QTL Mapping II:

Introduction

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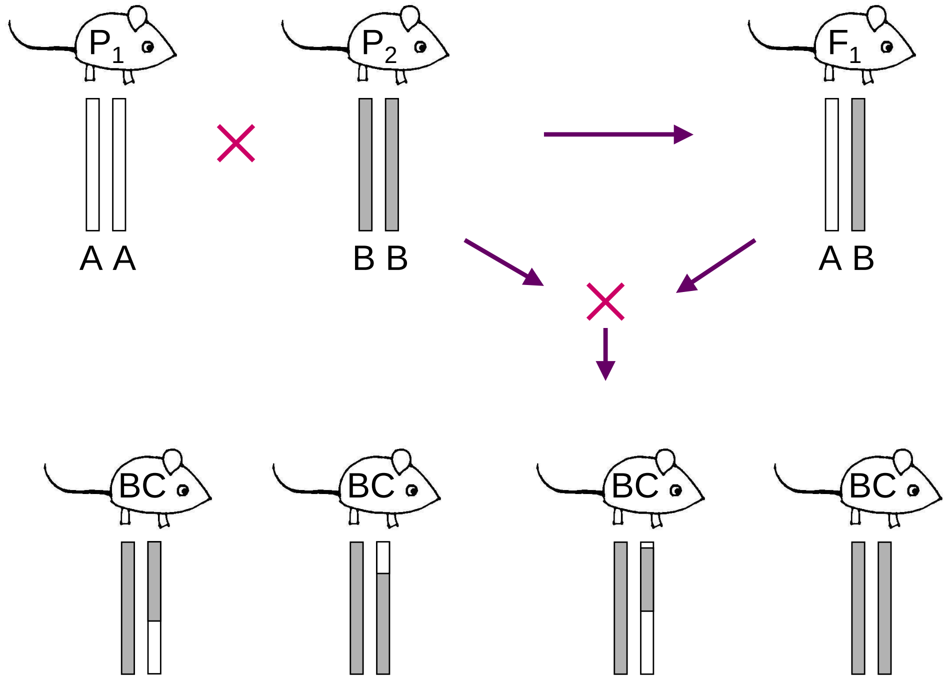
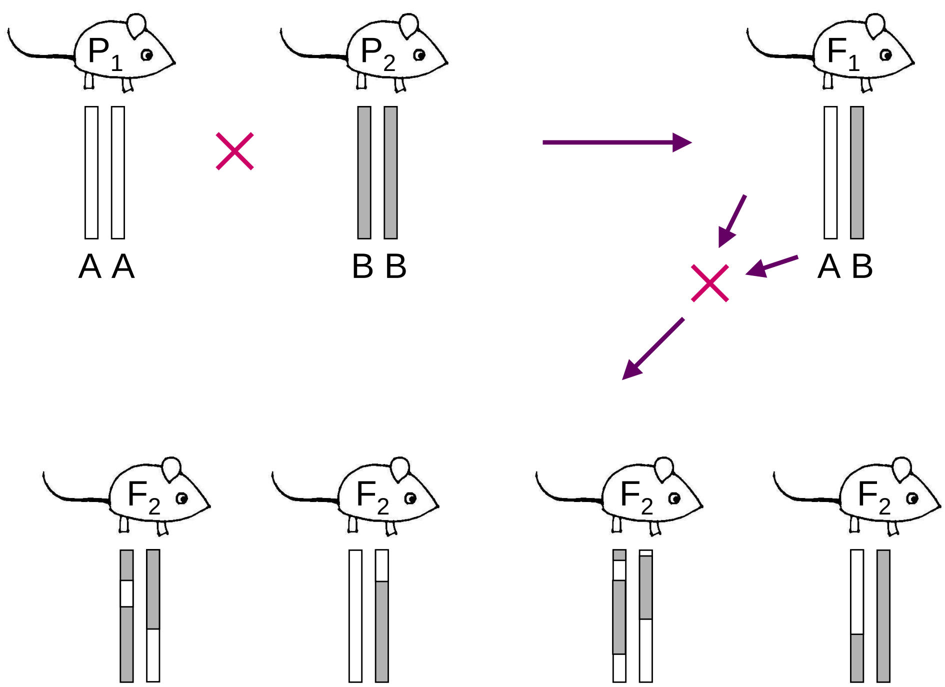
Outline

- Experiments and data
- Goals, statistical structure
- Models
- ANOVA and interval mapping
- LOD thresholds
- CIs for QTL location
- Selection bias
- Multiple QTLs, epistasis
- The X chromosome
- Selective genotyping
- Covariates
- Non-normal traits
- The need for good data

