Basic QTL mapping

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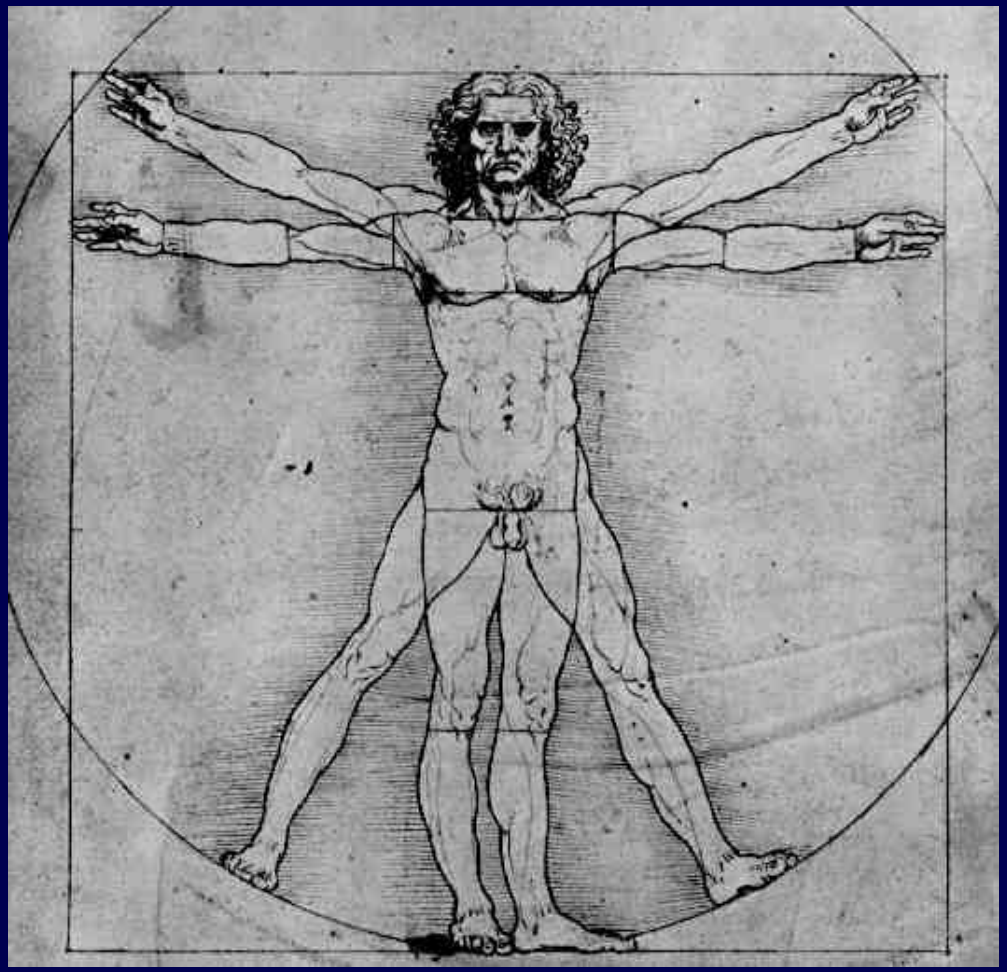
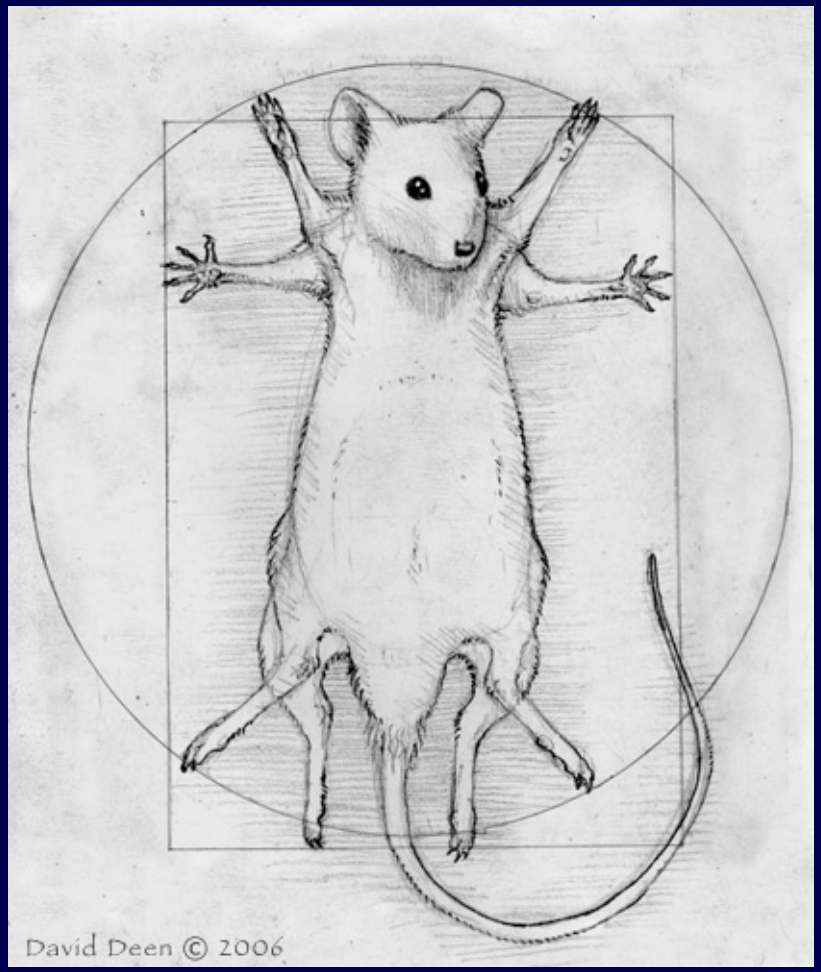
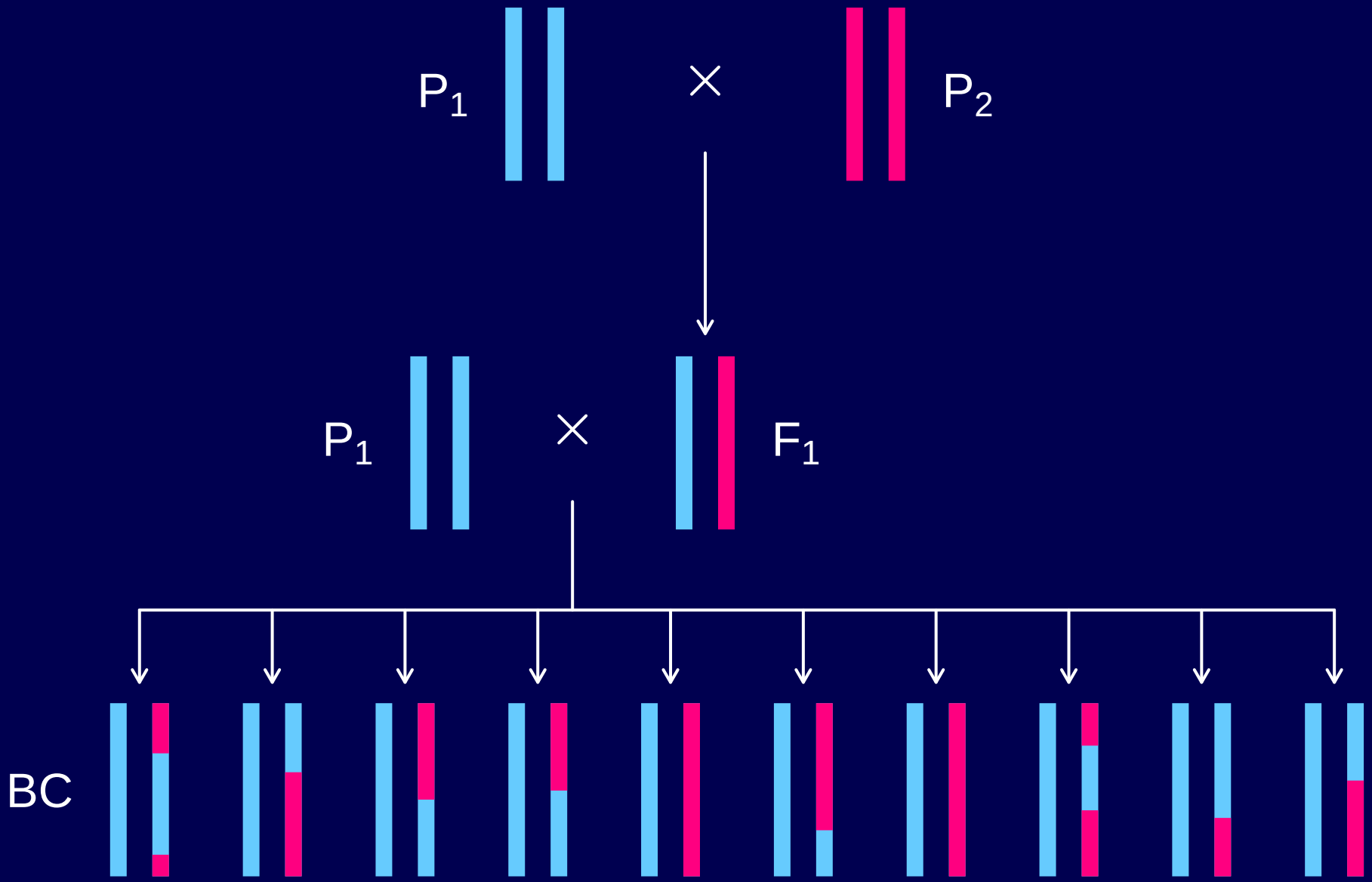
Human vs mouse



