QTL mapping in MAGIC populations

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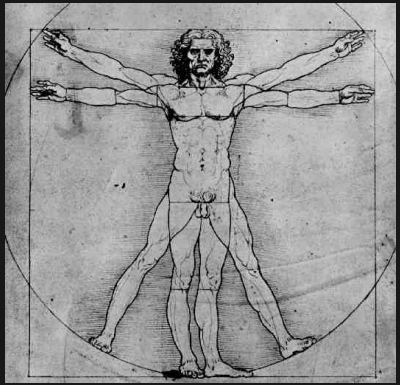
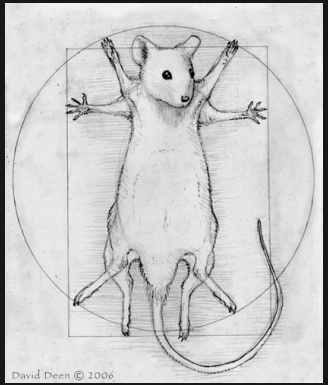
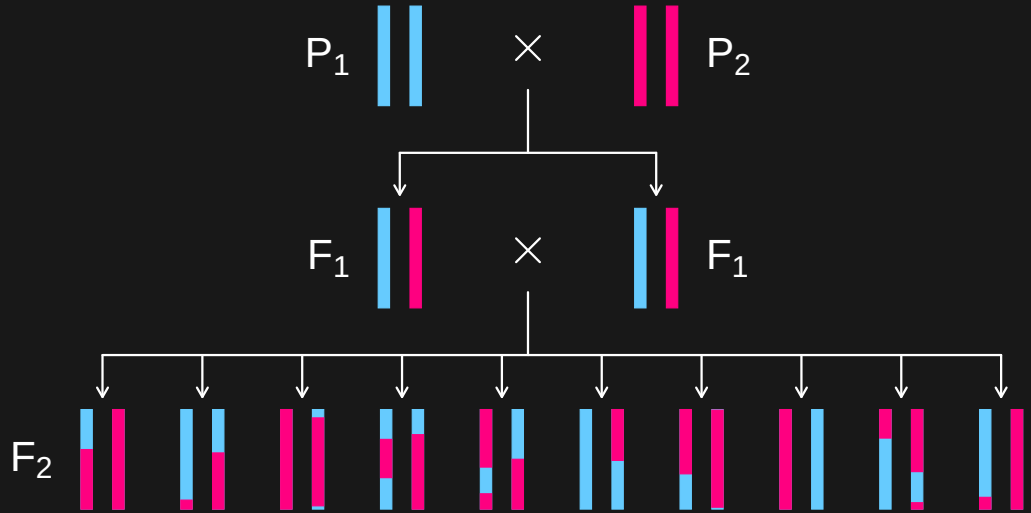
PUBLIC DOMAIN



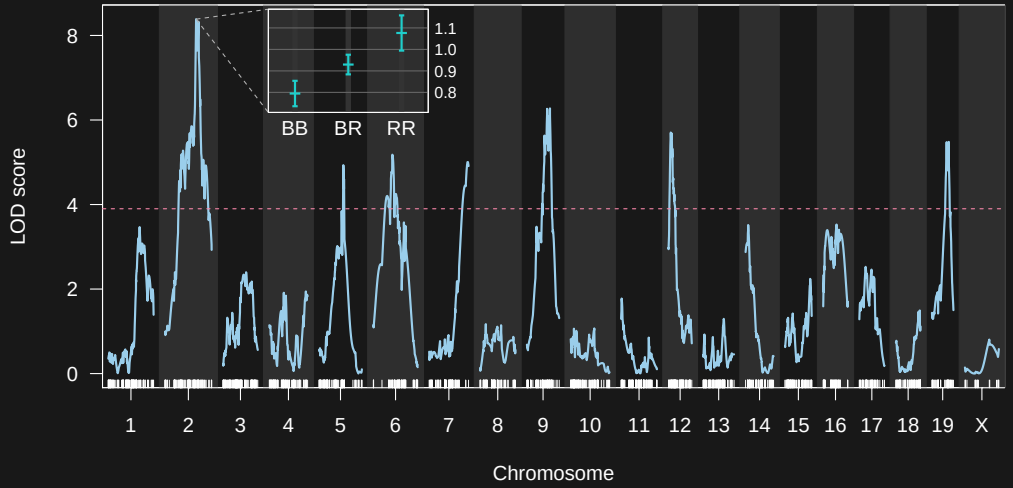


daviddeen.com

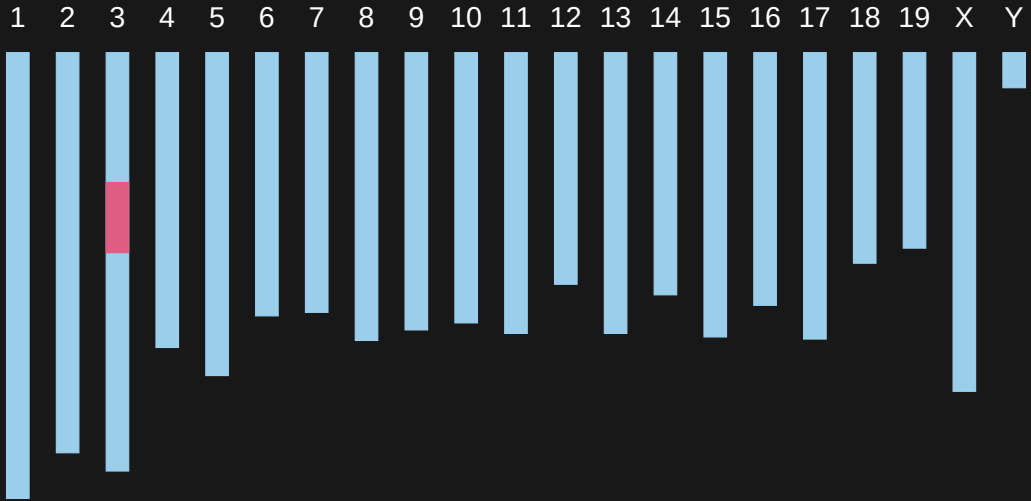
Intercross



QTL mapping


LOD score
0
2
4
6
8
1.1
1.0
0.9
0.8
BB
BR
RR
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
X
Chromosome