Backcross experiment

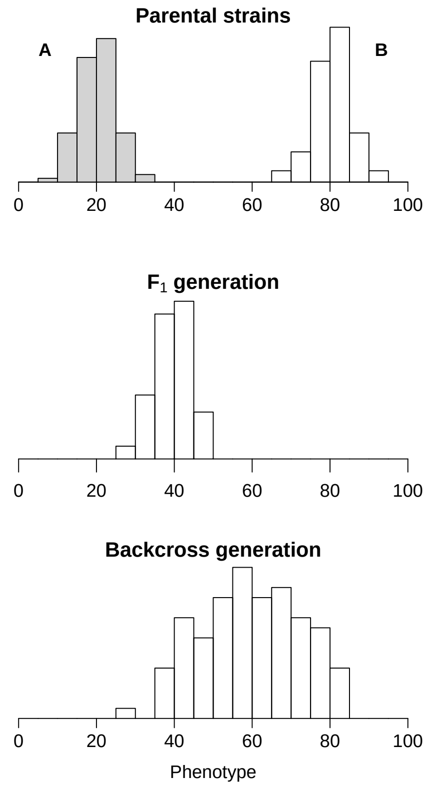


Intercross experiment



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.

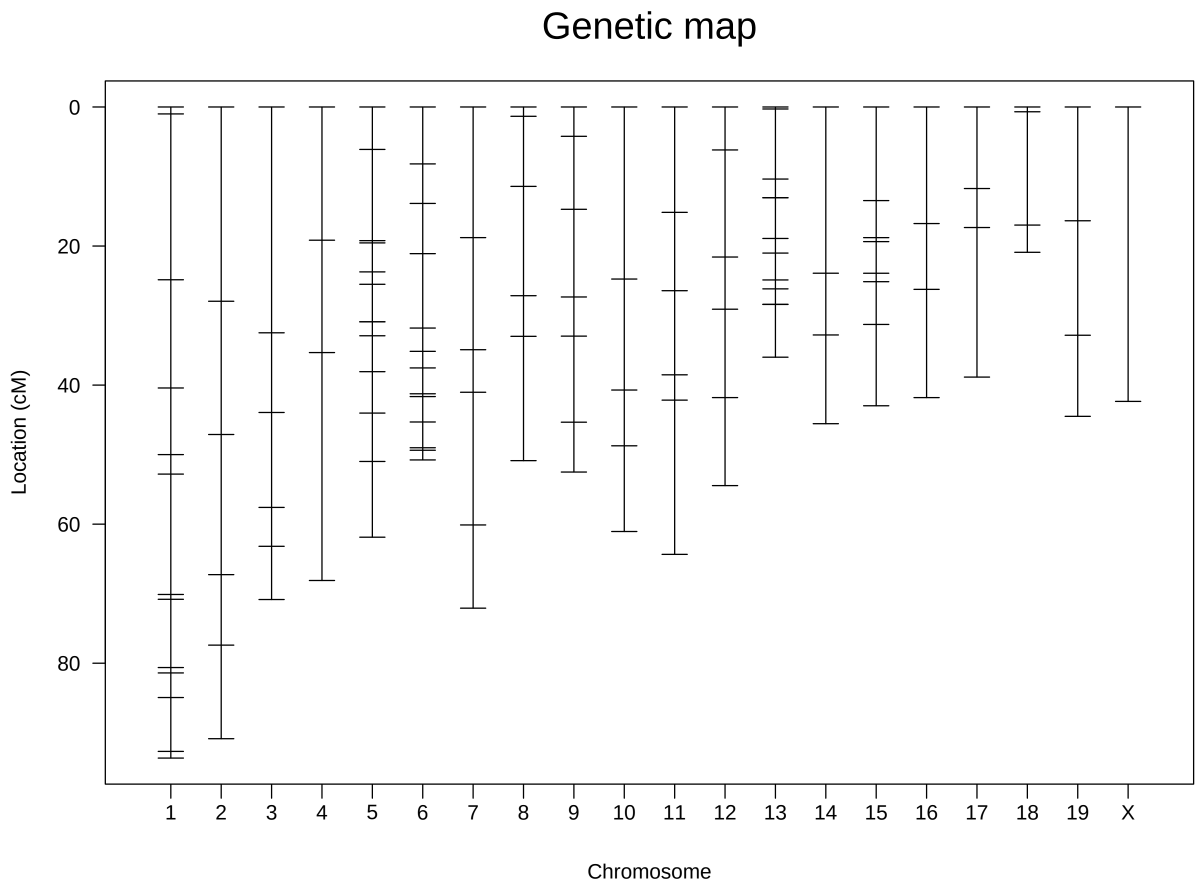


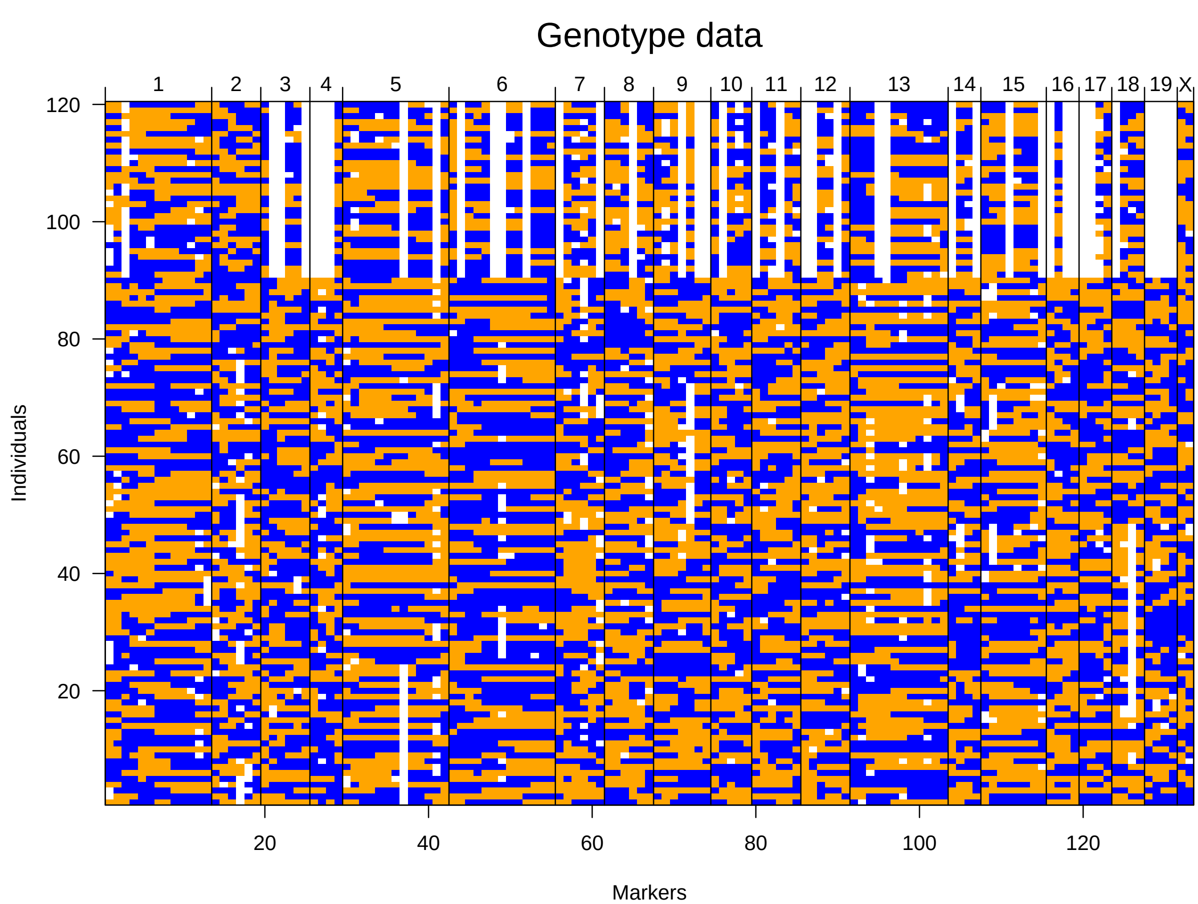
Data

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

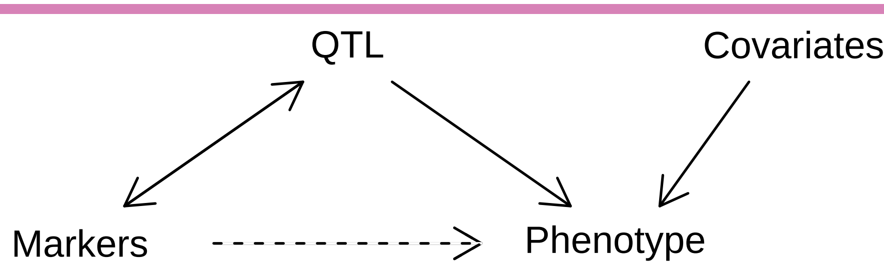

