Introduction to QTL mapping in model organisms

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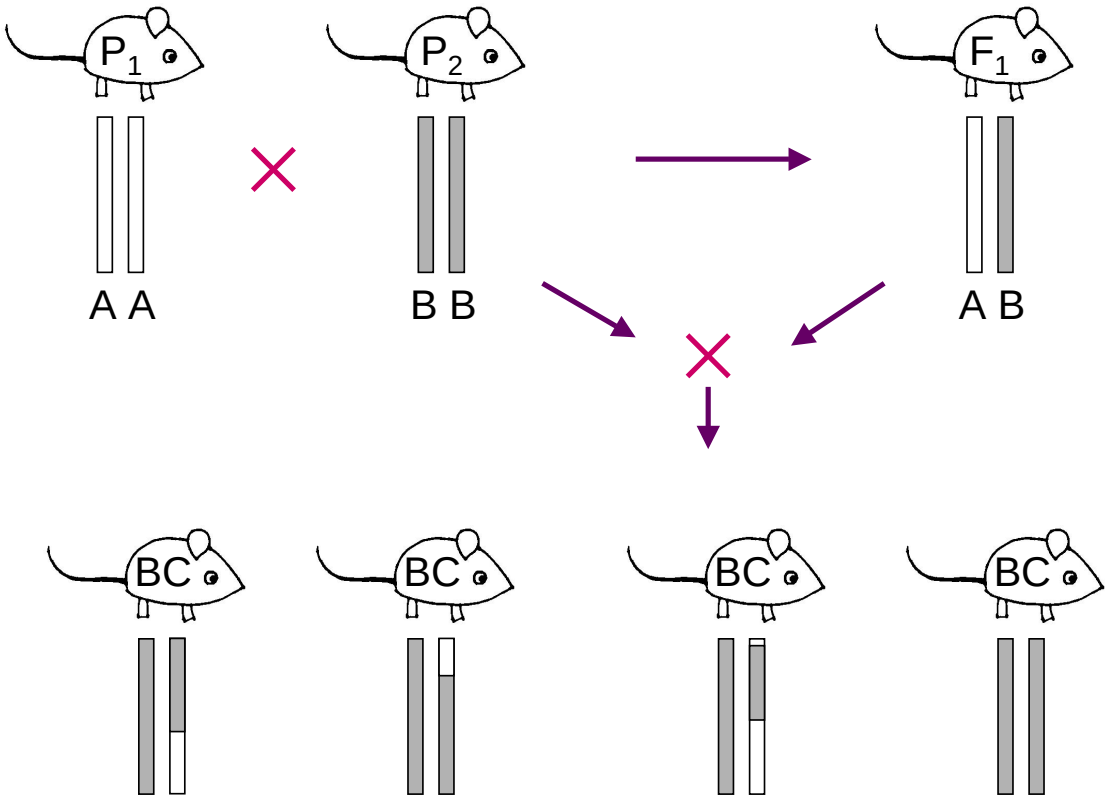
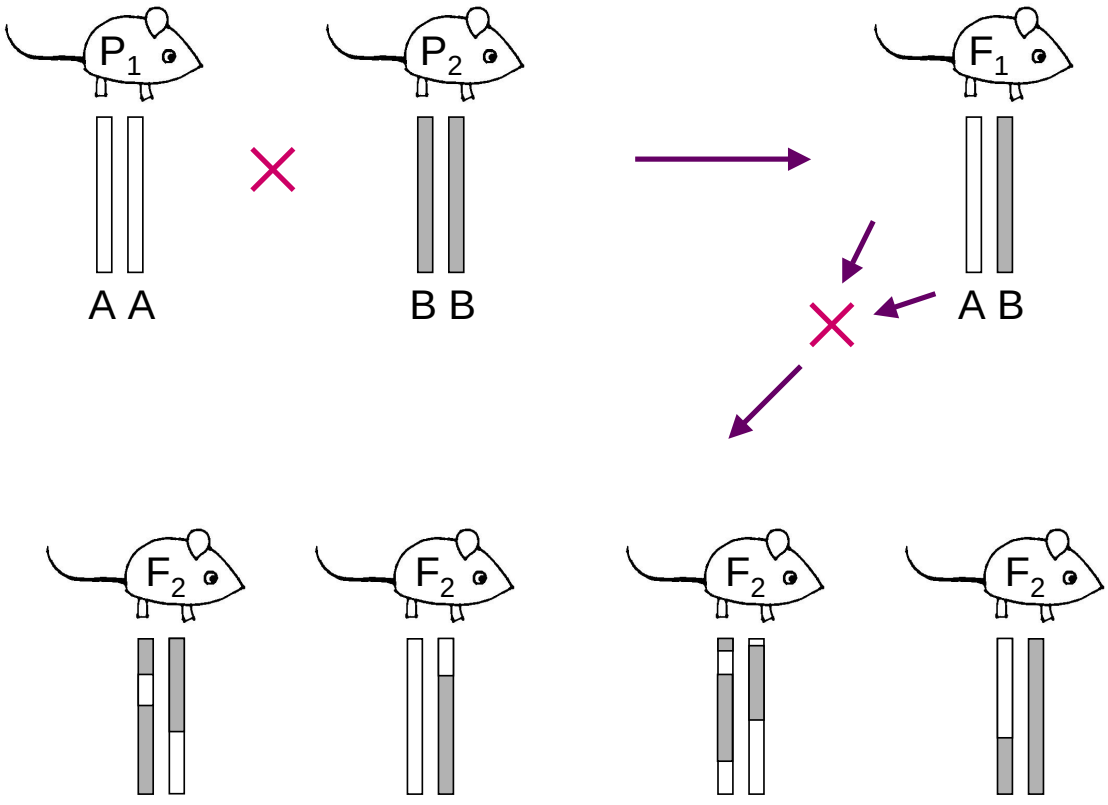
Outline

- Experiments and data
- Models
- ANOVA at marker loci
- Interval mapping
- Epistasis
- LOD thresholds
- CIs for QTL location
- Selection bias
- The X chromosome
- Selective genotyping
- Covariates
- Non-normal traits
- The need for good data