Congenic line/NIL



Improving precision

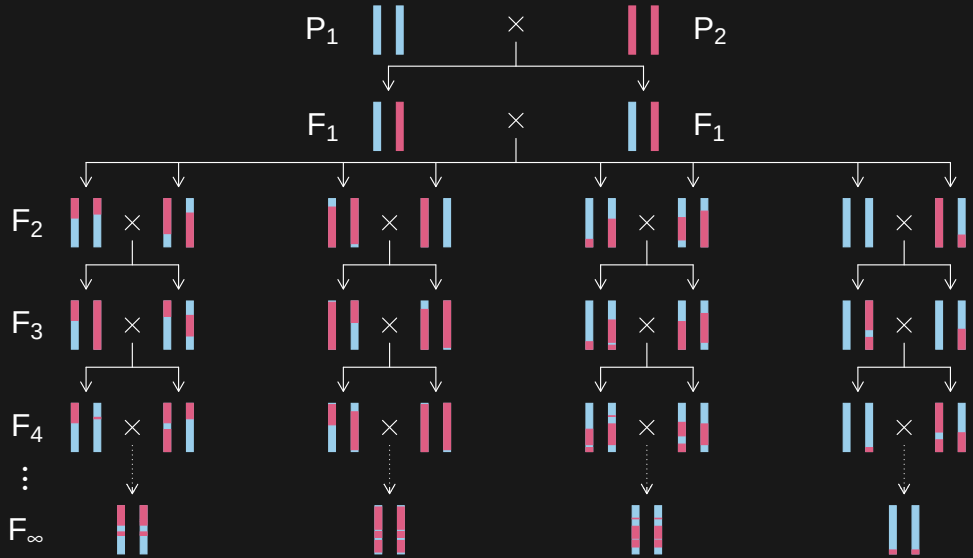
- more recombinations
- more individuals
- more precise phenotype
- lower-level phenotypes
  - transcripts, proteins, metabolites

