QTL mapping
in MAGIC populations
with R/qtl2

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kbroman.org
github.com/kbroman
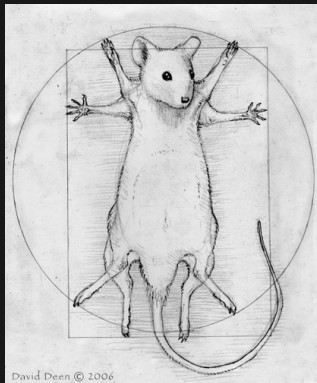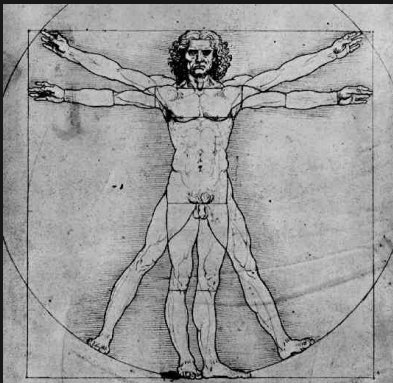@kwbroman
Slides: bit.ly/msu2019-12

PUBLIC DOMAIN
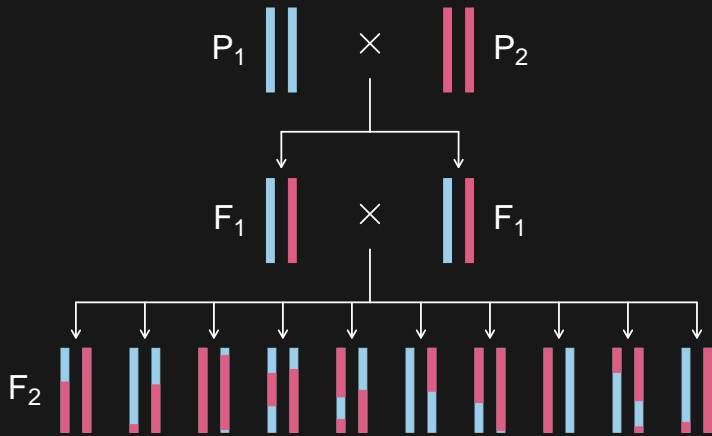
daviddeen.com

Intercross

QTL mapping
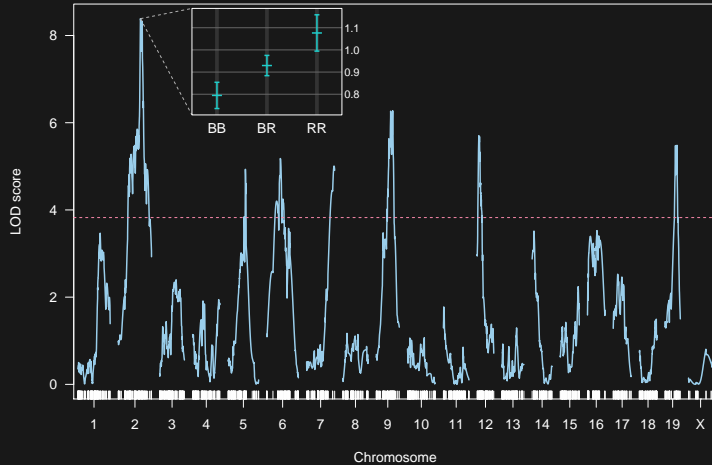
Congenic line/NIL
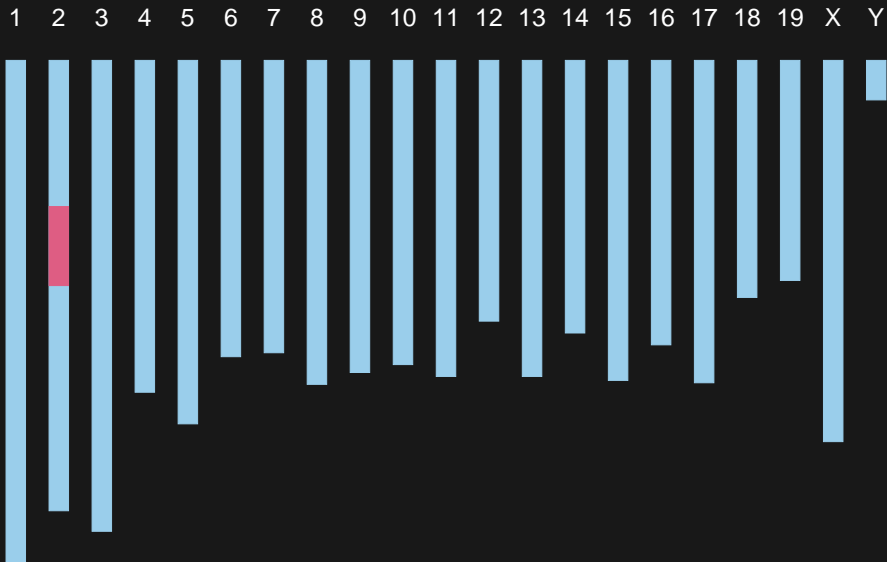
Improving precision

- ▶ more recombinations
- ▶ more individuals
- ▶ more precise phenotype
- ▶ lower-level phenotypes
  – transcripts, proteins, metabolites