Backcross experiment

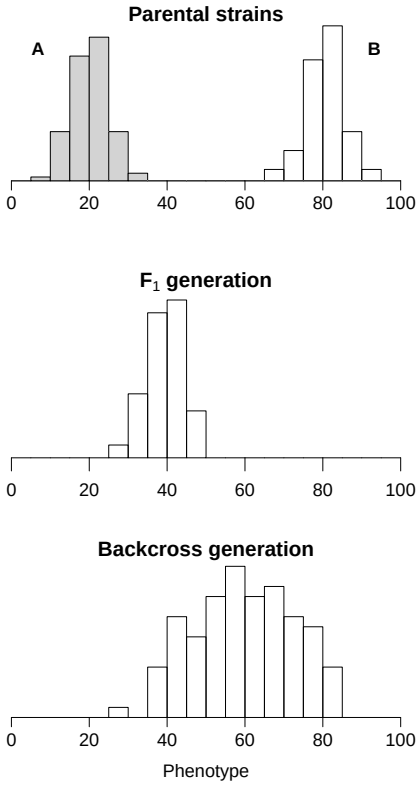


Intercross experiment



Phenotype distributions

- Within each of the parental and F_1 strains, individuals are genetically identical.
- Environmental variation may or may not be constant with genotype.
- The backcross generation exhibits genetic as well as environmental variation.

