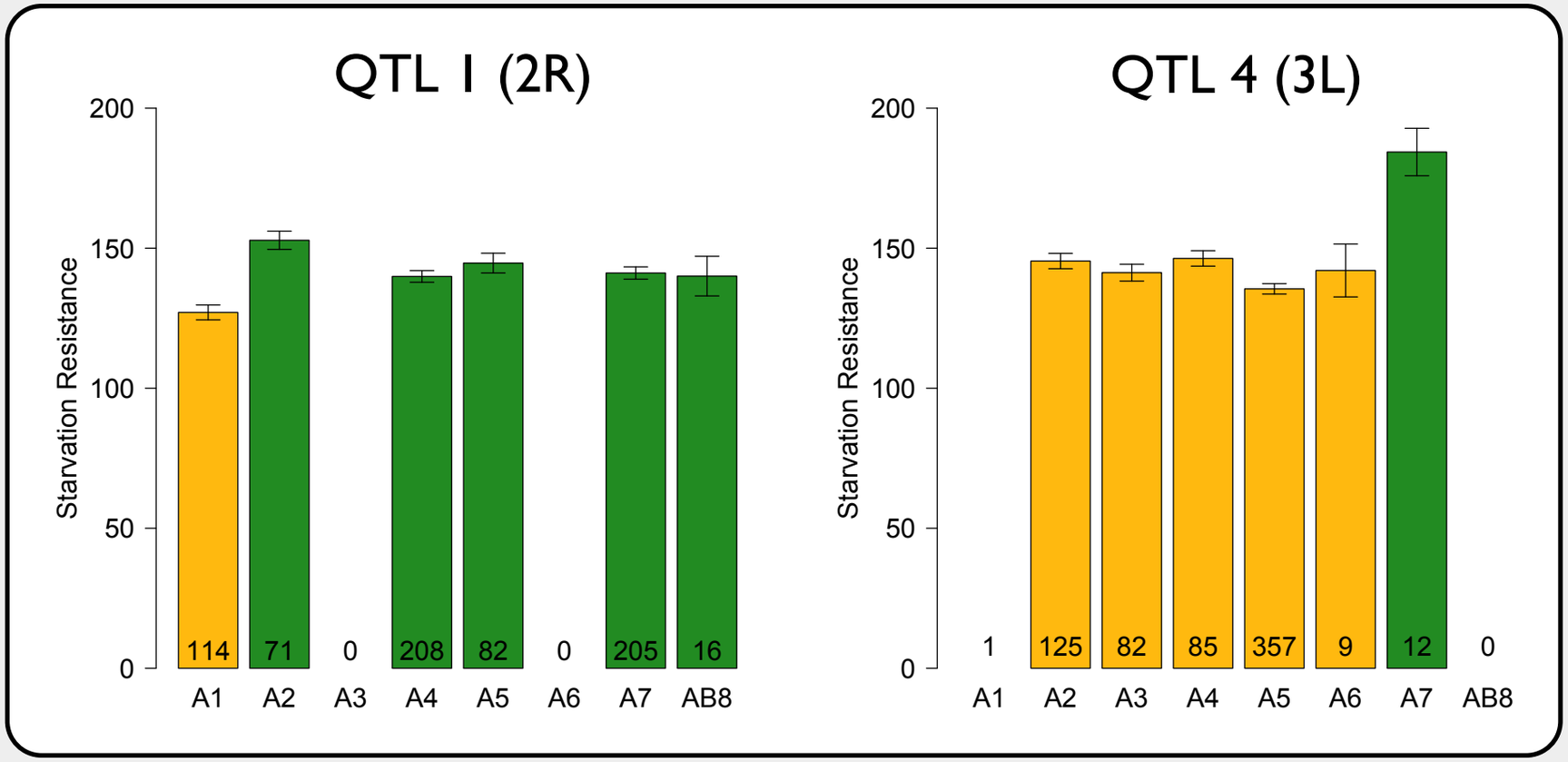


Female QTL Summary

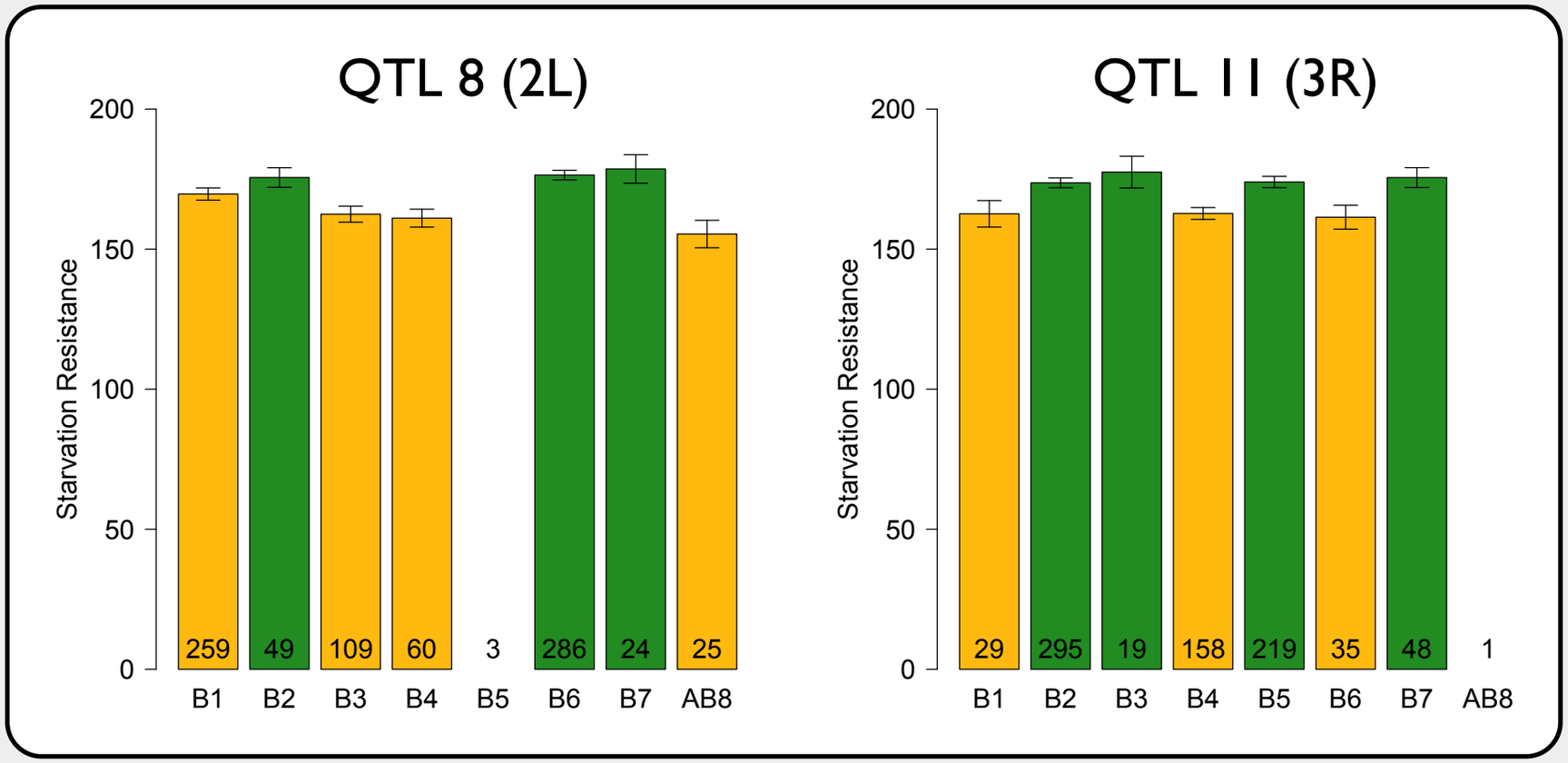
QTL	Popn	Chr	% Var Expl	Size (Mb)	# Genes	
1	A	2R	5.8	1.4	220	
2	A	2R	4.6	0.9	179	
3	A	2R	4.4	0.6	103	
4	A	3L	6.4	0.5	41	
5	A	3R	6.7	1.5	208	← centromeric
6	B	2L	4.6	0.4	28	
7	B	2L	4.3	0.5	40	
8	B	2L	7.1	2.5	243	← centromeric
9	B	3L	10.5	0.5	43	
10	B	3R	4.9	0.5	64	
11	B	3R	4.4	0.5	104	
			5.8 ± 1.87	0.9 ± 0.66	116 ± 81.8	

