QTL mapping in mice

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Outline

- Experiments, data, and goals
- Models
- ANOVA at marker loci
- Interval mapping
- LOD scores, LOD thresholds
- Mapping multiple QTLs
- Simulations
Backcross experiment

P1

A □A

P2

B □B

F1

A □B

BC

□□

□□

□□
Intercross experiment
Data and Goals

Phenotypes: \( y_i = \text{trait value for mouse } i \)

Genotypes: \( x_{ij} = 1/0 \) if mouse \( i \) is BB/AB at marker \( j \) (for a backcross)

Genetic map: Locations of markers

Goals:
- Identify the (or at least one) genomic regions (QTLs) that contribute to variation in the trait.
- Form confidence intervals for QTL locations.
- Estimate QTL effects.

Note: QTL = “quantitative trait locus”
Why?

Mice: Find gene

⇒ Drug targets, biochemical basis

Agronomy: Selection for improvement

Flies: Genetic architecture

⇒ Evolution
Genetic map

Chromosome Location (cM)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
X
Statistical structure

The missing data problem:
Markers $\leftrightarrow$ QTL

The model selection problem:
QTL, covariates $\rightarrow$ phenotype
Models: Recombination

We assume no crossover interference.

\[\rightarrow\] Points of exchange (crossovers) are according to a Poisson process.

\[\rightarrow\] The \(\{x_{ij}\}\) (marker genotypes) form a Markov chain.
Example
Models: Genotype $\leftrightarrow$ 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)

$$E(y|g) = \mu_{g_1,\ldots,g_p} \quad \text{var}(y|g) = \sigma^2_{g_1,\ldots,g_p}$$
Homoscedasticity (constant variance): \( \sigma^2_g \equiv \sigma^2 \)

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

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

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.
ANOVA at marker loci

Advantages

- Simple.
- Easily incorporate 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

![Graph showing LOD score against map position (cM)]
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 $\Pr(z = 1|\text{marker data})$:

<table>
<thead>
<tr>
<th>$M_1$</th>
<th>$M_2$</th>
<th>QTL genotype</th>
<th>$BB$</th>
<th>$AB$</th>
</tr>
</thead>
<tbody>
<tr>
<td>BB</td>
<td>BB</td>
<td>(1 − $r_L$)(1 − $r_R$)/(1 − $r$)</td>
<td>$r_Lr_R/(1 − r)$</td>
<td></td>
</tr>
<tr>
<td>BB</td>
<td>AB</td>
<td>(1 − $r_L$)$r_R/r$</td>
<td>$r_L(1 − r_R)/r$</td>
<td></td>
</tr>
<tr>
<td>AB</td>
<td>BB</td>
<td>$r_L(1 − r_R)/r$</td>
<td>(1 − $r_L$)$r_R/r$</td>
<td></td>
</tr>
<tr>
<td>AB</td>
<td>AB</td>
<td>$r_Lr_R/(1 − r)$</td>
<td>(1 − $r_L$)(1 − $r_R$)/(1 − $r$)</td>
<td></td>
</tr>
</tbody>
</table>
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 \( f(y; \mu, \sigma) = \text{density of normal distribution} \)

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 \( \mu_0, \mu_1, \sigma \):

EM algorithm.
LOD scores

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

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

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

\( \hat{\mu}_{0z}, \hat{\mu}_{1z}, \hat{\sigma}_z \) 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.
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 $\text{LOD}^*(z) \rightarrow M^* = \max_z \text{LOD}^*(z)$
- We wish to compare the observed $M$ to the distribution of $M^*$.
- $\Pr(M^* \geq M)$ is a genome-wide P-value.
- The 95th %ile of $M^*$ 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

95th percentile

maximum LOD score
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
Abstractions / simplifications

- Complete marker data
- QTLs are at the marker loci
- QTLs act additively
The problem

n backcross mice; M markers

\[ x_{ij} = \text{genotype } (1/0) \text{ of mouse } i \text{ at marker } j \]

\[ y_i = \text{phenotype (trait value) of mouse } i \]

\[ y_i = \mu + \sum_{j=1}^{M} \Delta_j x_{ij} + \epsilon_i \]

Which \( \Delta_j \neq 0? \)

\[ \rightarrow \quad \text{Model selection in regression} \]
How is this problem different?

- Relationship among the x’s
- Find a good model vs. minimize prediction error
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**
  - \( \text{BIC}_\delta(\gamma) = \log \text{RSS}(\gamma) + |\gamma| \left( \delta \frac{\log n}{n} \right) \)
  - Sequential permutation tests
  - Estimate of prediction error

- **Assess performance**
  - Maximize no. QTLs found; control false positive rate
Why $\text{BIC}_\delta$?

- For a fixed no. markers, letting $n \to \infty$, $\text{BIC}_\delta$ is consistent.

- There exists a prior (on models + coefficients) for which $\text{BIC}_\delta$ is the $-\log$ posterior.

- $\text{BIC}_\delta$ is essentially equivalent to use of a threshold on the conditional LOD score.

- It performs well.
Choice of $\delta$

Smaller $\delta$: include more loci; higher false positive rate
Larger $\delta$: include fewer loci; lower false positive rate

Let $L = 95\%$ genome-wide LOD threshold
(compare single-QTL models to the null model)

Choose $\delta = 2 \frac{L}{\log_{10} n}$

With this choice of $\delta$, in the absence of QTLs, we’ll include at least one extraneous locus, 5% of the time.
Simulations

- Backcross with n=250
- No crossover interference
- 9 chr, each 100 cM
- Markers at 10 cM spacing; complete genotype data
- 7 QTLs
  - One pair in coupling
  - One pair in repulsion
  - Three unlinked QTLs
- Heritability = 50%
- 2000 simulation replicates
Methods

- ANOVA at marker loci
- Composite interval mapping (CIM)
- Forward selection with permutation tests
- Forward selection with BIC$_\delta$
- Backward elimination with BIC$_\delta$
- FS followed by BE with BIC$_\delta$
- MCMC with BIC$_\delta$

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A selected marker is deemed correct if it is within 10 cM of a QTL (i.e., correct or adjacent)
QTLs linked in coupling

Ave no. chosen

ANOVA

CIM

fs, perm

fs

be

fs/be

mcmc

BIC
QTLs linked in repulsion

Ave no. chosen

ANOVA 3 5 7 9 11 CIM fs, perm fs be fs/be mcmc BIC
QTL mapping is a model selection problem.

Key issue: the comparison of models.

Large-scale simulations are important.

More refined procedures do not necessarily give improved results.

$\text{BIC}_\delta$ with forward selection followed by backward elimination works quite well (in the case of additive QTLs).
Acknowledgements

Terry Speed, University of California, Berkeley, and WEHI

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Saunak Sen, University of California, San Francisco
References

  Review for non-statisticians

  Older, more statistical review.

  The seminal paper.

  LOD thresholds by permutation tests.

  A reasonably good book on model selection in regression.

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


   Contains the simulation study described above.