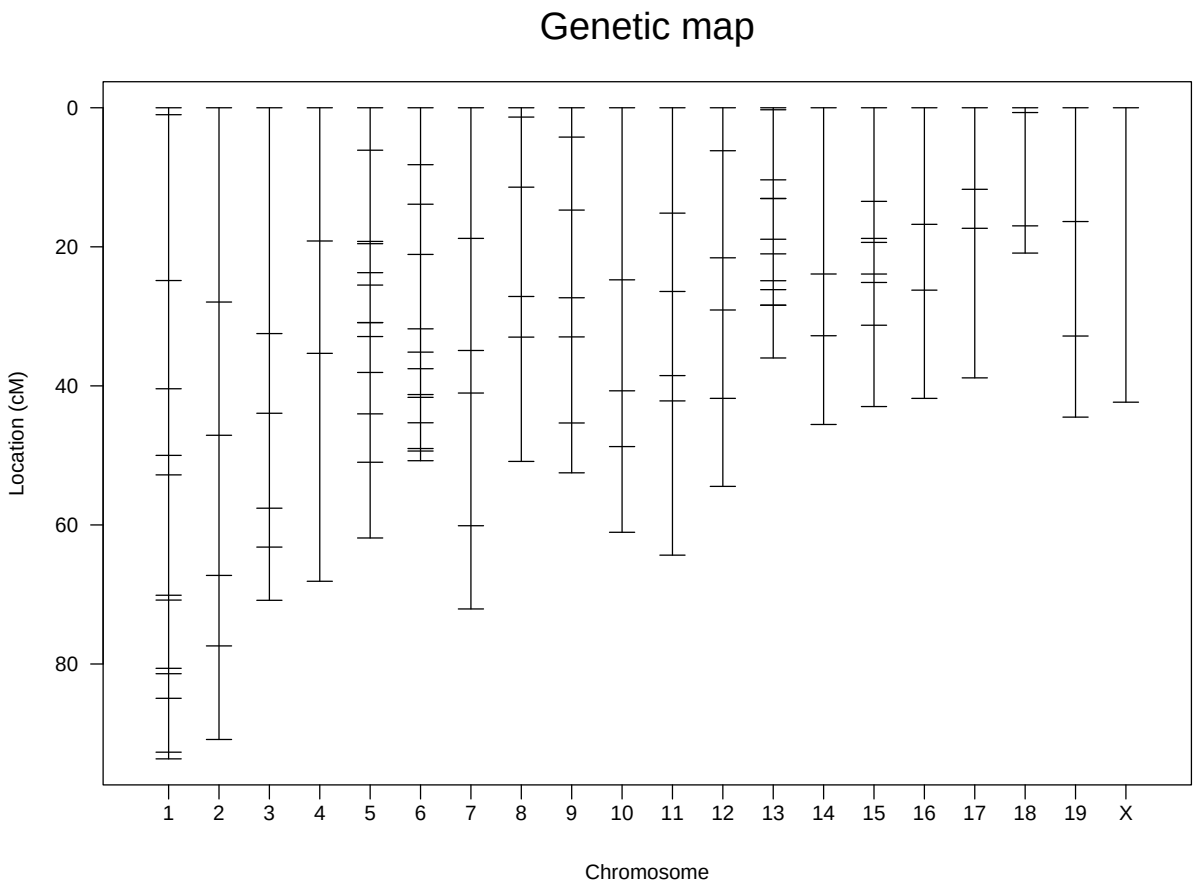


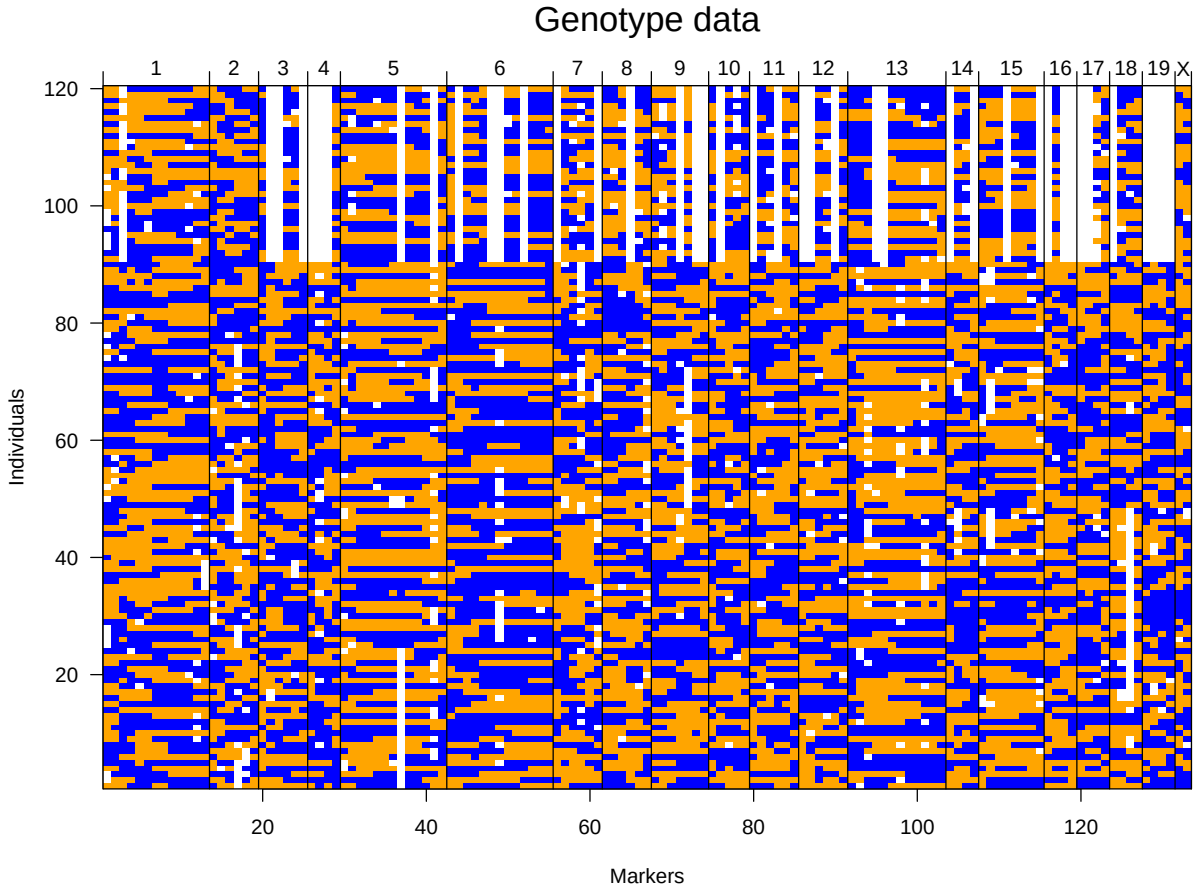
Data

Phenotypes: y_i = trait value for individual i

Genotypes: x_{ij} = 0/1 if mouse i is BB/AB at marker j (or 0/1/2, in an intercross)

Genetic map: Locations of markers

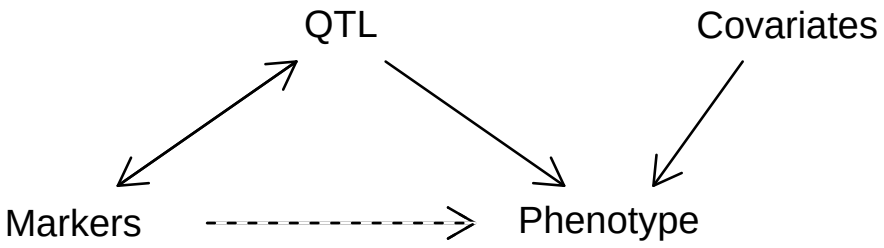

Genetic map
Location (cM)
0
20
40
60
80
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
X
Chromosome


Genotype data
Individuals
120
100
80
60
40
20
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
X
20
40
60
80
100
120
Markers

Goals

- Detect QTLs (and interactions between QTLs)
- Confidence intervals for QTL location
- Estimate QTL effects (effects of allelic substitution)

Statistical structure



The missing data problem:

$$\text{Markers} \longleftrightarrow \text{QTL}$$

The model selection problem:

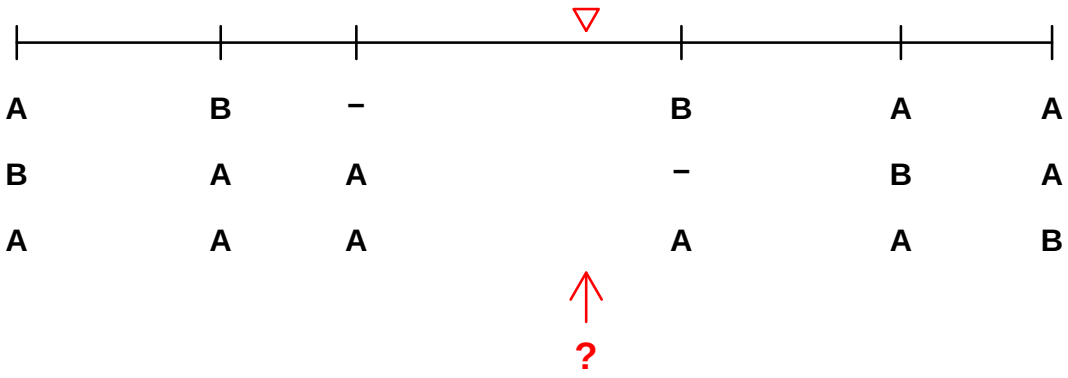
$$\text{QTL, covariates} \longrightarrow \text{phenotype}$$

Models: Recombination

We assume
- Mendel's rules
- no crossover interference

1. Pr(x_{ij} = 0) = Pr(x_{ij} = 1) = 1/2
2. Pr($x_{i,j+1}$ = 1 | x_{ij} = 0) = Pr($x_{i,j+1}$ = 0 | x_{ij} = 1) = r_j
   where r_i is the recombination fraction for the interval
3. The genotype at a position depends only on the genotypes at the nearest flanking typed markers

Example



Models: Genotype $\longleftrightarrow$ Phenotype

Let y = phenotype
g = whole genome genotype

Imagine a small number of QTLs with genotypes $g_1, \ldots, g_p$.
(2^p distinct genotypes)

$$\mathrm{E}(y|g) = \mu_{g_1,\ldots,g_p} \qquad \mathrm{var}(y|g) = \sigma^2_{g_1,\ldots,g_p}$$

Models: Genotype $\longleftrightarrow$ Phenotype

Homoscedasticity (constant variance): $\sigma^2_g \equiv \sigma^2$

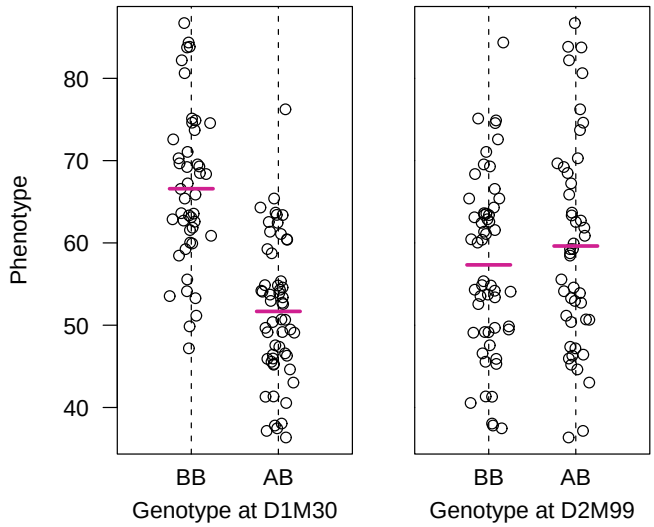
Normally distributed residual variation: $y|g \sim N(\mu_g, \sigma^2)$.