Genome-scale phenotypes

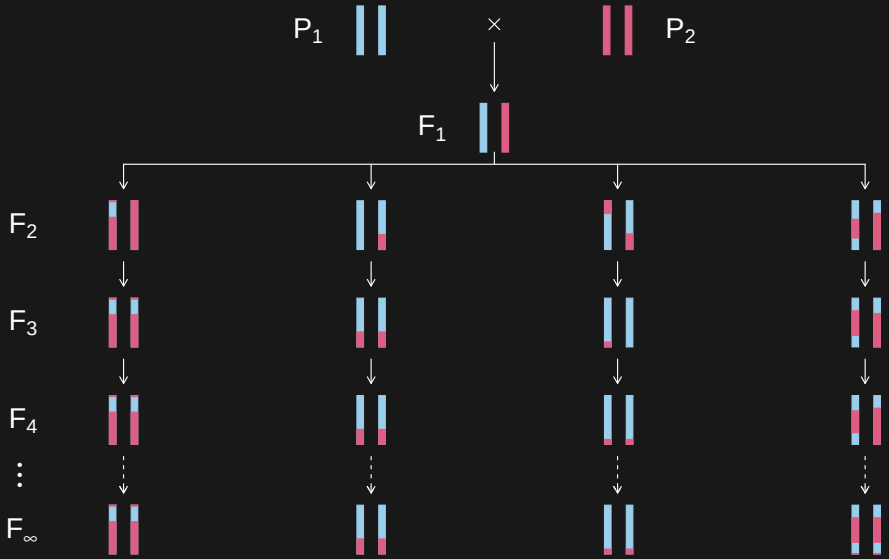
Alan Attie

Advanced intercross lines

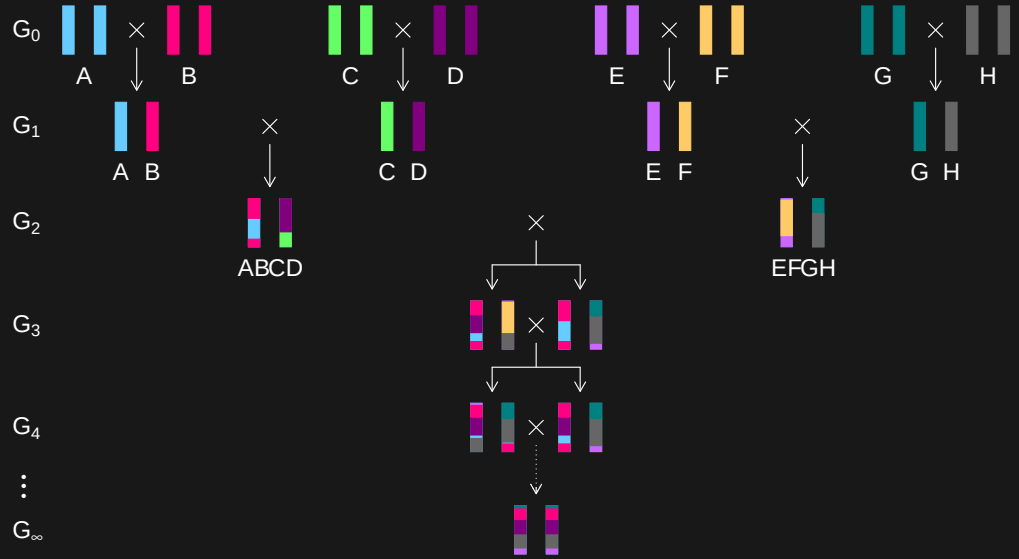
Recombinant inbred lines



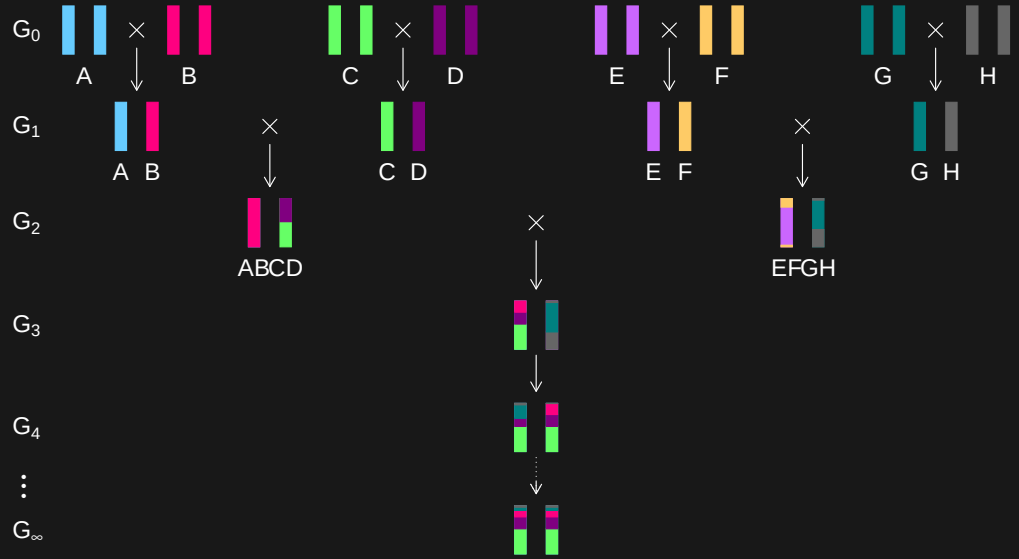
Recombinant inbred lines



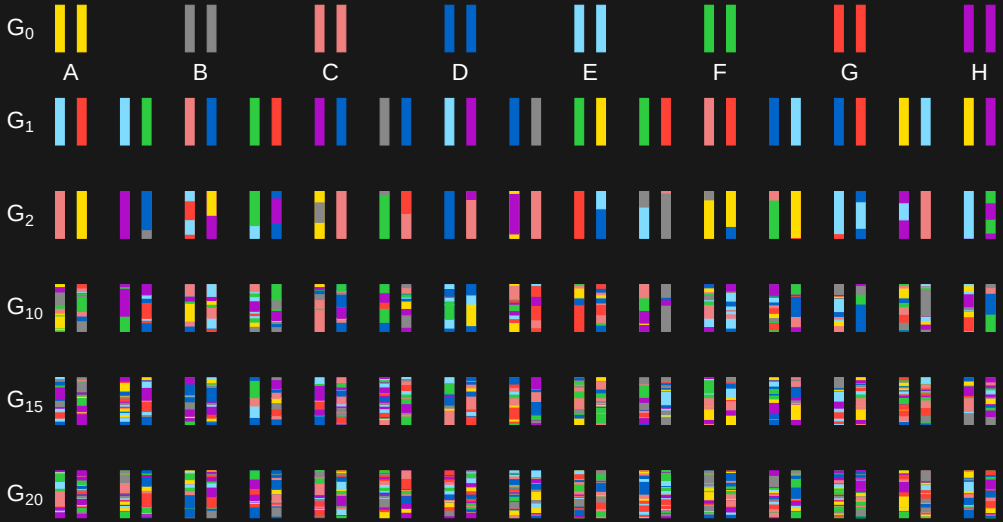
Collaborative Cross



MAGIC



Heterogeneous stock



MAGIC is magic

- Genetic diversity
- High-precision mapping
- Predictable linkage disequilibrium
- No rare alleles
- Phenotype replicates to reduce individual variation
- Pool phenotypes from multiple labs, environments, treatments
- Genotype once

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- Pool phenotypes from multiple labs, environments, treatments
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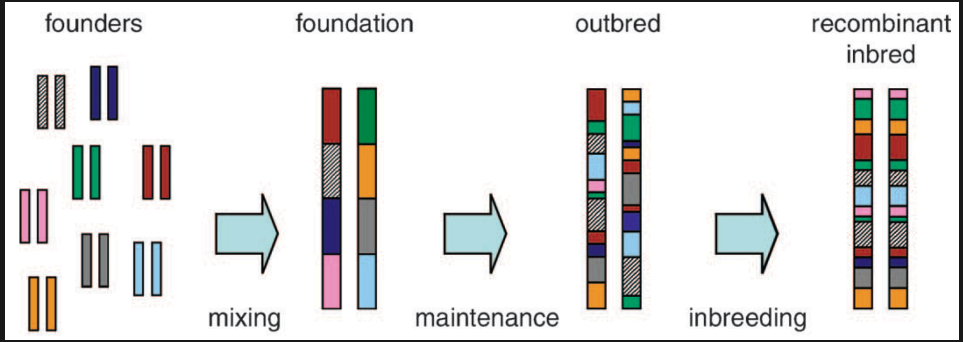
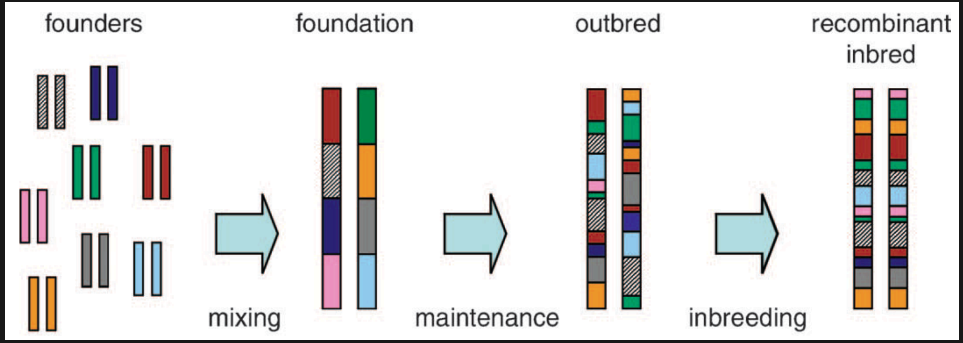
- Cool name

MAGIC lines



Valdar et al., Genetics 172:1783, 2006

MAGIC lines



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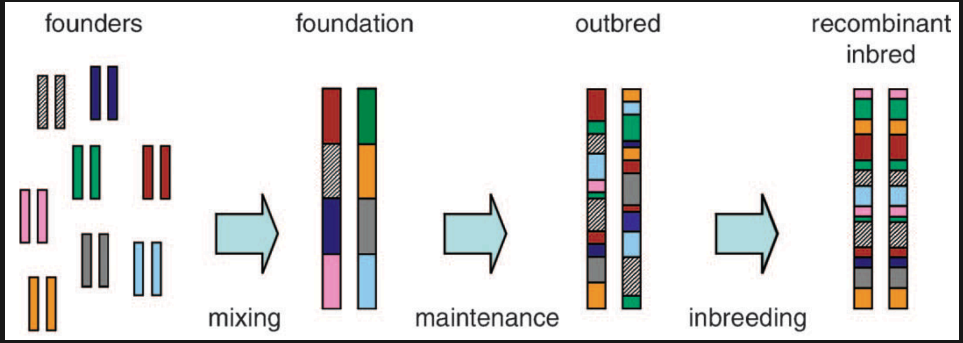
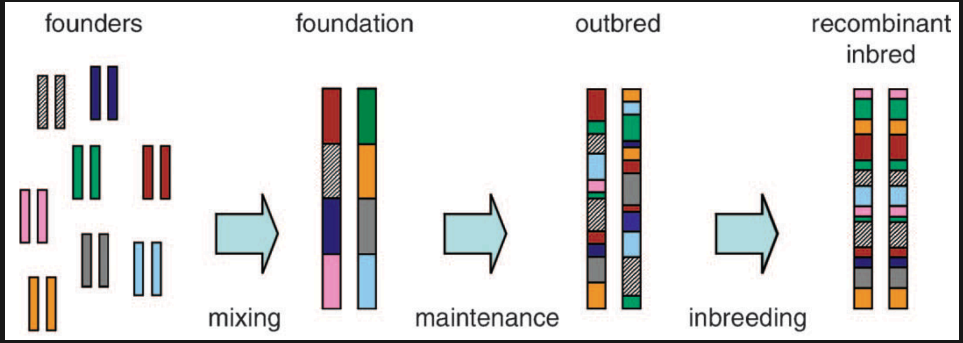


combine mix fix

How many?

