Introduction to QTL mapping
in model organisms

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Outline

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

Intercross experiment
• Within each of the parental and F₁ 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.

Phenotype distributions

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

Markers vs. Location (cM)

Genotype data

Individuals vs. 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:

Markers $\leftrightarrow$ QTL

The model selection problem:

QTL, covariates $\rightarrow$ phenotype
We assume no crossover interference.

⇒ Points of exchange (crossovers) are according to a Poisson process.

⇒ 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)

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

---

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

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

![Phenotype vs Genotype at D1M30 and D2M99](image)

**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

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>
</tr>
</thead>
<tbody>
<tr>
<td>BB</td>
<td>BB</td>
<td>$(1 - r_L)(1 - r_R)/(1 - r)$</td>
</tr>
<tr>
<td>BB</td>
<td>AB</td>
<td>$(1 - r_L)r_R/r$</td>
</tr>
<tr>
<td>AB</td>
<td>BB</td>
<td>$r_L(1 - r_R)/r$</td>
</tr>
<tr>
<td>AB</td>
<td>AB</td>
<td>$r_L r_R/(1 - r)$</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 shows 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 $f(y; \mu, \sigma) = \exp[-(y - \mu)^2/(2\sigma^2)]/\sqrt{2\pi\sigma^2}$

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

Dempster et al. (1977)

E step:
Let \( w^{(k+1)} = \Pr(z_i = 1|y_i, \text{marker data}, \hat{\mu}_0^{(k)}, \hat{\mu}_1^{(k)}, \hat{\sigma}^{(k)}) \)

\[
= \frac{p_i f(y_i; \hat{\mu}_1^{(k)}, \hat{\sigma}^{(k)})}{p_i f(y_i; \hat{\mu}_1^{(k)}, \hat{\sigma}^{(k)}) + (1 - p_i) f(y_i; \hat{\mu}_0^{(k)}, \hat{\sigma}^{(k)})}
\]

M step:
Let \( \hat{\mu}_1^{(k+1)} = \frac{\sum_i y_i w_i^{(k+1)}}{\sum_i w_i^{(k+1)}} \)

\( \hat{\mu}_0^{(k+1)} = \frac{\sum_i y_i (1 - w_i^{(k+1)})}{\sum_i (1 - w_i^{(k+1)})} \)

\( \hat{\sigma}^{(k+1)} = [\text{not worth writing down}] \)

The algorithm:
Start with \( w_i^{(1)} = p_i \); iterate the E & M steps until convergence.

Example

<table>
<thead>
<tr>
<th>Iteration</th>
<th>( \hat{\mu}_0 )</th>
<th>( \hat{\mu}_1 )</th>
<th>( \hat{\sigma} )</th>
<th>log likelihood</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.903</td>
<td>6.492</td>
<td>1.668</td>
<td>−770.752</td>
</tr>
<tr>
<td>2</td>
<td>5.835</td>
<td>6.562</td>
<td>1.654</td>
<td>−770.291</td>
</tr>
<tr>
<td>3</td>
<td>5.818</td>
<td>6.579</td>
<td>1.651</td>
<td>−770.264</td>
</tr>
<tr>
<td>4</td>
<td>5.815</td>
<td>6.583</td>
<td>1.650</td>
<td>−770.262</td>
</tr>
<tr>
<td>( \infty )</td>
<td>5.813</td>
<td>6.584</td>
<td>1.649</td>
<td>−770.262</td>
</tr>
</tbody>
</table>
LOD scores

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

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

\[
= \log_{10} \left\{ \frac{\Pr(y|\text{QTL at } \gamma, \hat{\mu}_0, \hat{\mu}_1, \hat{\sigma})}{\Pr(y|\text{no QTL, } \hat{\mu}, \hat{\sigma})} \right\}
\]

\(\hat{\mu}_0, \hat{\mu}_1, \hat{\sigma}\) are the MLEs, assuming a single QTL at position \(\gamma\).

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.
Haley-Knott regression

A quick approximation to Interval Mapping.

\[
E(y \mid QTL = q) = \mu + \beta 1\{q = AB\}
\]

\[
E(y \mid \text{marker data}) = \mu + \beta \Pr(QTL = AB \mid \text{marker data})
\]

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

\[
\text{LOD}(\gamma) = \left(\frac{n}{2}\right) \log_{10} \left\{ \frac{\text{RSS}_0}{\text{RSS}_a(\gamma)} \right\}
\]

Example
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 $\text{LOD}^*(z) \rightarrow M^* = \max_z \text{LOD}^*(z)$
- 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**

![Histogram of maximum LOD scores with 95th percentile indicated]
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

Two-dimensional genome scan

Consider each pair of positions, \((\gamma_1, \gamma_2)\)

Models
- Full
- Additive
- QTL 1
- QTL 2
- Null

Possible comparisons
- Full vs. null
- Full vs. additive
- Full vs. Best of QTL 1 & 2
- Add’ve vs. Best of QTL 1 & 2
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
  - $\text{BIC}_\delta(\gamma) = \log \text{RSS}(\gamma) + |\gamma| (\delta \log \frac{n}{n})$
  - Sequential permutation tests

- Assess performance
  - Maximize no. QTLs found; control false positive rate
What's different here?

- Association among the covariates.
- Missing covariate information.
- Find the model rather than minimize prediction error.

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.

\[(A \times B) \times (A \times B) \text{ or } (B \times A) \times (A \times B)\]

Females: \(X_{A\cdot B} X_A\)
Males: \(X_{A\cdot B} Y_B\)

\[(A \times B) \times (B \times A) \text{ or } (B \times A) \times (B \times A)\]

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

  A review for non-statisticians.
  A very recent review.
  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.
  Chapter on QTL mapping.
  The seminal paper.

  LOD thresholds by permutation tests.
  Non-parameteric interval mapping.
  QTL mapping with a special model for a non-normal phenotype.
  A good book on model selection in regression.
  An old but excellent general genetics textbook with a very interesting discussion of epistasis.
R/qtl: An extensible QTL mapping environment

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Why R/qtl?

• Interactive QTL mapping environment.
• Allow user to focus on modeling rather than computing.
• Embedded within general data analysis environment, R.
• Access to a variety of QTL mapping approaches, including sophisticated multiple QTL methods (soon, anyway).
• Includes functions for estimating genetic maps, identifying genotyping errors, and visualizing data.
• Easy extensibility for use with specialized crosses or specially-tailored models.
• Available for Unix, Windows, and MacOS.
About R

• **Open-source** implementation of the S language. (Like S-PLUS, and sort of like Matlab, but free.)

• Language and environment for statistical computing and graphics.

• Provides a wide variety of statistical and graphical techniques (including linear and nonlinear modelling, statistical tests, time series analysis, classification, clustering).

• Available for UNIX, Windows and MacOS.

Functionality

**Currently**

- Analysis of intercross, backcross and 4-way cross.
- One- and two-dimensional scans by interval mapping, imputation and Haley-Knott regression, with covariates.
- Permutation tests.
- Re-estimation of linkage map.
- “Ripple” marker order.
- Calculation of Lincoln & Lander error LOD scores.
- Visualization of genotype data.

**Soon**

- AILs, RIs, and more complex types of crosses.
- Analysis of multiple QTL models (by MIM or imputation).
- Sophisticated model search techniques.
- Advanced phenotype models, such as generalized linear models or Cox models
- Analysis of (and under) crossover interference.
- Graphical user interface (GUI)