Additivity: $\mu_{g_1,\ldots,g_p} = \mu + \sum_{j=1}^{p} \Delta_j \, g_j$ ($g_j = 1$ or 0)

Epistasis: Any deviations from additivity.

The simplest method: ANOVA

- 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.

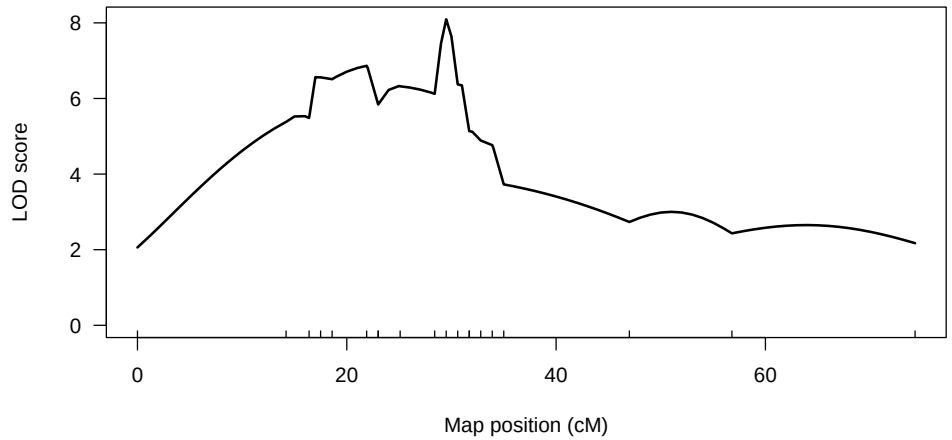
Disadvantages

- 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 (IM)

Lander & Botstein (1989)

- Take account of missing genotype data
- Interpolate between markers
- Maximum likelihood under a mixture model



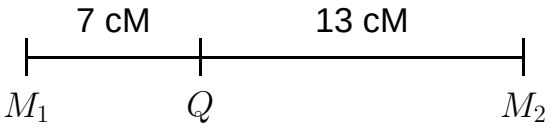
Interval mapping (IM)

Lander & Botstein (1989)

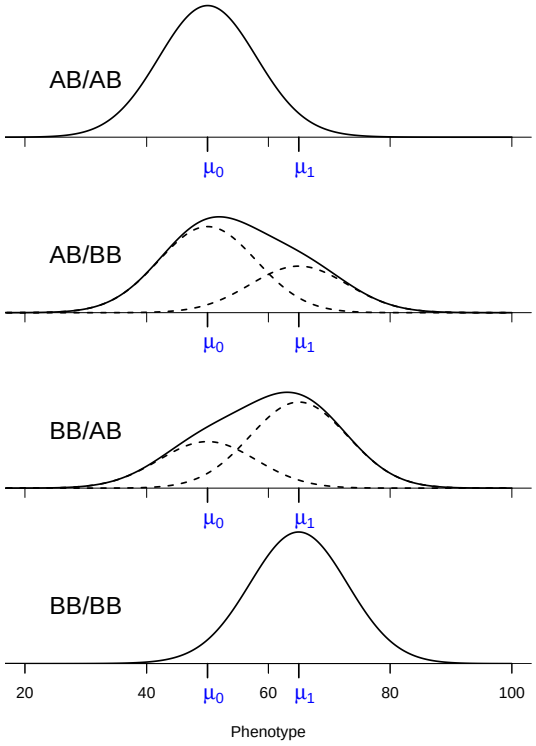
- Assume a single QTL model.
- Each position in the genome, one at a time, is posited as the putative QTL.
- Let $z = 1/0$ if the (unobserved) QTL genotype is BB/AB. Assume $y \sim N(\mu_z, \sigma)$
- Given genotypes at linked markers, $y \sim$ mixture of normal dist'ns with mixing proportion $\Pr(z = 1|\text{marker data})$:

		QTL genotype	
M_1	M_2	BB	AB
BB	BB	$(1-r_L)(1-r_R)/(1-r)$	$r_L r_R/(1-r)$
BB	AB	$(1-r_L)r_R/r$	$r_L(1-r_R)/r$
AB	BB	$r_L(1-r_R)/r$	$(1-r_L)r_R/r$
AB	AB	$r_L r_R/(1-r)$	$(1-r_L)(1-r_R)/(1-r)$

The normal mixtures



- Two markers separated by 20 cM, with the QTL closer to the left marker.
- The figure at right show the distributions of the phenotype conditional on the genotypes at the two markers.
- The dashed curves correspond to the components of the mixtures.



Interval mapping (continued)

Let $p_i = \Pr(z_i = 1|\text{marker data})$

$y_i|z_i \sim N(\mu_{z_i}, \sigma^2)$

$\Pr(y_i|\text{marker data}, \mu_0, \mu_1, \sigma) = p_i f(y_i; \mu_1, \sigma) + (1 - p_i) f(y_i; \mu_0, \sigma)$

where $f(y; \mu, \sigma)$ = density of normal distribution

Log likelihood: $l(\mu_0, \mu_1, \sigma) = \sum_i \log \Pr(y_i|\text{marker data}, \mu_0, \mu_1, \sigma)$

Maximum likelihood estimates (MLEs) of μ_0, μ_1, σ:

values for which $l(\mu_0, \mu_1, \sigma)$ is maximized.