Valdar et al., Genetics 172:1783, 2006

MAGIC lines



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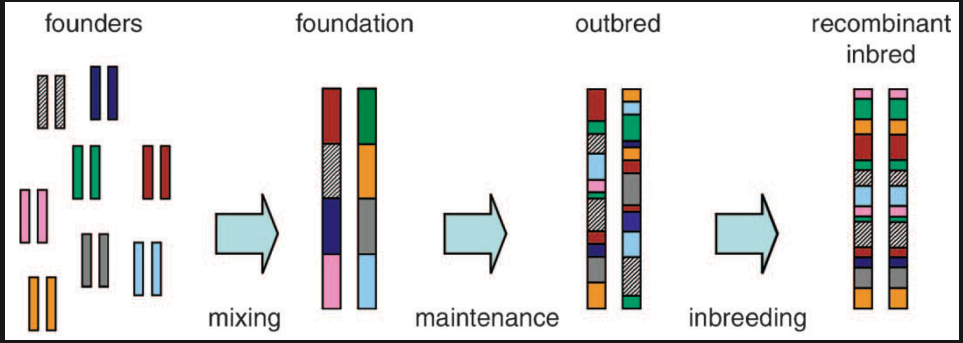

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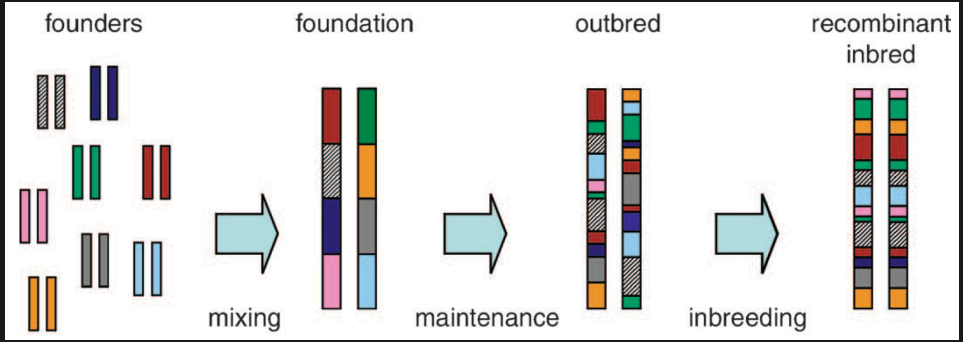
How many?

Which?

How long?

Valdar et al., Genetics 172:1783, 2006

MAGIC lines



combine mix fix

How many?

Which?

How long?

How?

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

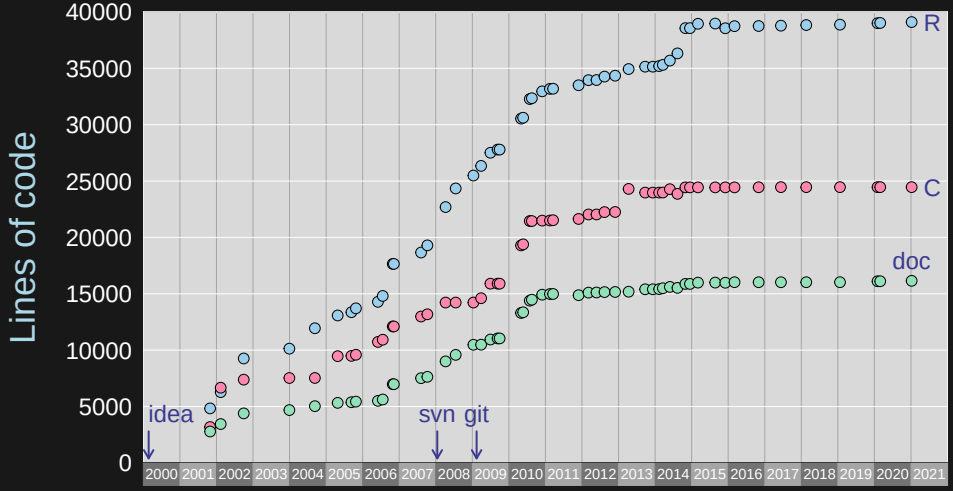
Sharing is also key

- 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

Summary

- How many founders?
  - Tradeoff between diversity and information about particular alleles
- Which founders?
  - Diverse, interesting, no breeding problems, star phylogeny
- How long to mix?
  - Tradeoff between power and precision
- How to fix?
  - Doubled haploids are great if feasible
- Let's share!
  - Lines, data, software, training

21 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/qt12
How in 3D

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
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14							
15							
16							

	A	B	C	D	E	F	G
1	marker	MAGIC.1	MAGIC.10	MAGIC.100	MAGIC.101	MAGIC.102	MAGIC.103
2	MN1_29291	A	A	A	B	B	A
3	MN1_29716	A	A	A	B	B	A
4	MN1_112907	A	A	A	B	B	A
5	MASC03771	A	B	A	A	B	A
6	MN1_197787	A	B	A	B	B	A
7	MN1_340810	A	B	A	A	A	A
8	MN1_395107	A	A	A	A	B	A
9	MN1_444820	A	A	−	A	A	A
10	MN1_494205	A	A	A	A	A	A
11	MN1_592863	A	B	A	A	A	A
12	MN1_592760	A	A	A	A	A	A
13	BKN118	A	A	A	A	A	A
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

	A	B	C	D	E	F	G
1	id	bolting_days	seed_weight	seed_area	ttl_seedspfruit	branches	height
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2							
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16							

	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	A	A	A	A
3	MN1_29716	B	A	A	B	A	A	B	A	A	A	B	B	A	B	A	A	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
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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	A	A	A	B	A	B	B	A
10	MN1_494205	B	A	A	A	B	A	A	A	A	A	A	A	A	A	A	A	A	B	A
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12	MN1_592760	B	A	A	A	B	A	A	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	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	A
16	CRY2_429	A	A	A	A	A	B	B	B	A	B	A	A	A	A	A	A	B	A	A
17	MASC07014	A	A	B	A	A	A	A	A	A	A	A	A	B	A	A	B	A	A	A
18	MASC03609	A	A	A	A	A	A	B	B	A	B	B	B	A	A	A	A	B	A	A
19	MN1_1296068	A	A	A	A	A	A	B	A	A	A	A	A	A	A	A	A	B	B	A
20	MN1_1399466	A	A	A	A	A	A	B	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	A	A	B	A	B
22	MASC07424	A	B	B	A	A	A	A	A	A	A	A	A	A	B	−	A	A	A	A

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13							
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15							
16							

	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	Ov	Po	Rsch	Sf	Tsu	Wil	Ws	Wu	Zu
2	MN1_292												B	A	B	A	A	A	A	A
3	MN1_297												B	A	B	A	A	A	A	A
4	MN1_112												B	A	B	B	A	A	A	A
5	MASC03												A	A	A	A	A	A	A	A
6	MN1_197												A	A	B	A	A	A	B	B
7	MN1_340												A	A	A	A	A	B	B	A
8	MN1_395												A	A	A	A	A	A	A	A
9	MN1_444												A	A	A	B	A	B	B	A
10	MN1_494												A	A	A	A	A	A	B	A
11	MN1_592												A	A	A	B	A	B	A	A
12	MN1_592												A	A	A	A	A	A	A	A
13	BKN11												A	A	A	A	A	A	A	A
14	MN1_1042												A	B	A	A	B	A	B	A
15	CRY2_10												A	A	A	A	A	A	A	A
16	CRY2_4												A	A	A	A	A	B	A	A
17	MASC07												A	B	A	A	B	A	A	A
18	MASC03												B	A	A	A	A	B	A	A
19	MN1_1296												A	A	A	A	A	B	B	A
20	MN1_1399												A	A	A	A	–	B	A	A
21	AXR1_3												A	A	A	A	A	B	A	B
22	MASC07												A	A	B	–	A	A	A	A