Strain Effects

Rare QTL



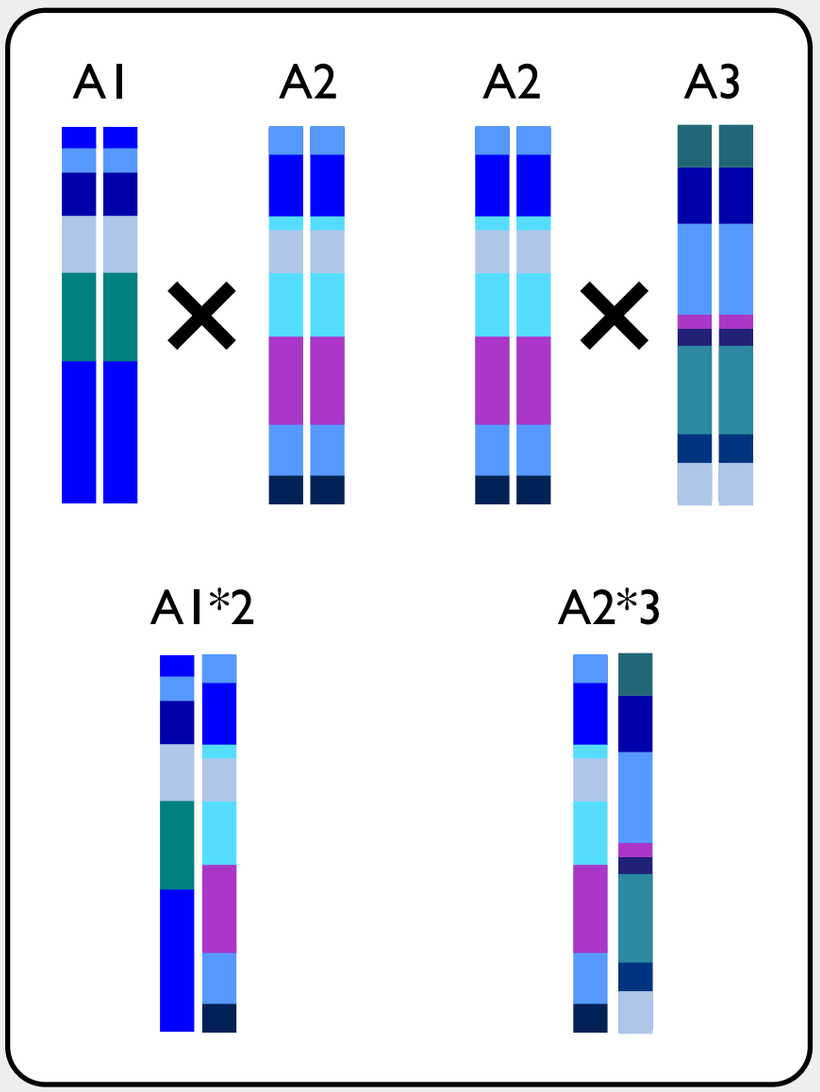
Common QTL



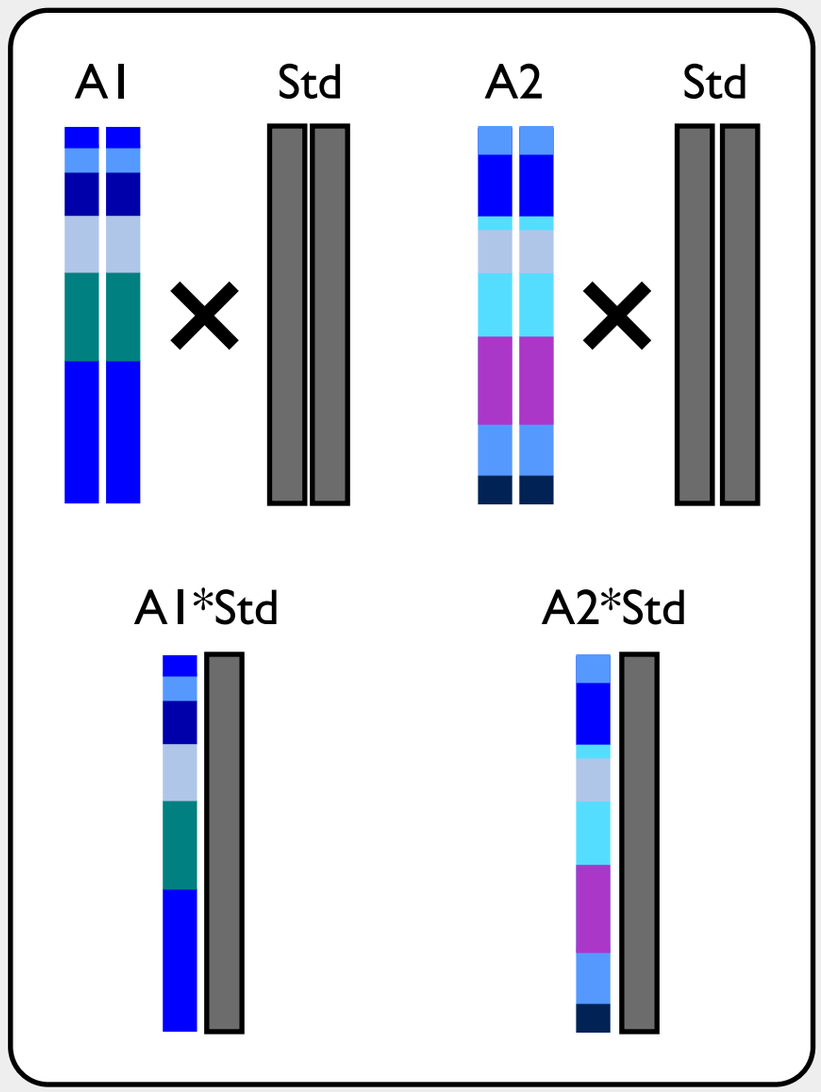
Heterozygous Mapping Designs

→ Map using *trans*-heterozygous cross progeny

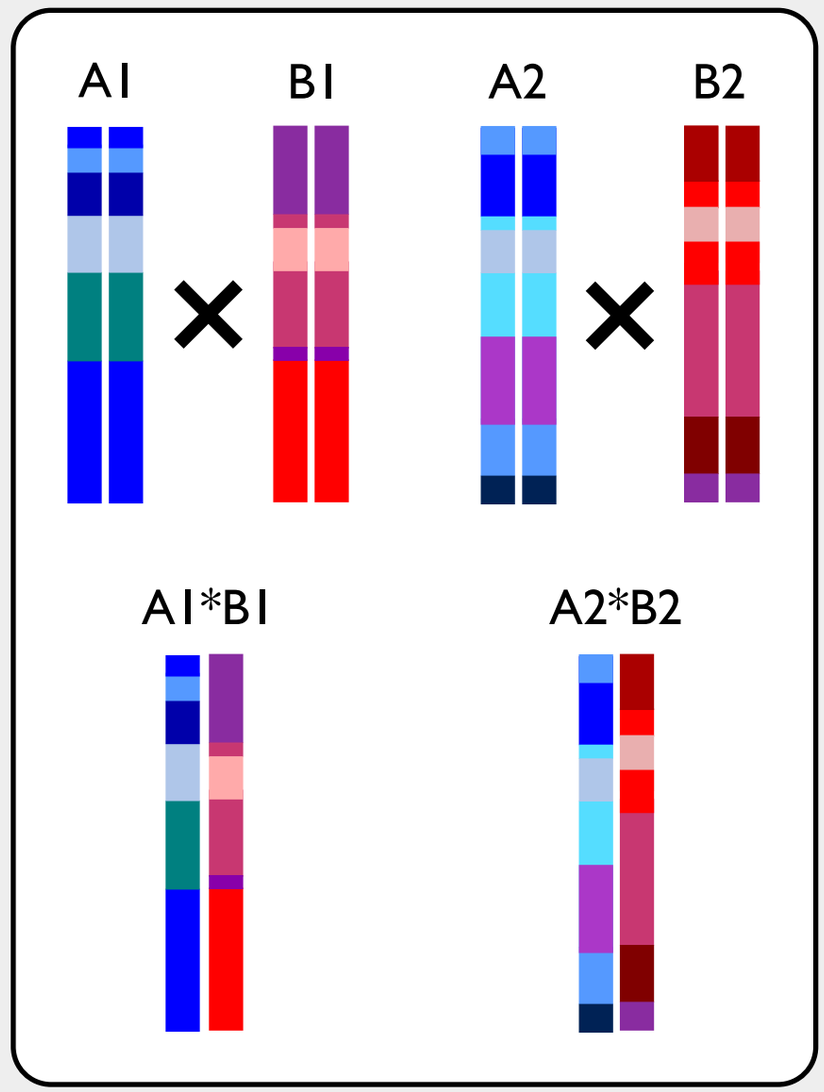
→ Minimizes inbreeding depression