LOD scores

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

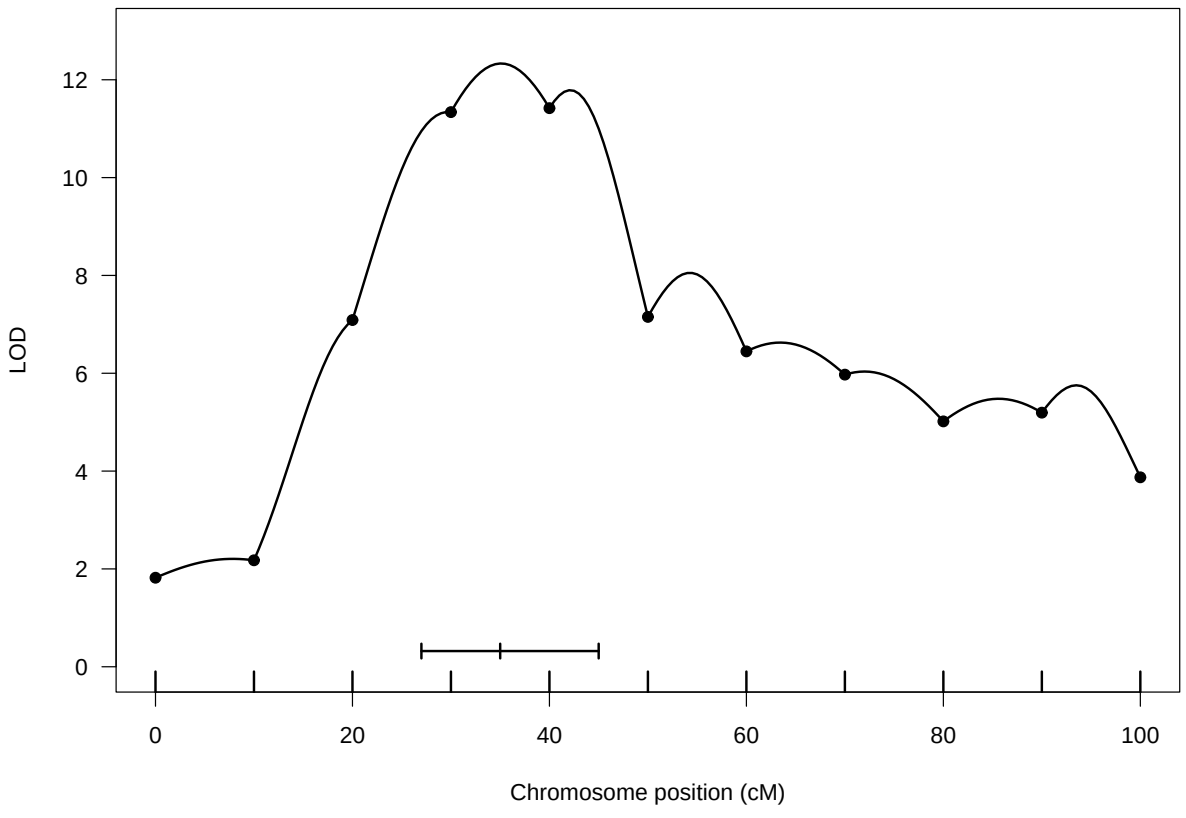
LOD$(z) = \log_{10}$ likelihood ratio comparing the hypothesis of a QTL at position z versus that of no QTL

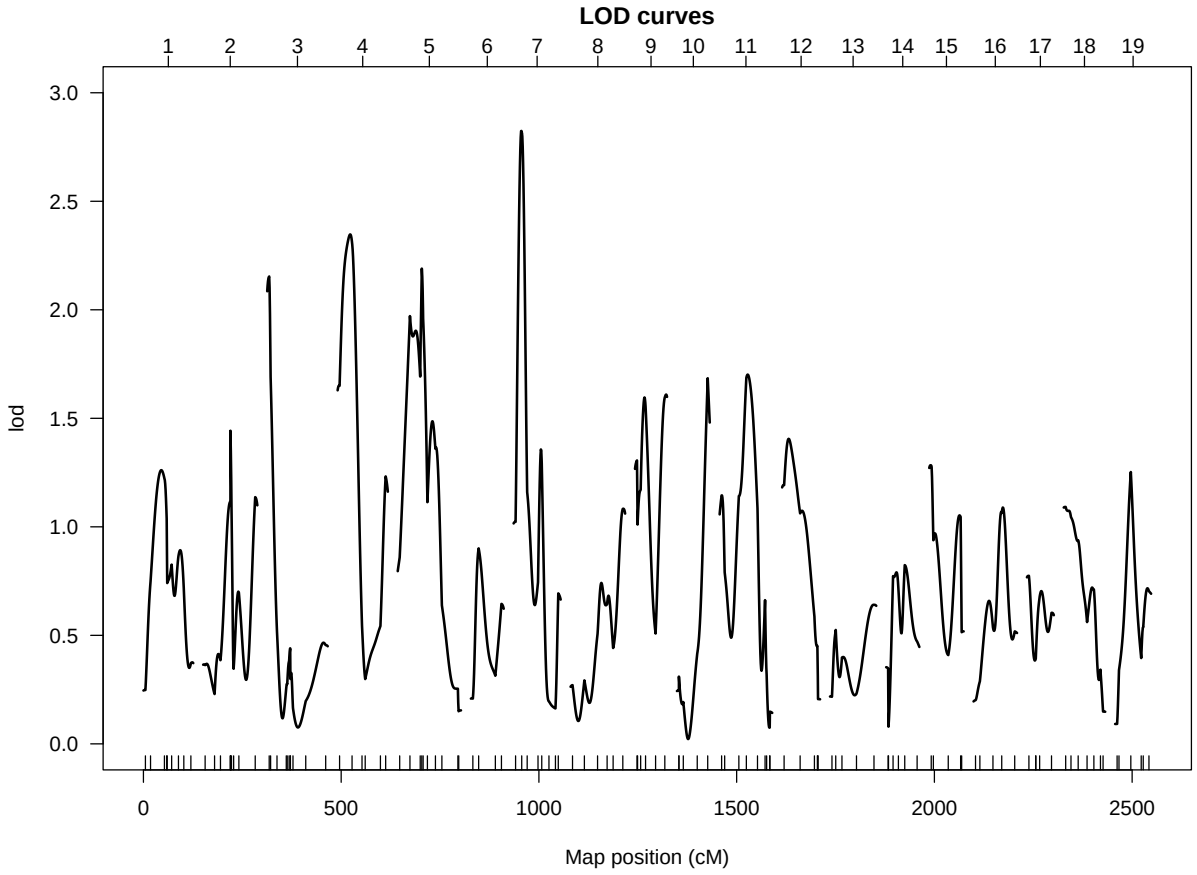
$$= \log_{10} \left\{ \frac{\Pr(y|\text{QTL at } z, \hat{\mu}_{0z}, \hat{\mu}_{1z}, \hat{\sigma}_z)}{\Pr(y|\text{no QTL}, \hat{\mu}, \hat{\sigma})} \right\}$$

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

No QTL model: The phenotypes are independent and identically distributed (iid) $N(\mu, \sigma^2)$.

An example LOD curve





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.