Genome-scale phenotypes

Alan Attie

Advanced intercross lines
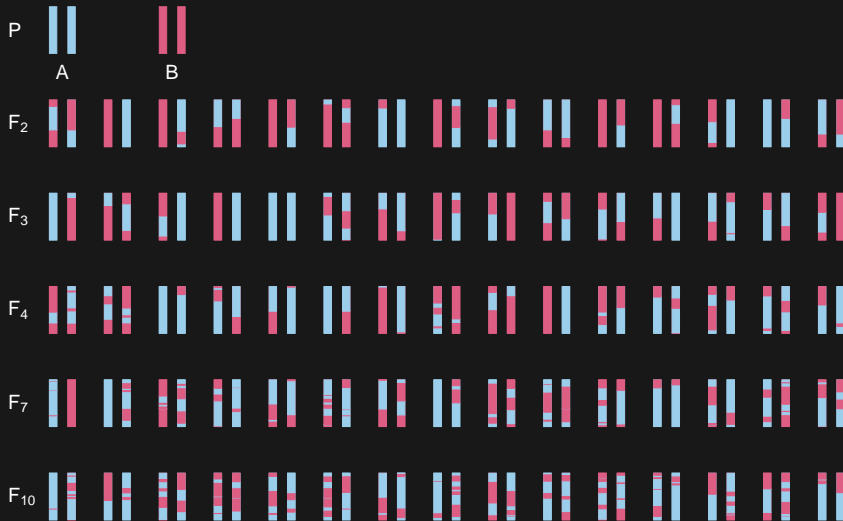
Recombinant inbred lines
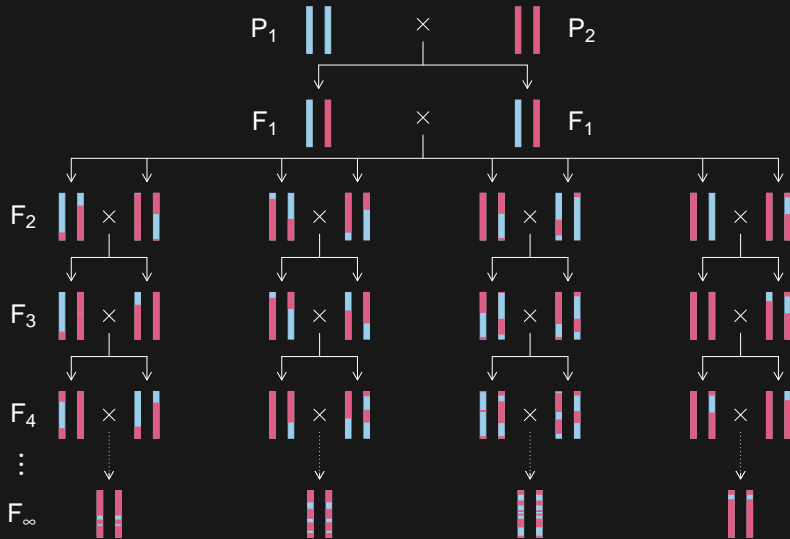
Collaborative Cross
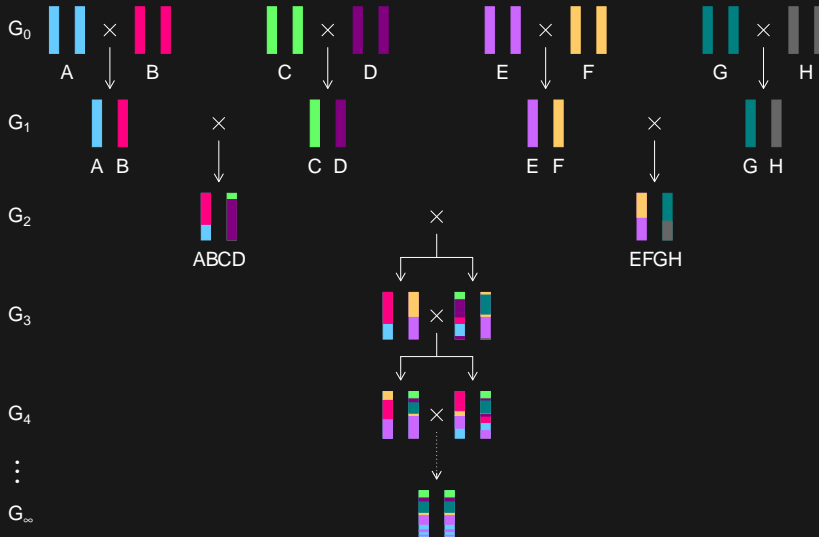
Heterogeneous stock
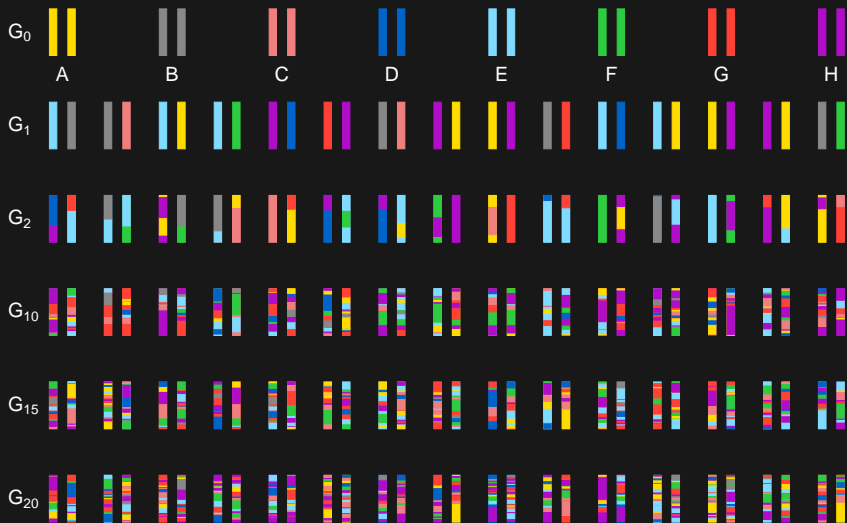
MAGIC is magic

- ► Genetic diversity
- ► High-precision mapping
- ► Predictable linkage disequilibrium
- ► Phenotype replicates to reduce individual variation
- ► Pool phenotypes from multiple labs, environments, treatments
- ► Genotype once

MAGIC is magic

- ▶ Genetic diversity
- ▶ High-precision mapping
- ▶ Predictable linkage disequilibrium
- ▶ Phenotype replicates to reduce individual variation
- ▶ Pool phenotypes from multiple labs, environments, treatments
- ▶ Genotype once
- ▶ Cool name

MAGIC lines
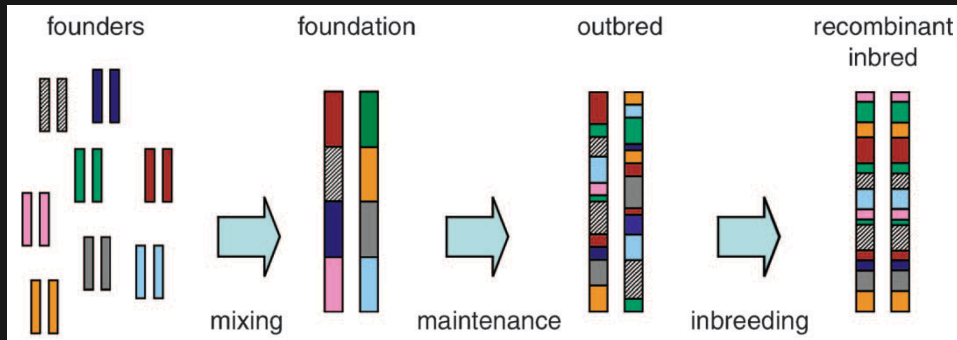
founders   foundation   outbred   recombinant inbred