	A	B	C
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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

Control file (json or yaml)

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  "comment.char": "#",
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  "gmap": "arabmagic_pmap_tair9.csv",
  "pmap": "arabmagic_pmap_tair9.csv",
  "pheno": "arabmagic_pheno.csv",
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    "H": 2
    "B": 3
  },
  "geno_transposed": true,
  "founder_geno_transposed": true
}
```

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  "pmap": "arabmagic_pmap_tair9.csv",
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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

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

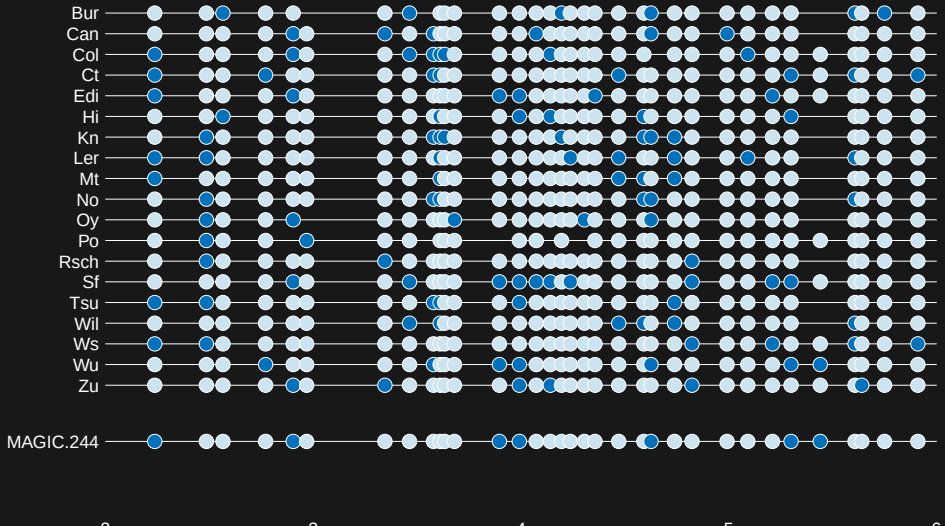
19-way Arabidopsis MAGIC
Kover et al. (2009) PLoS Genet
Gnan et al. (2014) Genetics
`github.com/rqtl/qtl2data`

Data diagnostics

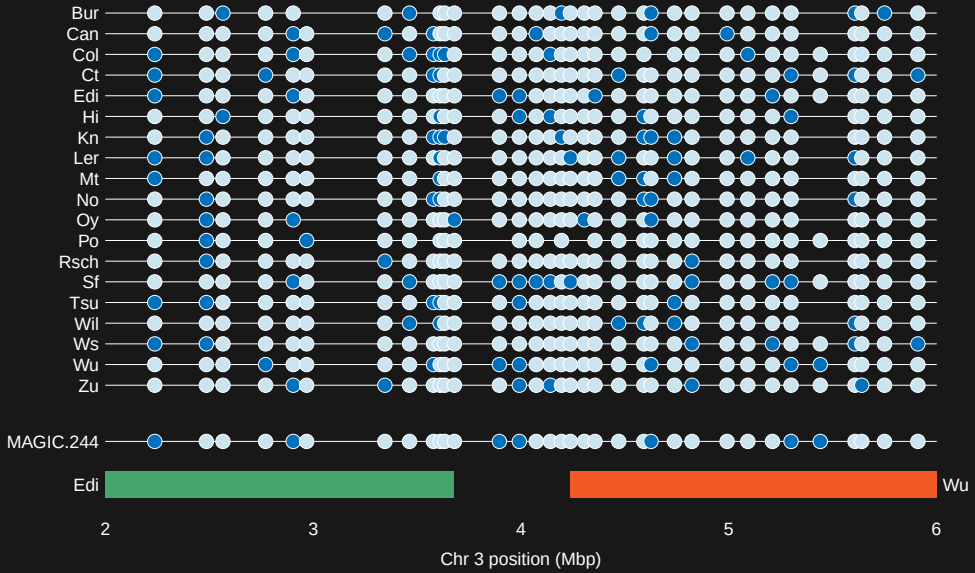
See Broman et al. (2019) Cleaning genotype data from Diversity Outbred mice. G3 9:1571–1579

doi: 10.1534/g3.119.400165

Genotype reconstruction



Genotype reconstruction

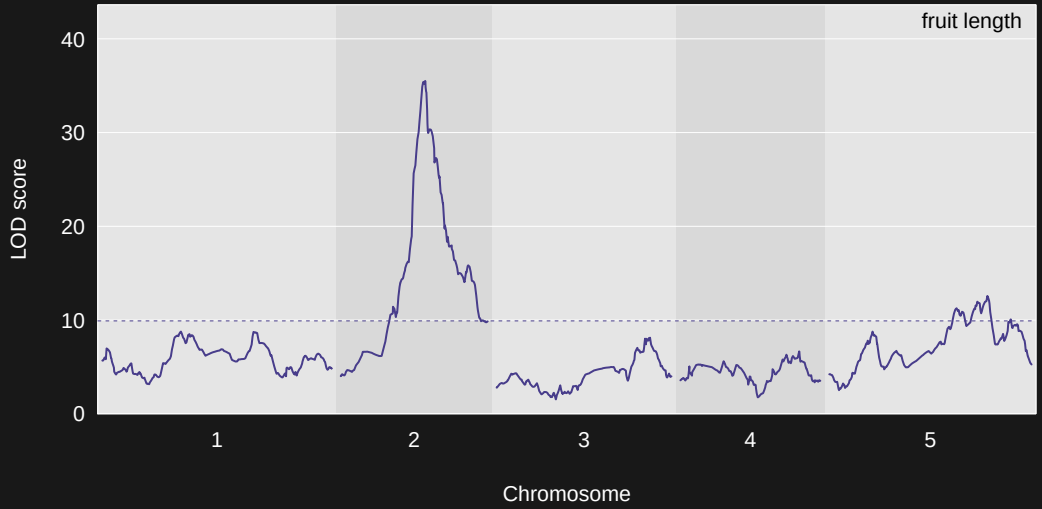


Genotype reconstruction

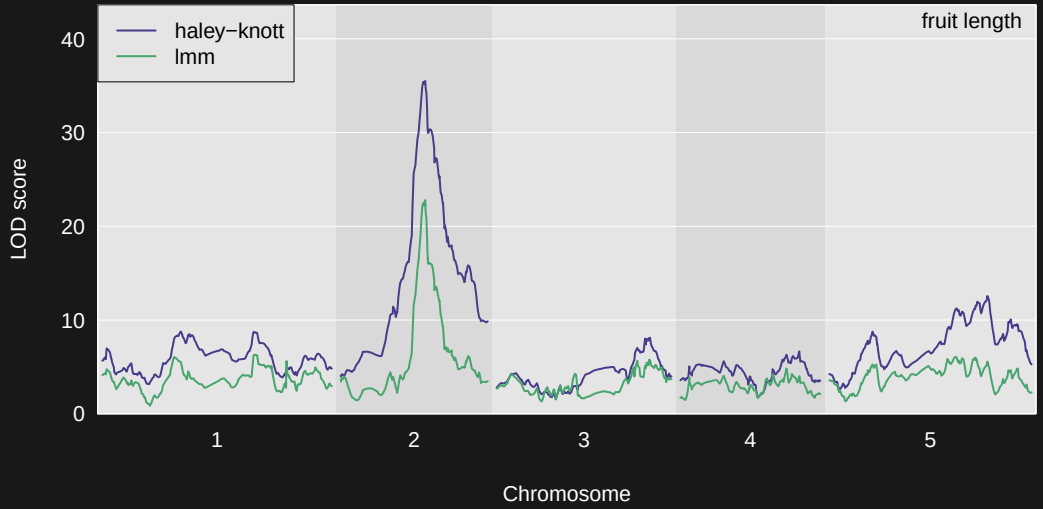
```
gmap <- insert_pseudomarkers(arab$gmap, step=0.2, stepwidth="max")
pmap <- interp_map(gmap, arab$gmap, arab$pmap)