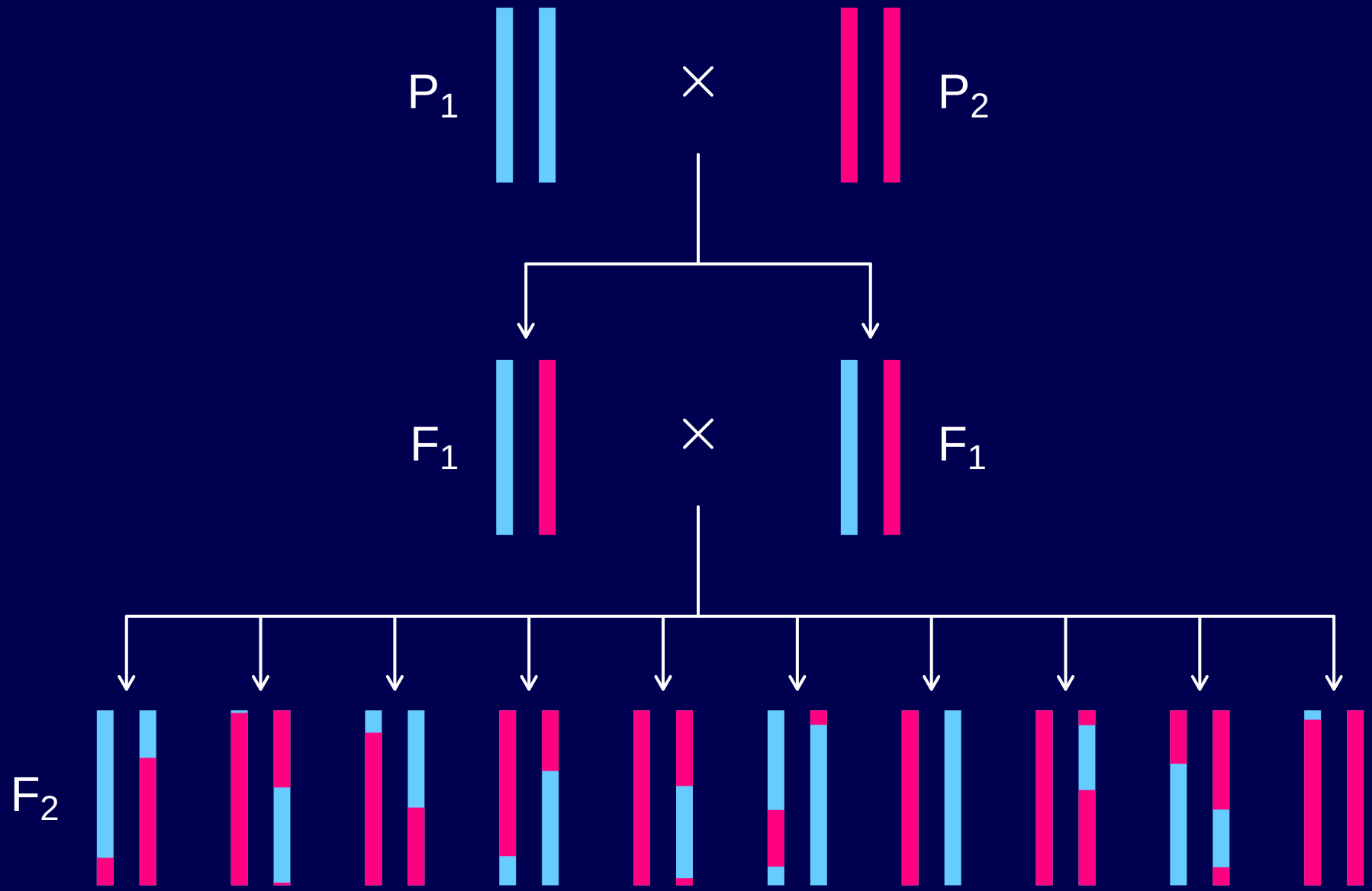


www.daviddeen.com

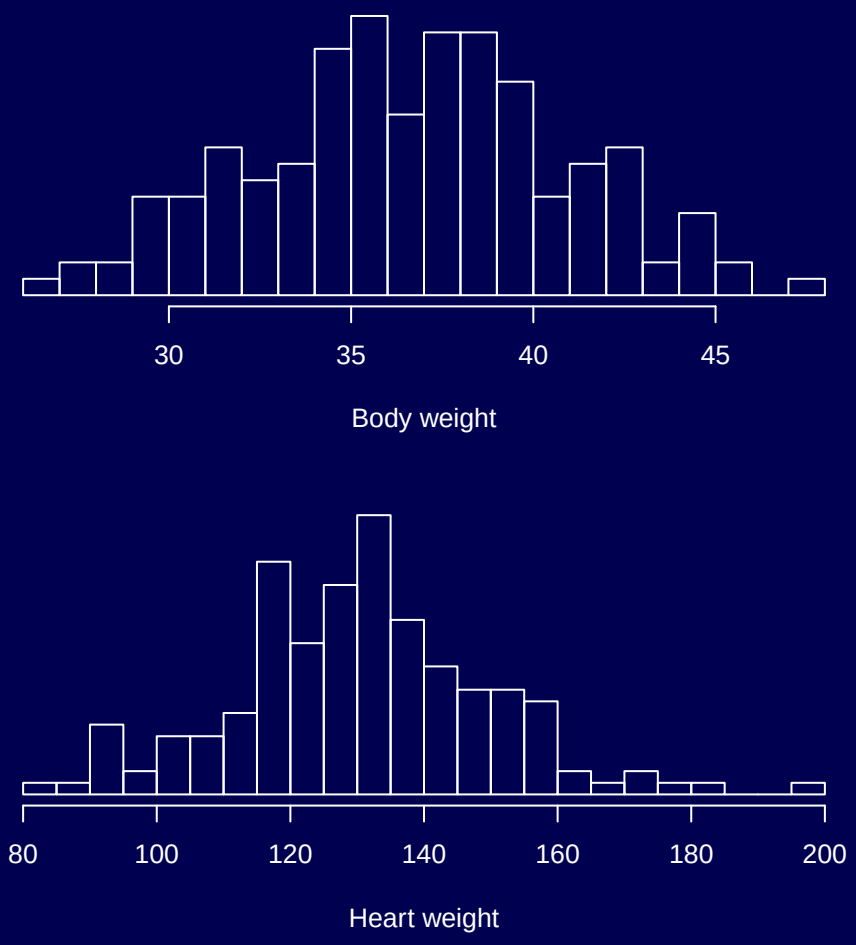
Backcross

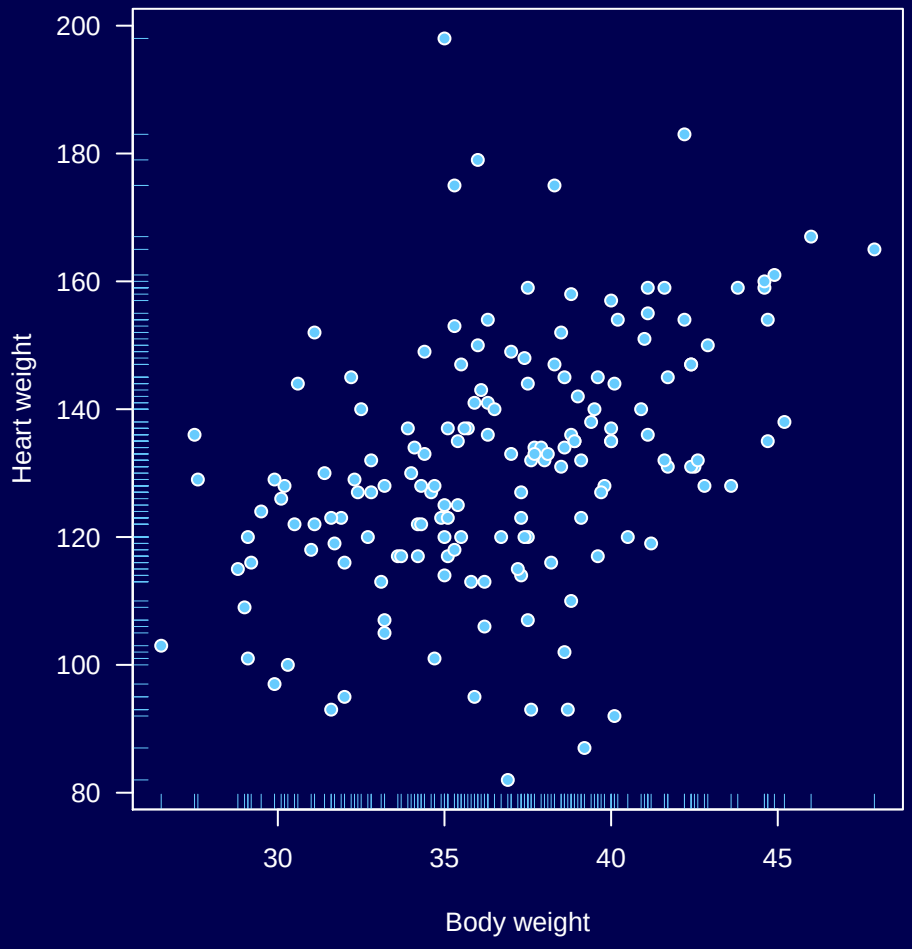


Intercross



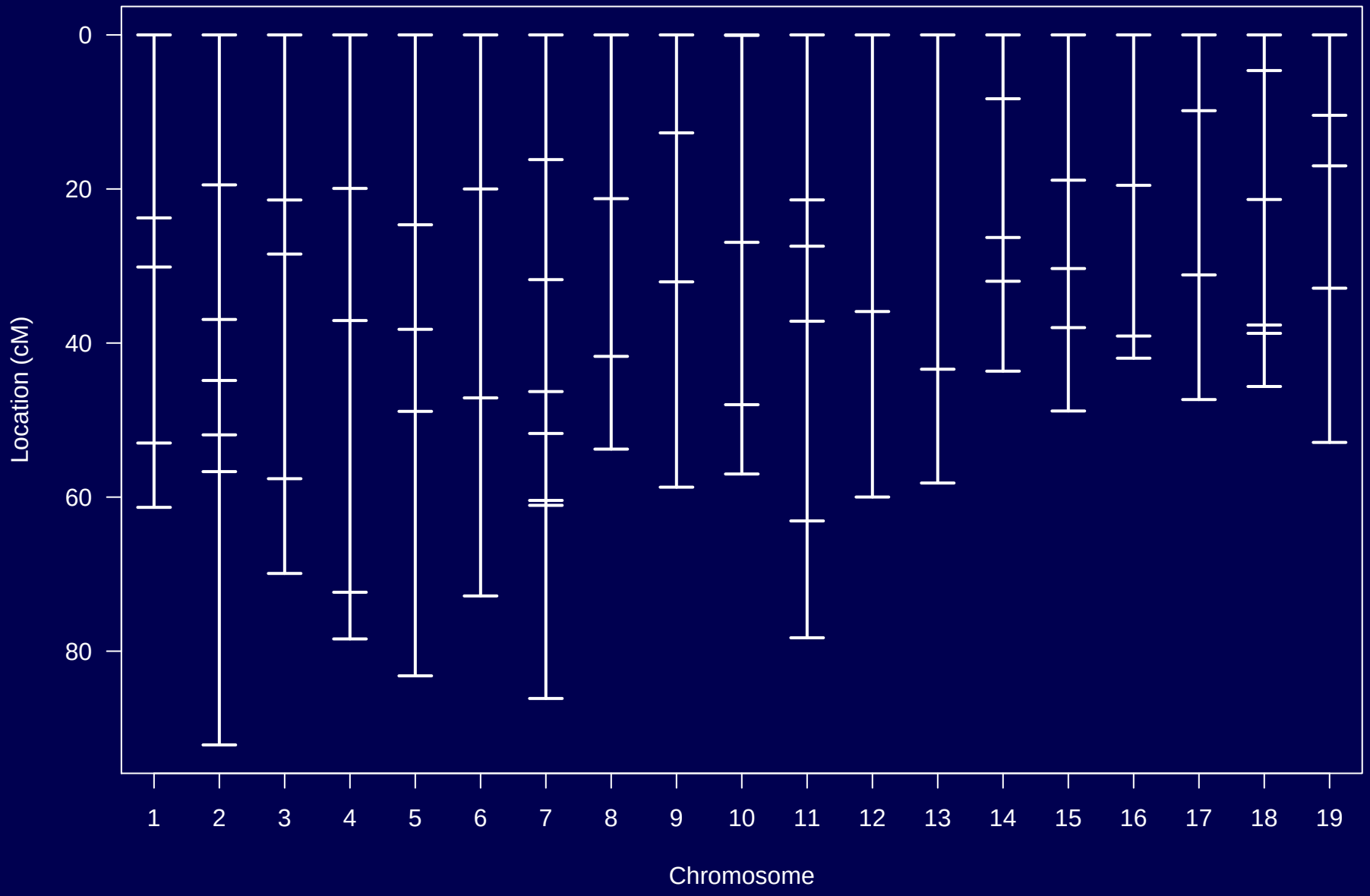
Phenotype data



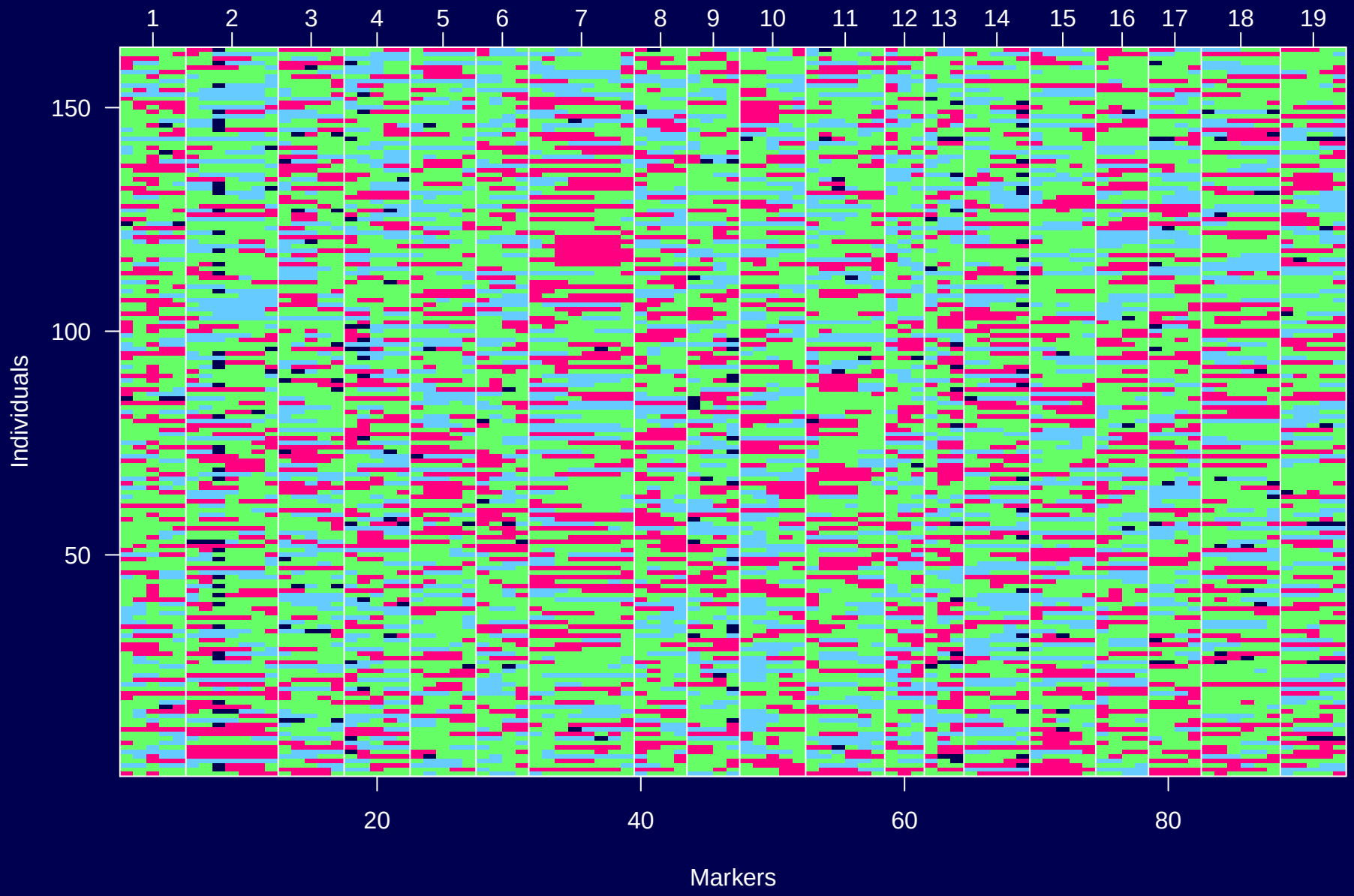


Sugiyama et al. (2002) Physiol Genomics 10:5–12

Genetic map


Location (cM)
0
20
40
60
80
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
Chromosome

