The genomes of recombinant inbred lines

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The intercross

Opportunities for improvement

- Each individual is unique.
  - Must genotype each mouse.
  - Unable to obtain multiple invasive phenotypes (e.g., in multiple environmental conditions) on the same genotype.
- Relatively low mapping precision.

→ Design a set of inbred mouse strains.
  - Genotype once.
  - Study multiple phenotypes on the same genotype.
Recombinant inbred lines
(by sibling mating)

Advantages
+ Each strain is a 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.

Disadvantages
- Time and expense.
- Available panels are generally too small (10-30 lines).
- Can learn only about 2 particular alleles.
- All individuals homozygous.
The RIX design

The Collaborative Cross

Complex Trait Consortium (2004)

Nat Genet 36:1133-1137
Genome of an 8-way RI

The Collaborative Cross

Advantages
+ Great mapping precision.
+ Eternal resource.
  + Genotype only once.
  + Study multiple invasive phenotypes on the same genotype.

Barriers
• Advantages not widely appreciated.
  • Ask one question at a time, or Ask many questions at once?
• Time.
• Expense.
• Requires large-scale collaboration.
The Collaborative Cross

The goal
(for the rest of this talk)

- 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.
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
Received August 9, 1930

Genetics 16:357-374

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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 Jenkins (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)

Markov chain

- Sequence of random variables \( \{X_0, X_1, X_2, \ldots\} \) satisfying
  \[
  \Pr(X_{n+1} | X_0, X_1, \ldots, X_n) = \Pr(X_{n+1} | X_n)
  \]
- Transition probabilities \( P_{ij} = \Pr(X_{n+1}=j | X_n=i) \)
- Here, \( X_n = \) “parental type” at generation \( n \)
- We are interested in absorption probabilities
  \[
  \Pr(X_n \rightarrow j | X_0)
  \]
Equations for selfing

\[ C_n, ABBB \] and \[ aabb \].
\[ D_n, AAbb \] and \[ aABb \].
\[ E_n, AABb, AaBB, Aabb, \] and \[ aabB \].
\[ F_n, AABB, \]
\[ G_n, AaBb. \]

We assume \[ 2C_n + 2D_n + 4E_n + F_n + G_n = 2 \], so that \[ C_1 = D_1 = E_1 = G_1 = 0 \],
and \[ F_1 = 2 \]. Clearly \[ E_n = F_n = G_n = 0 \], and \[ D_n \] is the final proportion of cross-over zygotes. Then considering the results of selfing each generation, we have:

\[
\begin{align*}
C_{n+1} &= C_n + \frac{1}{2}E_n + \frac{1}{2}(1 - \beta - \delta + \beta\delta)F_n + \frac{1}{2}\delta G_n \\
D_{n+1} &= D_n + \frac{1}{2}F_n + \frac{1}{2}(1 - \beta - \delta + \beta\delta)G_n \\
E_{n+1} &= E_n + \frac{1}{2}(\beta + \delta - 2\beta\delta)F_n + G_n \\
F_{n+1} &= \frac{1}{2}(1 - \beta - \delta + \beta\delta)E_n + \frac{1}{2}\delta G_n \\
G_{n+1} &= \frac{1}{2}\delta F_n + \frac{1}{2}(1 - \beta - \delta + \beta\delta)G_n
\end{align*}
\]

(1.1)

Put \( y = D_n \) (the final proportion of cross-over zygotes)

\[
\begin{align*}
\therefore C_{n+1} + D_{n+1} &= 1, C_n - D_n = c_n \quad \therefore y = \frac{1}{2}(1 - c_n)
\end{align*}
\]

(1.2)

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)

Equations for sib-mating
Omitting some rather tedious algebra, the solution of these equations is:

\[ i = \frac{q}{2 - 3q}, \quad \theta = \frac{-2q}{2 - 3q}, \quad \kappa = \frac{1}{2 - 3q}, \]

\[ \lambda = \frac{1 - 2q}{2 - 3q}, \quad \mu = \frac{1 - 2q}{2 - 3q}, \quad r = \frac{2q}{2 - 3q} \]

as may easily be verified.

\[ \therefore c_n = c_n + 2c_n + \frac{1}{1 + 6x} [(1 - 2x)(d_n + 2f_n + 2) + \frac{1}{2}k_n] \]

\[ + 2g_n + 4x(h_n + l_n)] \quad (3.4) \]

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

In the case considered, \( d_n = 1 \), \( c_n = 0 \), \( r_n = 1 - 2x/1 + 6x \). Hence the proportion of crossover zygotes \( y = 4x/1 + 6x \) (3.5). \( \square \)

The “Collaborative Cross”
8-way RILs

Autosomes
\[
\begin{align*}
\Pr(G_1 = i) &= 1/8 \\
\Pr(G_2 = j \mid G_1 = i) &= r / (1+6r) \quad \text{for } i \neq j \\
\Pr(G_2 \neq G_1) &= 7r / (1+6r)
\end{align*}
\]

X chromosome
\[
\begin{align*}
\Pr(G_1=A) &= \Pr(G_1=B) = \Pr(G_1=E) = \Pr(G_1=F) = 1/6 \\
\Pr(G_1=C) &= 1/3 \\
\Pr(G_2=B \mid G_1=A) &= r / (1+4r) \\
\Pr(G_2=C \mid G_1=A) &= 2r / (1+4r) \\
\Pr(G_2=A \mid G_1=C) &= r / (1+4r) \\
\Pr(G_2 \neq G_1) &= (14/3) \frac{r}{1+4r}
\end{align*}
\]

Computer simulations

[Graph showing two curves: \( R = \frac{7r}{1+6r} \) and \( R = \frac{(14/3)r}{1+4r} \).]
The X chromosome

• \( r_{ij} \) = recombination fraction for interval \( i,j \);
  assume \( r_{12} = r_{23} = r \)

• Coincidence = \( c = \frac{\Pr(\text{double recombinant})}{r^2} \)
  = \( \frac{\Pr(\text{rec’in 23} \mid \text{rec’in 12})}{\Pr(\text{rec’in 23})} \)

• 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

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

Coincidence

No interference

- Meiosis
- RILs by selfing
- RILs by sib-mating
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 $\rightarrow$ 22 states by symmetry
  - 3 points in 4-way RILs by sib-mating:
    2,164,240 parental types $\rightarrow$ 137,488 states
- Even counting the states was difficult.
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
No. gen’s to 99% fixation

Percent genome not fixed
Number of breakpoints

![Graph showing number of breakpoints with means labeled as 32.7, 65.3, and 114.4.](image)

Segment lengths

![Graph showing segment lengths with medians labeled as 8.5 cM, 12.9 cM, and 23.7 cM.](image)
Probability a segment is inherited intact

Length of smallest segment

95th %ile = 0.26 cM

95th %ile = 0.58 cM

95th %ile = 2.2 cM
No. segments < 1 cM

Summary

• 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: \( R = \frac{7}{1 + 6r} \).
• We’ve shown clustering of breakpoints in RILs by sib-mating, even in the presence of strong crossover interference.