mixing   maintenance   inbreeding

MAGIC lines

founders | foundation | outbred | recombinant inbred

mixing → combine

maintenance → mix

inbreeding → fix
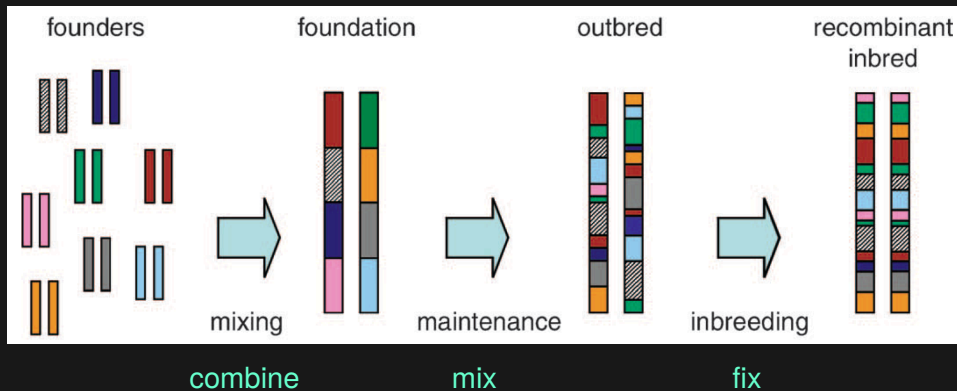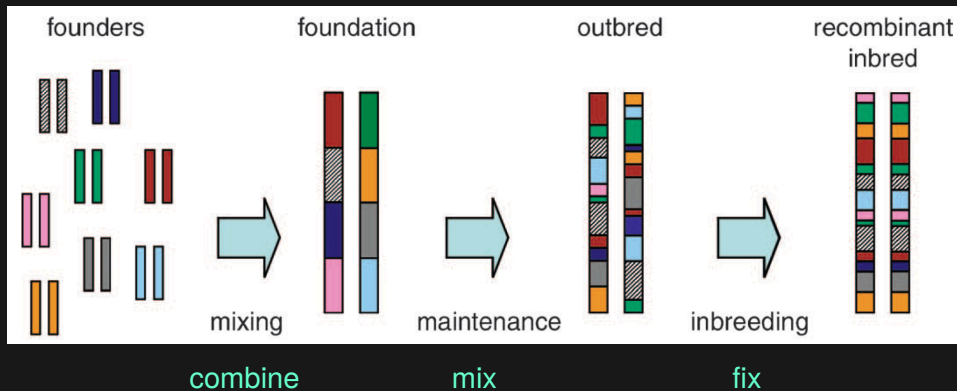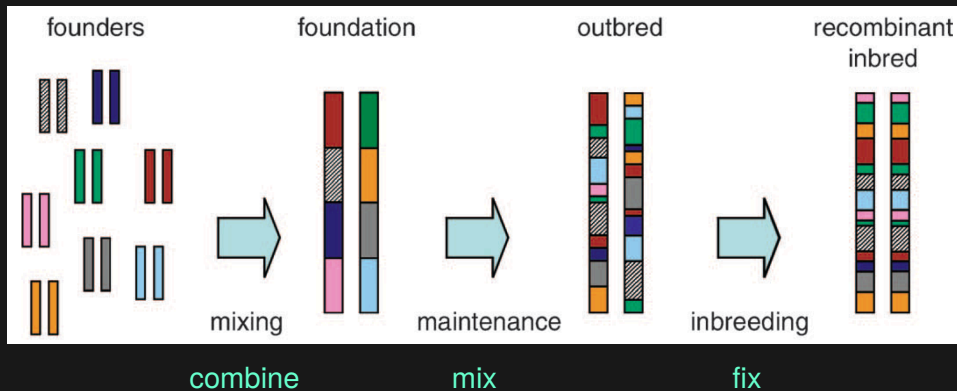
Valdar et al., Genetics 172:1783, 2006

MAGIC lines

founders · foundation · outbred · recombinant inbred

mixing → maintenance → inbreeding

combine · mix · fix

How many?

MAGIC lines

founders   foundation   outbred   recombinant inbred

mixing   maintenance   inbreeding

combine   mix   fix

How many?

Which?

MAGIC lines

founders | foundation | outbred | recombinant inbred

combine | mix | fix

mixing | maintenance | inbreeding
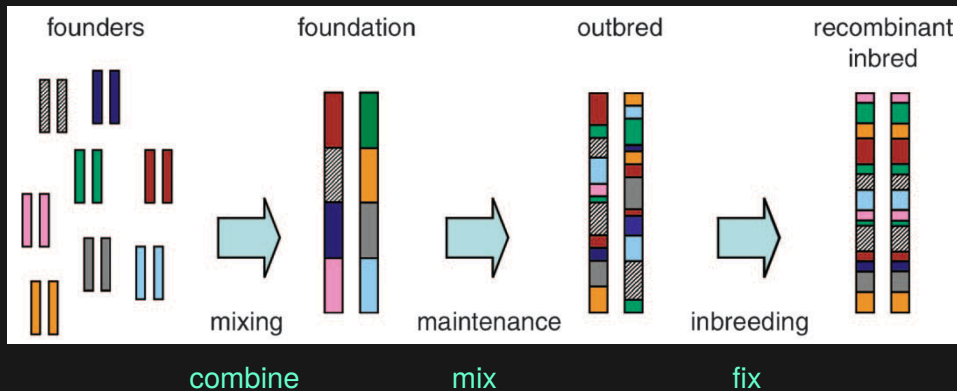
How many?　　　　How long?

Which?

Valdar et al., Genetics 172:1783, 2006

14

MAGIC lines

founders | foundation | outbred | recombinant inbred

combine | mix | fix

How many? | How long? | How?

Which?

Valdar et al., Genetics 172:1783, 2006

The goal

Identify QTL

- ▶ Power
- ▶ Mapping precision

The goal

Identify QTG

- ▶ Power
- ▶ Mapping precision

The goal

Identify QTG

- ▶ Power
- ▶ Mapping precision
- ▶ Estimate QTL allele frequencies

Principles

- ▶ Avoid population structure
- ▶ Tradeoff between power for *de novo* discovery and mapping precision
- ▶ More QTL to find $\Rightarrow$ more QTL getting in the way?
- ▶ More QTL alleles $\Rightarrow$ less information about each
- ▶ Are QTL alleles common or rare?

How many founders?

More

- ► More general use

- ► More QTL

- ► Greater precision

- ► Estimate allele frequencies

- ► Haplotype analysis in founders

Fewer

- ► Lower residual variance

- ► Greater power for a particular QTL?

- ► Better power for epistasis

- ► Rare alleles are less rare

Which founders?

- Diverse
- Interesting
- No breeding problems
- Balanced: star phylogeny

How much mixing?

- ▶ More mixing $\Rightarrow$ Greater mapping precision
- ▶ ...but lower power for *de novo* mapping
- ▶ Potential for population structure, missing alleles
- ▶ Random mating or curated mating?
- ▶ Start with many random cross directions?

Selfing or DH?

- ▶ Inbreeding gives added recombination
- ▶ But not so much as at the mixing stage
- ▶ If doubled haploids are feasible, use them

Sharing is also key

- ▶ The greatest power of MAGIC comes from sharing
  Pooling data, exploring multiple environments/treatments

- ▶ Common software needs
  Analysis software, database infrastructure

- ▶ Many students need to learn the same stuff
  Joint training opportunities

19 years of R/qtl
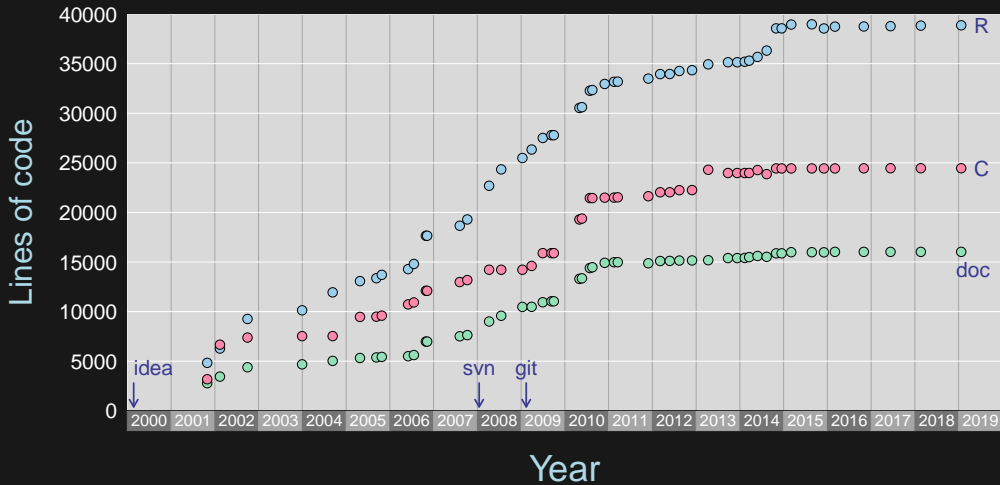
R/qtl cross types

- ► backcross, doubled haploids, haploid
- ► intercross
- ► 2-way RIL by selfing or sibling mating
- ► phase-known 4-way cross