Genotype data

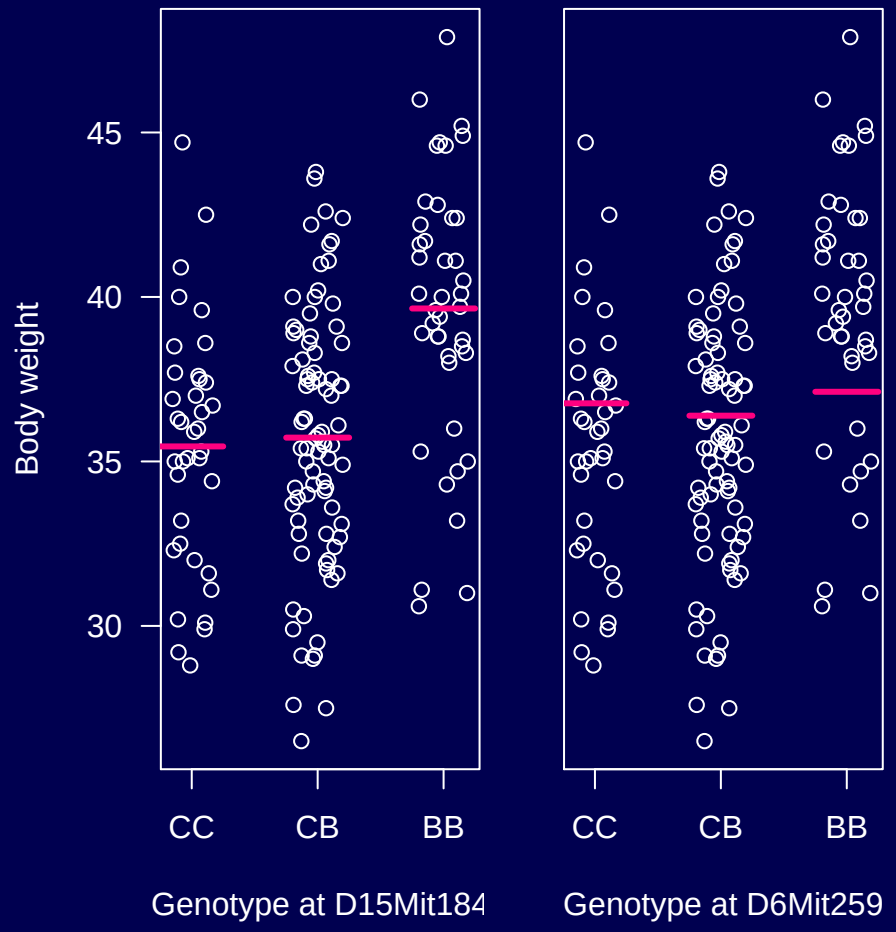


Goals

- Identify quantitative trait loci (QTL)
  (and interactions among QTL)
- Interval estimates of QTL location
- Estimated QTL effects

ANOVA at marker loci

- Also known as marker regression.
- Split mice into groups according to genotype at a marker.
- Do a t-test / ANOVA.
- Repeat for each marker.



ANOVA at marker loci

Advantages

- Simple.
- Easily incorporates covariates.
- Easily extended to more complex models.
- Doesn't require a genetic map.

Disadvantages

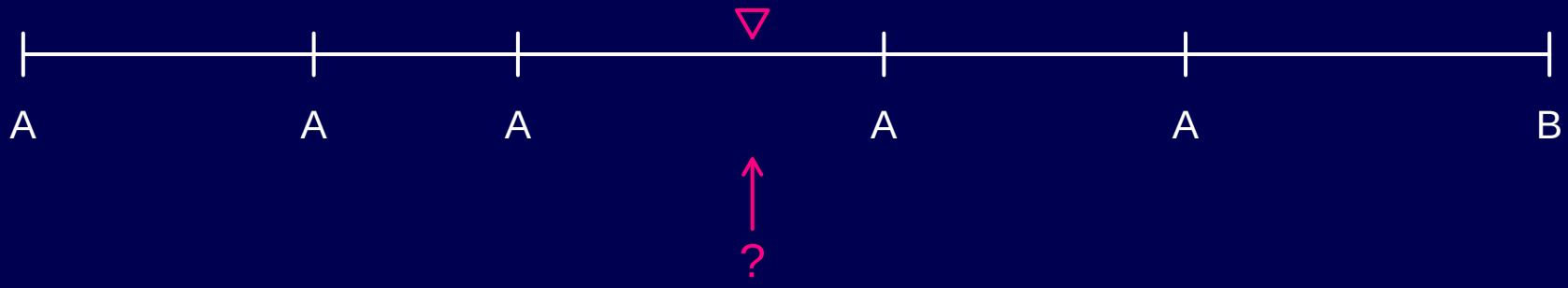
- Must exclude individuals with missing genotype data.
- Imperfect information about QTL location.
- Suffers in low density scans.
- Only considers one QTL at a time.

Interval mapping

Lander & Botstein (1989)

- Assume a single QTL model.
- Each position in the genome, one at a time, is posited as the putative QTL.
- Let $\mathsf{q} =$ the unobserved QTL genotype
  Assume $\mathsf{y}|\mathsf{q} \sim \mathsf{N}(\mu_{\mathsf{q}}, \sigma)$
- We don't know q, but we can calculate $\Pr(\mathsf{q} \mid \text{marker data})$
- Estimate μ_{q}, σ by *maximum likelihood* using an iterative EM algorithm

Genotype probabilities



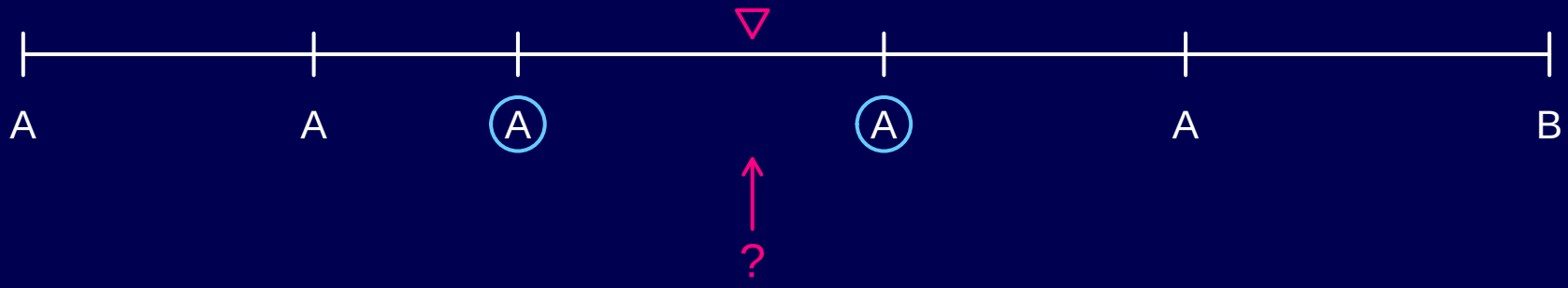
Calculate $\Pr(\mathrm{q} \mid \text{marker data})$, assuming

- No crossover interference
- No genotyping errors

Or use the hidden Markov model (HMM) technology

- To allow for genotyping errors
- To incorporate dominant markers
- (Still assume no crossover interference.)

Genotype probabilities



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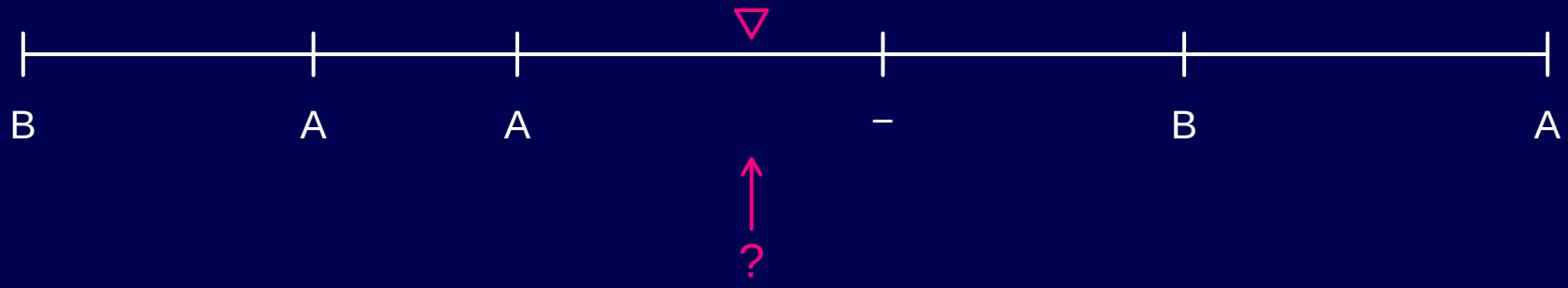

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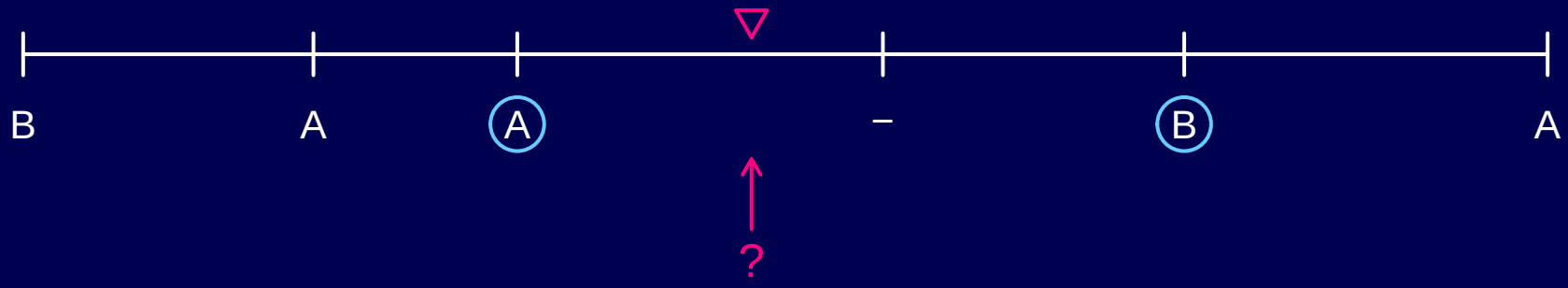

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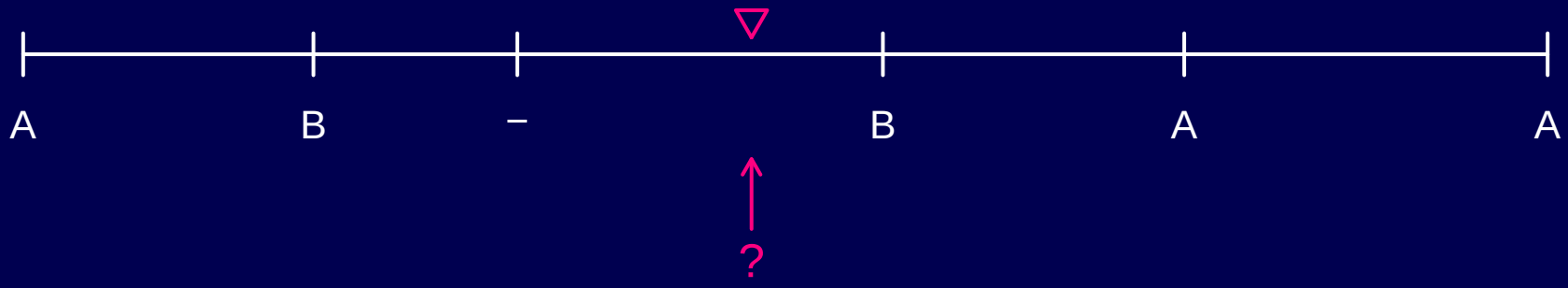

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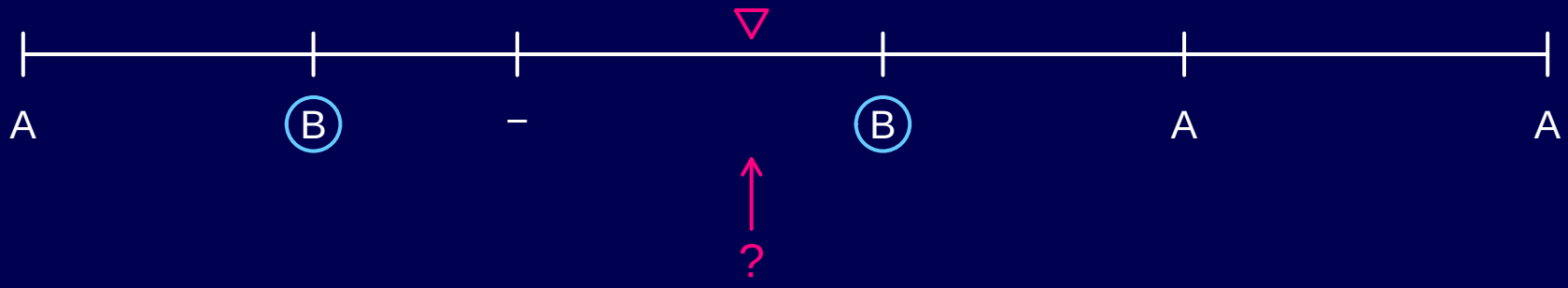
Genotype probabilities



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LOD scores

The LOD score is a measure of the strength of evidence for the presence of a QTL at a particular location.