p**A** RIL × p**A** RIL Crosses



p**A** RIL × Standard Crosses



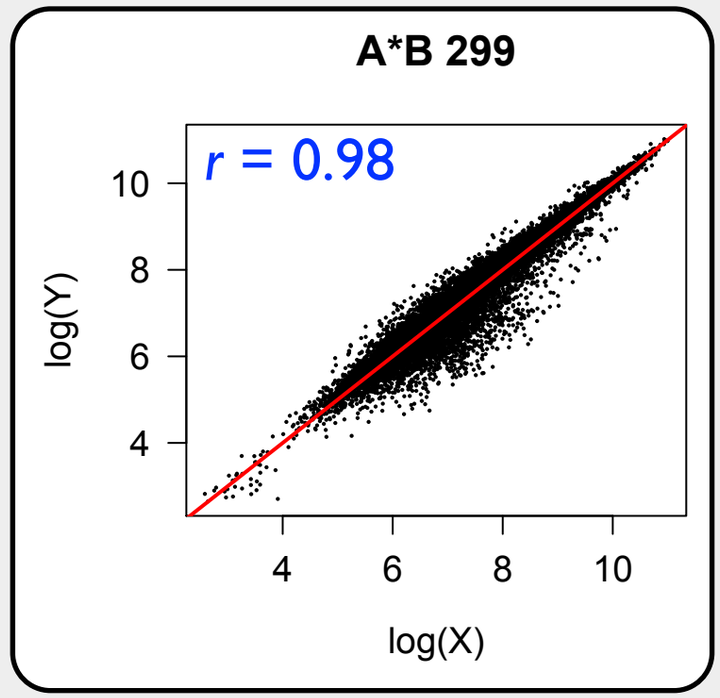
p**A** RIL × p**B** RIL Crosses

X
2
3
LOD
Homozygous
RIL
(*N* = 801)
Heterozygous
RIL × RIL
(*N* = 748)
Heterozygous
RIL × Std 1
(*N* = 788)
Heterozygous
RIL × Std 2
(*N* = 721)

