Traditional approach

F2/BC $\rightarrow$ QTL $\rightarrow$ congenic $\rightarrow$ subcongenics

$\rightarrow$ • coding/regulatory polymorphism
• expression/function difference
• knock-in / transgenic
• knock-out
• homology to other species

Issues: • Large QTL regions
• Time consuming and expensive
Advanced intercross lines

- Perform intercross with two inbred strains.
- Perform several generations of random mating, avoiding inbreeding.
- Genotype and phenotype individuals from the final generation.
Advanced intercross lines

AIL

Advantages
- Many more breakpoints $\implies$ more precise mapping
- Straightforward to create

Disadvantages
- Time and cost
- Each individual genetically distinct
- Useful largely for fine-mapping known loci
- Relationships among individuals in final generation
Sibships at F₈

AIL: percent matching genotypes
Analysis of AIL

- One generally treats the AIL as an intercross, but with an expanded genetic map. [Longer map $\implies$ larger significance threshold.]

- However, the relationships among the AIL individuals makes analysis tricky.

- To account (at least) for the sibships at the final generation:

  \[ y_{ij} = \mu + \beta q_{ij} + \epsilon_{ij} \]

  for sib \( j \) in sibship \( i \), with \( \text{cov}(\epsilon_{ij}, \epsilon_{ij}') = \tau^2 \) and \( \text{var}(\epsilon_{ij}) = \tau^2 + \sigma^2 \).

- Standard permutation tests are no longer appropriate.

  If possible, reconstruct the parental haplotypes (for the previous generation) and permute them. Alternatively, simulate the whole pedigree.
Imagine numerous, small-effect, unlinked, additive, background QTL.

\[ y_{ij} = \mu + \beta q_{ij} + \sum_k \alpha_k a_{ijk} + \epsilon_{ij} = \mu + \beta q_{ij} + \gamma_{ij} + \epsilon_{ij} \]

\( \gamma_{ij} = \text{combined effect of all background QTL} \)

→ correlated between siblings (or half-siblings)
RIL

Advantages
- High density of breakpoints
- Just genotype once
- Phenotype multiple individuals to reduce environmental/individual variation
- Multiple phenotypes on the same genomes
- Longitudinal phenotypes
- Genotype × environment interactions

Disadvantages
- Time-consuming, expensive to create
- Available panels generally too small
- Only homozygotes

Analysis of RIL

Available methods for analysis of RIL are rather rudimentary:

Treat like a backcross, working with the line averages.

Map expansion: $R = 4r/(1 + 6r)$

Let $y_{ij}$ be the phenotype for individual $j$ in line $i$.
Let $x_i = 1$ or 0, genotype of line $i$ at putative QTL.

Assume $y_{ij} = \mu + \beta x_i + \gamma_i + \epsilon_{ij}$ where $\epsilon_{ij} \sim N(0, \sigma_e^2)$, $\gamma_i \sim N(0, \sigma_p^2)$.

Residual heritability: $h_p^2 = \sigma_p^2/(\sigma_p^2 + \sigma_e^2)$

Then we can work with the strain averages, but they should be weighted by the sample sizes.

$\text{var}(\bar{y}_i) = \sigma_p^2 + \sigma_e^2/n_i \quad \implies \text{weight inversely proportional to } h_p^2 + (1 - h_p^2)/n_i$
**RIL: One QTL**

![Graph showing one QTL](image)

**RIL: Many QTL**

![Graph showing many QTL](image)
Analysis of RIX

- Correlations among RIX: shared paternal/maternal chromosomes
  \[\rightarrow\] Mixed-effects model, as with AIL (but it’s more clear here).

- Permutation test:

  Permute the parental genotypes and pass them down to the RIX.
Collaborative Cross

CC genome
Analysis of CC

• First, reconstruct haplotypes.

Map expansion: \( R = \frac{7r}{1 + 6r} \)

• Further analysis just like RIL, but now 8 alleles.

• Epistasis: consider 64 possible two-locus genotypes.

Random effects model?

Heterogeneous stock
Advantages
- Super-dense breakpoints
- Many alleles
- Heterozygous

Disadvantages
- Must be satisfied with what is available
- Inbreeding: loss of alleles
- Each individual unique
- Like AIL, maybe best for fine-mapping known loci
- Like AIL, relationships at last generations

Analysis of HS
- Reconstruction of haplotypes
- Treatment of the 8 alleles (and so 36 genotypes)
  - Additive alleles
  - Random effect
- Dealing with the relationships
- Establishing statistical significance?
Association mapping

- Phenotype available inbred strains
- Make use of available SNP data
- Need to account for the correlations among strains
- Likely want to work with haplotypes rather than just individual SNPs
- Be careful about wild-derived strains

Advantages
- Once you’ve done a strain survey, no further data needed
- Potentially very high resolution

Disadvantages
- All the usual problems with association mapping
- Power is unpredictable
- How to account for relationships among strains?
These approaches have many similarities.

Key differences:

- CC, HS: pattern of association along chromosomes by design
- HS: each individual unique
### CSS

#### Advantages
- Just phenotyping can get you to the chromosomes
- Eliminate the effects of other QTL
- Easy to create congenics

#### Disadvantages
- Time-consuming, expensive to create
- Lots of phenotyping required
- Cannot see interactions

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### Analysis of CSS

- Compare each CSS to recipient strain via t test.
- Account for multiple (dependent) tests
  - Easy with a permutation test
Summary

• Traditional approach

• Advanced intercross lines (AIL)

• Recombinant inbred lines (RIL)

• RIX

• Collaborative cross (CC)

• Heterogeneous stock (HS)

• Association mapping

• Chromosome substitution strains (CSS)

References

  A book everyone doing mouse genetics should own.

  Describes a view on sufficient proof that a candidate locus is responsible for the effect of a QTL.

  Proposed AIL.

  Discusses choice of number of individuals per strain to phenotype in RIL.

  Proposes the collaborative cross; discusses RIX.

• Tsaih SW, et al. (2005) Quantitative trait mapping in a diallel cross of recombinant inbred lines. Mamm Genome 16:344–355 Analysis issues concerning RIX.