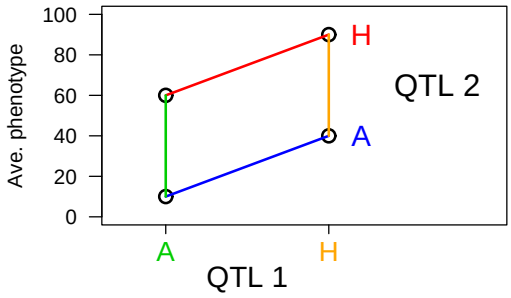
Multiple QTL methods

Why consider multiple QTLs at once?

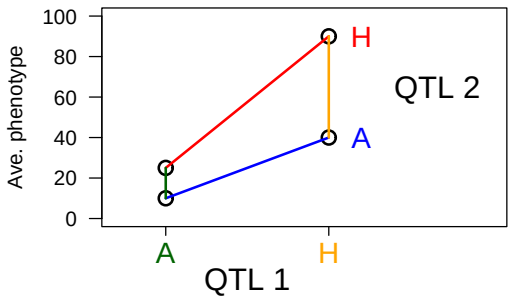
- Reduce residual variation.
- Separate linked QTLs.
- Investigate interactions between QTLs (epistasis).

Epistasis in a backcross

Additive QTLs

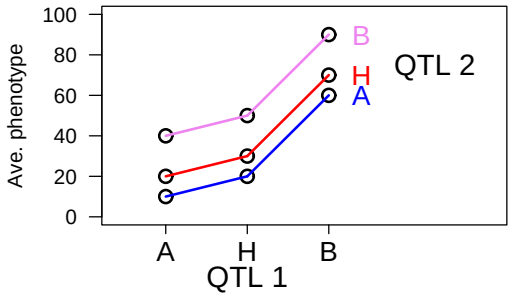


Interacting QTLs

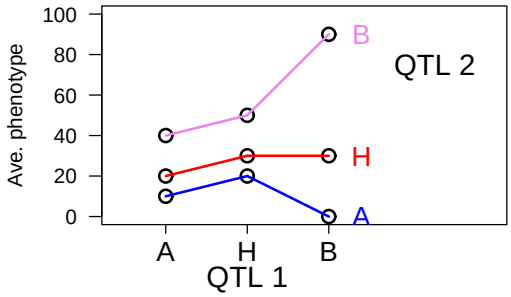


Epistasis in an intercross

Additive QTLs



Interacting QTLs



LOD thresholds

Large LOD scores indicate evidence for the presence of a QTL.

Q: How large is large?

→ We consider the distribution of the LOD score under the null hypothesis of no QTL.

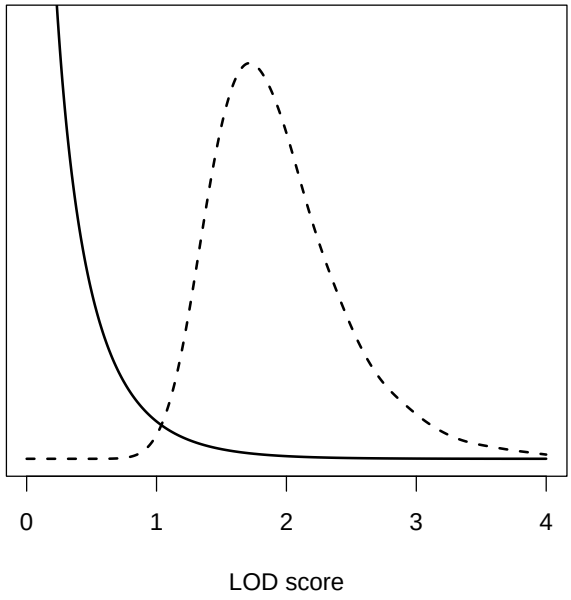
Key point: We must make some adjustment for our examination of multiple putative QTL locations.

→ We seek the distribution of the *maximum* LOD score, genome-wide. The 95th %ile of this distribution serves as a genome-wide LOD threshold.

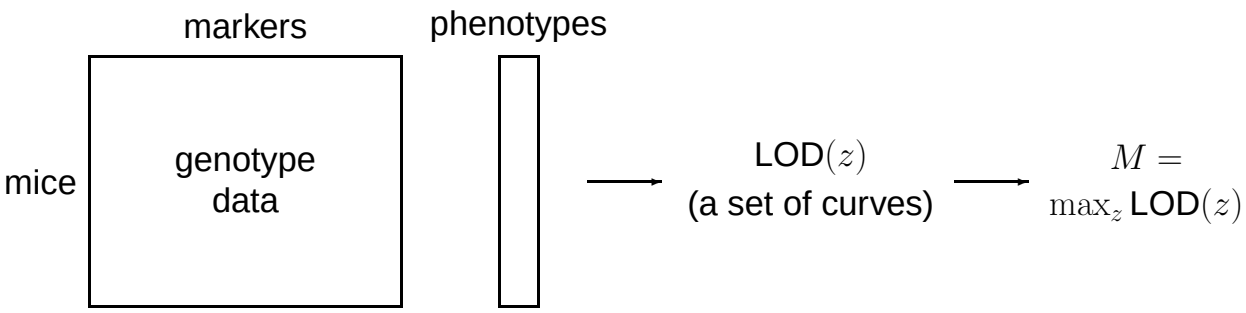
Estimating the threshold: simulations, analytical calculations, permutation (randomization) tests.

