QTL Mapping II:

Introduction

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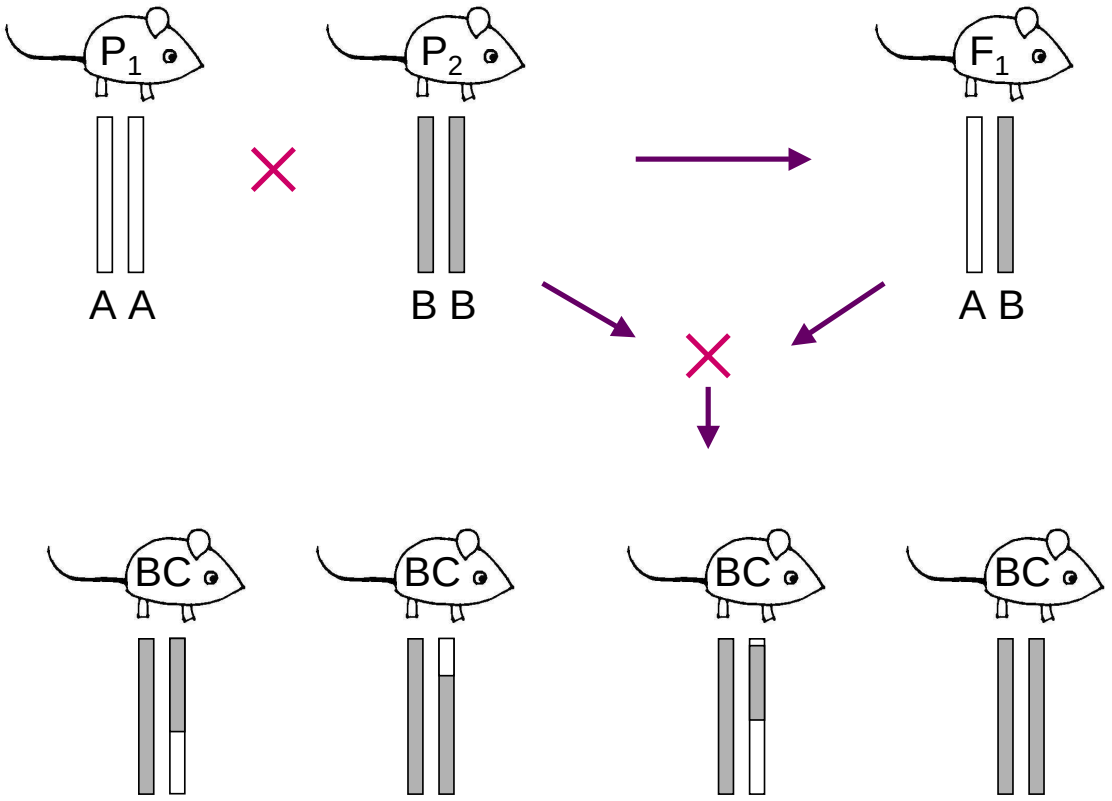
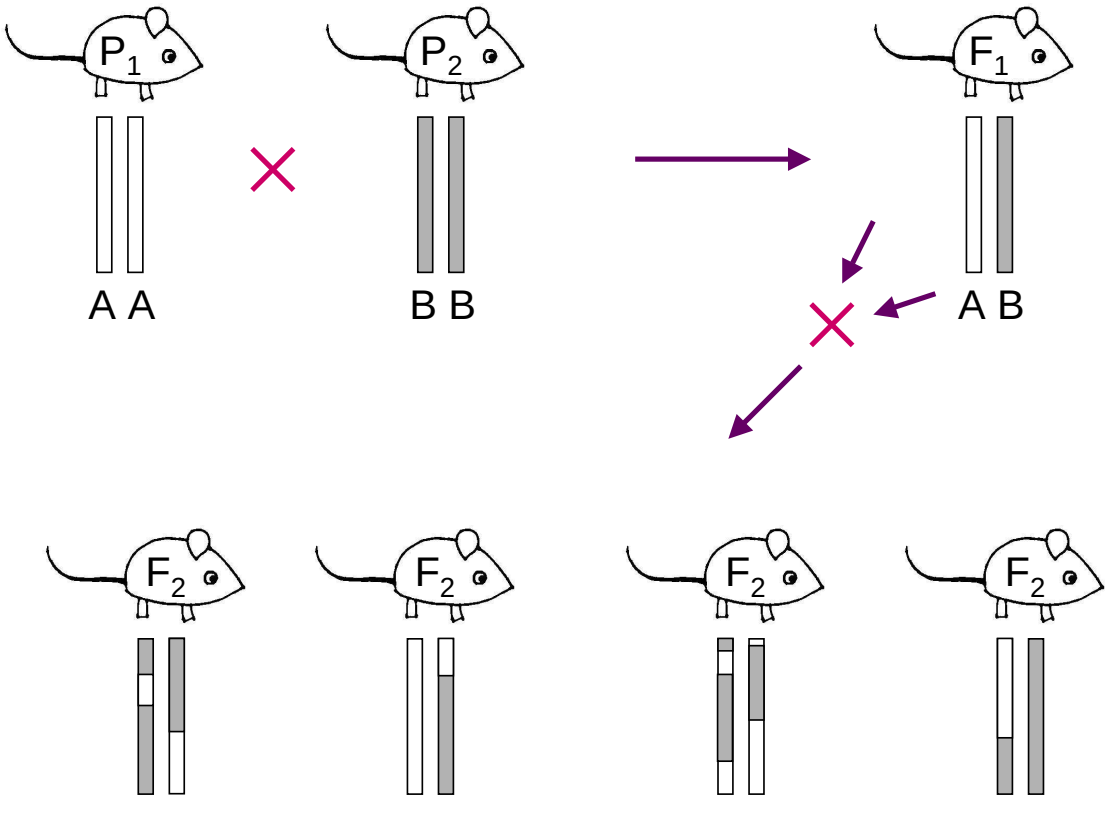
Outline

