The genomes of recombinant inbred lines

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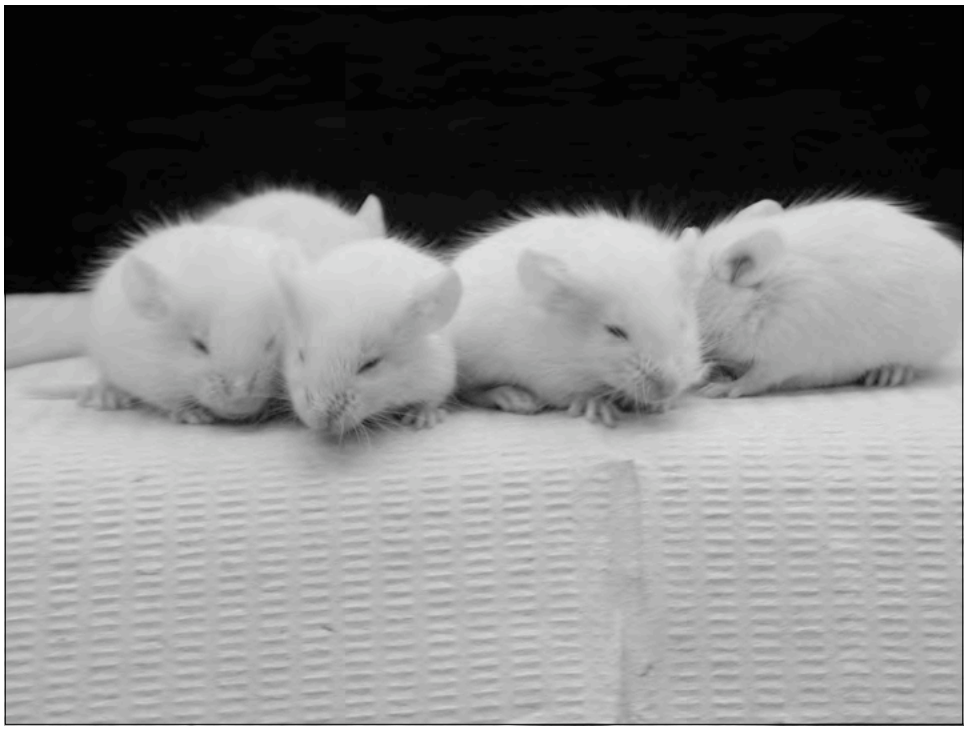


Why mice?

Advantages

+ Small and cheap
+ Inbred lines
+ Simpler genetic architecture
+ Controlled environment
+ Large, controlled crosses
+ Experimental interventions
+ Knock-outs and knock-ins

Disadvantages

– Is the model really at all like the corresponding human disease?
– Still not as small (or as fast at breeding) as a fly.

3

The mouse as a model

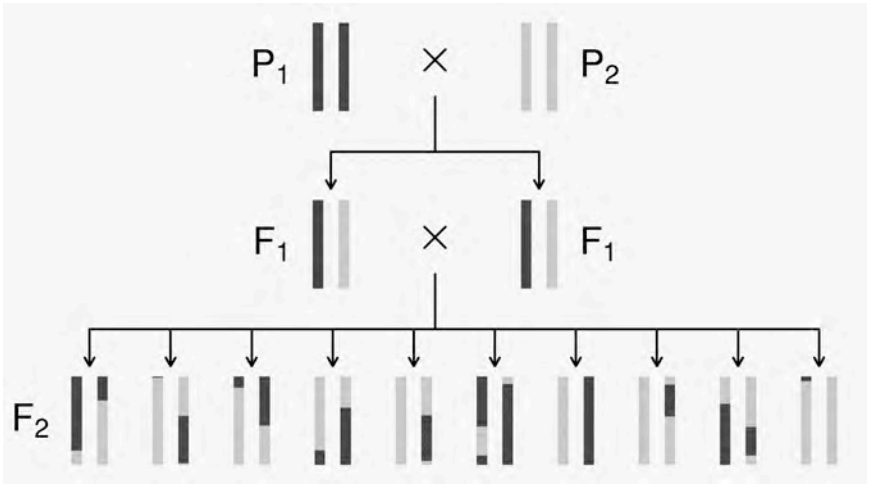
- Same genes?
  – The genes involved in a phenotype in the mouse may also be involved in similar phenotypes in the human.
- Similar complexity?
  – The complexity of the etiology underlying a mouse phenotype provides some indication of the complexity of similar human phenotypes.
- Transfer of statistical methods.
  – The statistical methods developed for gene mapping in the mouse serve as a basis for similar methods applicable in direct human studies.