Genetic map
Location (cM)
0
20
40
60
80
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 X
Chromosome


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

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

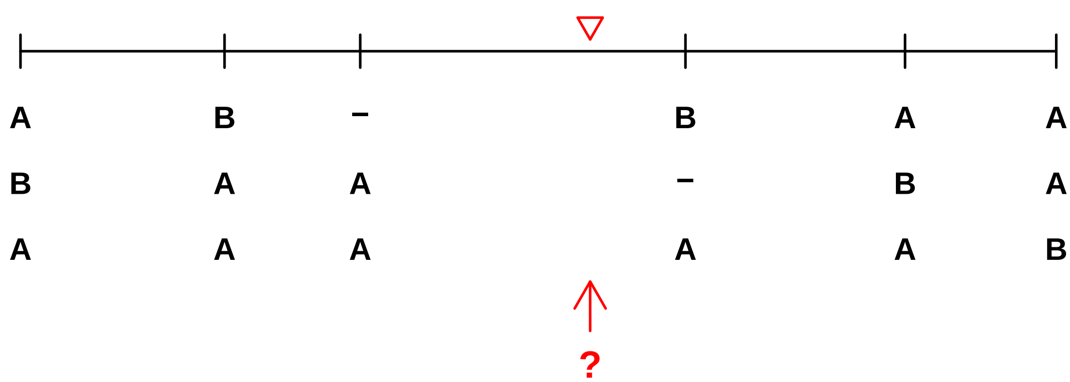
Models: Recombination

We assume no crossover interference.