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

Backcross experiment

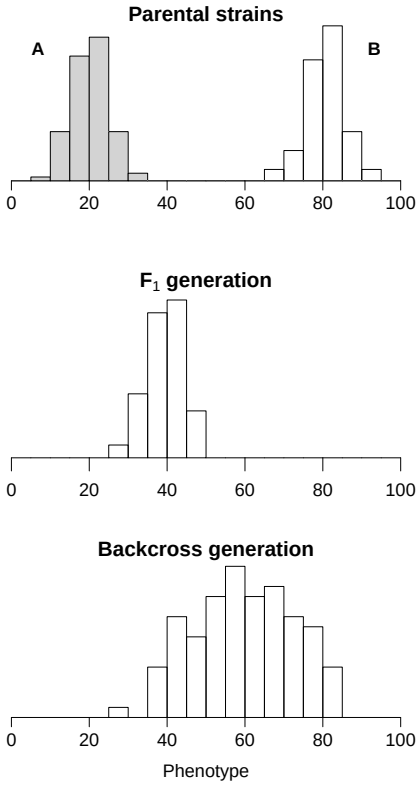


Intercross experiment



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.

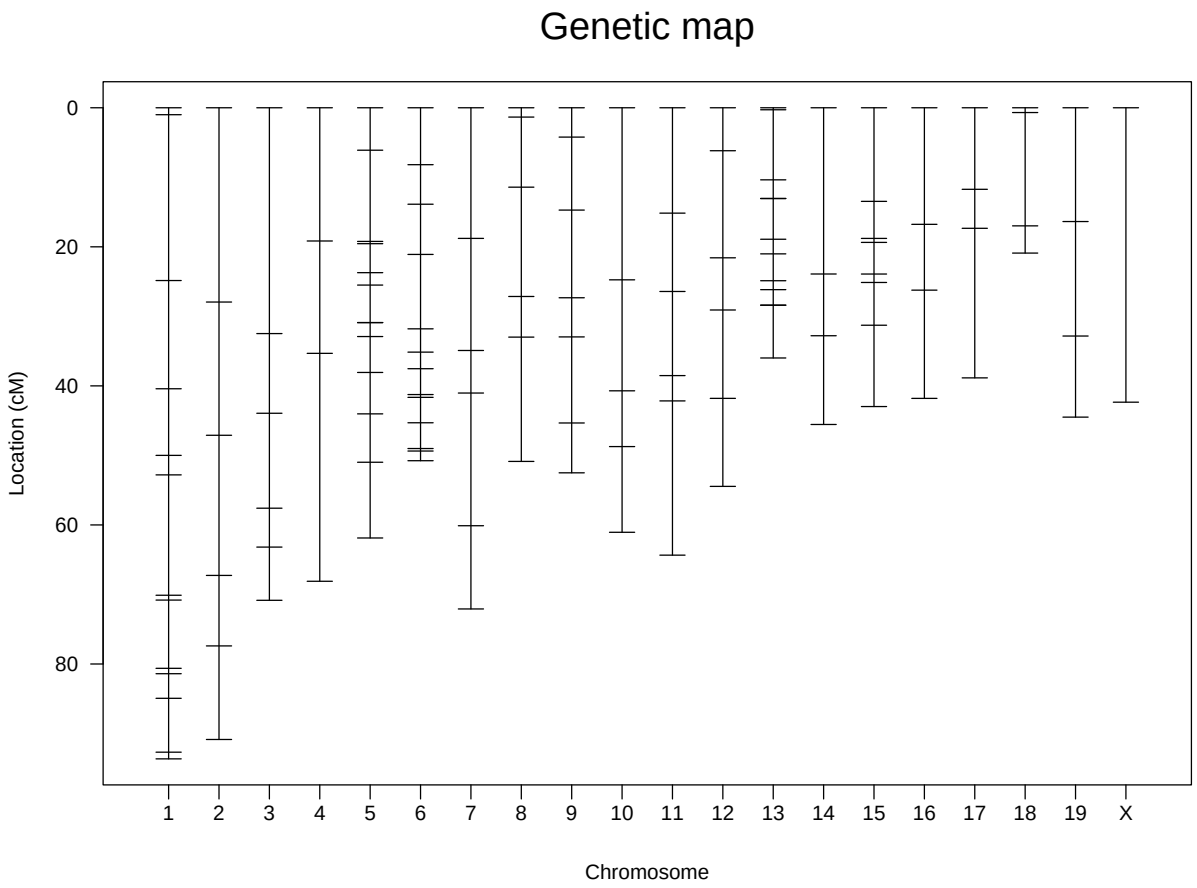


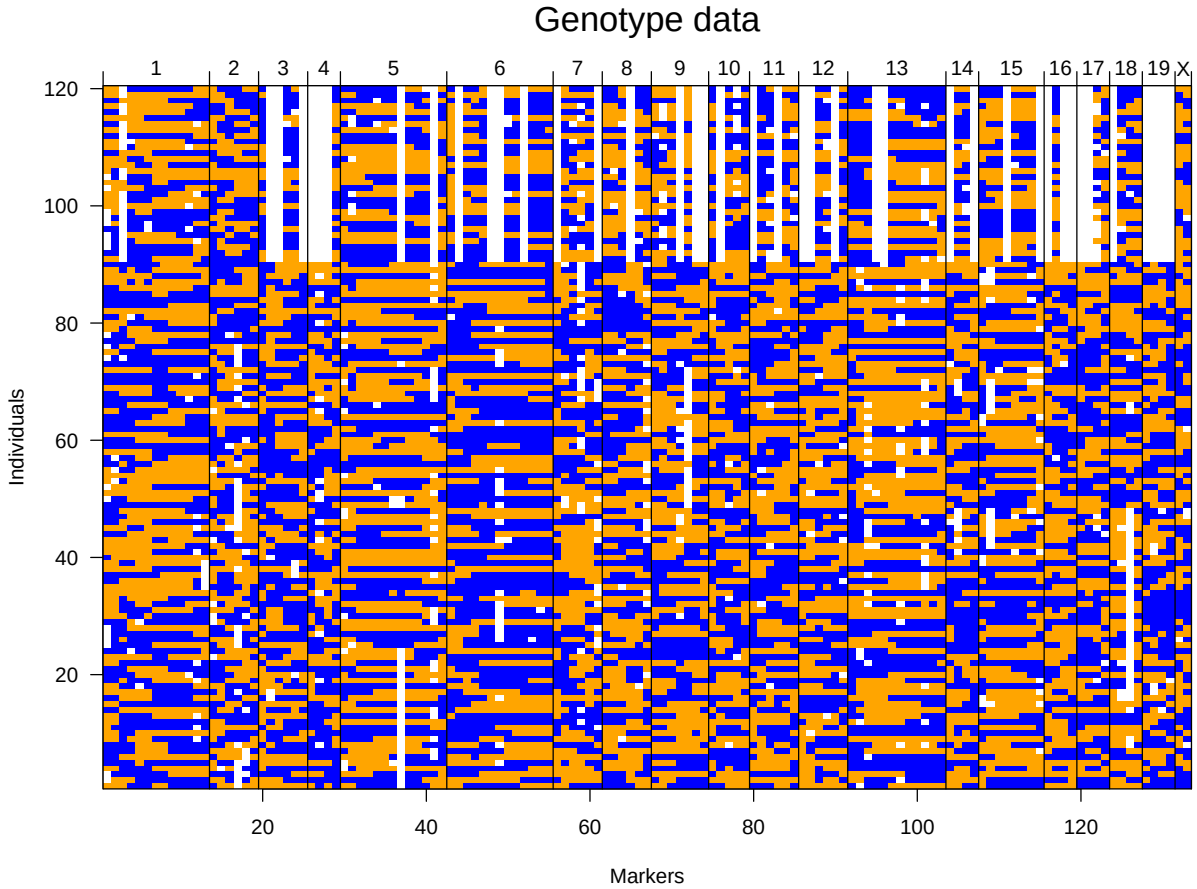
Data

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

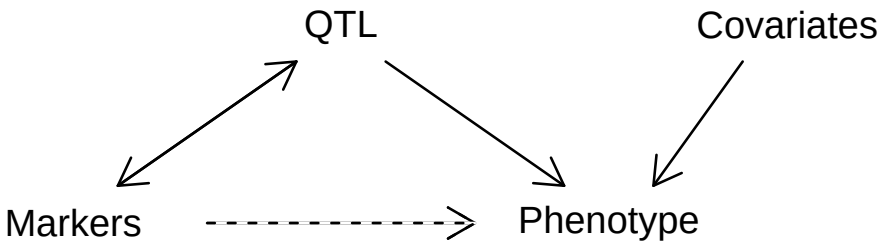

Genetic map
Location (cM)
0
20
40
60
80
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
X
Chromosome


Genotype data
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:

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

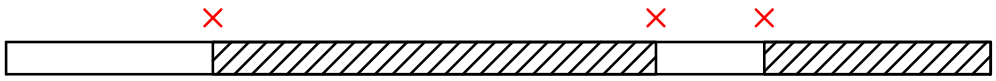
Models: Recombination

We assume no crossover interference.

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

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

Crossover locations



Crossover locations follow a Poisson process

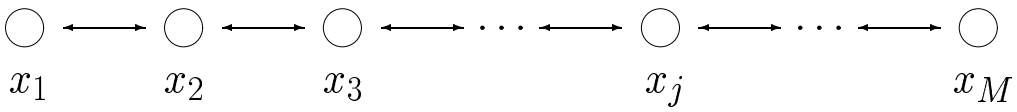
1. Number of XOs $\sim$ Poisson(L / 100)
   Locations of XOs (given number) $\sim$ iid uniform

2. Inter-XO distances are iid exponential(mean = 100 cM)

Note: In reality, crossovers are more spread out, but this no interference (NI) model is extremely convenient.