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

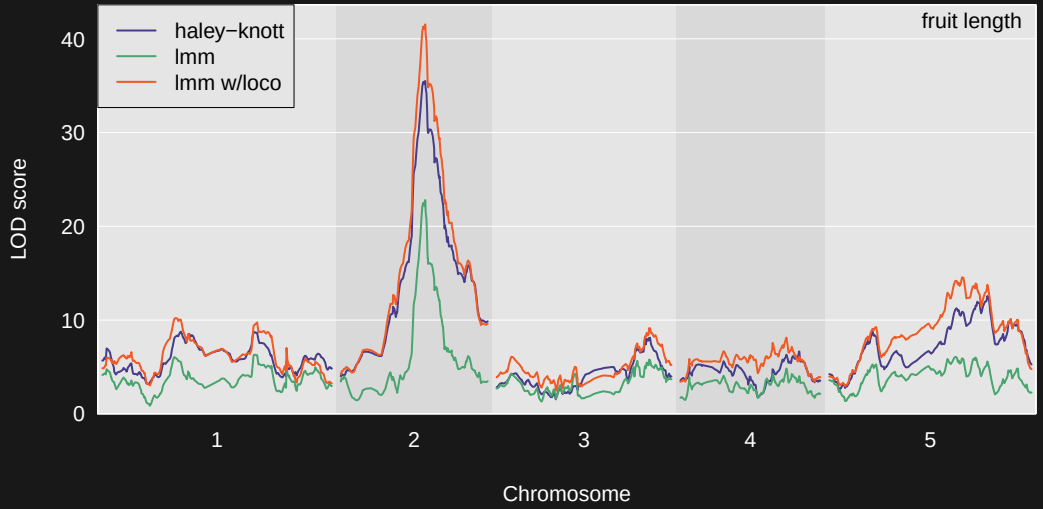
Genome scan



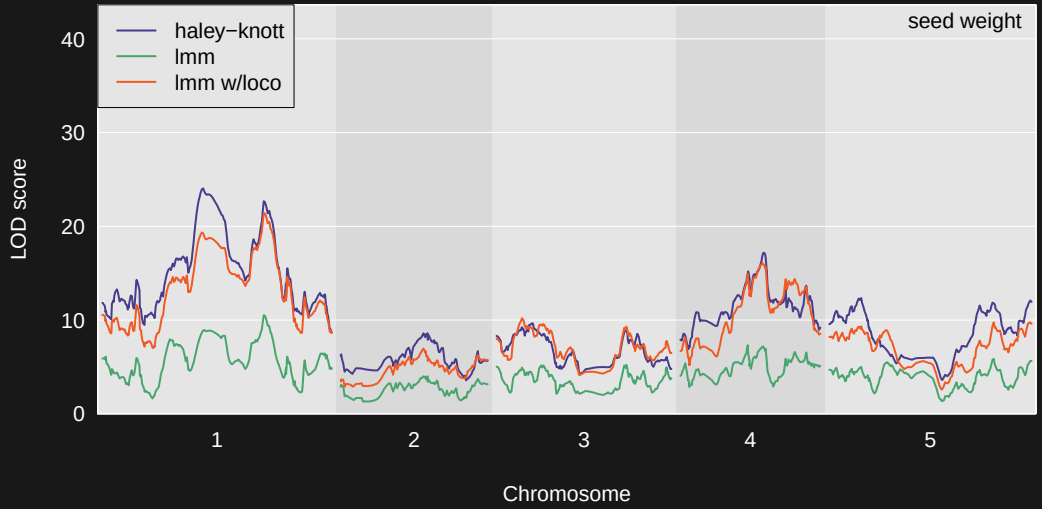
Genome scan


haley−knott
lmm
fruit length
LOD score
40
30
20
10
0
1
2
3
4
5
Chromosome

Genome scan


haley−knott
lmm
lmm w/loco
fruit length
LOD score
40
30
20
10
0
1
2
3
4
5
Chromosome

Genome scan


seed weight
haley−knott
lmm
lmm w/loco
LOD score
0
10
20
30
40
1
2
3
4
5
Chromosome