$\Longrightarrow$ Points of exchange (crossovers) are according to a Poisson process.

$\Longrightarrow$ The $\{x_{ij}\}$ (marker genotypes) form a Markov chain

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)

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

Models: Genotype $\longleftrightarrow$ Phenotype

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

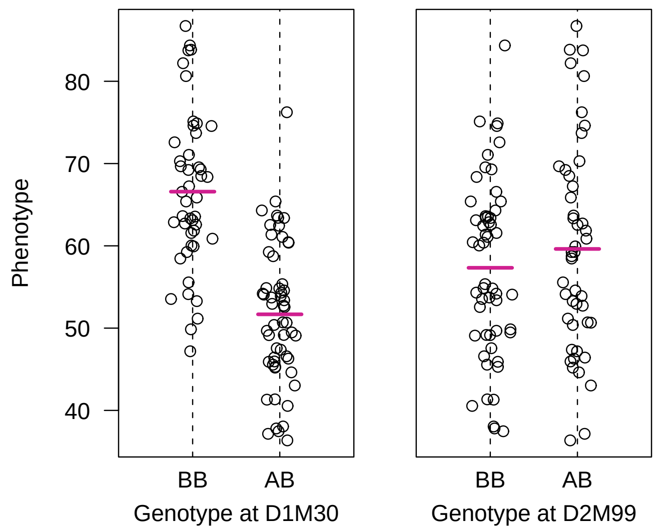
Normally distributed residual variation: $y|g \sim N(\mu_g, \sigma^2)$.

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

- Split mice into groups according to genotype at a marker.
- Do a t-test / ANOVA.
- Repeat for each marker.
- Adjust for multiple testing



ANOVA at marker loci

Advantages

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

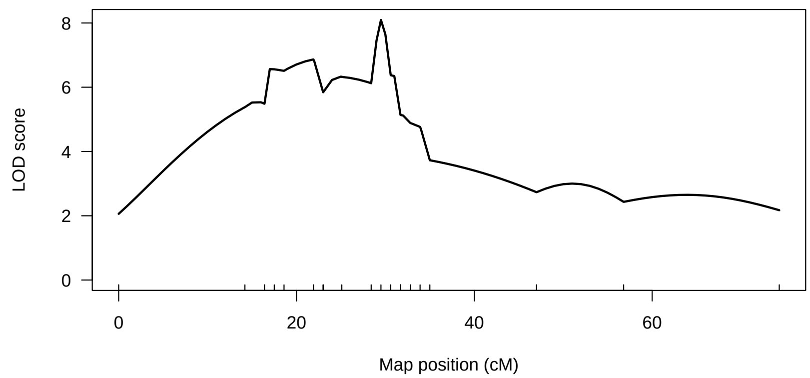
Disadvantages

- 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



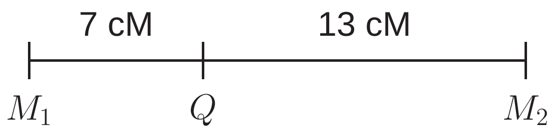
Interval mapping (IM)

Lander & Botstein (1989)

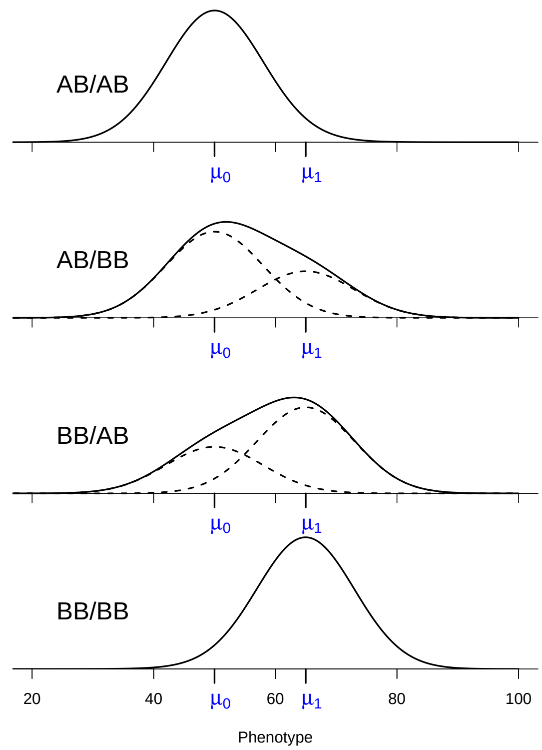
- Assume a single QTL model.
- Each position in the genome, one at a time, is posited as the putative QTL.
- Let $q =$ 1/0 if the (unobserved) QTL genotype is BB/AB. Assume $y \sim N(\mu_q, \sigma)$
- Given genotypes at linked markers, $y \sim$ mixture of normal dist'ns with mixing proportion $\Pr(q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