Marker genotypes

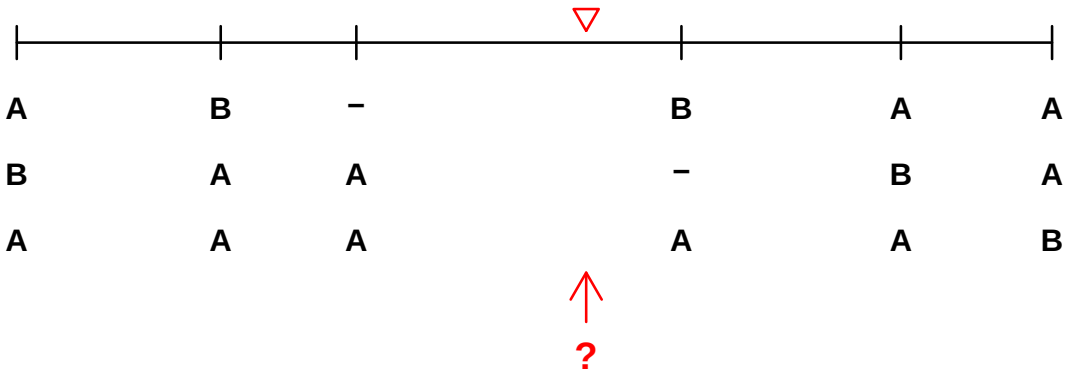
Under the no interference model,
the marker genotypes follow a Markov chain.



$$\Pr(x_{j+1} \mid x_j, x_{j-1}, \ldots, x_1) = \Pr(x_{j+1} \mid x_j)$$

The past and future are conditionally independent,
given the present.

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): $\sigma^2_g \equiv \sigma^2$

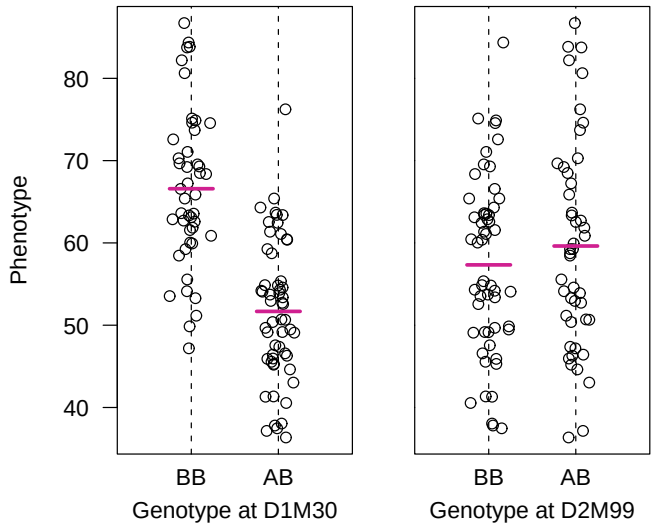
Normally distributed residual variation: $y|g \sim N(\mu_g, \sigma^2)$.

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

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



LOD score = $\log_{10}$ likelihood ratio comparing single-QTL model to "no QTL anywhere."