Expression Profiling

- RNA from ~300 3-5 d.o. mated female heads per genotype
- NimbleGen 12 × 135K microarrays

  16,637 target genes
  (8 probes/target)



Strong correlation between replicate arrays

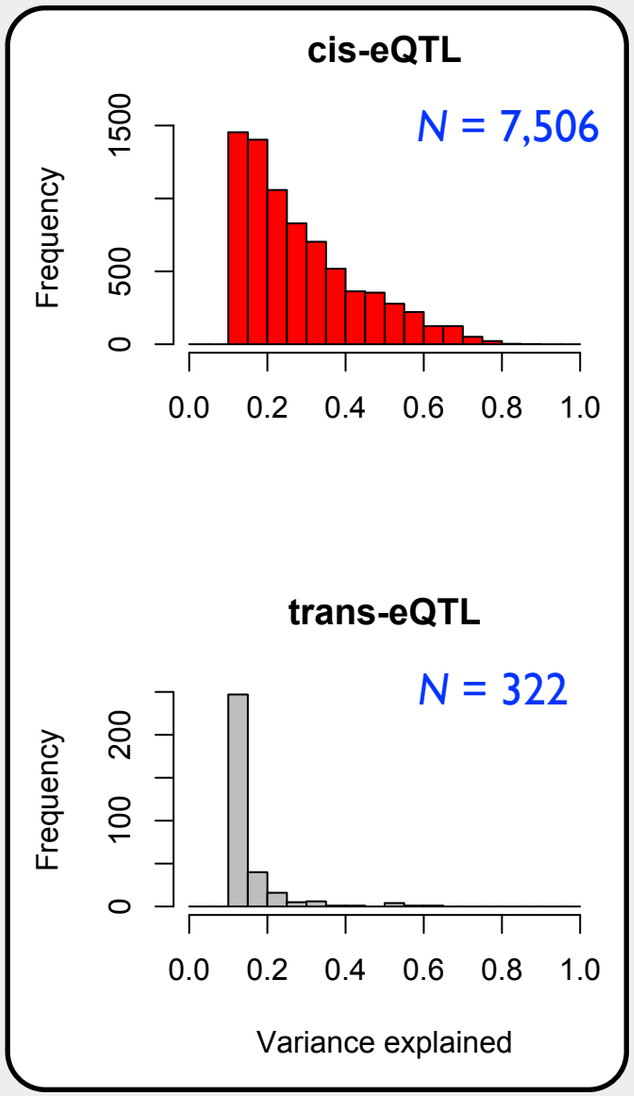
- Robust multi-array (RMA) analysis

  Account for mismatches in probes

Mapped eQTL

Symbol	Name	FlyBase #	eQTL
Clk	*Clock*	FBgn0023076	*cis*
tim	*timeless*	FBgn0014396	*cis*
rho	*rhomboid*	FBgn0004635	*cis*
cyc	*cycle*	FBgn0023094	*cis*
Oda	*Ornithine decarboxylase antizyme*	FBgn0014184	*trans*
Slob	*Slowpoke binding protein*	FBgn0264087	*trans*
DopR	*Dopamine receptor*	FBgn0011582	*cis*
Ssk	*Snakeskin*	FBgn0036945	*trans*
Pka-R2	*cAMP-dependent protein kinase R2*	FBgn0022382	*cis*
5-HT1B	*Serotonin receptor 1B*	FBgn0263116	*cis*