LOD(λ) $= \log_{10}$ likelihood ratio comparing the hypothesis of a QTL at position λ versus that of no QTL

$$= \log_{10} \left\{ \frac{\Pr(\text{y}|\text{QTL at } \lambda, \hat{\mu}_{0\lambda}, \hat{\mu}_{1\lambda}, \hat{\sigma}_\lambda)}{\Pr(\text{y}|\text{no QTL}, \hat{\mu}, \hat{\sigma})} \right\}$$

$\hat{\mu}_{0\lambda}, \hat{\mu}_{1\lambda}, \hat{\sigma}_\lambda$ are the MLEs, assuming a single QTL at position λ.

No QTL model: The phenotypes are independent and identically distributed (iid) N(μ, σ^2).

→ R

- `read.cross()`
- `summary()`, `plot()`
- `nind()`, `nmar()`, `totmar()`, `nchr()`, `nphe()`
- `calc.genoprob()`
- `scanone()`
- `iplotScanone()` from R/qtlcharts

Interval mapping

Advantages

- Takes proper account of missing data.
- Allows examination of positions between markers.
- Gives improved estimates of QTL effects.
- Provides pretty graphs.

Disadvantages

- Increased computation time.
- Requires specialized software.
- Difficult to generalize.
- Only considers one QTL at a time.

LOD thresholds

Large LOD scores indicate evidence for the presence of a QTL

Question: How large is large?

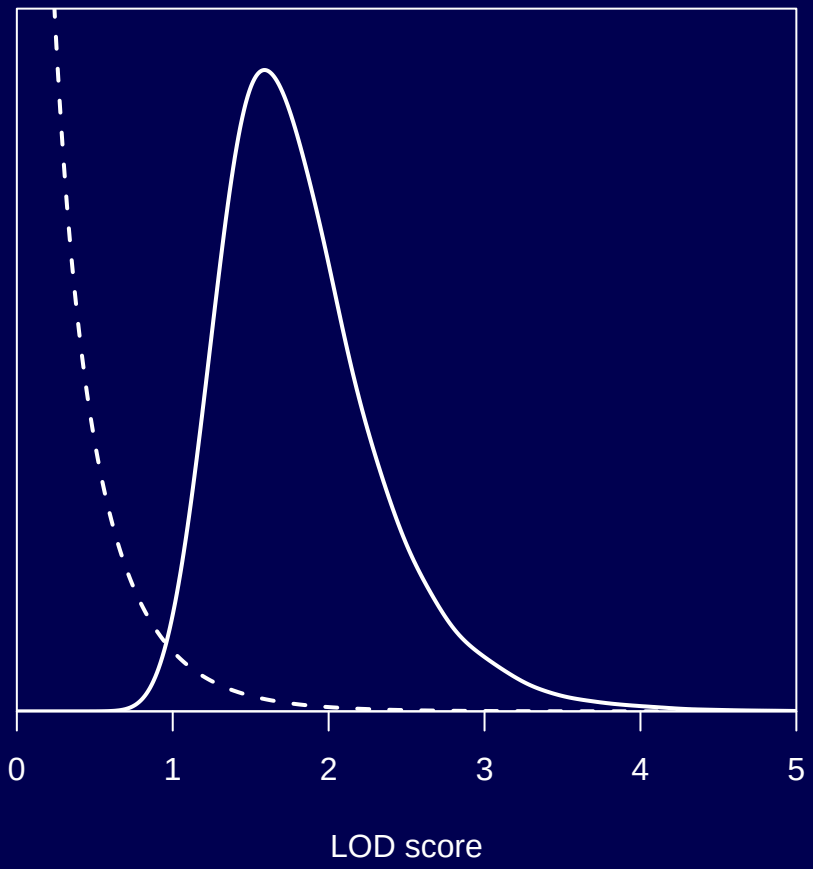
LOD threshold = 95 %ile of distr'n of max LOD, genome-wide, if there are no QTLs anywhere

Derivation:

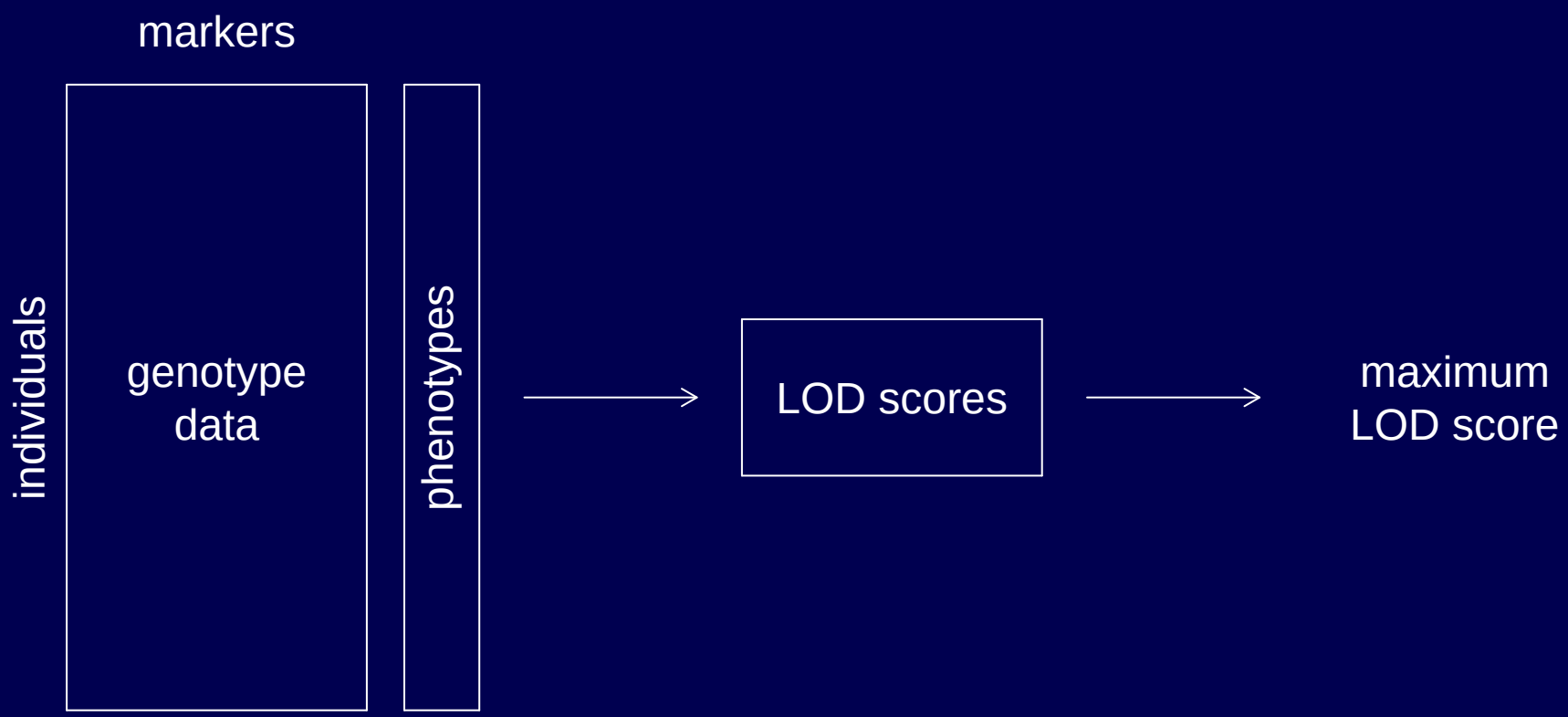
- Analytical calculations (L & B 1989)
- Simulations (L & B 1989)
- Permutation tests (Churchill & Doerge 1994)

Null distribution of the LOD score

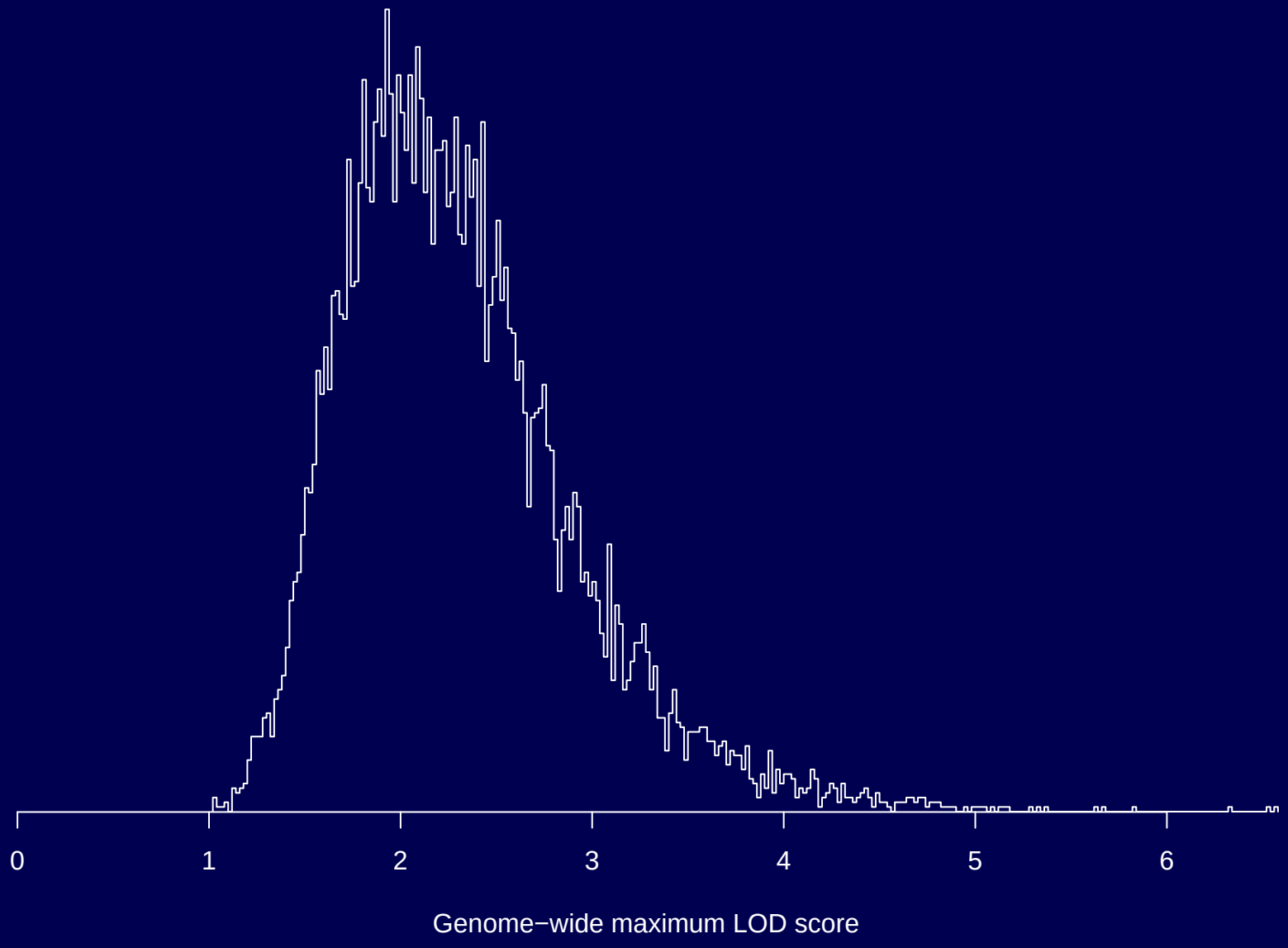
- Null distribution derived by computer simulation of backcross with genome of typical size.
- Dashed curve: distribution of LOD score at any one point.
- Solid curve: distribution of maximum LOD score, genome-wide.