ANOVA at marker loci

Advantages

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

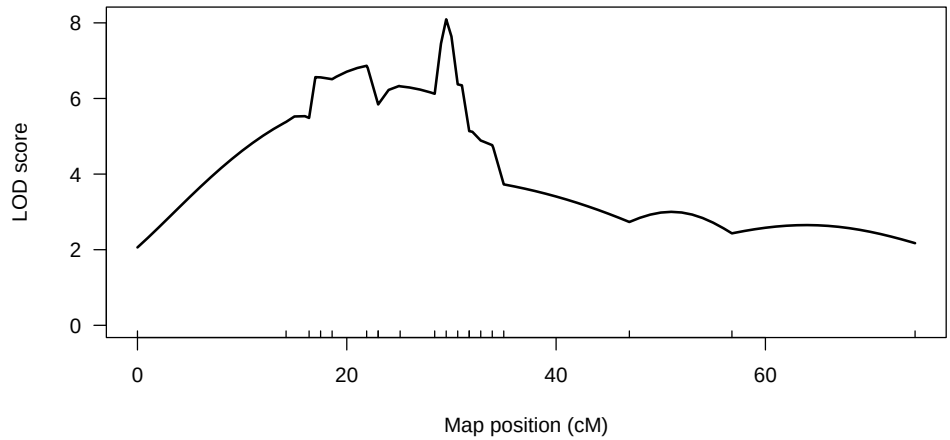
Disadvantages

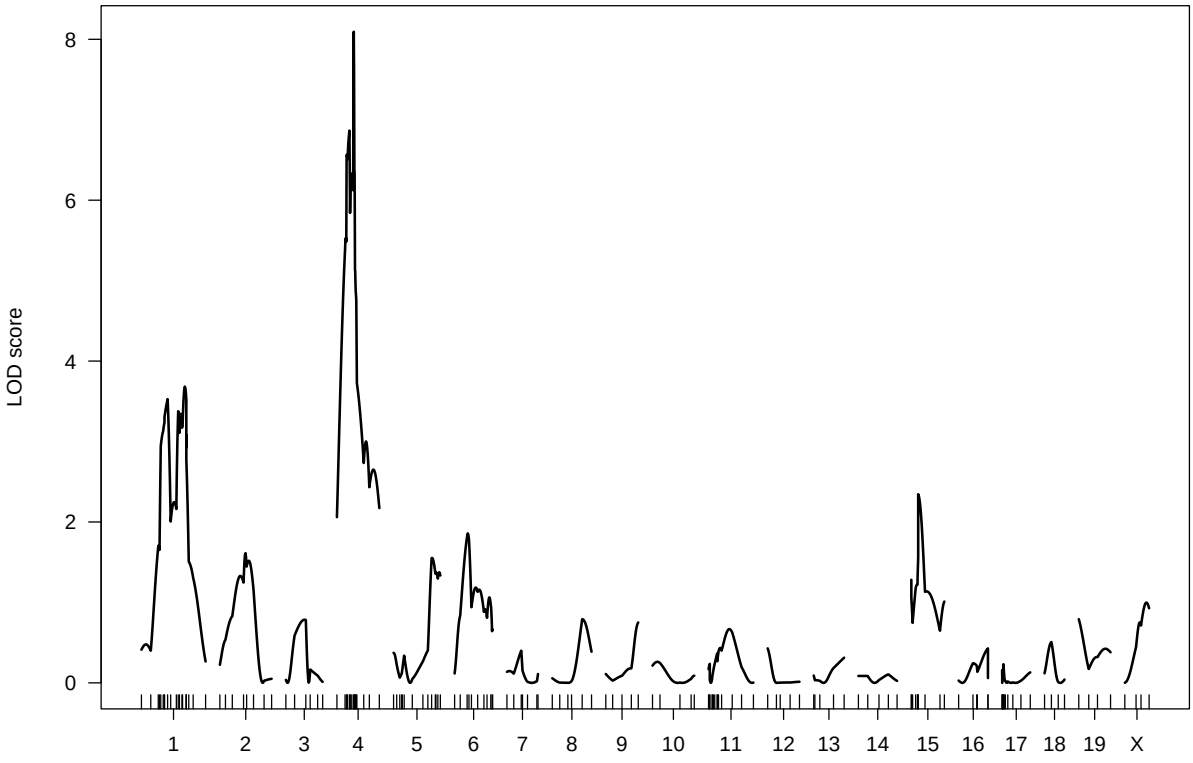
- Must exclude individuals with missing genotype data.
- Imperfect information about QTL location.
- Suffers in low density scans.
- Only considers one QTL at a time.

Interval mapping (IM)

Lander & Botstein (1989)

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





Interval mapping

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

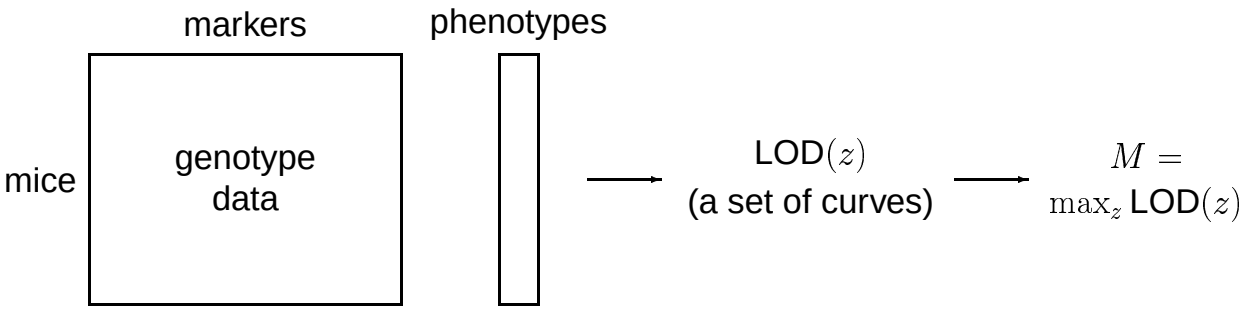
Large LOD scores indicate evidence for the presence of a QTL

Question: How large is large?

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

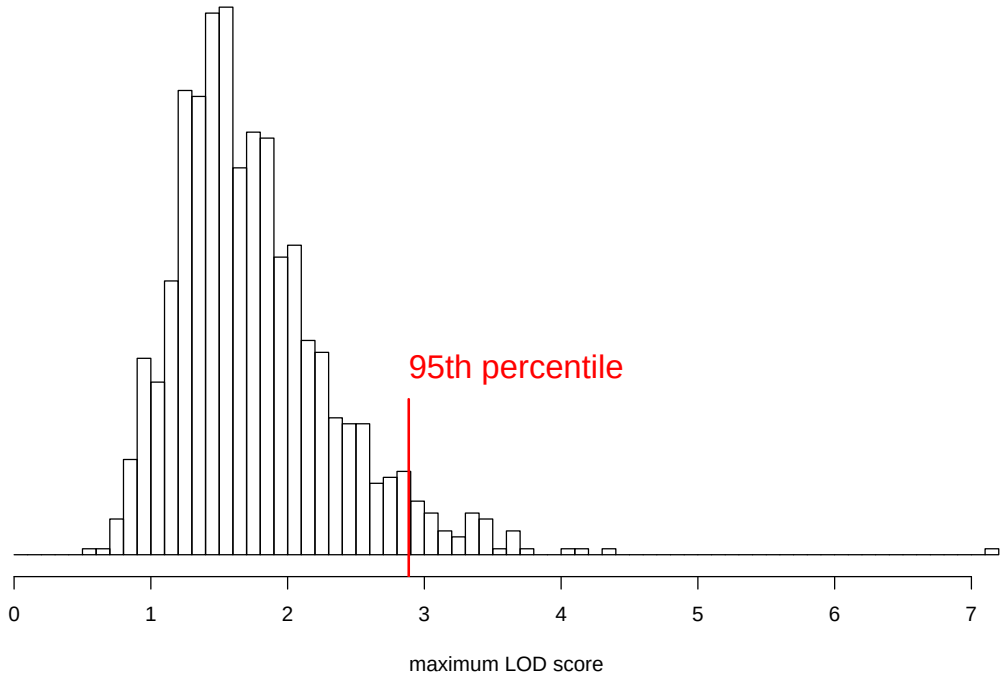
Derivation:
- Analytical calculations (L & B 1989)
- Simulations (L & B 1989)
- Permutation tests (Churchill & Doerge 1994)