Genome scan

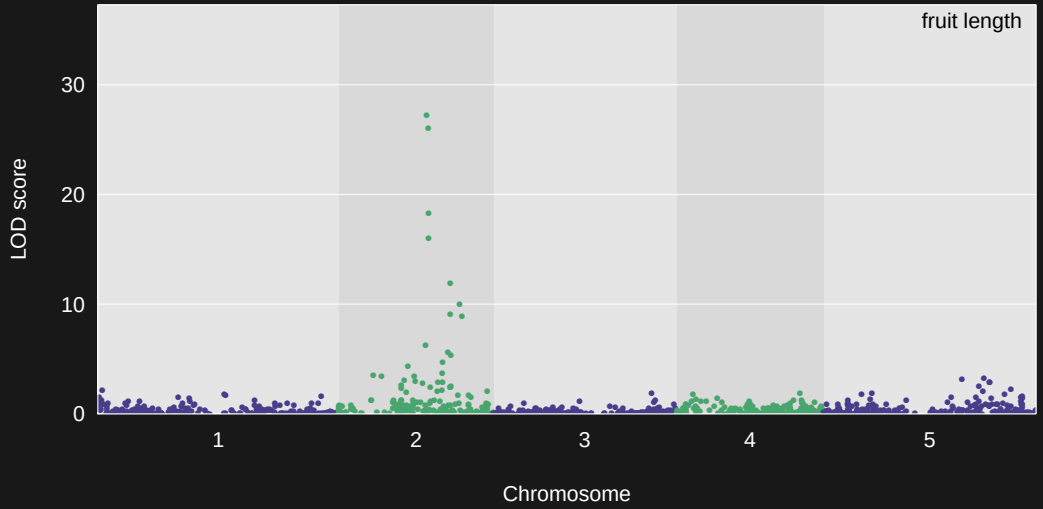
```
out_hk <- scan1(pr, arab$pheno, cores=24)

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

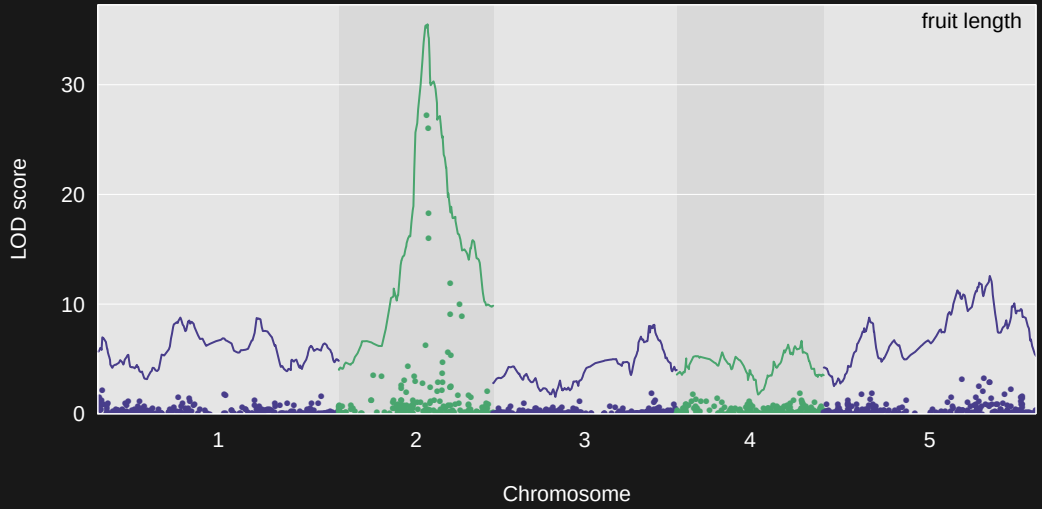
k <- calc_kinship(pr, cores=24)
out_lmm <- scan1(pr, arab$pheno, k, cores=24)

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

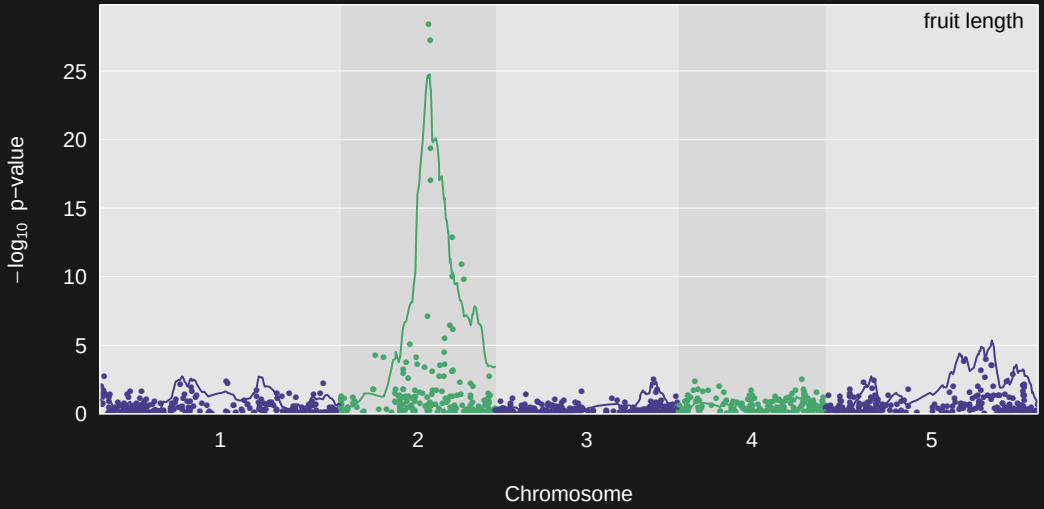
SNP association scan



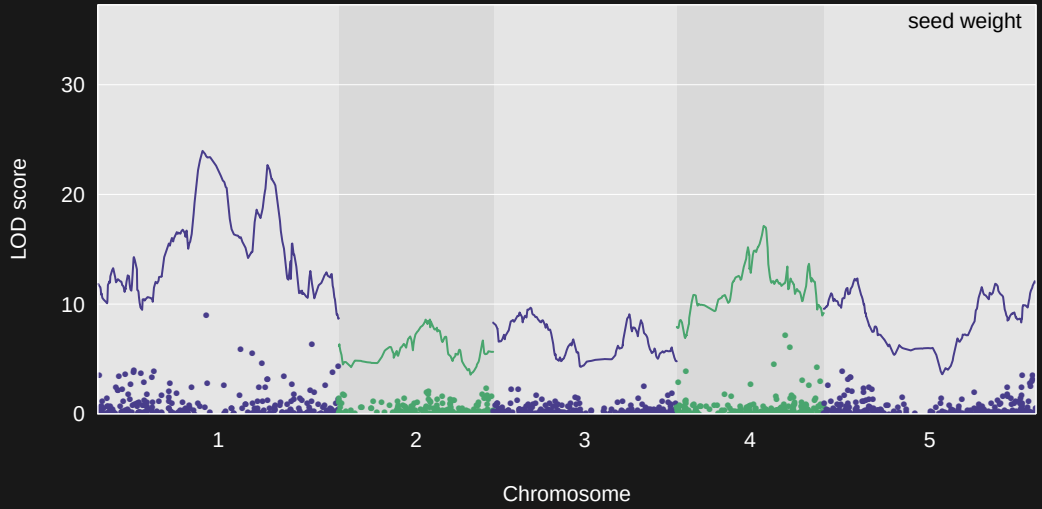
SNP association scan



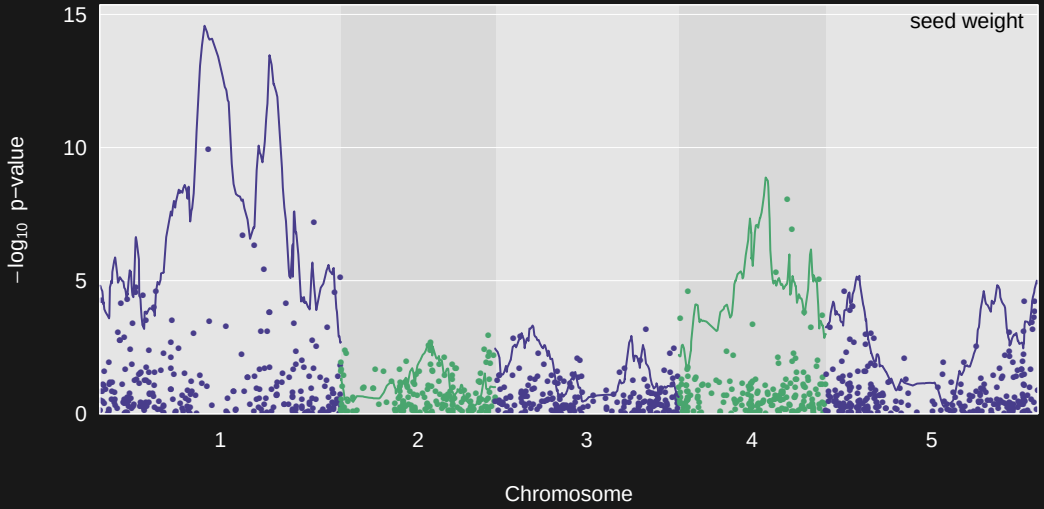
SNP association scan



SNP association scan



SNP association scan

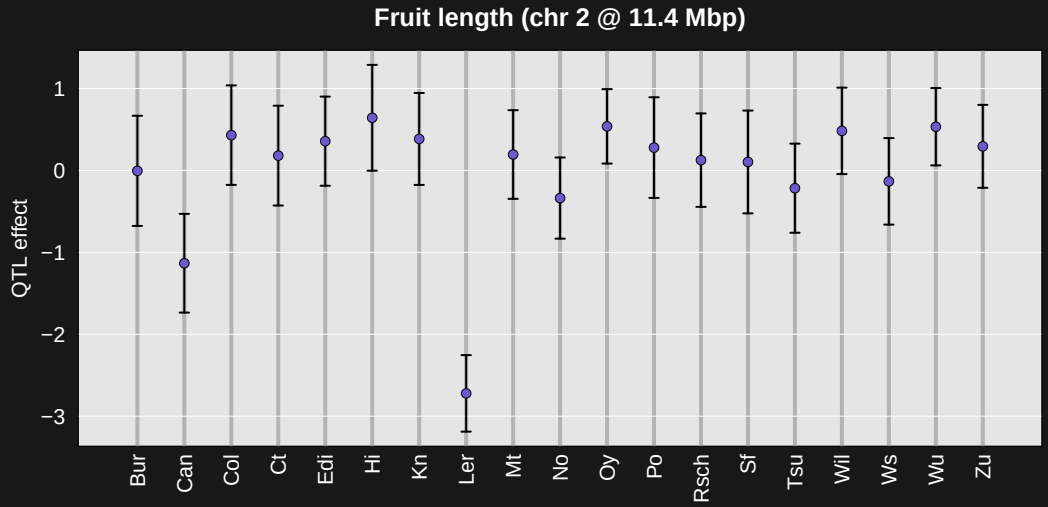


SNP association scan

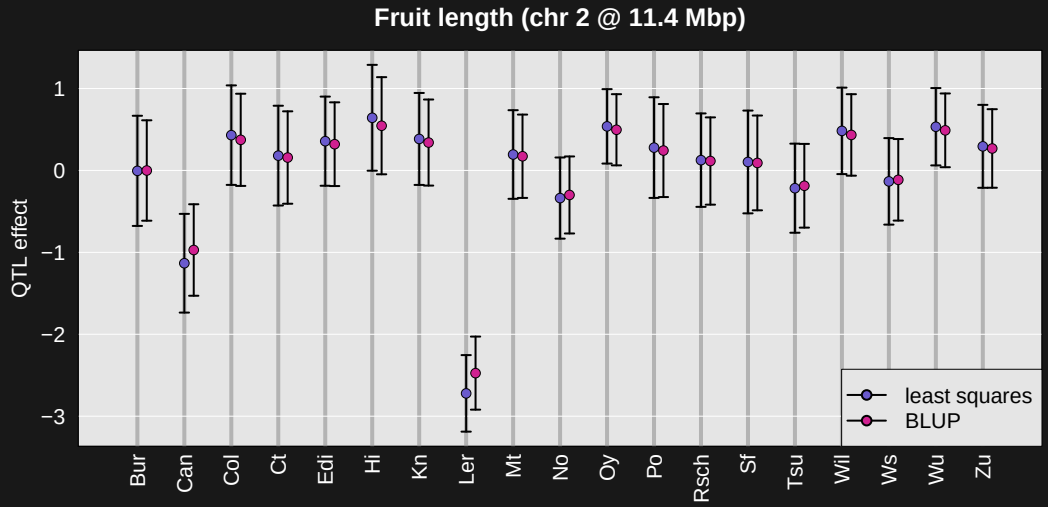
```
snp_pr <- genoprob_to_snpprob(pr, arab)