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

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

where $f(y; \mu, \sigma) = \exp[-(y - \mu)^2/(2\sigma^2)]/\sqrt{2\pi\sigma^2}$

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

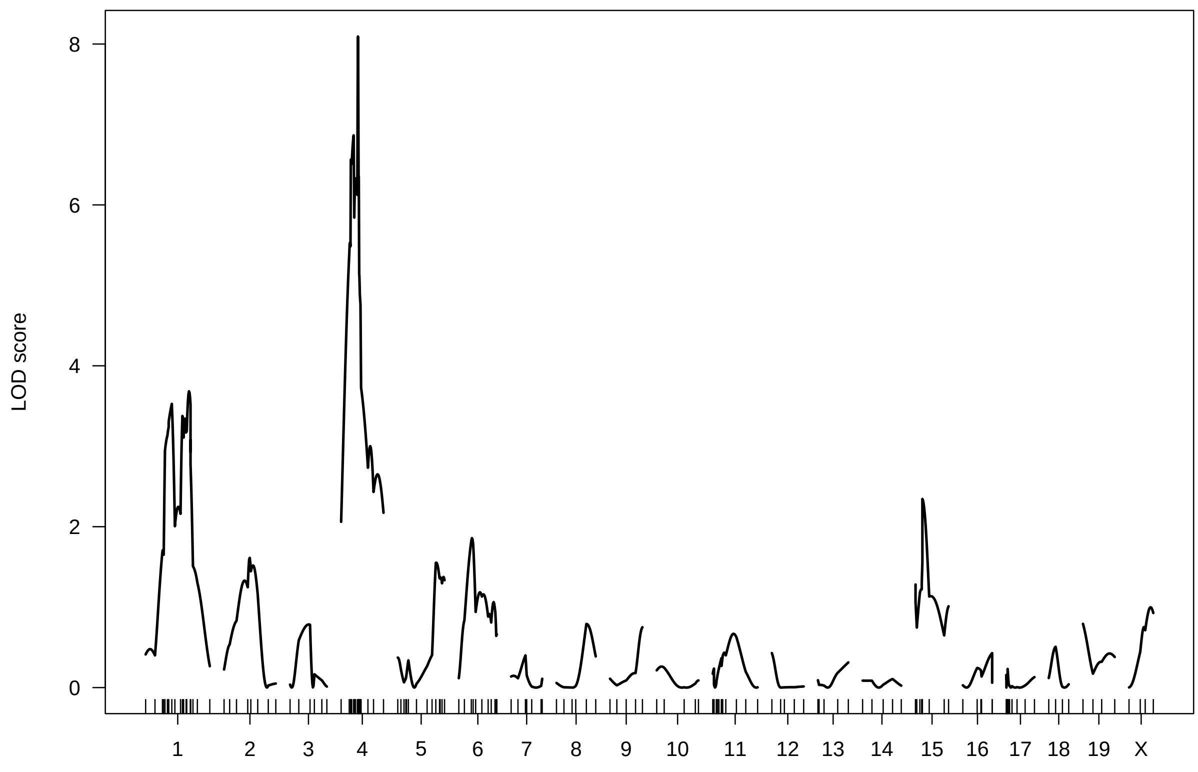
The LOD score is a measure of the strength of evidence for the presence of a QTL at a particular location.

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

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

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

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



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.

Haley-Knott regression

A quick approximation to Interval Mapping.