Permutation tests



- Permute/shuffle the phenotypes; keep the genotype data intact.
- Calculate LOD$^\star(z) \longrightarrow M^\star = \max_z$ 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



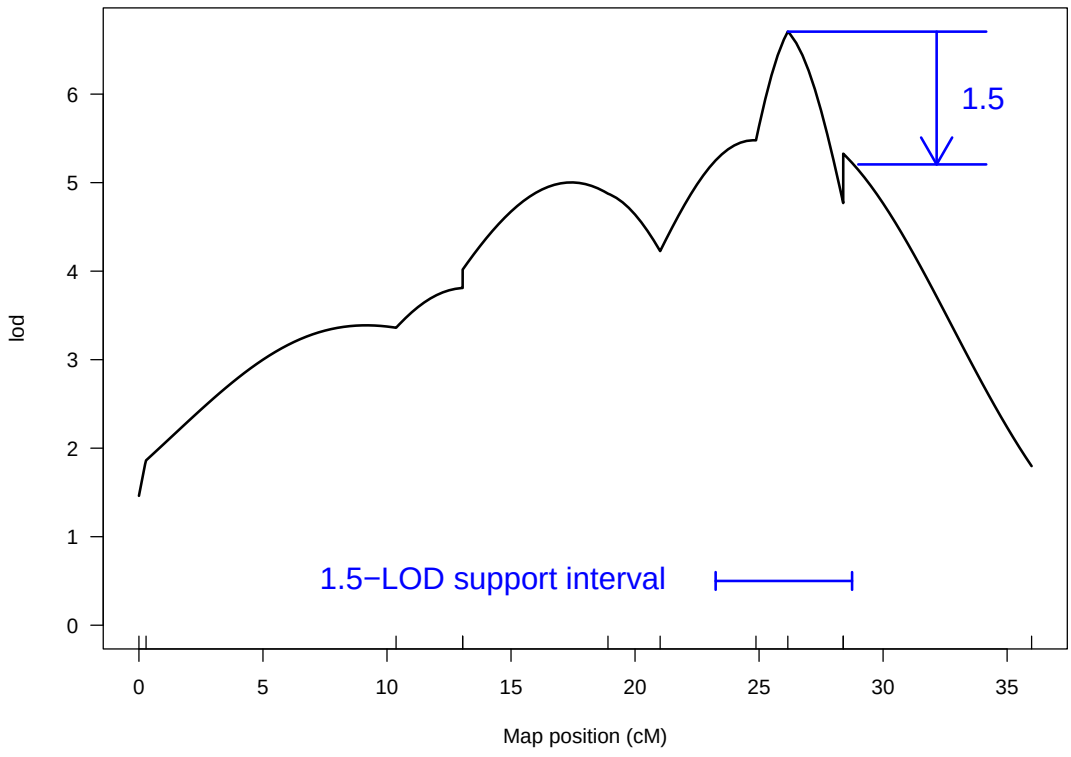
Confidence intervals for QTL location

Confidence interval indicates plausible location of a QTL.

Derivation:

- LOD support intervals
  (I prefer 1.5-LOD intervals.)
- Bootstrap
  (Resample individuals with replacement)