4

C57BL/6

5

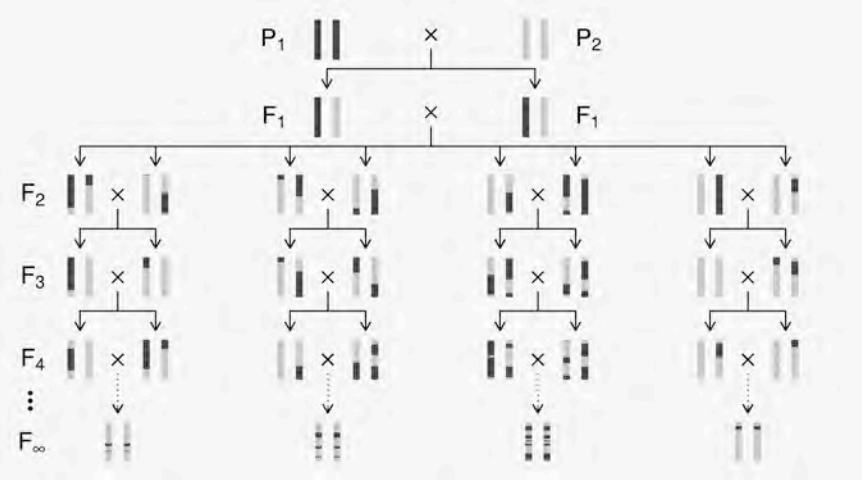
The intercross



6

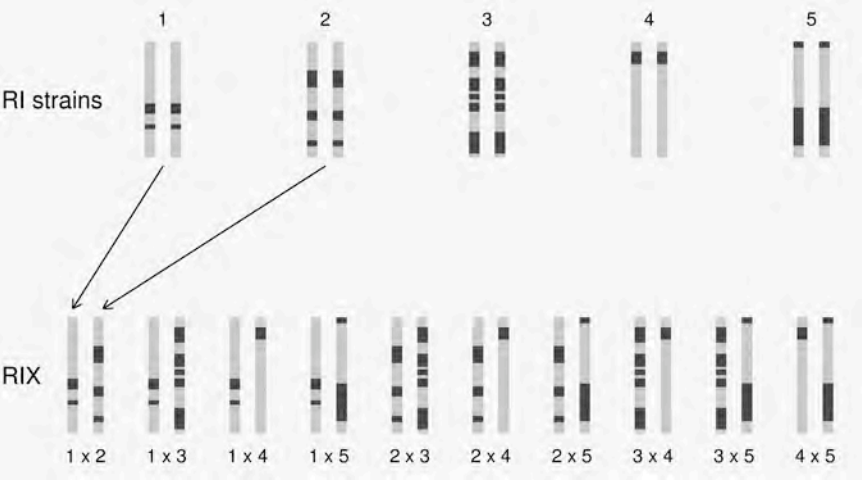
Recombinant inbred lines

(by sibling mating)