out_snps <- scan1(snp_pr, arab$pheno, cores=24)
```

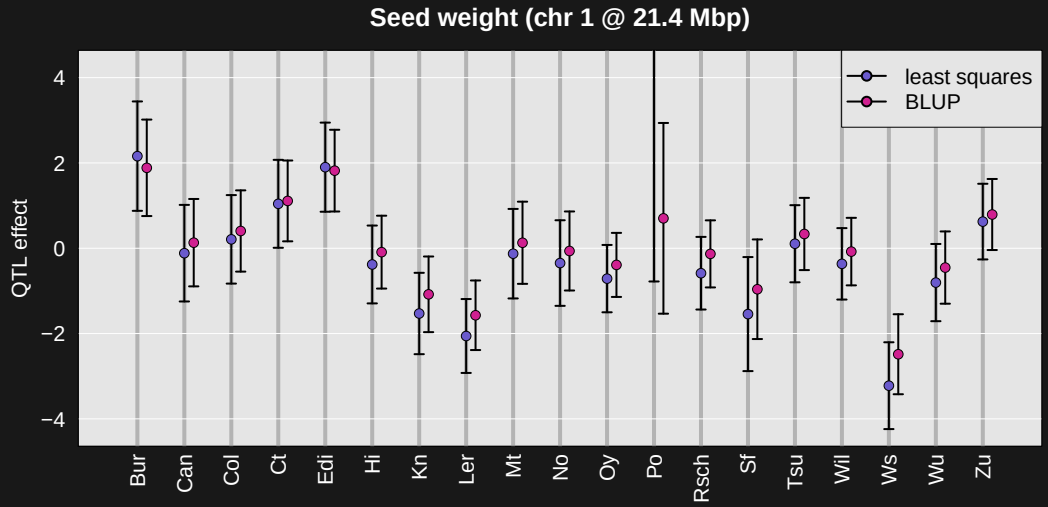
QTL effects


Fruit length (chr 2 @ 11.4 Mbp)
QTL effect
1
0
−1
−2
−3
Bur
Can
Col
Ct
Edi
Hi
Kn
Ler
Mt
No
Oy
Po
Rsch
Sf
Tsu
Wil
Ws
Wu
Zu

QTL effects


Fruit length (chr 2 @ 11.4 Mbp)
QTL effect
1
0
−1
−2
−3
Bur
Can
Col
Ct
Edi
Hi
Kn
Ler
Mt
No
Oy
Po
Rsch
Sf
Tsu
Wil
Ws
Wu
Zu
least squares
BLUP

QTL effects


Seed weight (chr 1 @ 21.4 Mbp)
QTL effect
4
2
0
−2
−4
least squares
BLUP
Bur
Can
Col
Ct
Edi
Hi
Kn
Ler
Mt
No
Oy
Po
Rsch
Sf
Tsu
Wil
Ws
Wu
Zu

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
- Sequencing-based genotype data
- Multiple-QTL models
- QTL $\times$ environment interactions
- Interactive data visualization

Slides: kbroman.org/Talk_MAGIC2021

kbroman.org

kbroman.org/qtl2

github.com/kbroman

@kwbroman