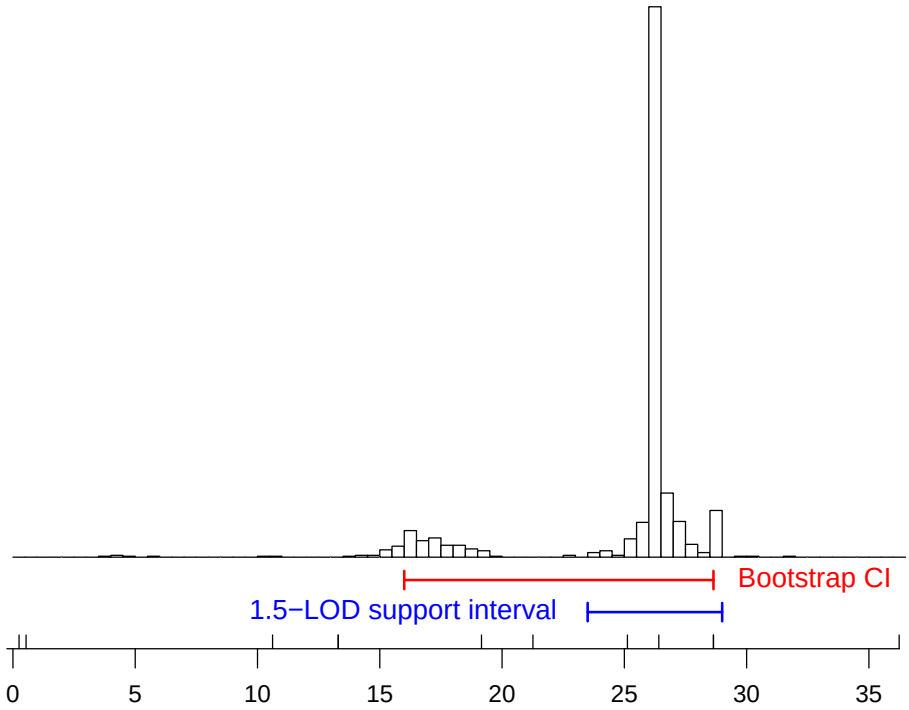
1.5-LOD support interval



Bootstrap-based confidence intervals

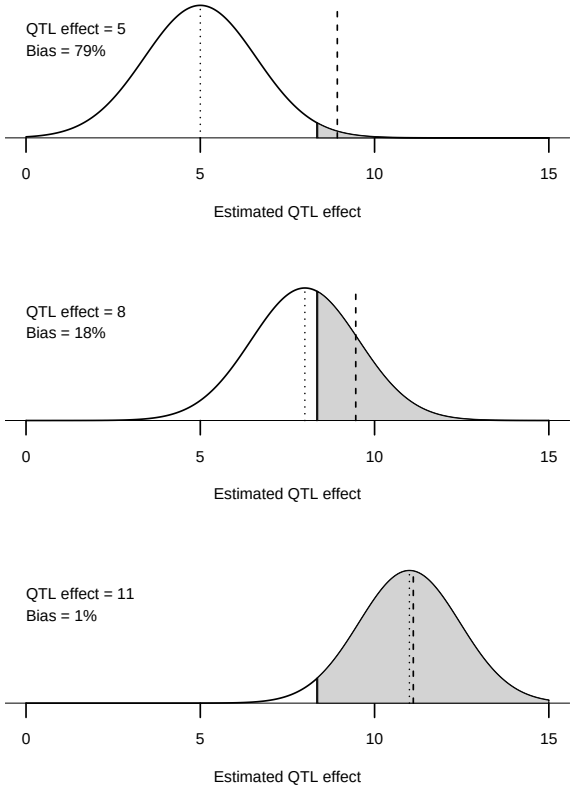
- Consider a set of n individuals (e.g., n = 124)
- Sample, with replacement, n individuals
  - Some individuals duplicated
  - Some individuals missing
- Using the sampled individuals, re-calculate the LOD curve for the chromosome of interest
- Identify the location of the maximum LOD score
- Repeat many times (e.g., 250)
- Bootstrap CI = (2.5 %ile to 97.5 %ile) of locations of maximum LOD.

Bootstrap confidence 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

Data structure

y = phenotypes

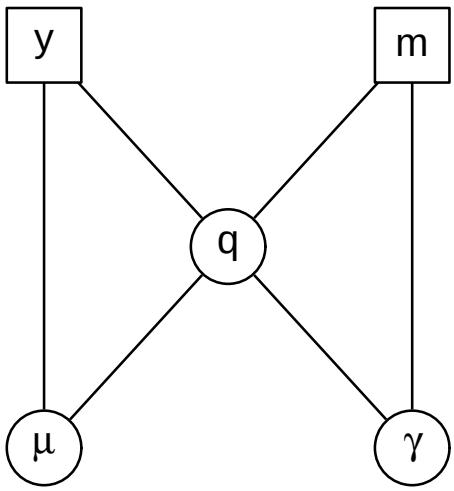
m = observed marker genotypes

q = unobserved QTL genotypes

μ = model parameters

γ = QTL locations

H = QTL model



Likelihood vs. Bayes

$$l(H, \mu, \gamma \mid y, m) = \sum_q \Pr(y, q \mid m, \mu, \gamma, H)$$

Likelihood:

$$l(H \mid y, m) = \max_{\mu,\gamma} l(H, \mu, \gamma \mid y, m)$$

Bayes:

$$\Pr(H, \mu, \gamma \mid y, m) \propto l(H, \mu, \gamma \mid y, m) \Pr(\mu, \gamma \mid H) \Pr(H)$$

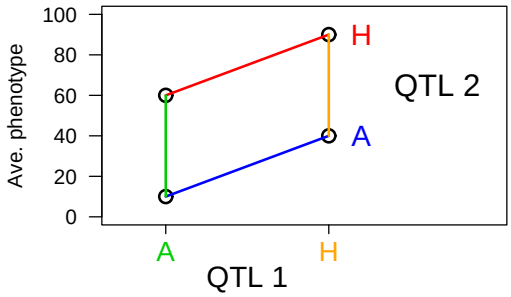
$$\Pr(H \mid y, m) = \iint \Pr(H, \mu, \gamma \mid y, m)\, d\mu\, d\gamma$$