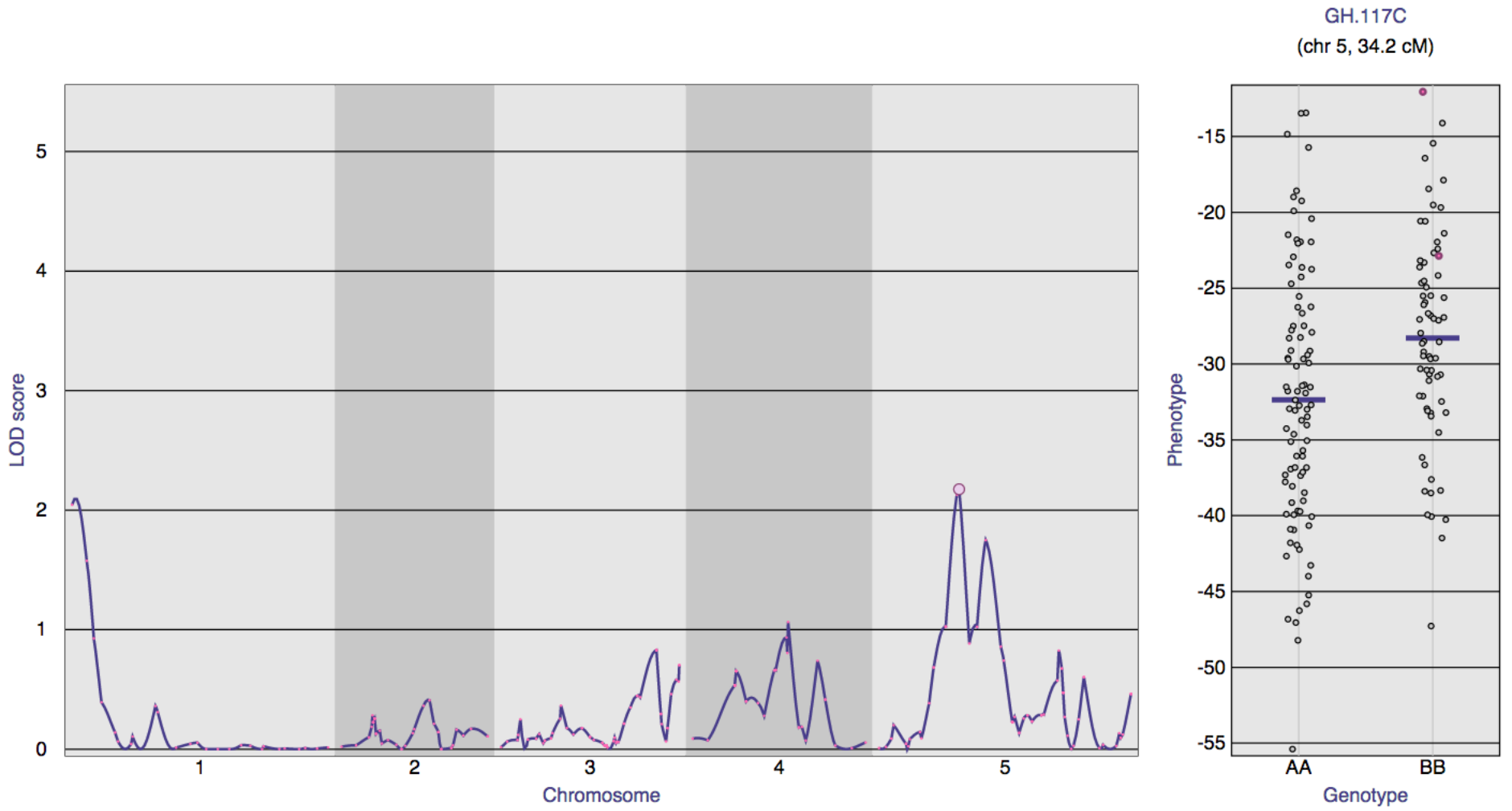
Permutation test



Permutation results



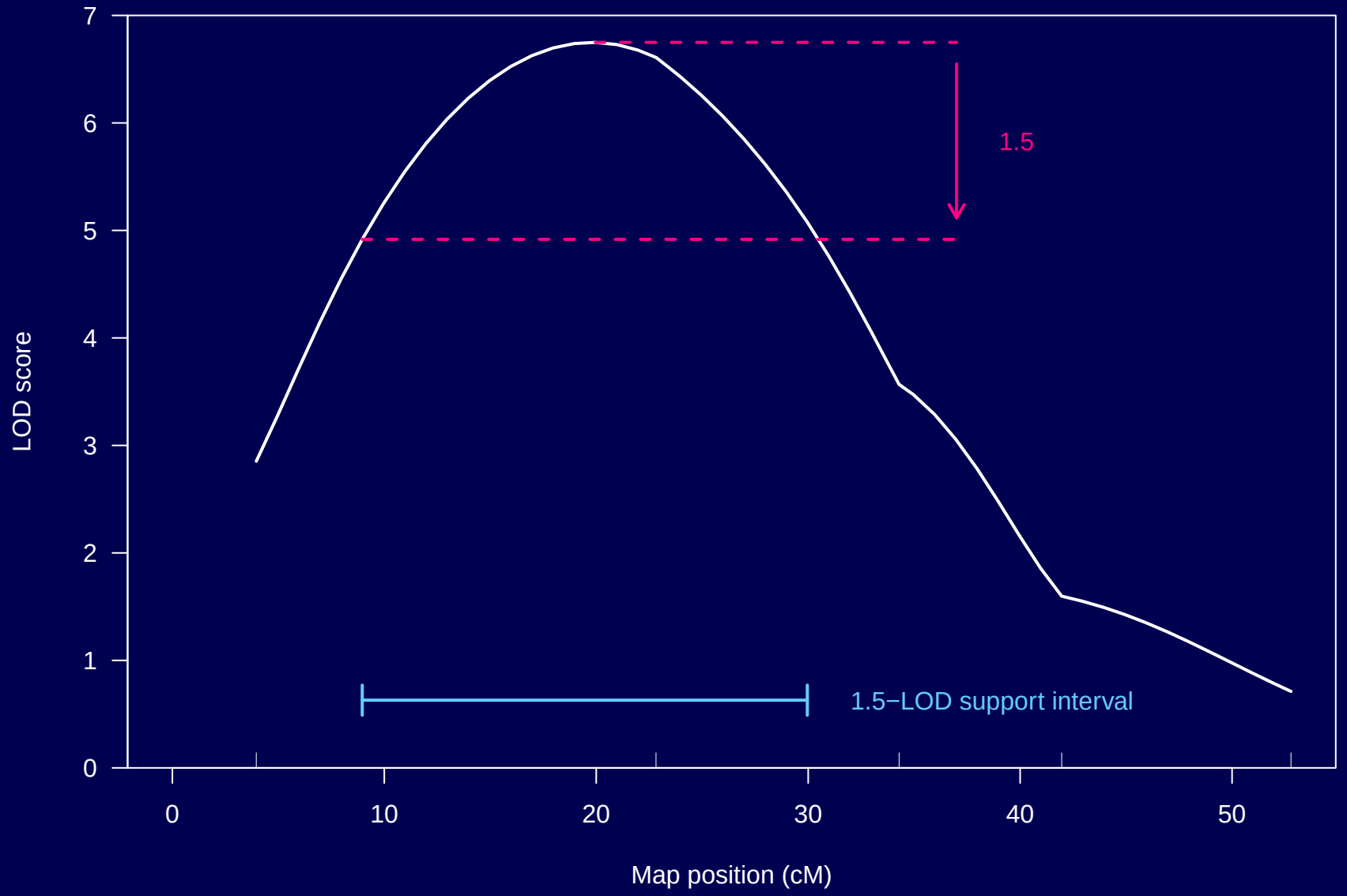
Interactive plot



→ R

- `scanone()` for permutations

LOD support intervals



→ R

- `lodint()`
- `bayesint()`

Haley-Knott regression

A quick approximation to Interval Mapping.

$$
\begin{aligned}
E(y_i|q_i) &= \mu_q \\
E(y_i|M_i) &= E[\, E(y_i|q_i) \,|M_i] = \textstyle\sum_j \Pr(q = j|M_i)\mu_j \\
&= \textstyle\sum_j p_{ij}\mu_j
\end{aligned}
$$

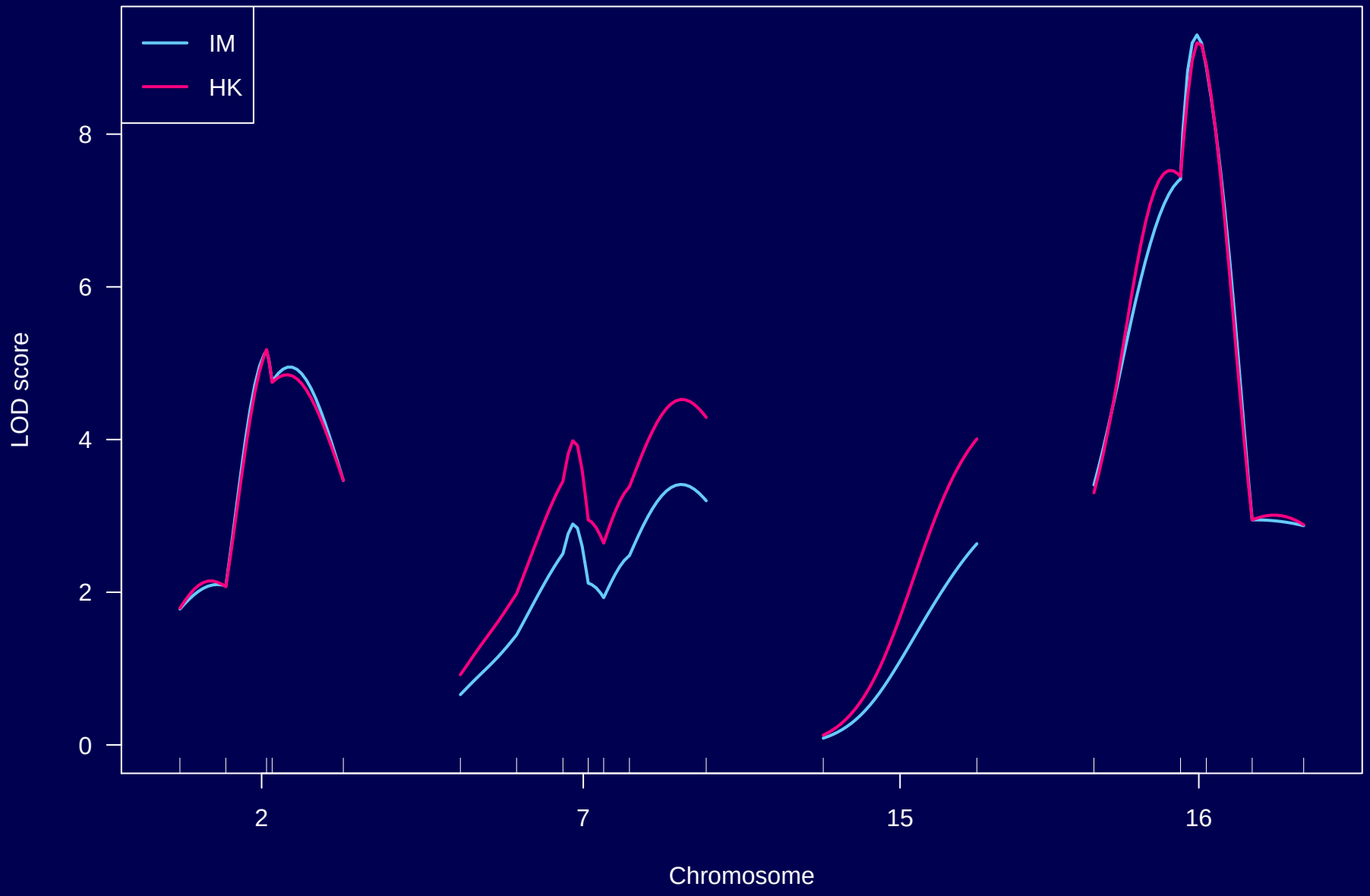
Regress y on p_i, pretending the residual variation is normally distributed (with constant variance).

$$
\text{LOD} = \frac{n}{2}\log_{10}\left(\frac{\text{RSS}_0}{\text{RSS}_1}\right)
$$