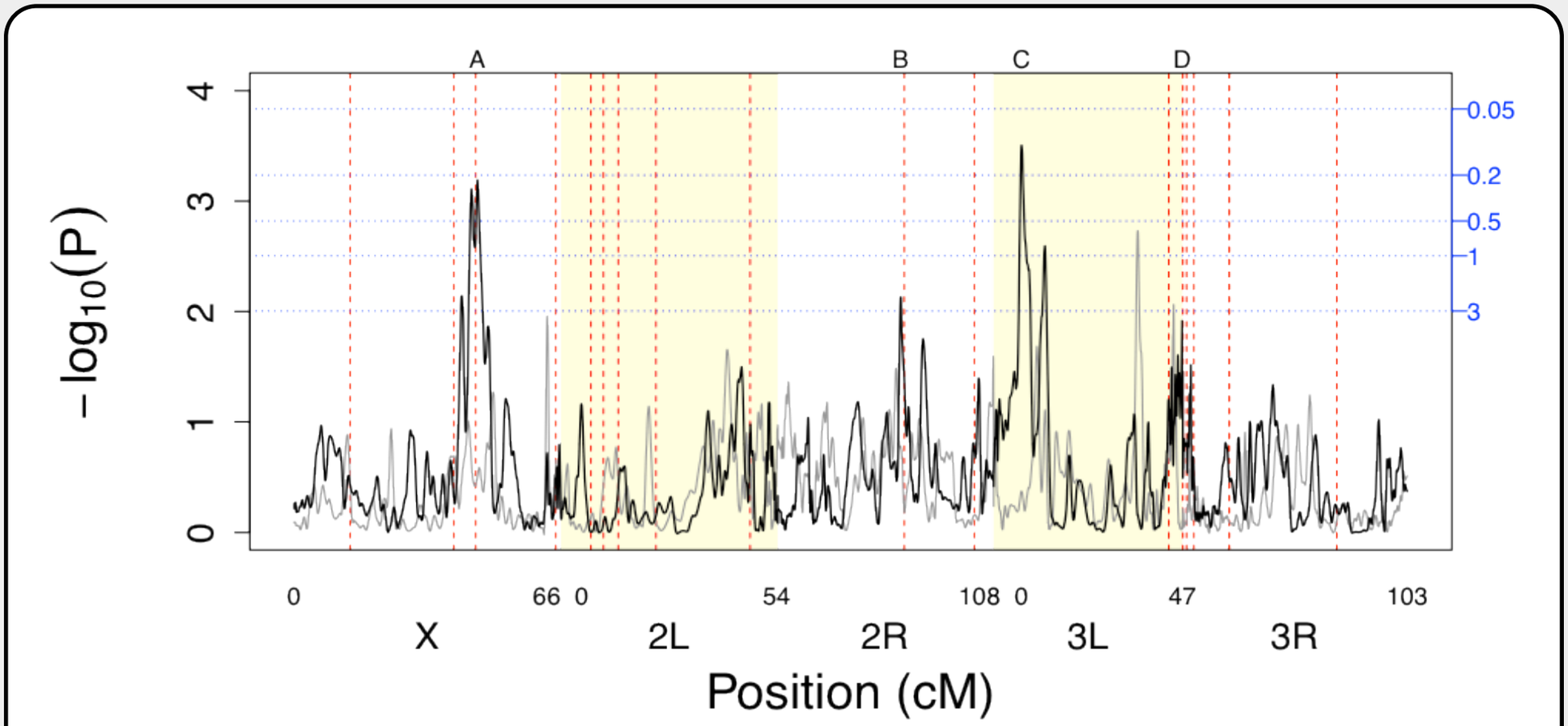
Behavioral / Neural Gene eQTL



7,828 eQTL mapped for 7,422 target gene expression measures

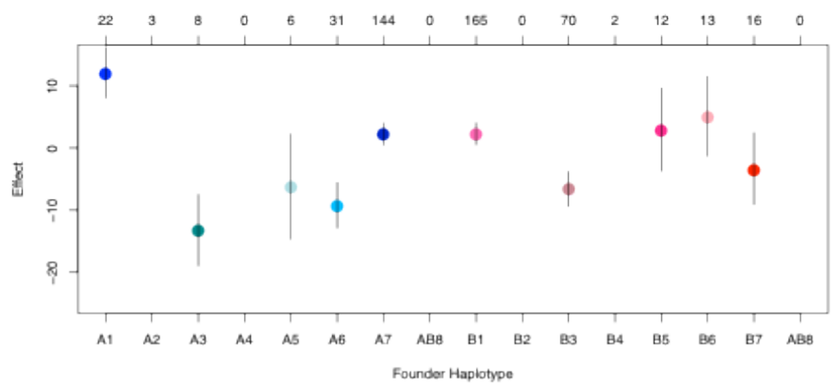
Multiallelic QTL ?