Null distribution of the LOD score

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

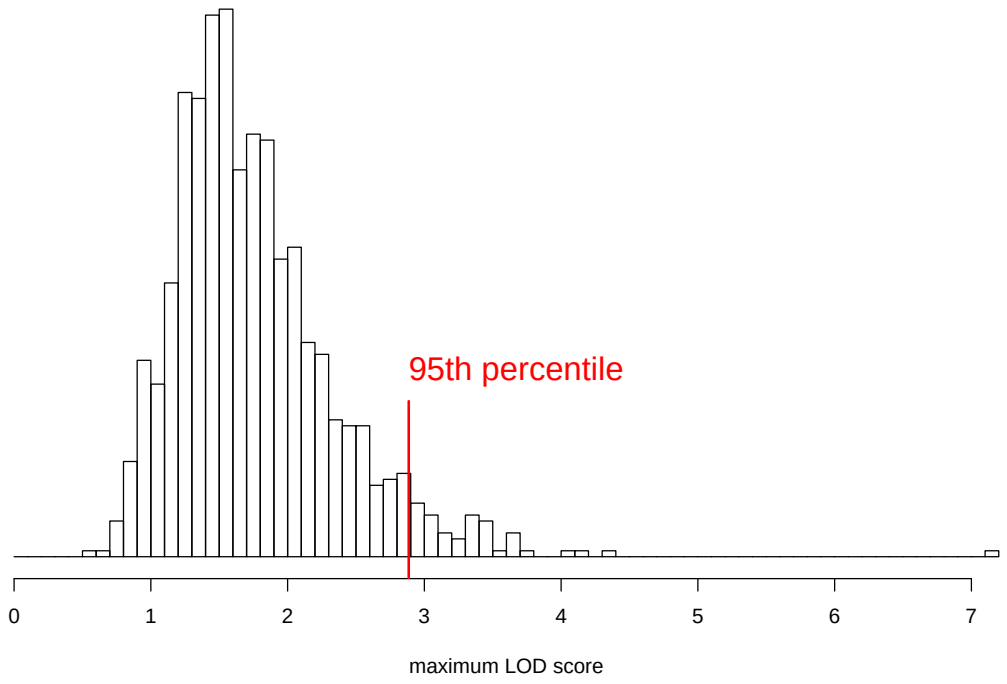


Permutation tests

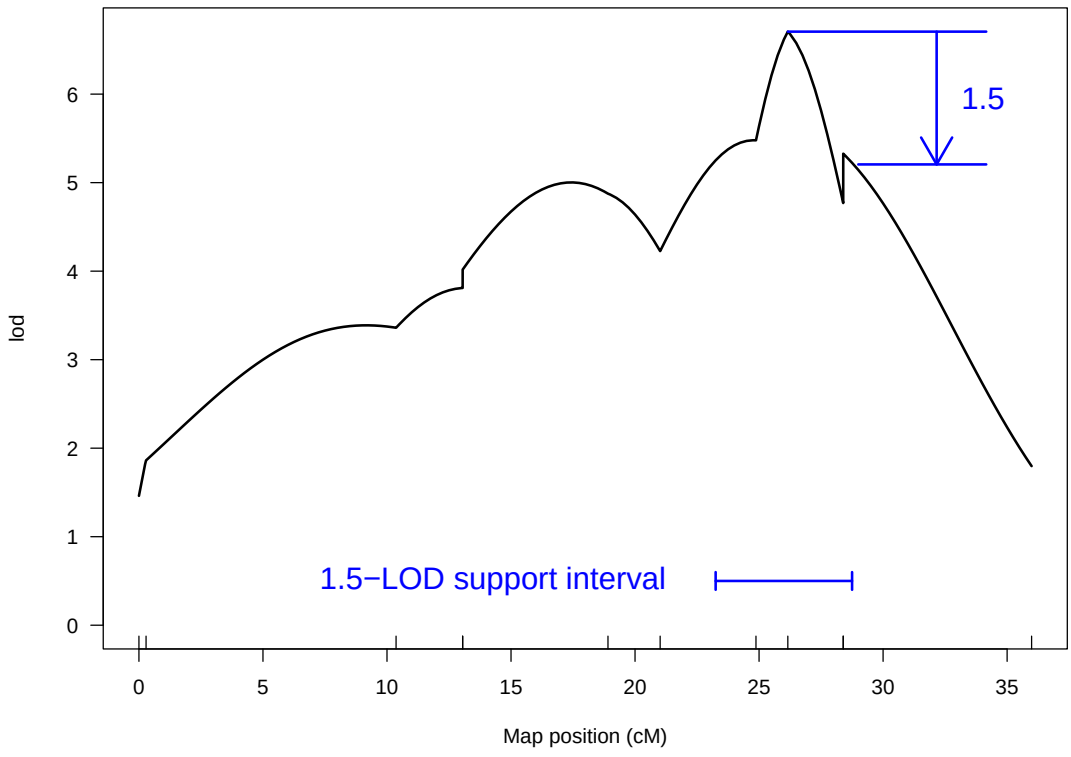


- Permute/shuffle the phenotypes; keep the genotype data intact.
- Calculate $\text{LOD}^\star(z) \longrightarrow M^\star = \max_z \text{LOD}^\star(z)$
- We wish to compare the observed M to the distribution of $M^\star$.
- $\Pr(M^\star \geq M)$ is a genome-wide P-value.
- The 95th %ile of $M^\star$ is a genome-wide LOD threshold.
- We can't look at all $n!$ possible permutations, but a random set of 1000 is feasible and provides reasonable estimates of P-values and thresholds.
- Value: conditions on observed phenotypes, marker density, and pattern of missing data; doesn't rely on normality assumptions or asymptotics.

Permutation distribution

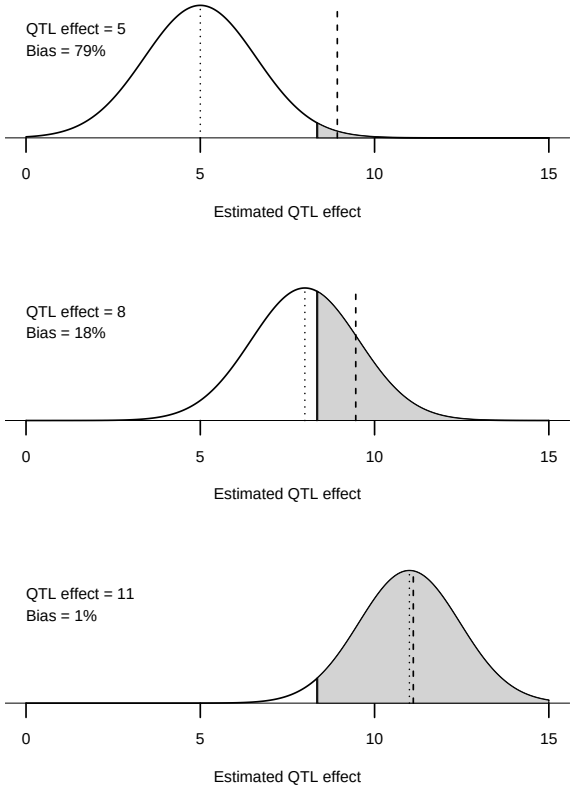


1.5-LOD support interval



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 of selection bias

- Estimated % variance explained by identified QTLs
- Repeating an experiment
- Congenics
- Marker-assisted selection

The X chromosome

In a backcross, the X chromosome may or may not be segregating.

