The genomes of recombinant inbred lines

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C57BL/6
Recombinant inbred lines
(by sibling mating)

Advantages of RI lines

• Each strain is an eternal resource.
  – Only need to genotype once.
  – Reduce individual variation by phenotyping multiple individuals from each strain.
  – Study multiple phenotypes on the same genotype.

• Greater mapping precision.
  – More dense breakpoints on the RI chromosomes.
Recombinant inbred lines
(by sibling mating)

Recombinant inbred lines
(by selfing)
The “Collaborative Cross”

Genome of an 8-way RI
The goal

• Characterize the breakpoint process along a chromosome in 8-way RILs.
  – Understand the two-point haplotype probabilities.
  – Study the clustering of the breakpoints, as a function of crossover interference in meiosis.

Why?

• It’s interesting.
• Later statistical analyses will require:
  – The two-point probabilities.
  – A model for the whole process.

Actually, we’ll probably just assume that:
  – The breakpoints follow a Poisson process.
  – The genotypes follow a Markov chain.
2 points in an RIL

1  2

- \( r \) = recombination fraction = probability of a recombination in the interval in a random meiotic product.

- \( R \) = analogous thing for the RIL = probability of different alleles at the two loci on a random RIL chromosome.

Haldane & Waddington 1931

INBREEDING AND LINKAGE

J. B. S. HALDANE AND C. H. WADDINGTON
John Innes Horticultural Institution, London, England

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When a heterozygous population is self-fertilized or inbred the ultimate result (apart from effects of mutation) is complete homozygosis. The final proportions of the various genotypes are usually independent of the system of inbreeding adopted, although, as Jennings (1916) and others have shown, the speed at which equilibrium is approached is greater in the case of self-fertilization than of brother-sister mating, and so on.
Recombinant inbred lines
(by selfing)

Equations for selfing

\[ C_n = C_0 + \frac{1}{4} E_0 + \frac{1}{4} (1 - \beta - \delta + \delta) F_0 + \frac{1}{4} \delta G_0 \]
\[ D_n = D_0 + \frac{1}{4} E_0 + \frac{1}{4} \delta F_0 + \frac{1}{4} (1 - \beta - \delta + \delta) G_0 \]
\[ E_n = \frac{1}{4} E_0 + \frac{1}{4} (\delta - \delta + \delta - \delta) (F_0 + G_0) \]
\[ F_n = \frac{1}{2} (1 - \beta - \delta + \delta) F_0 + \frac{1}{4} \delta G_0 \]
\[ G_n = \frac{1}{2} \delta F_0 + \frac{1}{4} (1 - \beta - \delta + \delta) G_0 \]

Put \( y = D_n \) (the final proportion of crossover zygotes)
\[ \therefore C_n + D_n = 1, C_n - D_n = c_n \quad \therefore y = \frac{1}{2} (1 - c_n) \]
\[ \therefore y = \frac{-2x}{1 + 2x} \]
Absorption probabilities

Let \( P_{ij} = \Pr(X_{n+1} = j \mid X_n = i) \) where \( X_n \) = state at generation \( n \).

Consider the case of absorption into the state AA|AA.

Let \( h_i = \) probability, starting at \( i \), eventually absorbed into AA|AA.

Then \( h_{AA|AA} = 1 \) and \( h_{AB|AB} = 0 \).

Condition on the first step: \( h_i = \sum_k P_{ik} h_k \)

For selfing, this gives a system of 3 linear equations.

Recombinant inbred lines
(by sibling mating)

[Diagram of recombinant inbred lines]
Equations for sib-mating

\[
\begin{align*}
C_{a1} &= a_1 + \frac{1}{6}(a_1^2 + a_2^2 + 2a_1a_2) + \frac{1}{6}(b_1^2 + b_2^2 + 2b_1b_2)
\end{align*}
\]

Result for sib-mating

Omitting some rather tedious algebra, the solution of these equations is:

\[
\begin{align*}
\lambda &= \frac{q}{2 - 3q}, \\
\mu &= \frac{-2q}{2 - 3q}, \\
\theta &= \frac{2q}{2 - 3q}, \\
\kappa &= \frac{1}{2 - 3q}
\end{align*}
\]

as may easily be verified.

\[
\begin{align*}
c &= c_s + 2a + \frac{1}{6}(1 - 2x)(d_3 + 2L + 2l_3 + \frac{1}{2}k_4) \\
&\quad + 2g + 4x(b_4 + l_4)
\end{align*}
\]

and \(y = \frac{1}{2}(1 - c_s)\).