R/qtl2 cross types

- backcross, doubled haploids, haploid
- intercross
- 2-, 4-, 8-, 16-way RIL by selfing
- 2-, 4-, 8-way RIL by sibling mating
- 2-, 3-, 8-way advanced intercross
- 6- and 19-way MAGIC
- Diversity Outbred (DO) mice
- F_1 of DO $\times$ inbred
- general RIL or AIL

Data files

	A	B	C	D	E	F	G
1	id	bolting_days	seed_weight	seed_area	ttl_seedspfruit	branches	height
2	MAGIC.1	15.33	17.15	0.64	45.11	10.5	NA
3	MAGIC.2	22	22.71	0.75	49.11	4.33	42.33
4	MAGIC.3	23	21.03	0.68	57	4.67	50
5	MAGIC.4	18.67	22.45	0.74	54.33	6.33	NA
6	MAGIC.5	18.67	25.36	0.82	38.33	5.67	42.25
7	MAGIC.6	25	21.53	0.71	52	4.33	NA
8	MAGIC.7	15.33	20.92	0.71	39	4	37.35
9	MAGIC.8	14.33	24.2	0.79	50.56	7.33	43.23
10	MAGIC.9	16.33	18.86	0.63	75.78	5.33	42.4
11	MAGIC.10	30	30.46	0.91	56.33	3	48.5
12	MAGIC.11	14	20.58	0.66	41.56	5.67	36.25
13	MAGIC.12	21.33	19.05	0.67	52.33	4.67	49.35
14	MAGIC.14	18.67	21.14	0.72	49.78	6	48.5
15	MAGIC.15	15	21.71	0.73	49	6.33	41.9
16	MAGIC.16	18.33	21.37	0.71	66.67	3.33	18.17

Data files

	A	B	C	D	E	F	G
1	id	bolting_days	seed_weight	seed_area	ttl_seedspfruit	branches	height
2							

	A	B	C	D	E	F	G
3 / **1**	marker	MAGIC.1	MAGIC.10	MAGIC.100	MAGIC.101	MAGIC.102	MAGIC.103
4 / **2**	MN1_29291	A	A	A	B	B	A
5 / **3**	MN1_29716	A	A	A	B	B	A
6 / **4**	MN1_112907	A	A	A	B	B	A
7 / **5**	MASC03771	A	B	A	A	B	A
8 / **6**	MN1_197787	A	B	A	B	B	A
9 / **7**	MN1_340810	A	B	A	A	A	A
10 / **8**	MN1_395107	A	A	A	A	B	A
11 / **9**	MN1_444820	A	A	–	A	A	A
12 / **10**	MN1_494205	A	A	A	A	A	A
13 / **11**	MN1_592863	A	B	A	A	A	A
14 / **12**	MN1_592760	A	A	A	A	A	A
15 / **13**	BKN118	A	A	A	A	A	A
16 / **14**	MN1_1042427	A	B	B	A	B	B
15	CRY2_1021	A	A	A	A	A	A
16	CRY2_429	A	A	A	A	B	A

Data files

Table 1

	A	B	C	D	E	F	G
1	id	bolting_days	seed_weight	seed_area	ttl_seedspfruit	branches	height
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							
16							

Table 2

	A	B	C	D	E	F	G
1	marker	MAGIC.1	MAGIC.10	MAGIC.100	MAGIC.101	MAGIC.102	MAGIC.103
2	1						
3	2						
4	3						
5	4						
6	5						
7	6						
8	7						
9	8						
10	9						
11	10						
12	11						
13	12						
14	13						
15	14						
	15						
	16						

Table 3

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
1	marker	Bur	Can	Col	Ct	Edi	Hi	Kn	Ler	Mt	No	Oy	Po	Rsch	Sf	Tsu	Wil	Ws	Wu	Zu
2	MN1_29291	B	A	A	B	A	A	B	A	A	A	B	B	A	B	A	B	A	A	A
3	MN1_29716	B	A	A	A	A	A	A	A	A	A	B	B	A	B	A	B	A	A	A
4	MN1_112907	B	A	A	A	B	B	B	B	A	A	B	B	A	B	B	A	A	A	A
5	MASC03771	A	B	A	A	B	A	B	A	A	A	A	A	A	A	A	A	A	A	A
6	MN1_197787	B	B	A	A	A	B	B	A	A	A	A	B	A	A	A	A	A	B	B
7	MN1_340810	B	B	A	B	A	B	A	A	A	A	A	A	A	A	A	A	B	B	A
8	MN1_395107	A	A	A	A	A	A	B	A	A	A	A	A	A	A	A	A	A	A	A
9	MN1_444820	B	A	A	B	B	A	A	A	A	A	A	B	A	B	B	B	B	A
10	MN1_494205	B	A	A	A	B	A	A	A	A	A	A	A	A	A	A	A	B	A
11	MN1_592863	A	A	B	A	A	B	A	B	A	A	A	A	B	A	B	A	A
12	MN1_592760	A	A	A	A	A	B	A	A	A	A	A	A	A	A	A	A	A	A
13	BKN118	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
14	MN1_1042427	A	B	A	B	A	B	B	A	B	A	A	A	B	A	A	B	A	B	A
15	CRY2_1021	A	A	A	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A
16	CRY2_429	A	A	A	A	A	B	B	A	B	A	B	A	A	A	A	A	B	A	A
17	MASC07014	A	A	B	A	A	A	A	A	A	A	B	A	A	B	A	B	A	A	A
18	MASC03609	A	A	A	A	A	A	B	B	B	B	A	A	A	A	A	A	A	A
19	MN1_1296068	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	A
20	MN1_1399466	A	A	A	A	A	A	A	A	A	A	A	A	−	B	A	A
21	AXR1_381	A	B	A	A	A	B	A	A	A	B	A	A	A	A	B	A	A	B	B
22	MASC07424	A	B	B	A	A	A	A	A	A	A	A	B	−	A	A	A

Data files

Phenotype file (columns A–G):

	A	B	C	D	E	F	G
1	id	bolting_days	seed_weight	seed_area	ttl_seedspfruit	branches	height
2	1						
3	2						
4	3						
5	4						
6	5						
7	6						
8	7						
9	8						
10	9						
11	10						
12	11						
13	12						
14	13						
15	14						
16	15						
	16						

Genotype / MAGIC file (columns A–G):

	A	B	C	D	E	F	G
1	marker	MAGIC.1	MAGIC.10	MAGIC.100	MAGIC.101	MAGIC.102	MAGIC.103

Marker position file (columns A–C):

	A	B	C
1	marker	chr	pos
2	MN1_29291	1	0.029291
3	MN1_29716	1	0.029757
4	MN1_112907	1	0.112907
5	MASC03771	1	0.174605
6	MN1_197787	1	0.197787
7	MN1_340810	1	0.34081
8	MN1_395107	1	0.395107
9	MN1_444820	1	0.444764
10	MN1_494205	1	0.494205
11	MN1_592863	1	0.592867
12	MN1_592760	1	0.592984
13	BKN118	1	0.761584
14	MN1_1042427	1	1.042428
15	CRY2_1021	1	1.187841
16	CRY2_429	1	1.188433
17	MASC07014	1	1.189374
18	MASC03609	1	1.22655