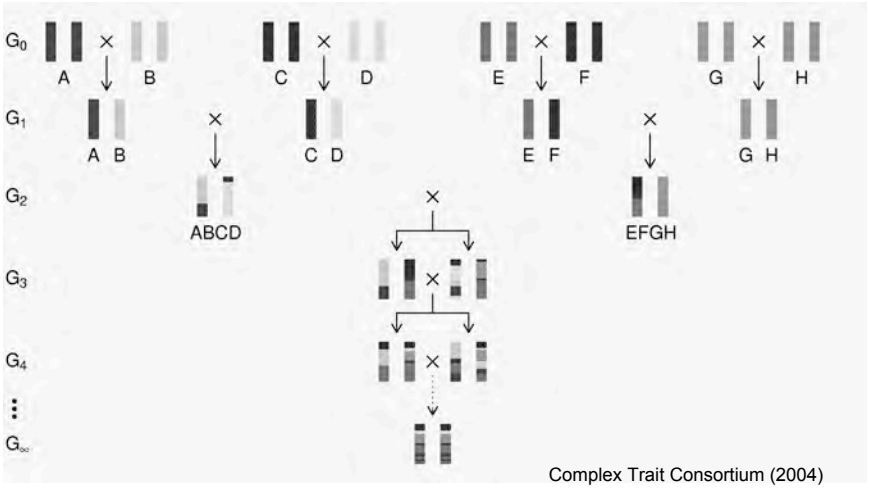
7

The RIX design



8

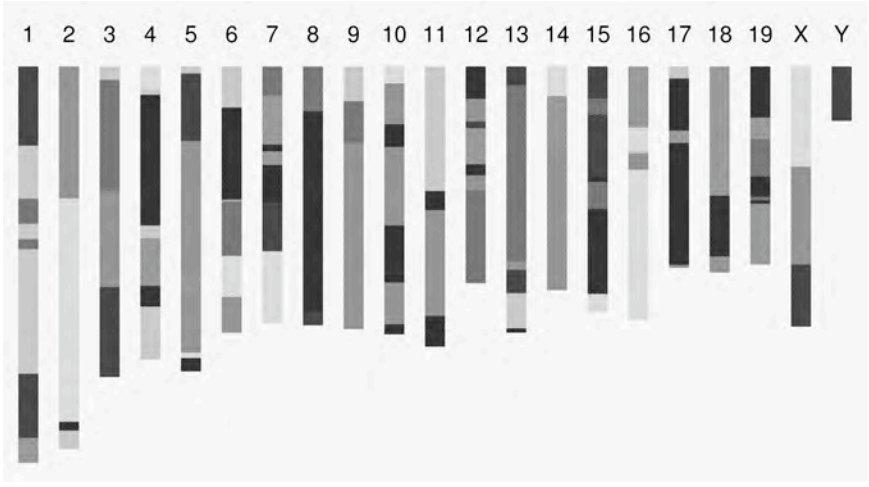
The Collaborative Cross



Complex Trait Consortium (2004)
Nat Genet 36:1133-1137

9

Genome of an 8-way RI



10

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.

11

2 points in an RIL

- 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.

12

Haldane & Waddington 1931

INBREEDING AND LINKAGE*

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

Received August 9, 1930

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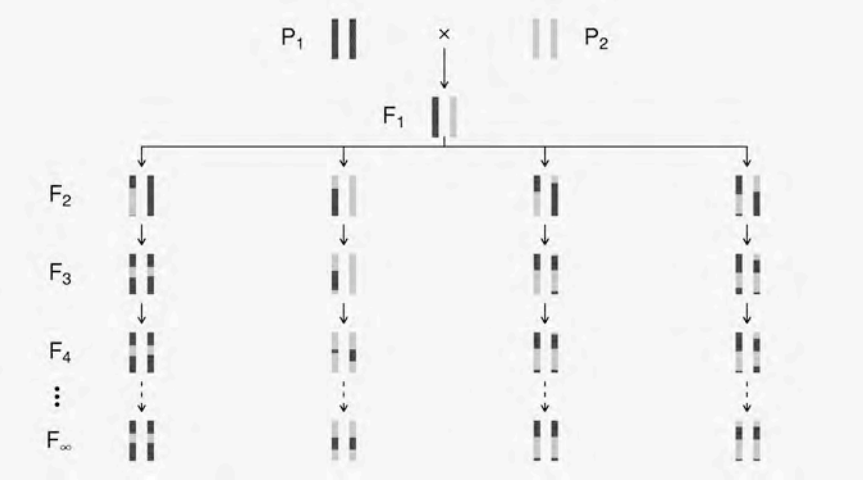
TABLE OF CONTENTS

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.