QTL for methotrexate toxicity

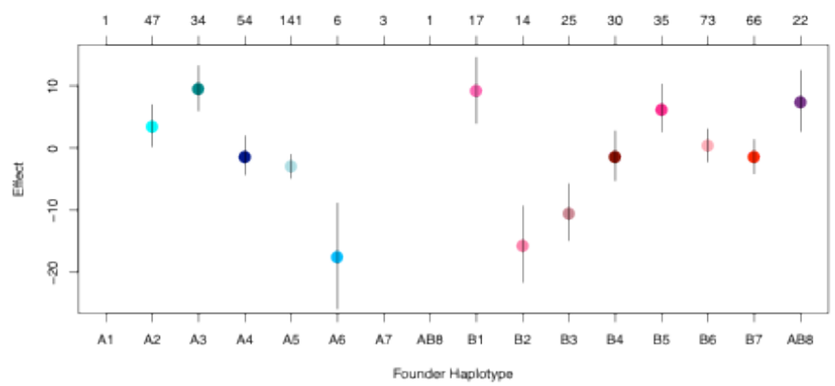


No clear biallelic pattern of strain effects

QTL *A*

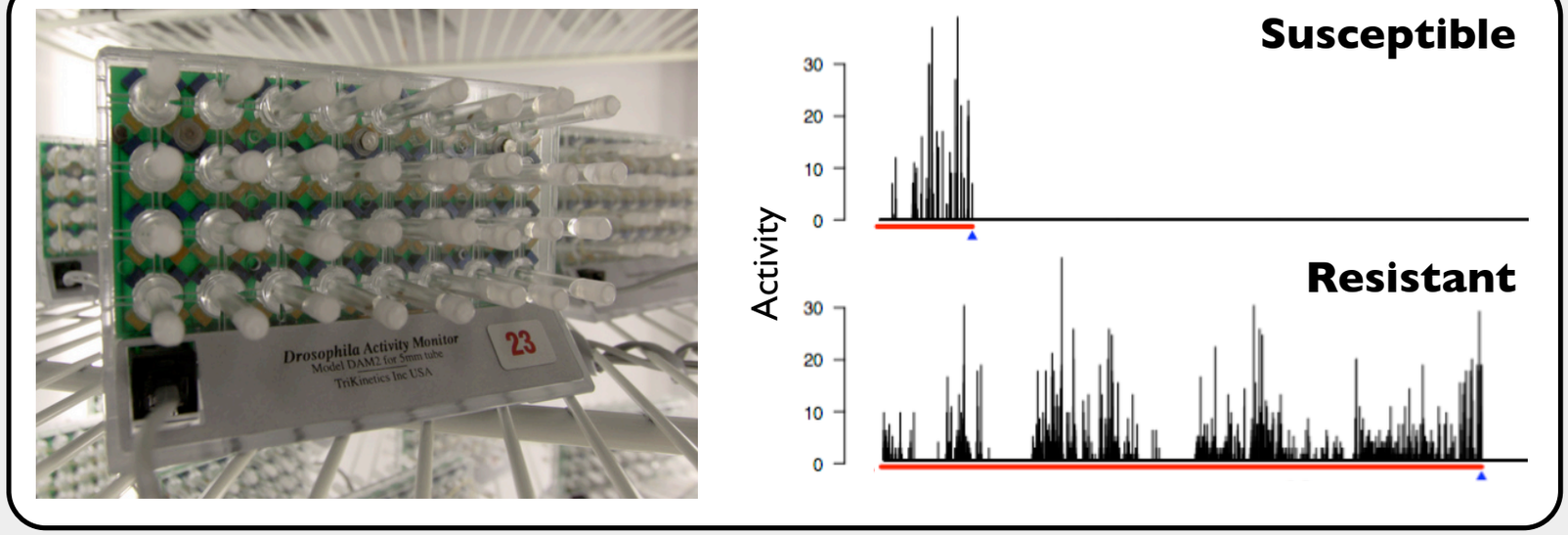


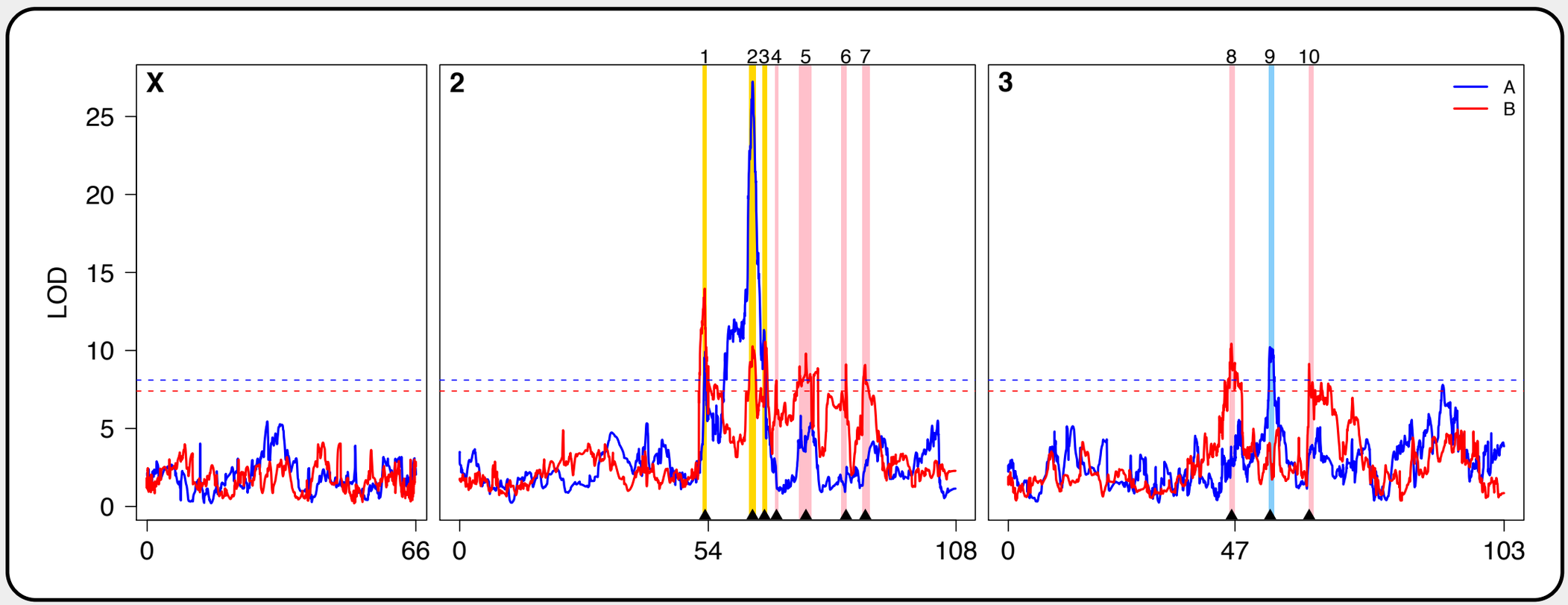
QTL *C*



Kislukhin, King *et al.*, G3 2013

Caffeine Resistance QTL





Najarro *et al.*, unpublished

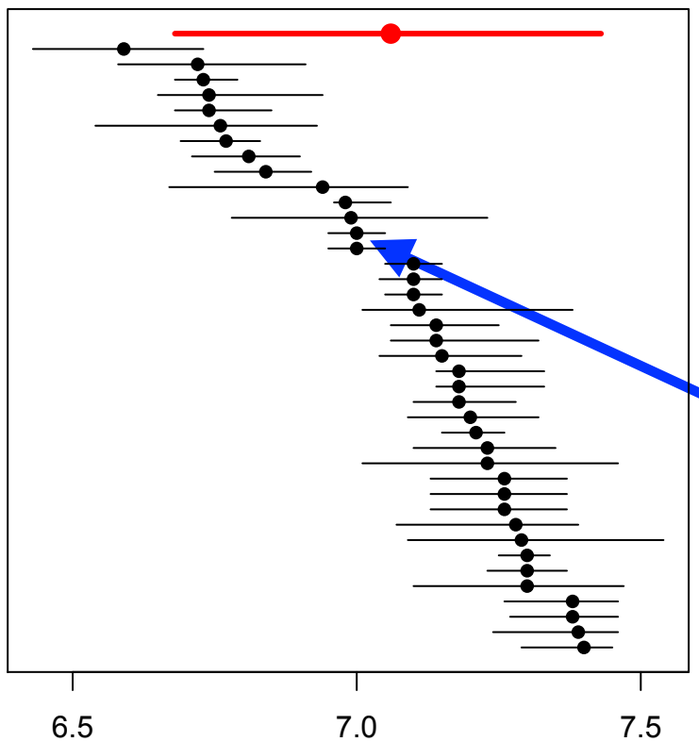
pQTL-eQTL Overlap