Founder genotype table (columns A–T):

	A	Bur	Can	Col	Ct	Edi	Hi	Kn	Ler	Mt	No	Ov	Po	Rsch	Sf	Tsu	Wil	Ws	Wu	Zu
													B	A	B	A	A	A	A	A
													B	A	B	B	A	A	A	A
													A	A	A	A	A	A	A	A
													A	A	B	A	A	A	B	B
													A	A	A	A	A	B	B	A
													A	A	A	B	A	B	A	A
													A	A	A	A	A	A	A	A
													A	A	B	A	B	B	B	A
													A	B	A	A	B	A	B	A
													A	A	A	A	A	A	A	A
													A	A	A	A	A	B	B	A
													A	B	A	A	B	A	A	A
													B	A	A	A	B	A	A	A
													A	A	A	A	B	B	B	A
													A	A	A	−	B	A	A	A
													A	A	B	A	A	B	A	B
													A	A	B	−	A	A	A	A

Control file (json or yaml)

```json
{
  "description": "Arabidopsis MAGIC data, Gnan et al (2014)",
  "crosstype": "magic19",
  "sep": ",",
  "na.strings": ["-", "NA"],
  "comment.char": "#",
  "geno": "arabmagic_geno.csv",
  "founder_geno": "arabmagic_foundergeno.csv",
  "gmap": "arabmagic_pmap_tair9.csv",
  "pmap": "arabmagic_pmap_tair9.csv",
  "pheno": "arabmagic_pheno.csv",
  "genotypes":
    "A": 1
    "H": 2
    "B": 3
  },
  "geno_transposed": true,
  "founder_geno_transposed": true
}
```

Control file (json or yaml)

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{
  "description": "Arabidopsis MAGIC data, Gnan et al (2014)",
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  "gmap": "arabmagic_pmap_tair9.csv",
  "pmap": "arabmagic_pmap_tair9.csv",
  "pheno": "arabmagic_pheno.csv",
  "genotypes":
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    "B": 3
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  "founder_geno": "arabmagic_foundergeno.csv",
  "gmap": "arabmagic_pmap_tair9.csv",
  "pmap": "arabmagic_pmap_tair9.csv",
  "pheno": "arabmagic_pheno.csv",
  "genotypes":
    "A": 1
    "H": 2
    "B": 3
  },
  "geno_transposed": true,
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}
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{
  "description": "Arabidopsis MAGIC data, Gnan et al (2014)",
  "crosstype": "magic19",
  "sep": ",",
  "na.strings": ["-", "NA"],
  "comment.char": "#",
  "geno": "arabmagic_geno.csv",
  "founder_geno": "arabmagic_foundergeno.csv",
  "gmap": "arabmagic_pmap_tair9.csv",
  "pmap": "arabmagic_pmap_tair9.csv",
  "pheno": "arabmagic_pheno.csv",
  "genotypes":
    "A": 1
    "H": 2
    "B": 3
  },
  "geno_transposed": true,
  "founder_geno_transposed": true
}
```

Control file (json or yaml)

```
{
  "description": "Arabidopsis MAGIC data, Gnan et al (2014)",
  "crosstype": "magic19",
  "sep": ",",
  "na.strings": ["-", "NA"],
  "comment.char": "#",
  "geno": "arabmagic_geno.csv",
  "founder_geno": "arabmagic_foundergeno.csv",
  "gmap": "arabmagic_pmap_tair9.csv",
  "pmap": "arabmagic_pmap_tair9.csv",
  "pheno": "arabmagic_pheno.csv",
  "genotypes":
    "A": 1
    "H": 2
    "B": 3
  },
  "geno_transposed": true,
  "founder_geno_transposed": true
}
```

Reading data into R

```
library(qtl2)
arab <- read_cross2("arab_magic.json")
```

Reading data into R

```
library(qtl2)
arab <- read_cross2("arab_magic.json")
```

19-way Arabidopsis MAGIC
Kover et al. (2009) PLoS Genet
Gnan et al. (2014) Genetics
github.com/rqtl/qtl2data

Data diagnostics

See Broman et al. (2019) Cleaning genotype data from
Diversity Outbred mice. G3 9:1571–1579

doi: 10.1534/g3.119.400165

Genotype reconstruction
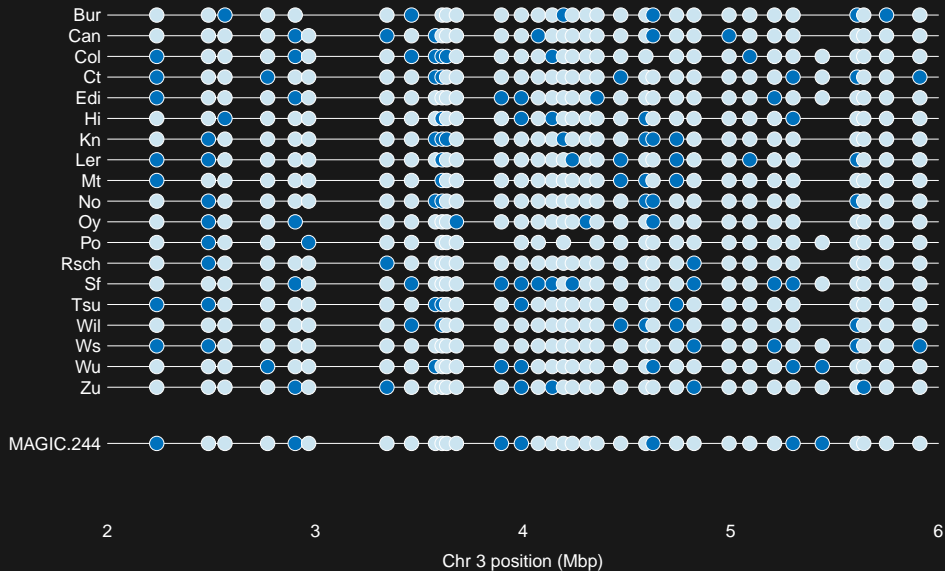
Genotype reconstruction
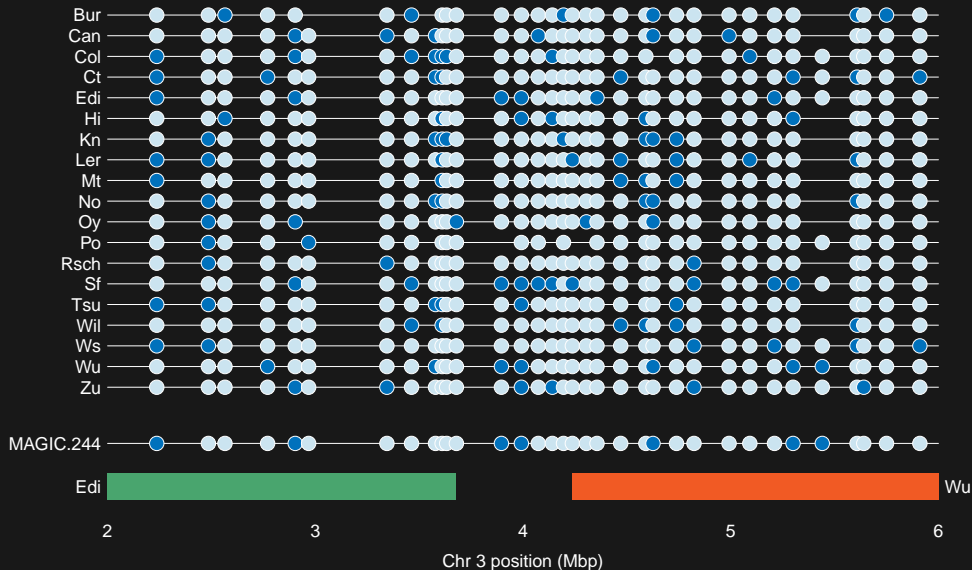
Genotype reconstruction