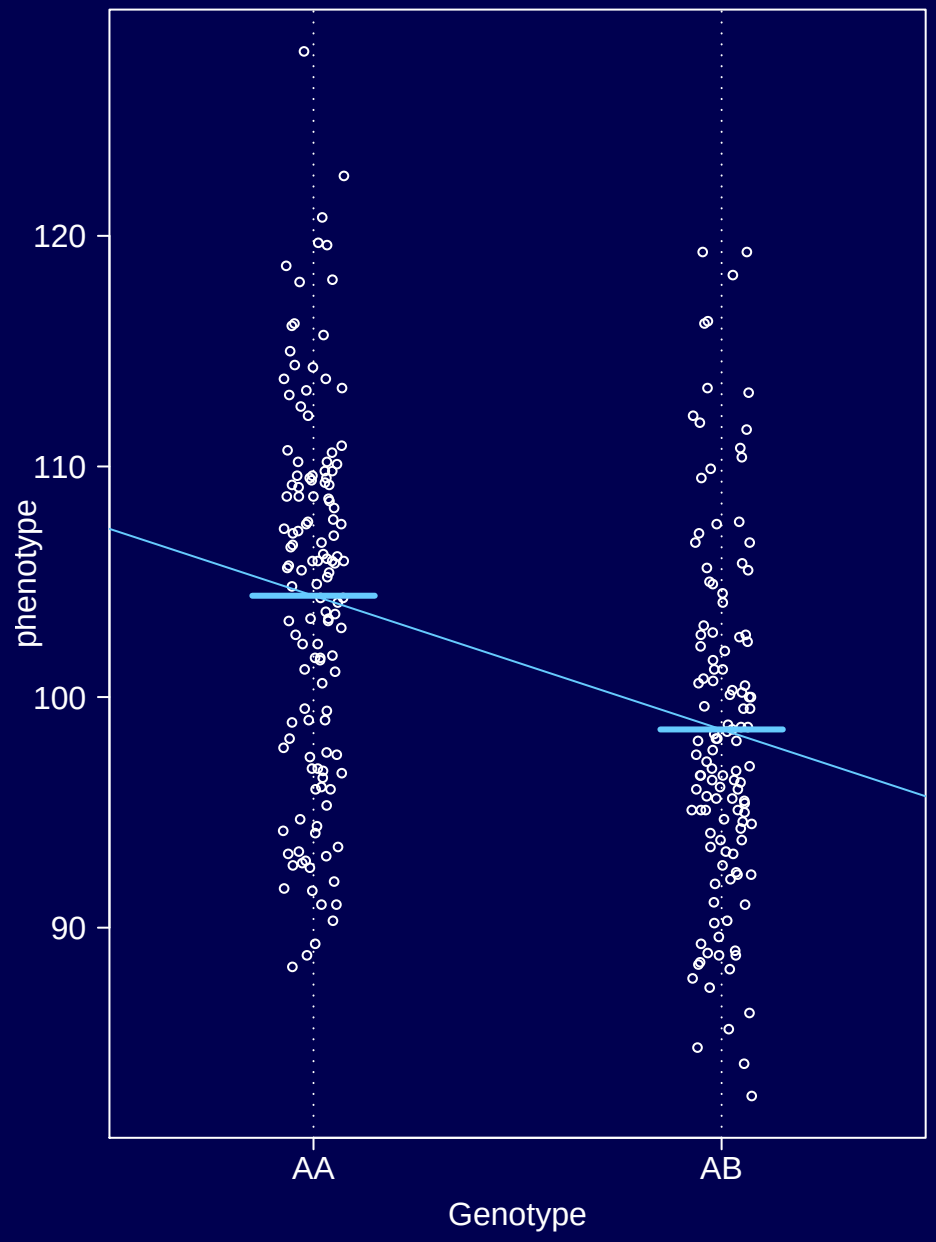
→ R

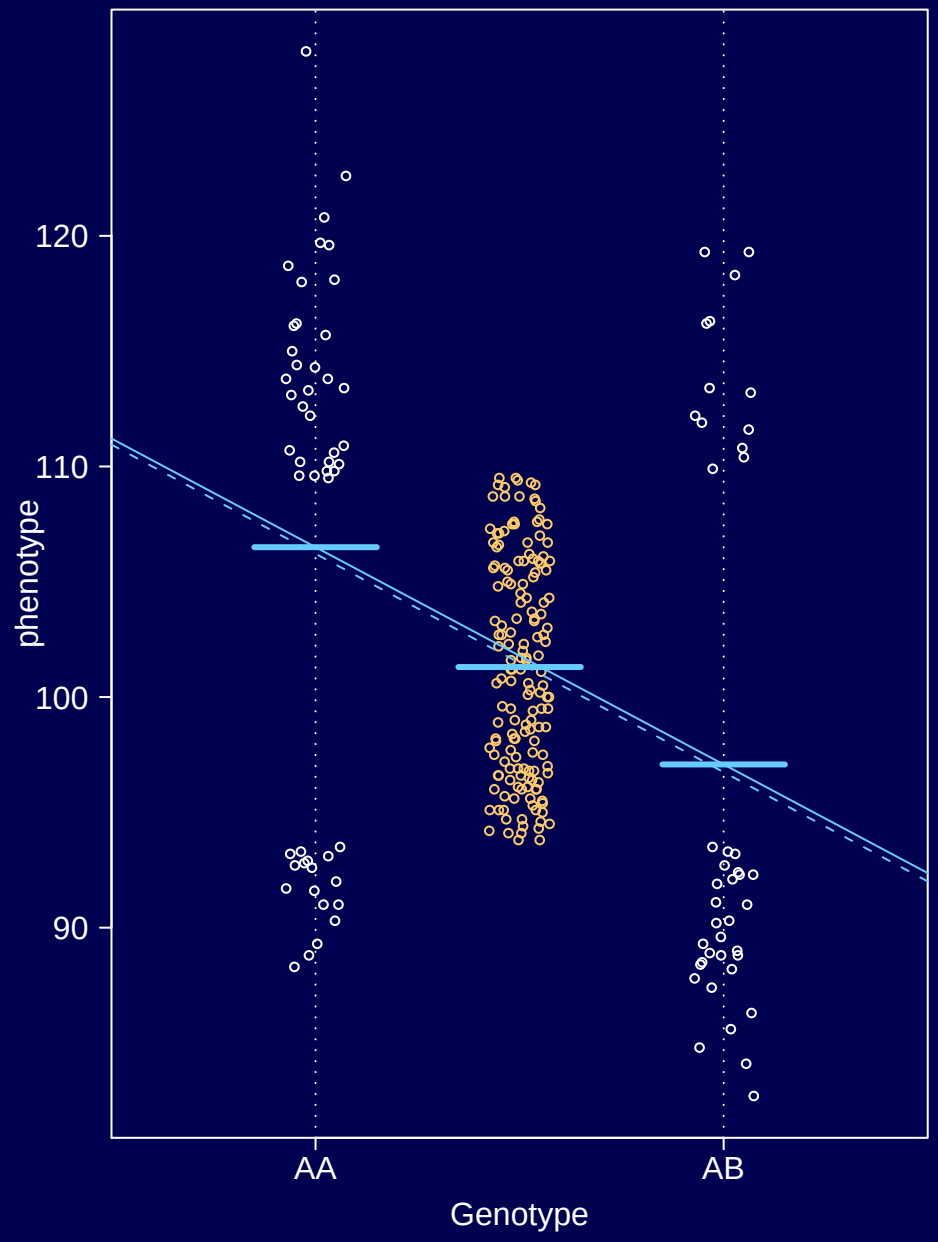
- `scanone()` with `method="hk"`

Haley-Knott results

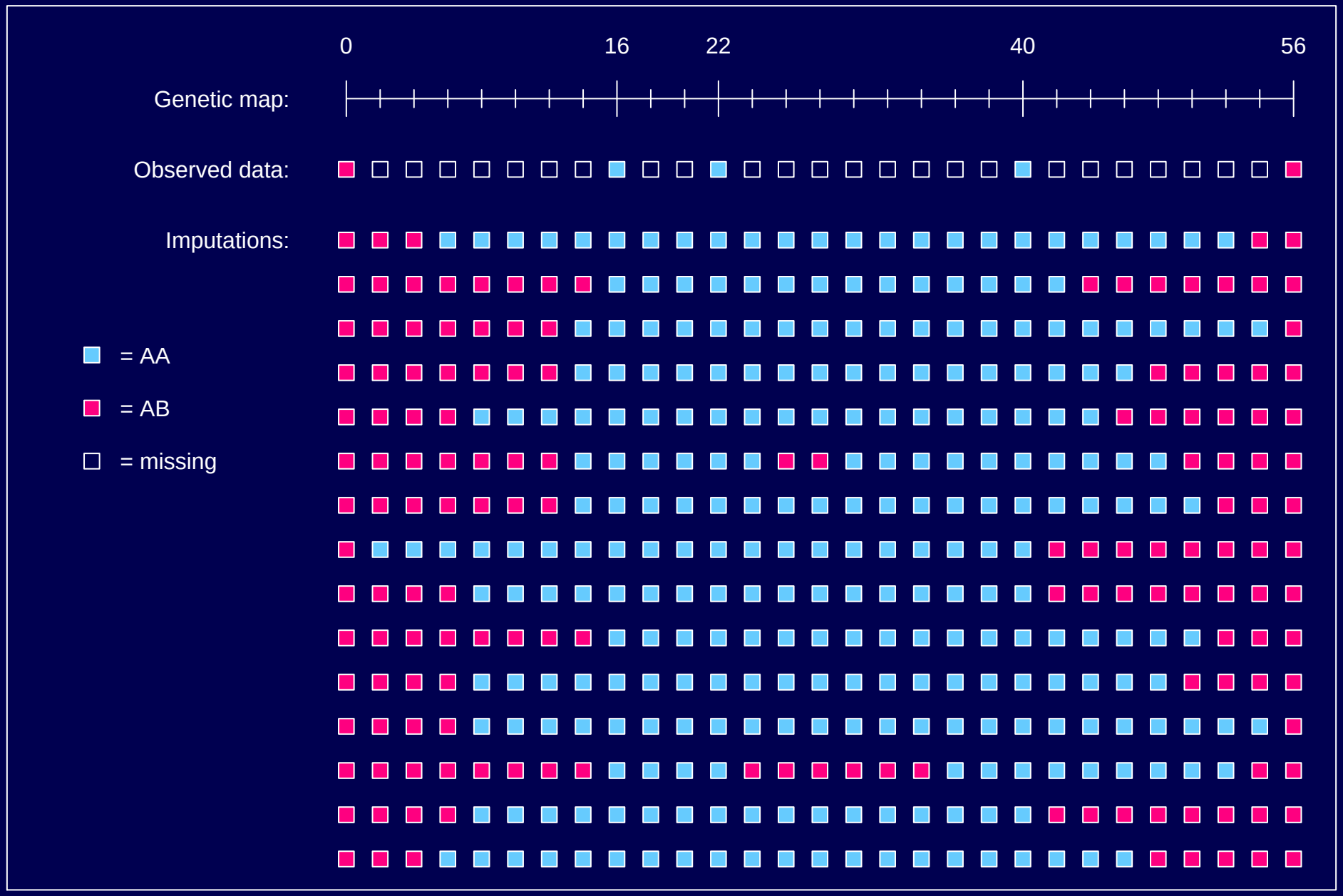


H-K with selective genotyping

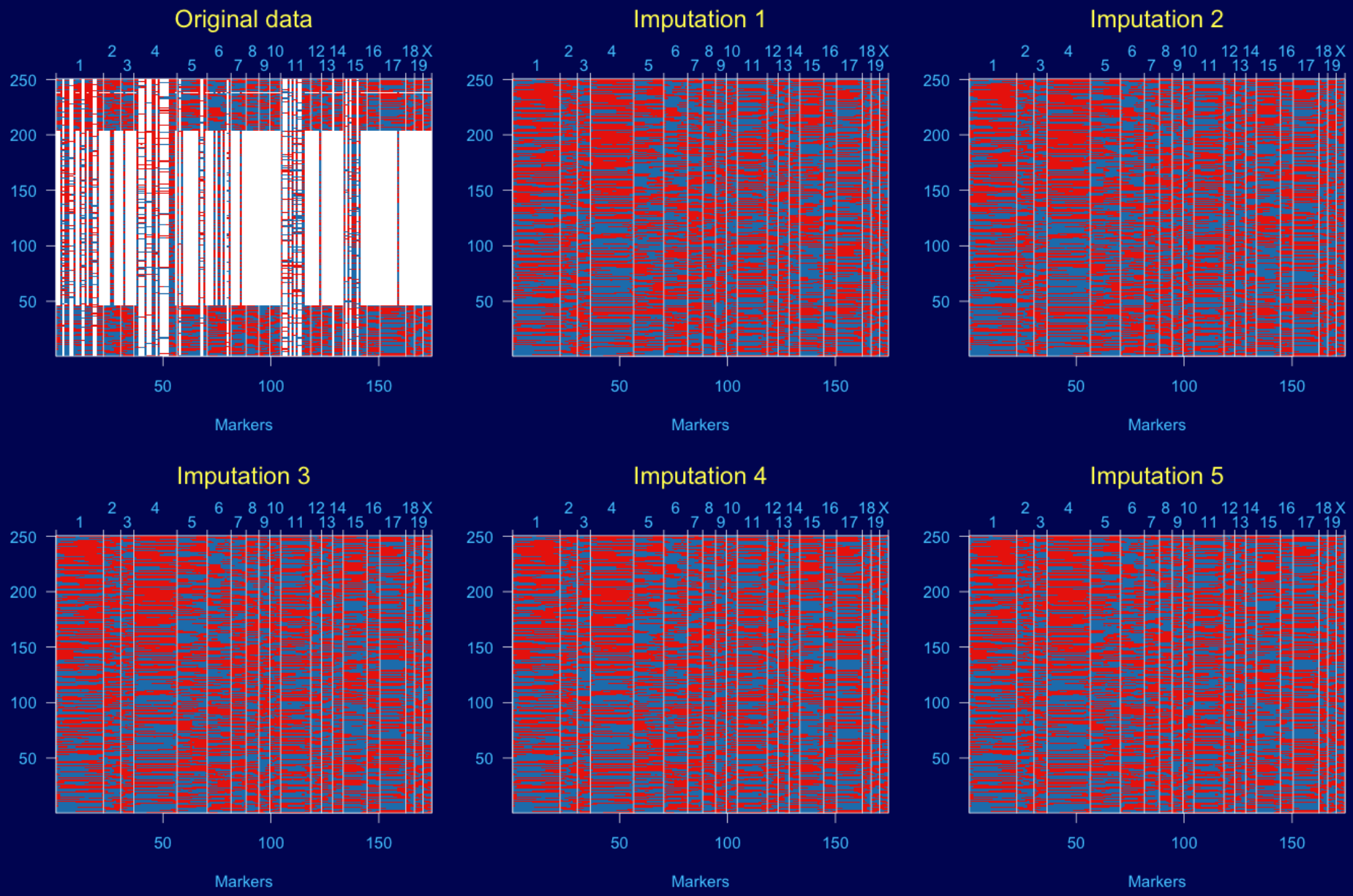




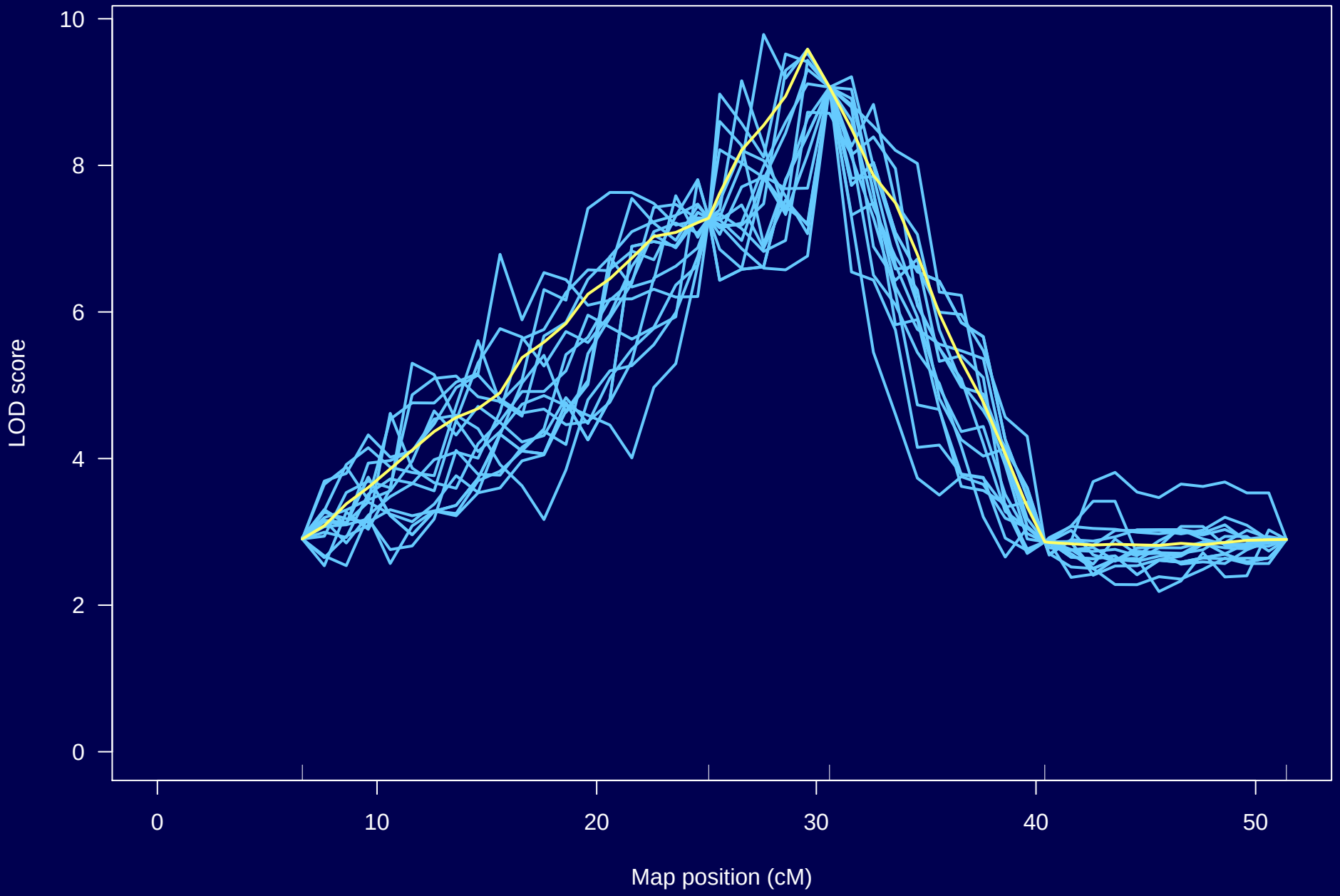
Multiple imputation



Multiple imputations



Imputation LOD curves



→ R

- `sim.geno()`
- `scanone()` with `method="imp"`

Summary comparison

Approach	Speed	Extensibility	Stability	Missing data	Parallelization
HK	++	+	+	−	++
EM	+	−	−	+	−
Imputation	−	+	+	+	+

Non-normal traits

- Standard interval mapping assumes normally distributed residual variation. (Thus the phenotype distribution is a mixture of normals.)
- In reality: we see dichotomous traits, counts, skewed distributions, outliers, and all sorts of odd things.
- Interval mapping, with LOD thresholds derived from permutation tests, generally performs just fine anyway.
- Alternatives to consider:
  - Nonparametric approaches (Kruglyak & Lander 1995)
  - Transformations (*e.g.*, log, square root, normal quantiles)