→ Combine pQTL and eQTL to help define loci contributing to phenotypic variation

Q2.2R.AB

Implicates 128 genes

44 have *cis*-eQTL



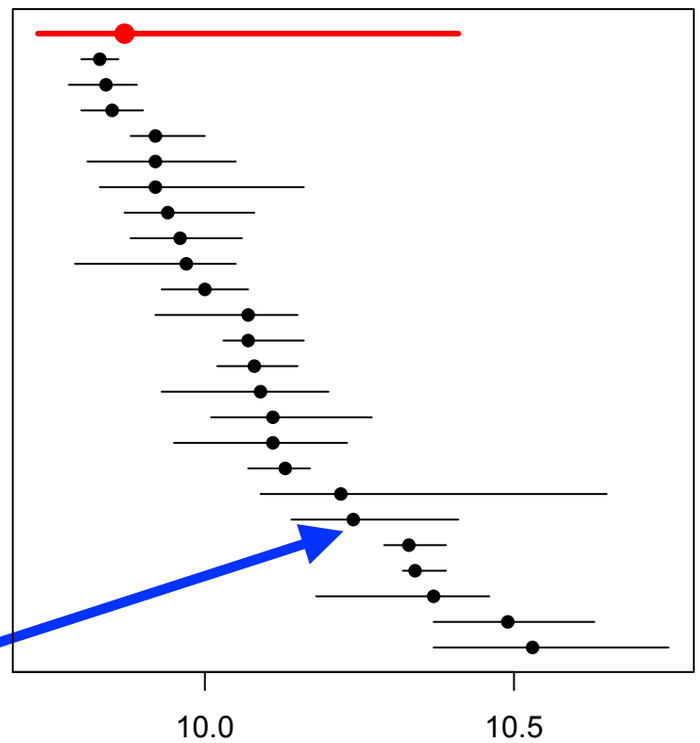
Cyp12d1-d

Cyp313a1

Q9.3R.A

Implicates 81 genes

24 have *cis*-eQTL



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Current Lab Members

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