```
gmap <- insert_pseudomarkers(arab$gmap, step=0.2, stepwidth="max")
pmap <- interp_map(gmap, arab$gmap, arab$pmap)

pr <- calc_genoprob(arab, gmap, error_prob=0.002, cores=24)
```

Genome scan
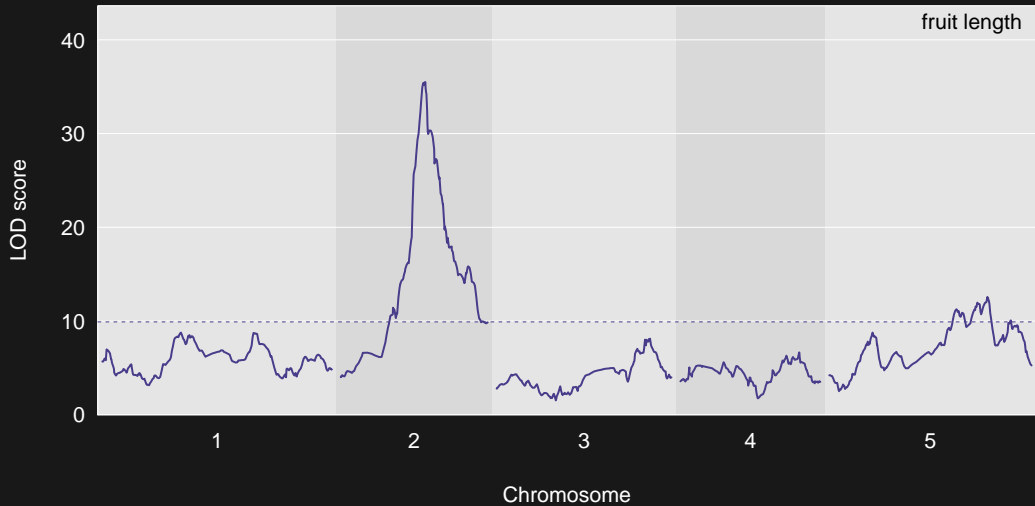
Genome scan
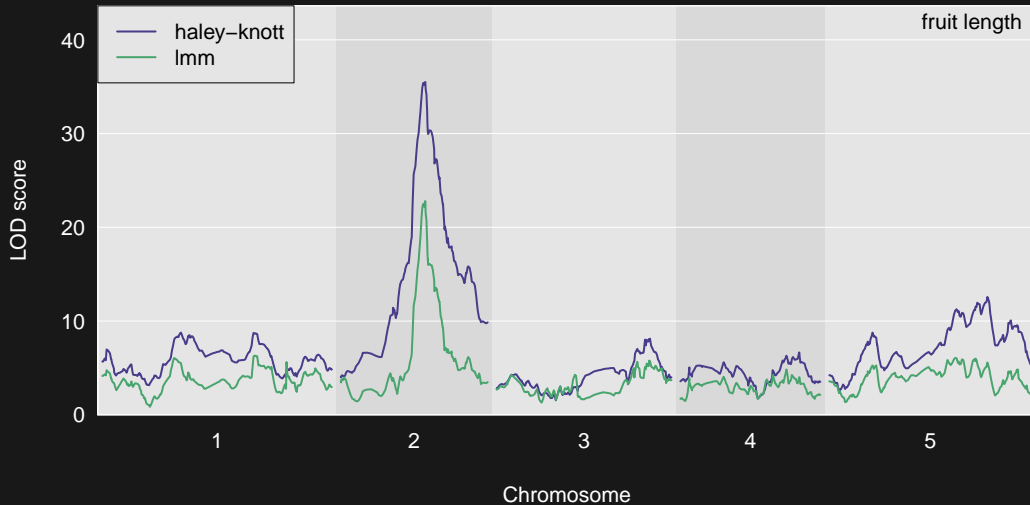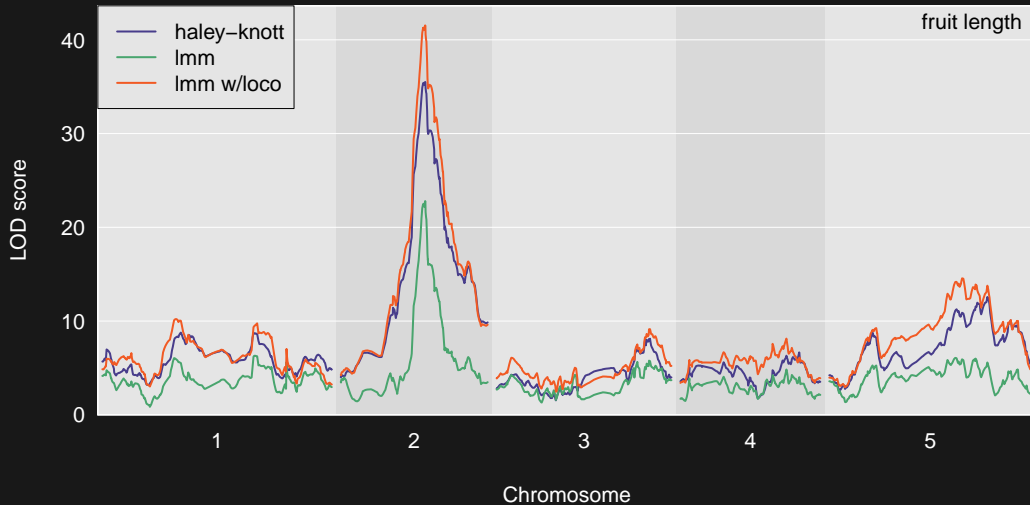
Genome scan
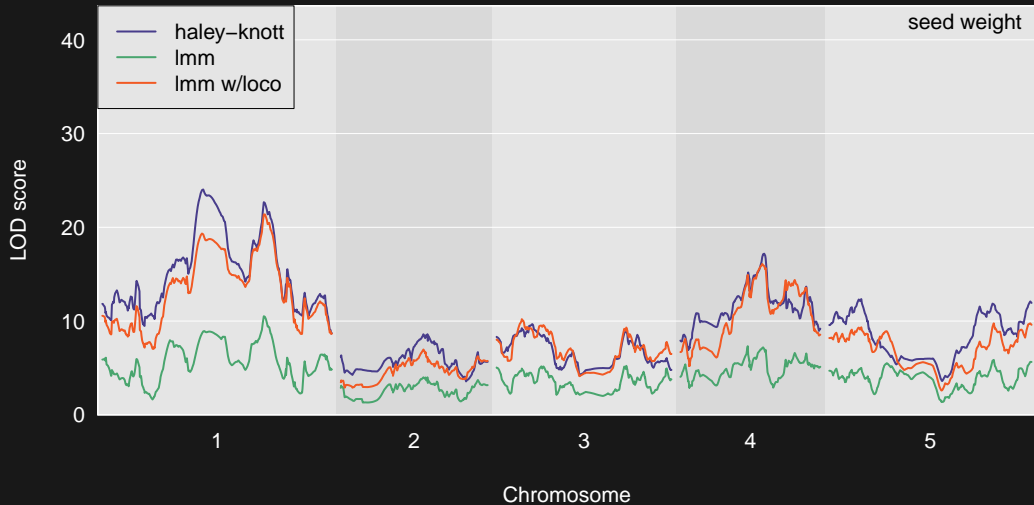
Genome scan

Genome scan