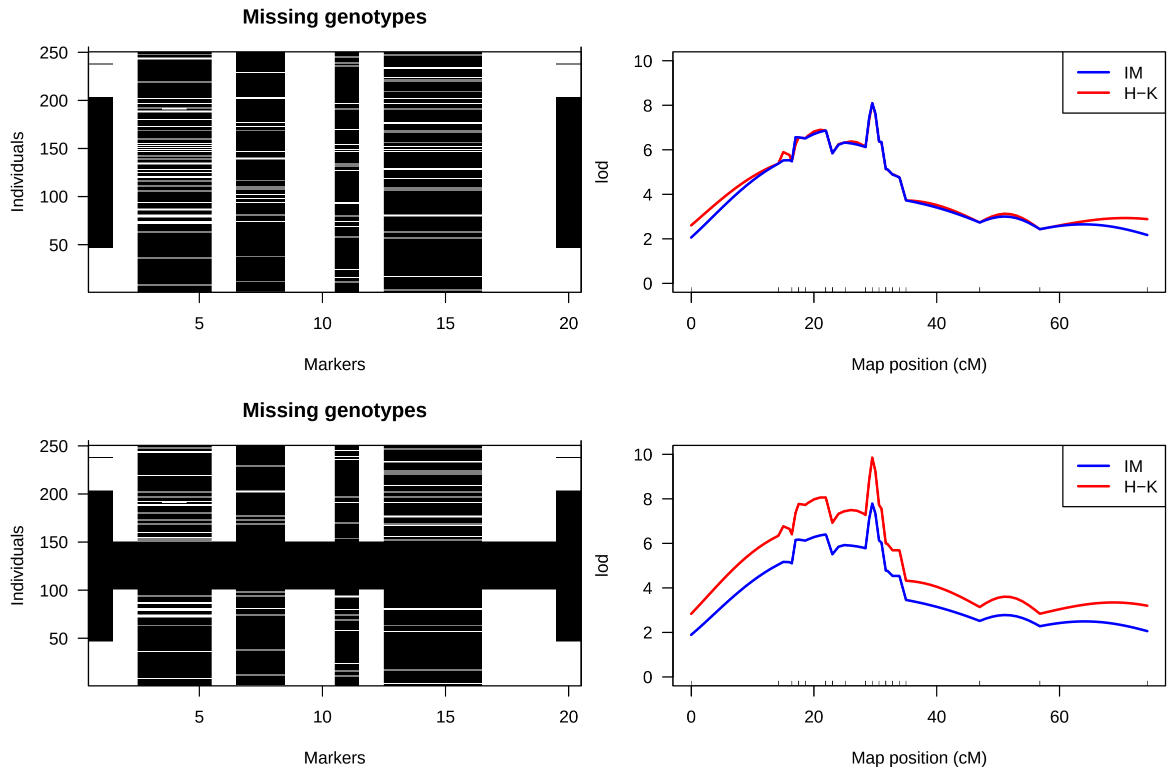
$$\text{E}(y \mid \text{QTL} = q) \;=\; \mu_0 + (\mu_1 - \mu_0)\; 1\{q = \text{AB}\}$$

$$\text{E}(y \mid \text{marker data}) \;=\; \mu_0 + (\mu_1 - \mu_0)\; \text{Pr}(\text{QTL} = \text{AB} \mid \text{marker data})$$

- Regress y on $\text{Pr}(\text{QTL} = \text{AB} \mid \text{marker data})$.
- Pretend that the residual variation is normally distributed.
- Calculate

$$\text{LOD}(\lambda) = (n/2)\; \log_{10}\left\{\frac{\text{RSS}_0}{\text{RSS}_a(\lambda)}\right\}$$

Example



LOD thresholds

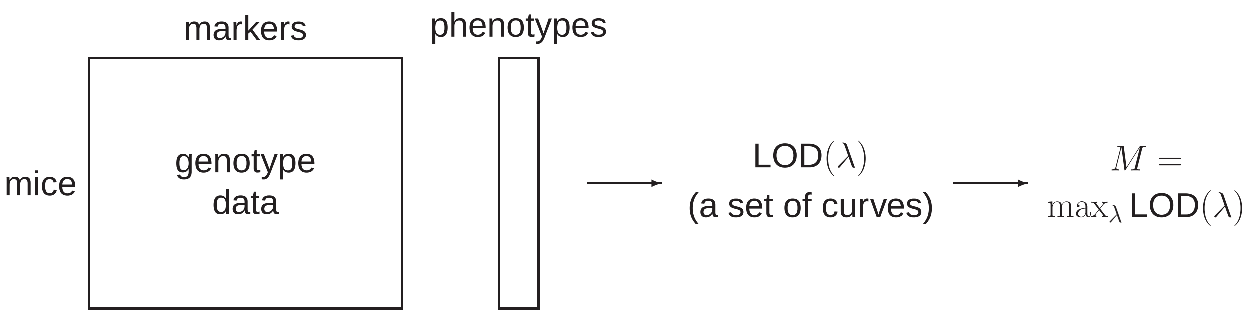
Large LOD scores indicate evidence for the presence of a QTL

Question: How large is large?