  - Specially-tailored models (*e.g.*, a generalized linear model, the Cox proportional hazard model, and the two-part model in Broman 2003)

→ R

- `nqrank()`
- `scanone()` with `model="binary"` or `model="np"`

Covariates

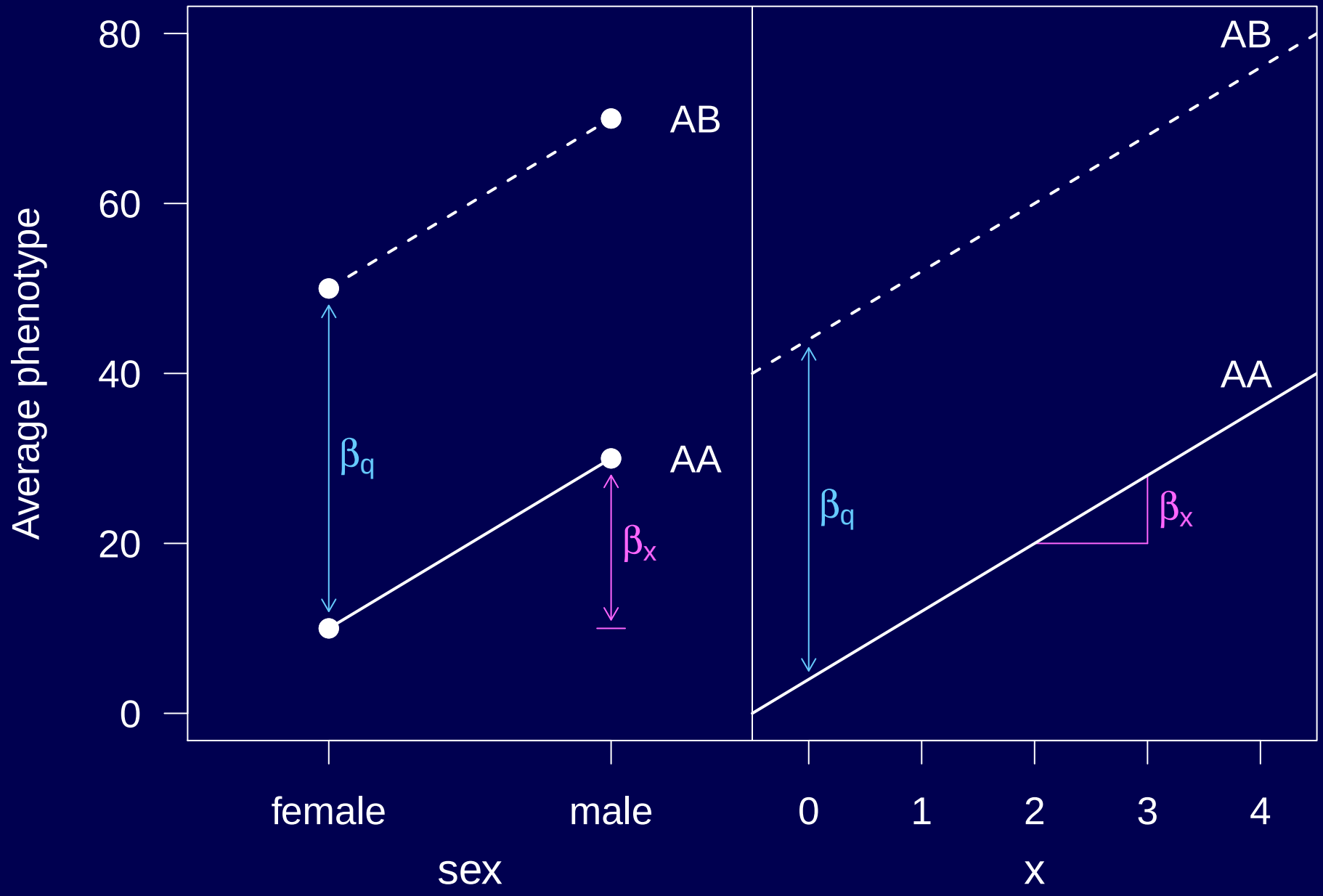
- Examples: treatment, sex, age, weight
- Control residual variation $\rightarrow$ increase power
- Look for QTL $\times$ covariate interactions

Additive covariate

$$\mathsf{H}_0 : y = \mu + \beta_x x + \epsilon$$
$$\mathsf{H}_a : y = \mu + \beta_x x + \beta_q q + \epsilon$$

- If covariate has strong effect on the phenotype, accounting for it can give improved power to detect QTL.
- In permutations, keep phenotype and covariate together
- Use care when the covariate is another phenotype

Additive covariate

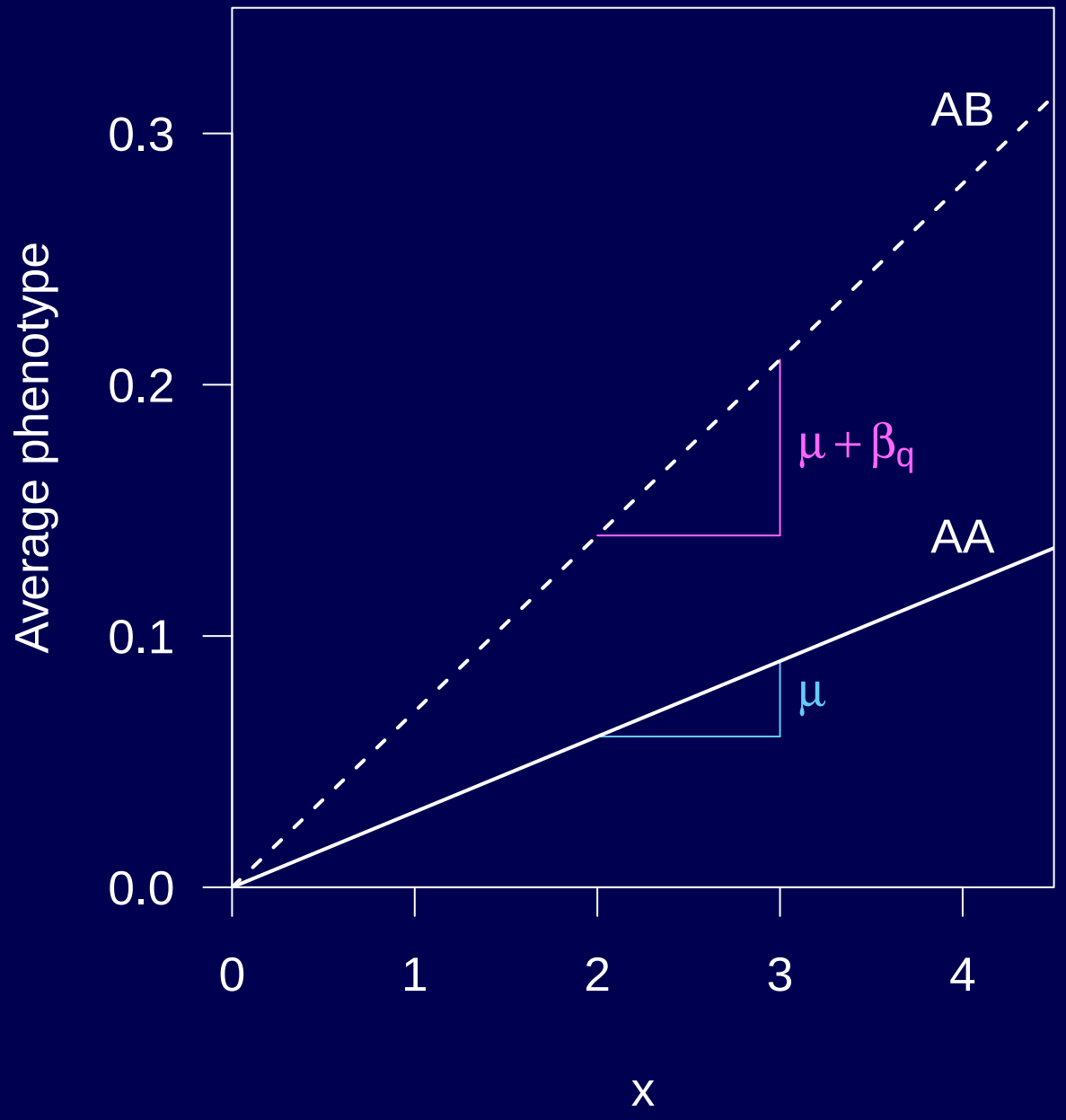


Adjust then scan?

- Consider adjusted phenotype $y' = y/x$
- The QTL model is $(y/x) = \mu + \beta_q q + \epsilon$
- Equivalently

$$y = \begin{cases} \mu\, x + \epsilon' & \text{if } q = 0 \\ (\mu + \beta_q)x + \epsilon' & \text{if } q = 1 \end{cases}$$

Adjust then scan?