```
out_hk <- scan1(pr, arab$pheno, cores=24)

operm_hk <- scan1perm(pr, arab$pheno, n_perm=1000, cores=24)

k <- calc_kinship(pr, cores=24)
out_lmm <- scan1(pr, arab$pheno, k, cores=24)

k_loco <- calc_kinship(pr, "loco", cores=24)
out_loco <- scan1(pr, arab$pheno, k_loco, cores=24)
```

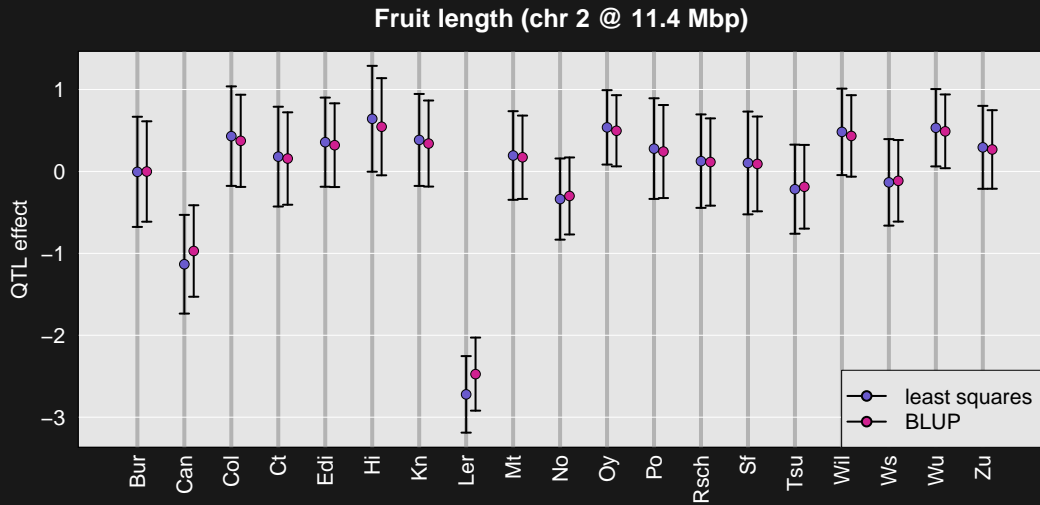# SNP association scan
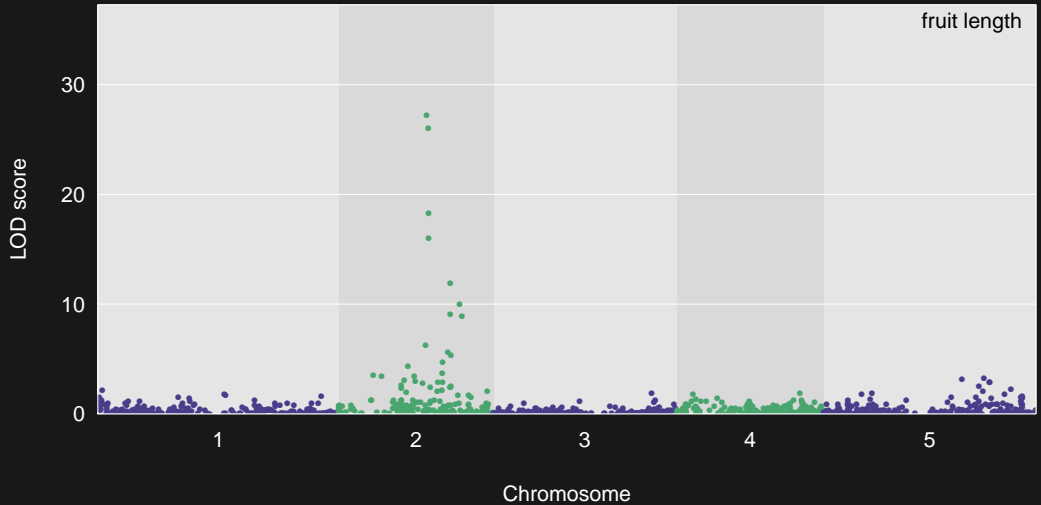
SNP association scan

SNP association scan
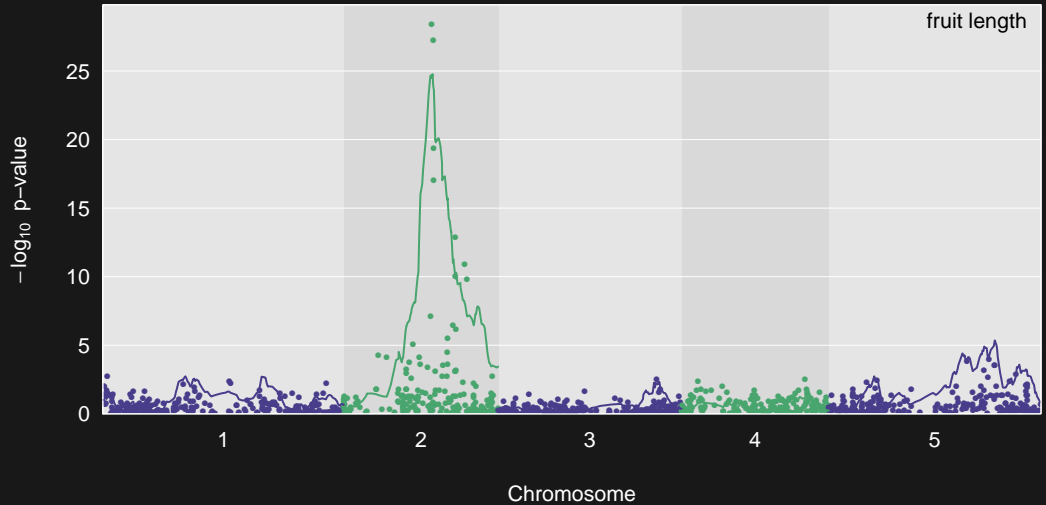
fruit length

SNP association scan
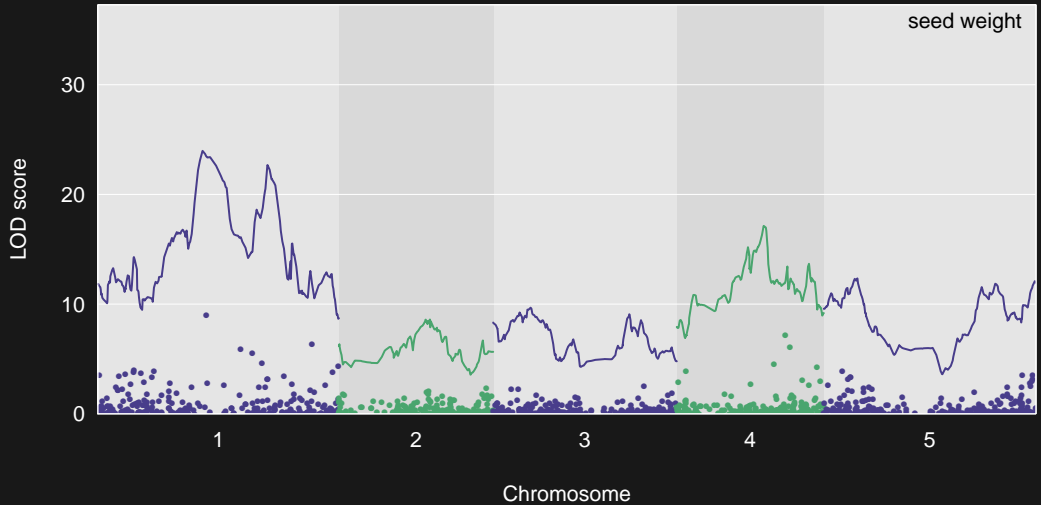
SNP association scan
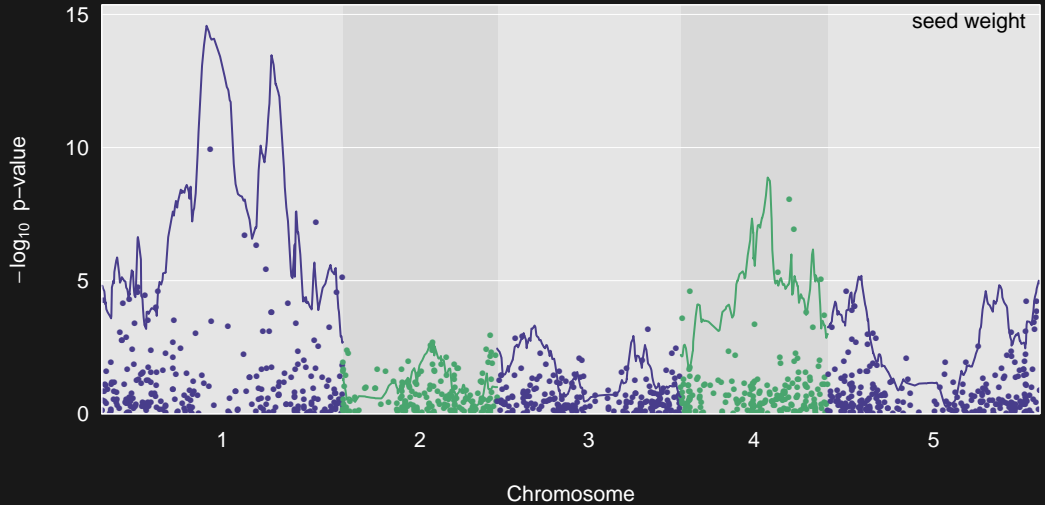
SNP association scan