In the case considered, \(d_3 = 1\); \(c_s = c_d = 1 - 2x/1 + 6x\). Hence the proportion of crossover zygotes \(y = 4x/1 + 6x\).
The “Collaborative Cross”

8-way RILs

**Autosomes**

- Pr(G₁ = i) = 1/8
- Pr(G₂ = j | G₁ = i) = r / (1+6r) for i ≠ j
- Pr(G₂ ≠ G₁) = 7r / (1+6r)

**X chromosome**

- Pr(G₁=A) = Pr(G₁=B) = Pr(G₁=E) = Pr(G₁=F) = 1/6
- Pr(G₁=C) = 1/3
- Pr(G₂=B | G₁=A) = r / (1+4r)
- Pr(G₂=C | G₁=A) = 2r / (1+4r)
- Pr(G₂=A | G₁=C) = r / (1+4r)
- Pr(G₂ ≠ G₁) = (14/3) r / (1+4r)
Computer simulations

\[ R = \frac{7r}{(1+6r)} \]

\[ R = \frac{(14/3)r}{(1+4r)} \]

The X chromosome
3-point coincidence

\[ \begin{array}{c|c|c} 1 & 2 & 3 \\ \hline \end{array} \]

- \( r_{ij} = \) recombination fraction for interval \( i,j \);
  - assume \( r_{12} = r_{23} = r \)
- Coincidence = \( c = \frac{\text{Pr(double recombinant)}}{r^2} = \frac{\text{Pr(rec'\text{'}n in 23 | rec'\text{'}n in 12)}}{\text{Pr(rec'\text{'}n in 23)}} \)
- No interference \( \rightarrow = 1 \)
  - Positive interference \( \rightarrow < 1 \)
  - Negative interference \( \rightarrow > 1 \)
- Generally \( c \) is a function of \( r \).

3-points in 2-way RILs

\[ \begin{array}{c|c|c} 1 & 2 & 3 \\ \hline \end{array} \]

- \( r_{13} = 2 r (1 - c r) \)
- \( R = f(r); \quad R_{13} = f(r_{13}) \)
- \( \text{Pr(double recombinant in RIL)} = \frac{\{ R + R - R_{13} \}}{2} \)
- \( \text{Coincidence (in 2-way RIL)} = \frac{\{ 2 R - R_{13} \}}{\{ 2 R^2 \}} \)
Coincidence

No interference

Coincidence

Coincidence
Why the clustering of breakpoints?

- The really close breakpoints occur in different generations.
- Breakpoints in later generations can occur only in regions that are not yet fixed.
- The regions of heterozygosity are, of course, surrounded by breakpoints.

Coincidence in 8-way RILs

- The trick that allowed us to get the coincidence for 2-way RILs doesn’t work for 8-way RILs.
- It’s sufficient to consider 4-way RILs.
- Calculations for 3 points in 4-way RILs is still astoundingly complex.
  - 2 points in 2-way RILs by sib-mating: 55 parental types → 22 states by symmetry
  - 3 points in 4-way RILs by sib-mating: 2,164,240 parental types → 137,488 states
- Even counting the states was difficult.
Coincidence

![Graph showing coincidence over R values](image)

Whole genome simulations

- 2-way selfing, 2-way sib-mating, 8-way sib-mating
- Mouse-like genome, 1665 cM
- Strong positive crossover interference
- Inbreed to complete fixation
- 10,000 simulation replicates
No. generations to fixation

- 2-way selfing
- 2-way sib-mating
- 8-way sib-mating

mean = 10.5
mean = 35.6
mean = 38.9

No. gen’s to 99% fixation

- 2-way selfing
- 2-way sib-mating
- 8-way sib-mating

mean = 8.0
mean = 23.5
mean = 26.7
Percent genome not fixed

Number of segments
Segment lengths

median = 6.5 cM

median = 12.9 cM

median = 23.7 cM

2-way selfing
2-way sib-mating
8-way sib-mating

Two chromosomes
X chromosome

Probability a segment is inherited intact

2-way selfing
2-way sib-mating
8-way sib-mating

Length of segment (cM)

Probability segment inherited intact

0.0
0.2
0.4
0.6
0.8
1.0

0 20 40 60 80 100 120
Length of smallest segment

No. segments < 1 cM
Summary

• RILs are useful.
• The Collaborative Cross could provide “one-stop shopping” for gene mapping in the mouse.
• Use of such 8-way RILs requires an understanding of the breakpoint process.
• We’ve extended Haldane & Waddington’s results to the case of 8-way RILs.
• We’ve shown clustering of breakpoints in RILs by sib-mating, even in the presence of strong crossover interference.
• Formulae for the 3-point problem in 8-way RILs elude us, but we can obtain numerical results.
• We used simulations to study other features of RILs.

The key points

• \( R = \frac{7r}{1 + 6r} \)
• 2-point probabilities, for the autosomes of 8-way RILs, have all off-diagonal elements identical.
• 3-point coincidence on 8-way RIL is near 1.