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

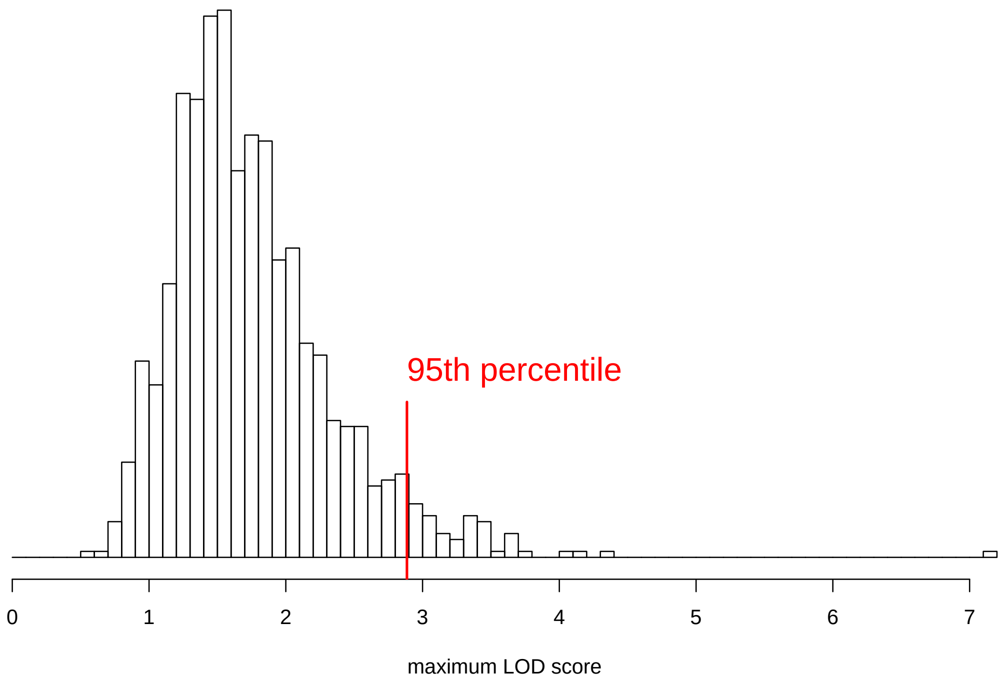
Derivation:
- Analytical calculations (L & B 1989)
- Simulations (L & B 1989)
- Permutation tests (Churchill & Doerge 1994)

Permutation tests

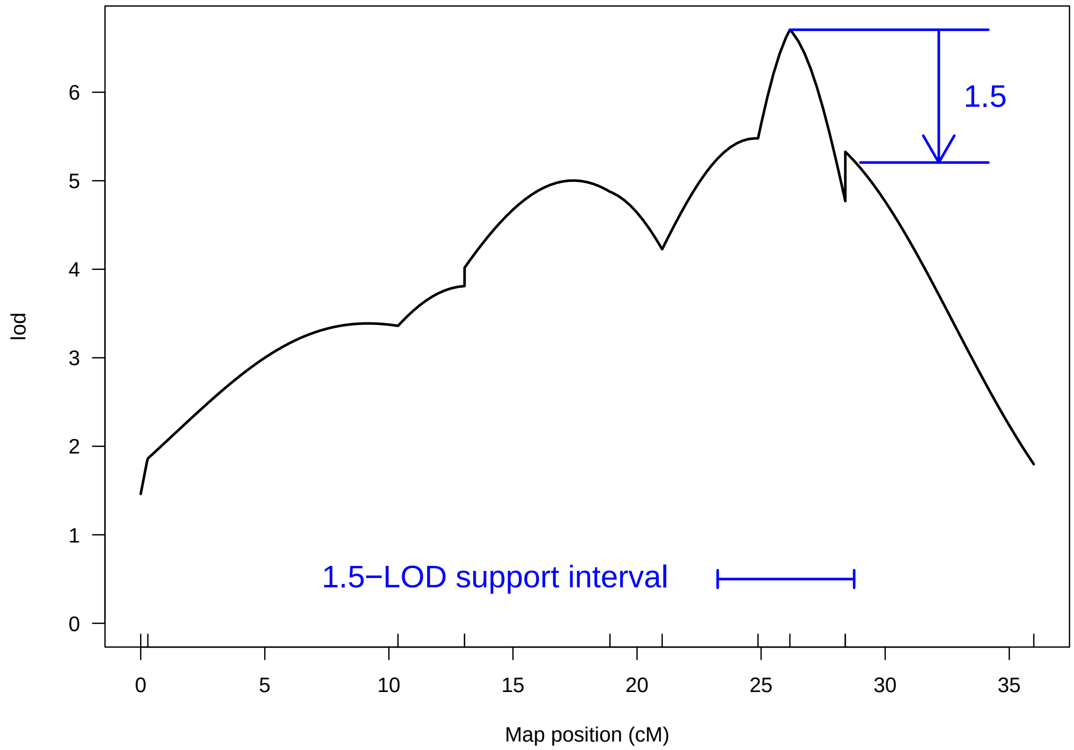


- Permute/shuffle the phenotypes; keep the genotype data intact.
- Calculate LOD$^\star(\lambda)$ $\longrightarrow$ $M^\star = \max_\lambda$ LOD$^\star(\lambda)$
- We wish to compare the observed M to the distribution of $M^\star$.
- $\Pr(M^\star \geq M)$ is a genome-wide P-value.
- The 95th %ile of $M^\star$ 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