```
snp_pr <- genoprob_to_snpprob(pr, arab)

out_snps <- scan1(snp_pr, arab$fruit, cores=24)
```
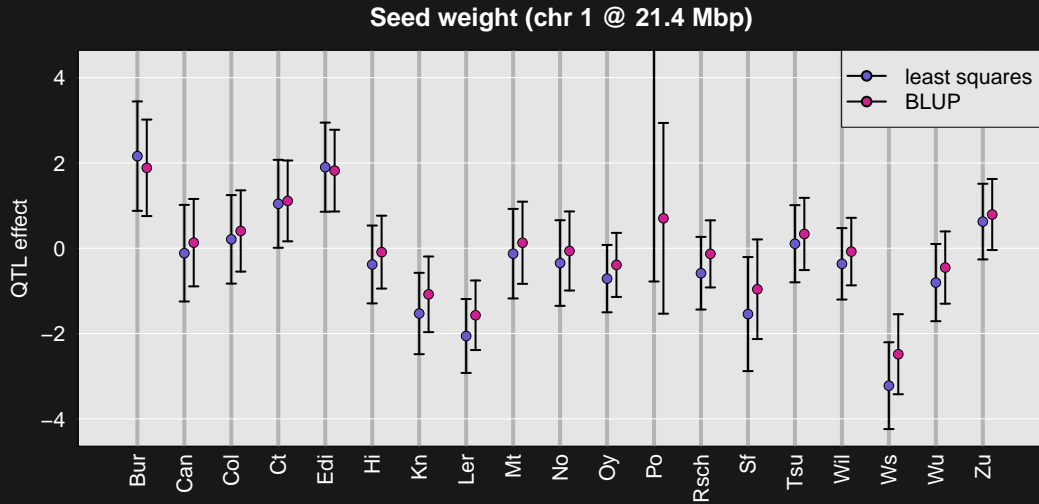
QTL effects

Fruit length (chr 2 @ 11.4 Mbp)

QTL effects

Fruit length (chr 2 @ 11.4 Mbp)

QTL effects

Seed weight (chr 1 @ 21.4 Mbp)
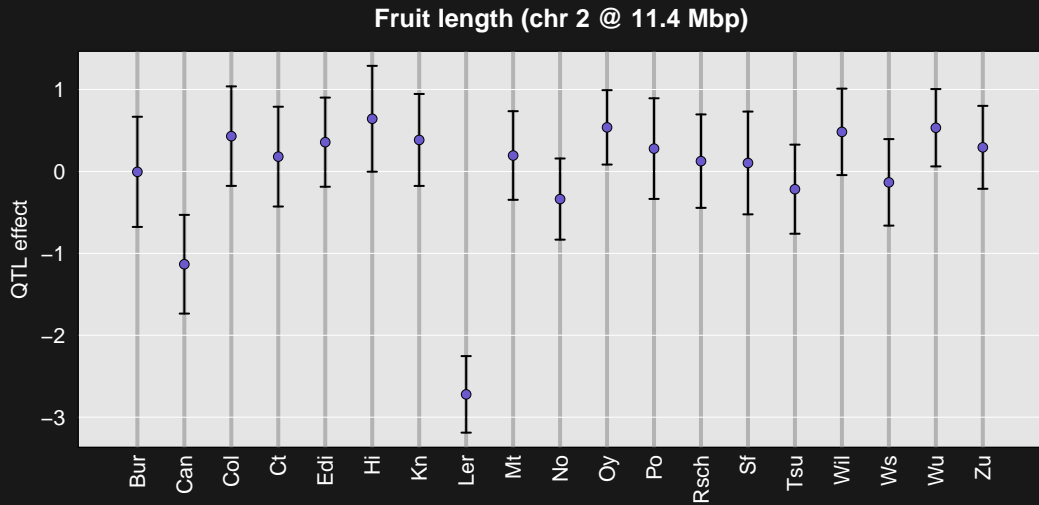
QTL effects

```
fl_peak <- max(out_hk, pmap, lodcolumn="fruit_length")
fl_pr <- pull_genoprobpos(pr, pmap, fl_peak$chr, fl_peak$pos)

fl_fit1 <- fit1(fl_pr, arab$pheno[,"fruit_length"])
fl_blup <- fit1(fl_pr, arab$pheno[,"fruit_length"], blup=TRUE)
```

Goals

- ▶ Genotype reconstructions from external software

- ▶ General models for RIL and AIL

- ▶ Sequencing-based genotype data

- ▶ Multiple-QTL models

- ▶ QTL $\times$ environment interactions

- ▶ Interactive data visualization

Slides: `bit.ly/msu2019-12` 🄯 PUBLIC DOMAIN

`kbroman.org`

`kbroman.org/qtl2`

`github.com/kbroman`

`@kwbroman`