$(A \times B) \times A$

Females: $X_{A \cdot B}\ X_A$

Males: $X_{A \cdot B}\ Y_A$

$A \times (A \times B)$

Females: $X_A\ X_A$

Males: $X_A\ Y_B$

The X chromosome

In an intercross, one must pay attention to the paternal grandmother's genotype.

$(A \times B) \times (A \times B)$ or $(B \times A) \times (A \times B)$

Females: $X_{A \cdot B}\ X_A$

Males: $X_{A \cdot B}\ Y_B$

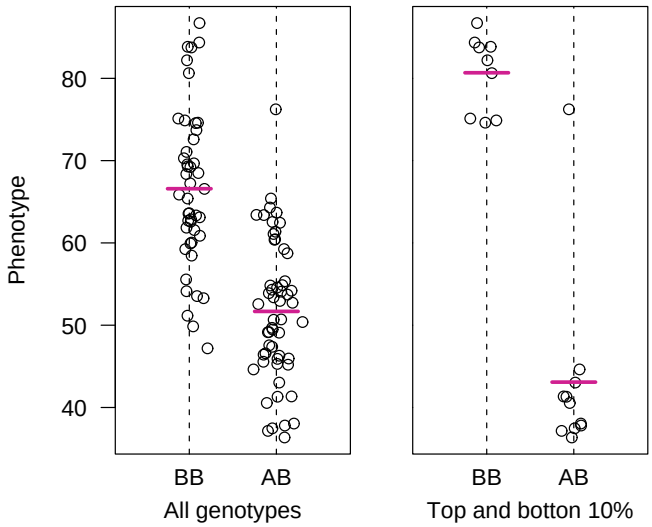
$(A \times B) \times (B \times A)$ or $(B \times A) \times (B \times A)$

Females: $X_{A \cdot B}\ X_B$

Males: $X_{A \cdot B}\ Y_A$

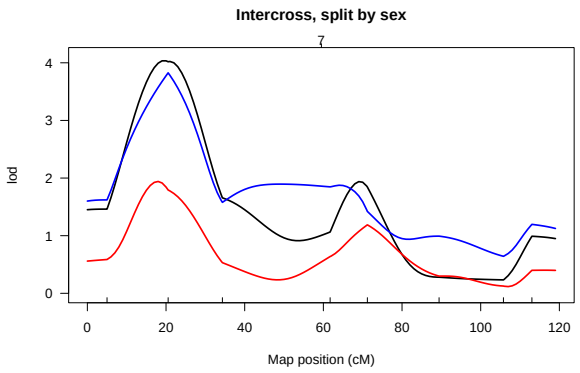
Selective genotyping

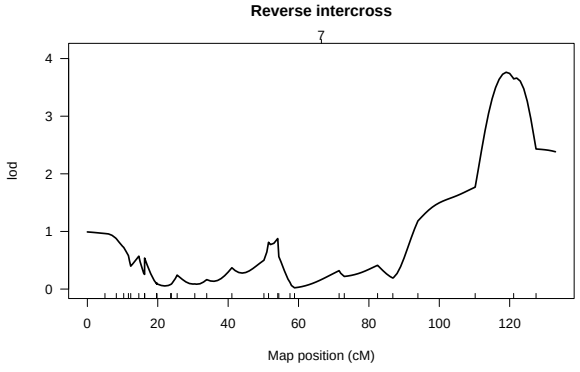
- Save effort by only typing the most informative individuals (say, top & bottom 10%).
- Useful in context of a single, inexpensive trait.
- Tricky to estimate the effects of QTLs: use IM with all phenotypes.
- Can't get at interactions.
- Likely better to also genotype some random portion of the rest of the individuals.



Covariates

- Examples: treatment, sex, litter, lab, age.
- Control residual variation.
- Avoid confounding.
- Look for QTL × environ't interactions
- Adjust before interval mapping (IM) versus adjust within IM.





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)
  - Specially-tailored models (*e.g.*, a generalized linear model, the Cox proportional hazard model, and the model in Broman et al. 2000)

Check data integrity

The success of QTL mapping depends crucially on the integrity of the data.

- Segregation distortion
- Genetic maps / marker positions
- Genotyping errors (tight double crossovers)
- Phenotype distribution / outliers
- Residual analysis

Summary I

- ANOVA at marker loci (aka marker regression) is simple and easily extended to include covariates or accommodate complex models.
- Interval mapping improves on ANOVA by allowing inference of QTLs to positions between markers and taking proper account of missing genotype data.
- ANOVA and IM consider only single-QTL models. Multiple QTL methods allow the better separation of linked QTLs and are necessary for the investigation of epistasis.
- Statistical significance of LOD peaks requires consideration of the maximum LOD score, genome-wide, under the null hypothesis of no QTLs. Permutation tests are extremely useful for this.
- 1.5-LOD support intervals indicate the plausible location of a QTL.
- Estimates of QTL effects are subject to selection bias. Such estimated effects are often too large.

Summary II

- The X chromosome must be dealt with specially, and can be tricky.
- Study your data. Look for errors in the genetic map, genotyping errors and phenotype outliers. But don't worry about them too much.
- Selective genotyping can save you time and money, but proceed with caution.
- Study your data. The consideration of covariates may reveal extremely interesting phenomena.
- Interval mapping works reasonably well even with non-normal traits. But consider transformations or specially-tailored models. If interval mapping software is not available for your preferred model, start with some version of ANOVA.

References

- Broman KW (2001) Review of statistical methods for QTL mapping in experimental crosses. Lab Animal 30(7):44–52

  A review for non-statisticians.

- Doerge RW (2002) Mapping and analysis of quantitative trait loci in experimental populations. Nat Rev Genet 3:43–52

  A very recent review.

- Doerge RW, Zeng Z-B, Weir BS (1997) Statistical issues in the search for genes affecting quantitative traits in experimental populations. Statistical Science 12:195–219

  Review paper.

- Jansen RC (2001) Quantitative trait loci in inbred lines. In Balding DJ et al., *Handbook of statistical genetics*, John Wiley & Sons, New York, chapter 21

  Review in an expensive but rather comprehensive and likely useful book.

- 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.

- Kruglyak L, Lander ES (1995) A nonparametric approach for mapping quantitative trait loci. Genetics 139:1421–1428

  Non-parameteric interval mapping.

- Boyartchuk VL, Broman KW, Mosher RE, D'Orazio S, Starnbach M, Dietrich WF (2001) Multigenic control of Listeria monocytogenes susceptibility in mice. Nat Genet 27:259–260

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

  QTL mapping with a special model for a non-normal phenotype.