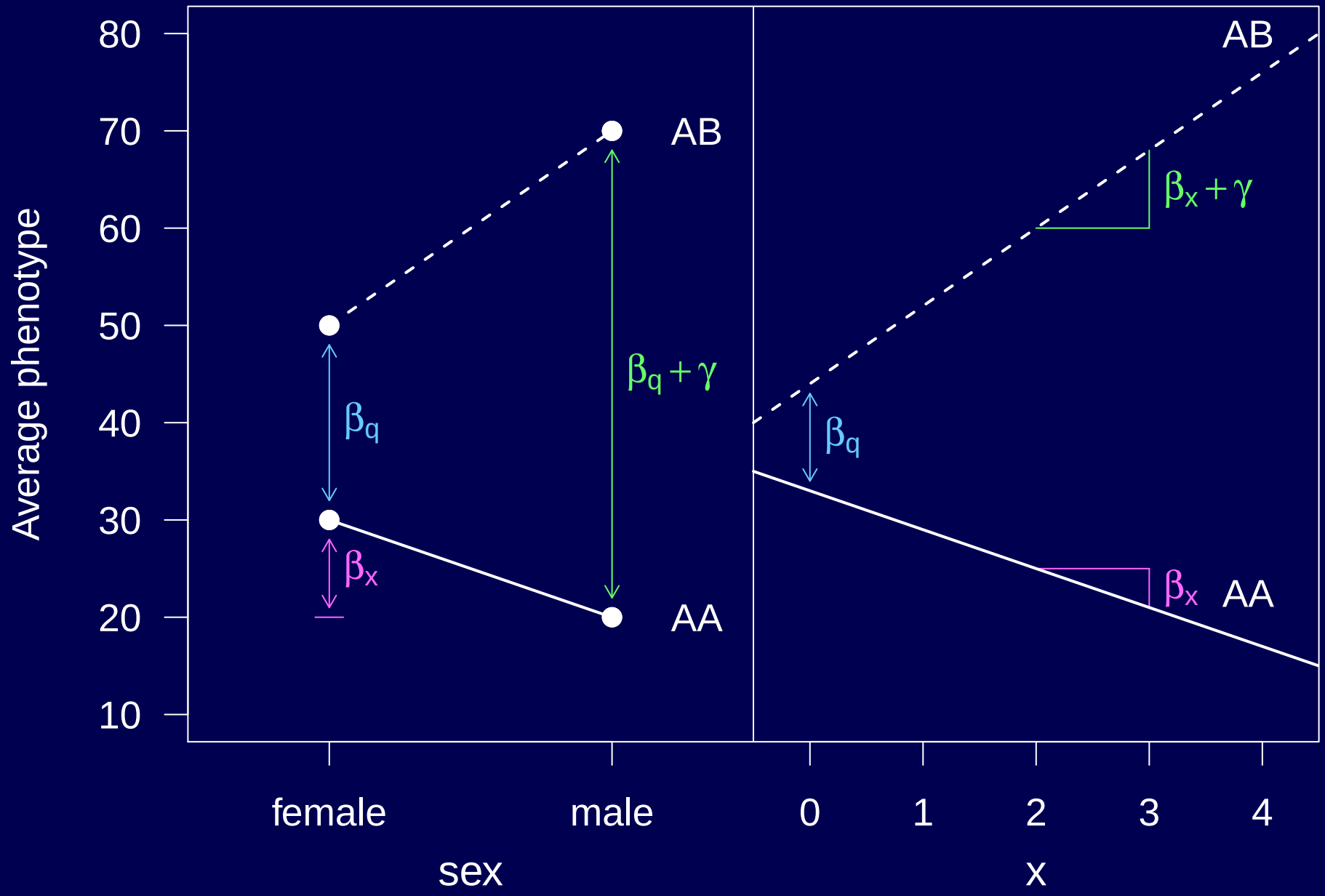
Interactive covariate

$$\begin{aligned} \mathsf{H}_0 : y &= \mu + \beta_x x + \epsilon \\ \mathsf{H}_a : y &= \mu + \beta_x x + \beta_q q + \epsilon \\ \mathsf{H}_i : y &= \mu + \beta_x x + \beta_q q + \gamma x q + \epsilon \end{aligned}$$

Can consider 3 LOD scores:

- LOD_a comparing H_a and H_0
- LOD_f comparing H_i and H_0
- LOD_i comparing H_i and H_a

Interactive covariate

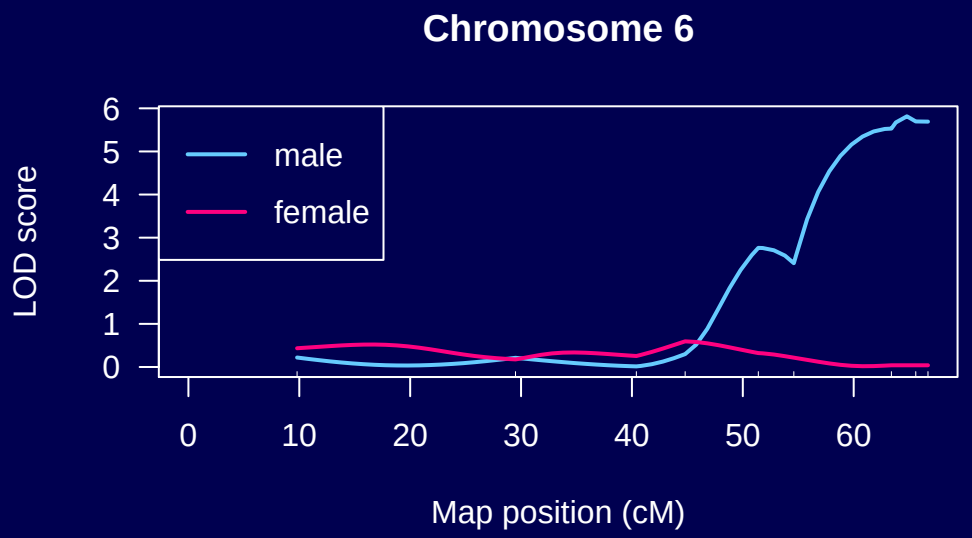


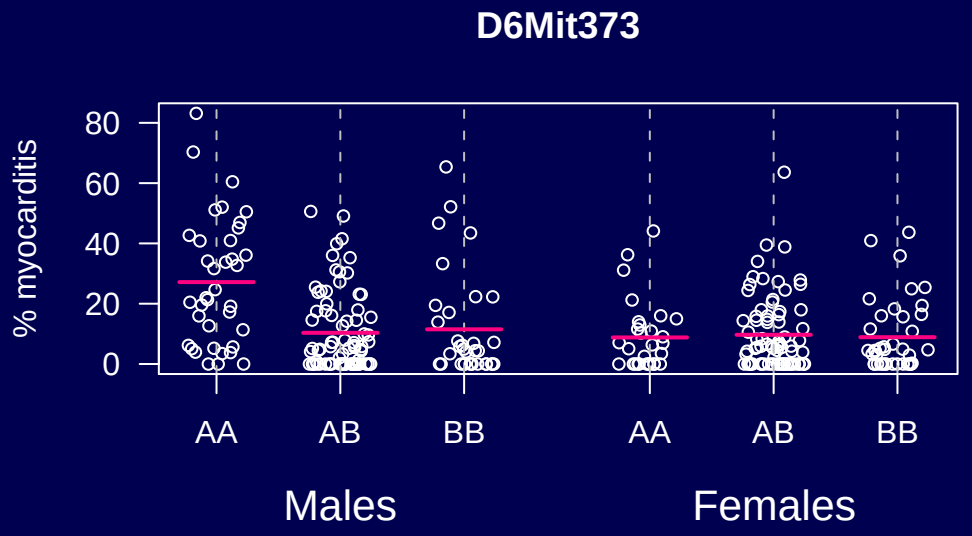
→ R

- `scanone()` with `addcovar` and `intcovar`
- `set.seed()` to do permutations

Split on sex?

- Informative, understandable
- But tempting to falsely conclude "sex-specific QTL"
- Absence of evidence is not *evidence of absence*.
- Use explicit test of QTL $\times$ sex interaction





→ R

- `subset()` to split on sex

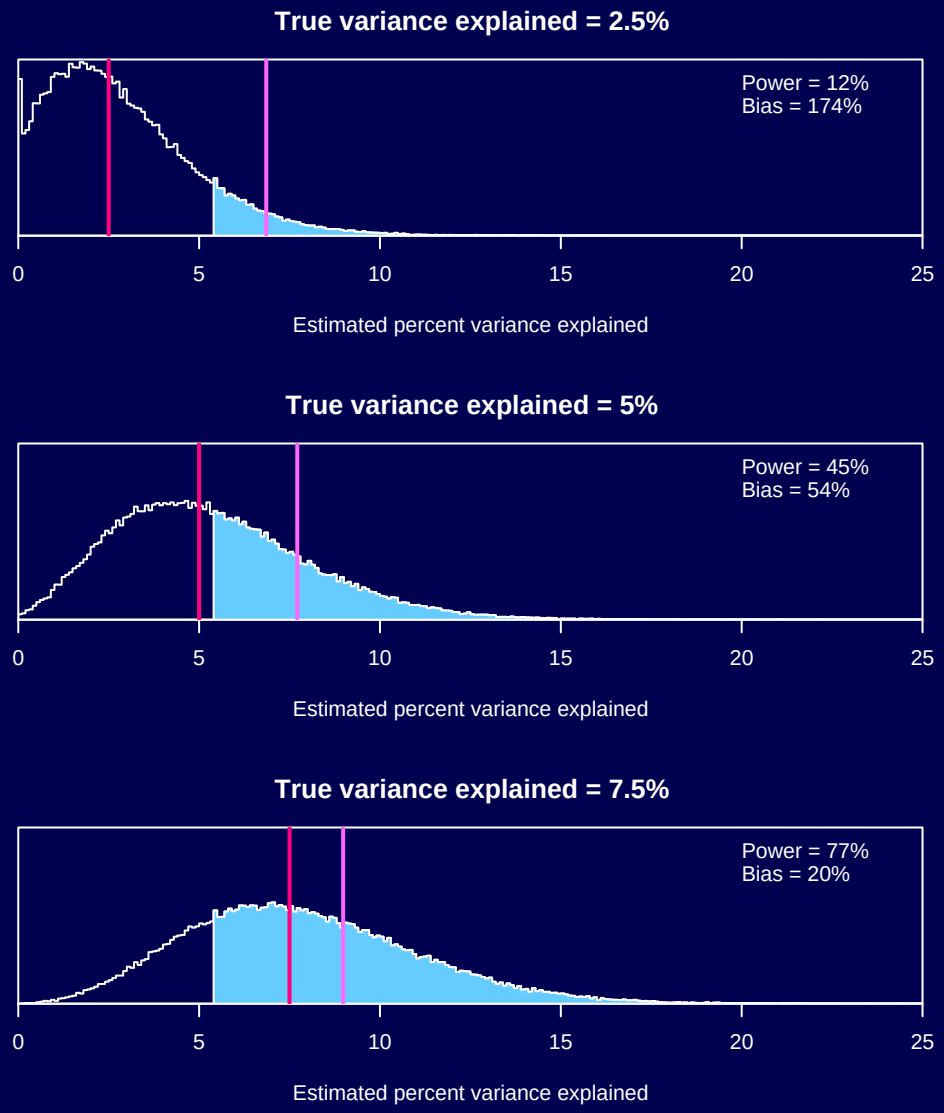
Data diagnostics

- Plot phenotypes
- Look for sample duplicates
- Look for excessive missing data
- Investigate segregation distortion
- Verify genetic maps/marker positions
- Look for genotyping errors
- Look at counts of crossovers

See Ch 3 in the R/qtl book, `rqtl.org/book`

Selection bias

- The estimated effect of a QTL will vary somewhat from its true effect.
- Only when the estimated effect is large will the QTL be detected.
- Among those experiments in which the QTL is detected, the estimated QTL effect will be, on average, larger than its true effect.
- This is selection bias.
- Selection bias is largest in QTLs with small or moderate effects.
- The true effects of QTLs that we identify are likely smaller than was observed.



Implications

- Estimated % variance explained by identified QTLs
- Repeating an experiment
- Congenics (aka near isogenic lines)
- Marker-assisted selection

References

- Broman KW (2001) Review of statistical methods for QTL mapping in experimental crosses. Lab Animal 30:44–52

  A review for non-statisticians.

- Lynch M, Walsh B (1998) *Genetics and analysis of quantitative traits*. Sinauer Associates, Sunderland, MA, chapter 15

  Chapter on QTL mapping.

- Lander ES, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185–199

  The seminal paper.

- Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics 138:963–971

  LOD thresholds by permutation tests.

References

- Beavis WD (1994). The power and deceit of QTL experiments: Lessons from comparative QTL studies. In DB Wilkinson, (ed) 49th Ann Corn Sorghum Res Conf, pp 252–268. Amer Seed Trade Asso, Washington, DC.

  Discusses selection bias in estimated QTL effects.

- Broman KW (2003) Mapping quantitative trait loci in the case of a spike in the phenotype distribution. Genetics 163:1169–1175

  Two-part model; also discusses binary traits and non-parametric QTL mapping.

- Haley CS, Knott SA (1992) A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. Heredity 69: 315–324

  Haley-Knott regression

- Sen S, Churchill GA (2001) A statistical framework for quantitative trait mapping. Genetics 159: 371–387

  Multiple imputation

- Solberg LC, et al. (2004) Sex- and line-specific lineage inheritance of depression-like behavior in the rat. Mamm Genome 15:648–662

  Additive and interactive covariates.

- Broman KW et al (2006) The X chromosome in quantitative trait locus mapping. Genetics 174:2151–2158

References

- Broman KW, Speed TP (2002) A model selection approach for the identification of quantitative trait loci in experimental crosses. J Roy Stat Soc B 64:641–656

  Multiple-QTL model selection with additive QTL.

- Manichaikul A, Moon JY, Sen Ś, Yandell BS, Broman KW (2009) A model selection approach for the identification of quantitative trait loci in experimental crosses, allowing epistasis. Genetics 181:1077–1086

  Also account for epistasis.