Model selection

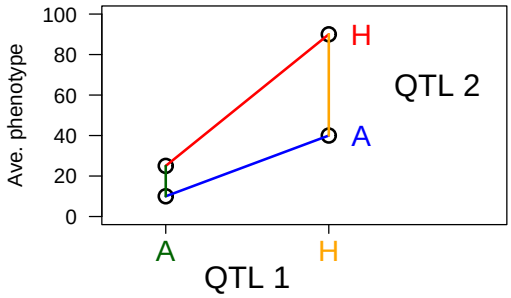
- Select class of models
  - Additive models
  - Add've plus pairwise interactions
  - Regression trees
- Search model space
  - Forward selection (FS)
  - Backward elimination (BE)
  - FS followed by BE
  - MCMC
- Compare models
  - Penalized likelihood (e.g., AIC, BIC)
  - Sequential permutation tests
  - Bayes (posterior probability)
- Assess performance
  - Maximize no. QTLs found; control false positive rate

Epistasis in a backcross

Additive QTLs

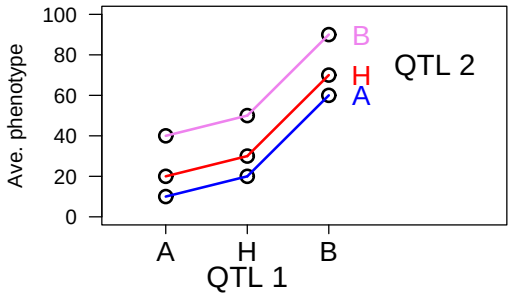


Interacting QTLs

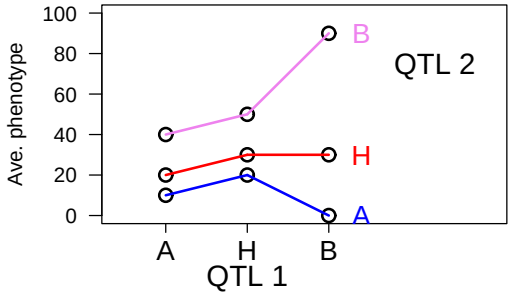


Epistasis in an intercross

Additive QTLs



Interacting QTLs



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

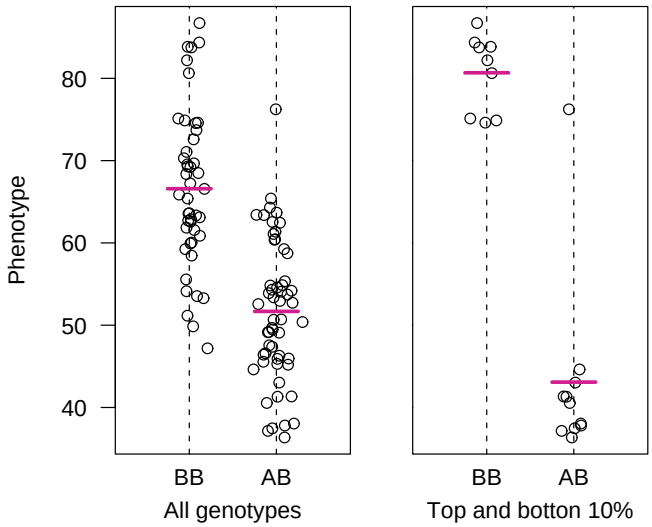
$(A \times B) \times (B \times A)$ or $(B \times A) \times (B \times A)$

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

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

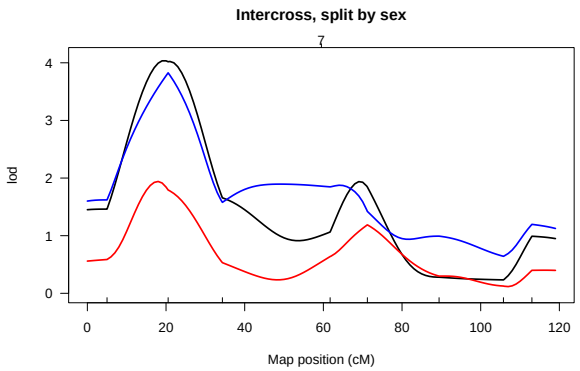
Selective genotyping

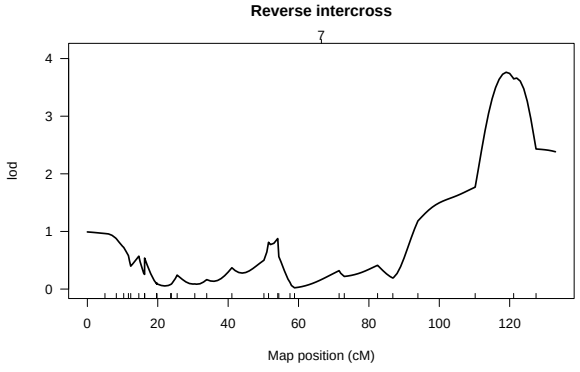
- Save effort by only typing the most informative individuals (say, top & bottom 10%).
- Useful in context of a single, inexpensive trait.
- Tricky to estimate the effects of QTLs: use IM with all phenotypes.
- Can't get at interactions.
- Likely better to also genotype some random portion of the rest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