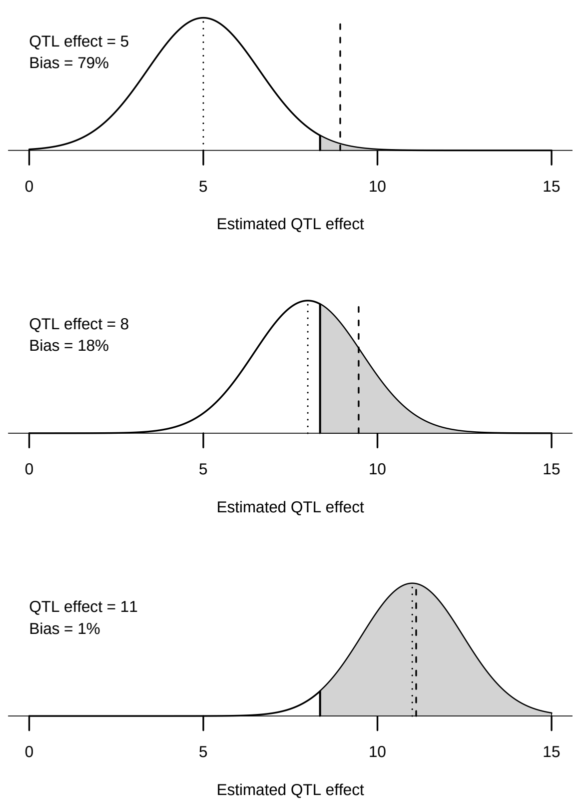


1.5-LOD support interval



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

Multiple QTL methods

Why consider multiple QTLs at once?

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

Issues:

- Missing genotype information
- The model selection problem

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

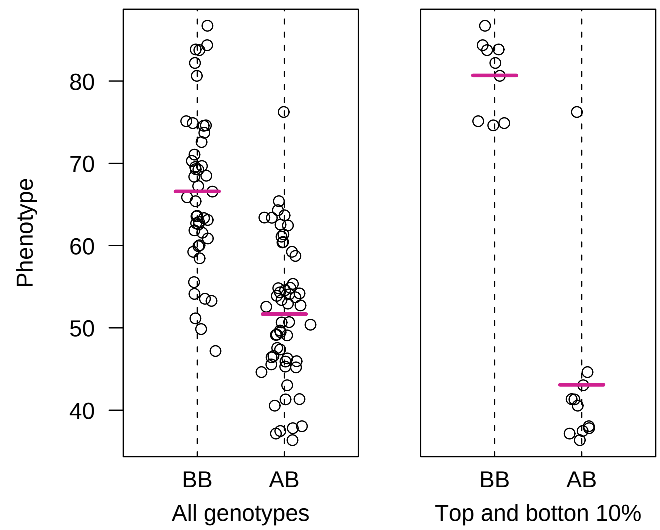
$(A \times B) \times (B \times A)$ or $(B \times A) \times (B \times A)$

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

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

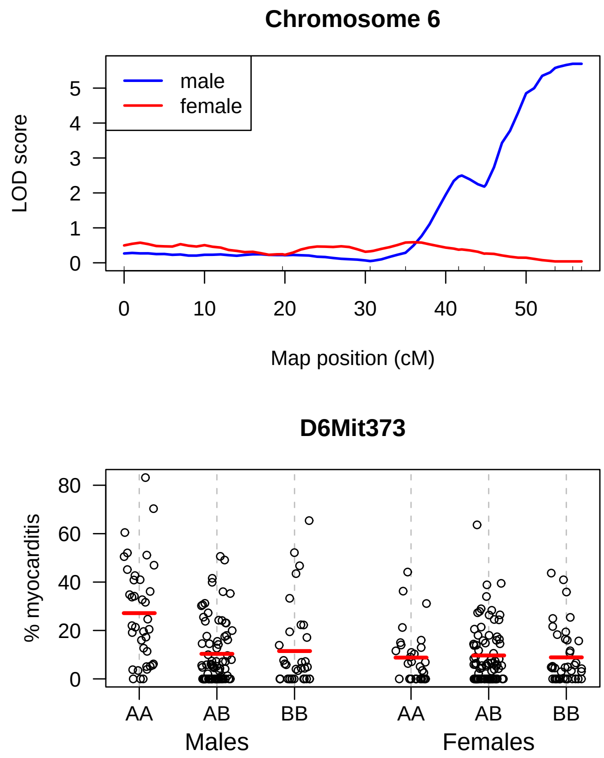
Selective genotyping

- 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 $\times$ 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 (2003))

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.

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  An old but excellent general genetics textbook with a very interesting discussion of epistasis.