13

Recombinant inbred lines

(by selfing)



14

Markov chain

- Sequence of random variables $\{X_0, X_1, X_2, \ldots\}$ satisfying
  $$\Pr(X_{n+1} \mid X_0, X_1, \ldots, X_n) = \Pr(X_{n+1} \mid X_n)$$
- Transition probabilities $P_{ij} = \Pr(X_{n+1}=j \mid X_n=i)$
- Here, X_n = "parental type" at generation n
- We are interested in absorption probabilities
  $$\Pr(X_n \to j \mid X_0)$$

15

Equations for selfing

C_n $AABB$ and $aabb$.
D_n $AAbb$ and $aaBB$.
E_n $AABb$, $AaBB$, $Aabb$, and $aaBb$.
F_n $AB.ab$.
G_n $Ab.aB$.

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 $E_\infty=F_\infty=G_\infty=0$, and D_∞ is the final proportion of crossover zygotes. Then considering the results of selfing each generation, we have:

$$\left.\begin{aligned}
C_{n+1} &= C_n + \tfrac{1}{2}E_n + \tfrac{1}{4}(1-\beta-\delta+\beta\delta)F_n + \tfrac{1}{4}\beta\delta G_n \\
D_{n+1} &= D_n + \tfrac{1}{2}E_n + \tfrac{1}{4}\beta\delta F_n + \tfrac{1}{4}(1-\beta-\delta+\beta\delta)G_n \\
E_{n+1} &= \tfrac{1}{2}E_n + \tfrac{1}{4}(\beta+\delta-2\beta\delta)(F_n+G_n) \\
F_{n+1} &= \tfrac{1}{2}(1-\beta-\delta+\beta\delta)F_n + \tfrac{1}{2}\beta\delta G_n \\
G_{n+1} &= \tfrac{1}{2}\beta\delta F_n + \tfrac{1}{2}(1-\beta-\delta+\beta\delta)G_n
\end{aligned}\right\} \quad (1.1)$$

or C_{n+1}, D_{n+1}, and F_{n+1}, G_{n+1},

d_n } (1.2)

r all values of n.
$- 2x)d_n$

$= \dfrac{1-2x}{1+2x}$.

Put $y=D_\infty$ (the final proportion of crossover zygotes)

$\therefore\ C_\infty + D_\infty = 1,\ C_\infty - D_\infty = c_\infty\ \therefore\ y = \tfrac{1}{2}(1-c_\infty).$

$$\therefore\ y = \frac{2x}{1+2x}. \quad (1.3)$$

16

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 $h_{AB|AB} = 0$.

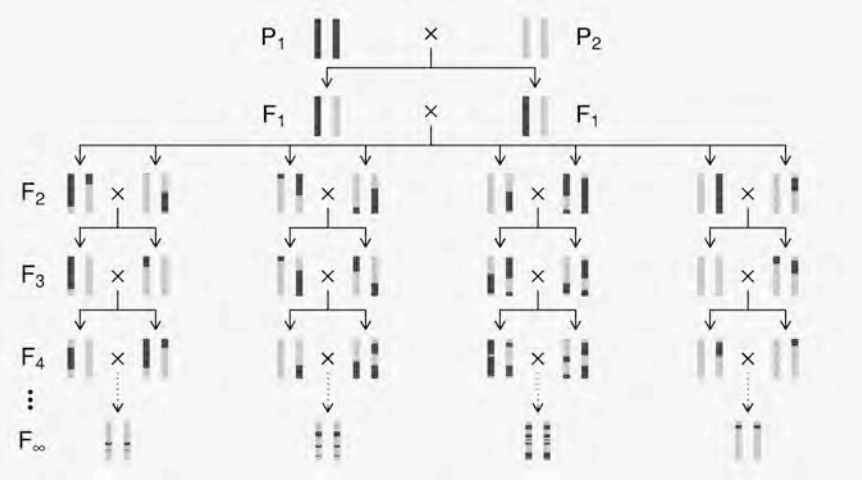
Condition on the first step: $h_i = \sum_k P_{ik} h_k$

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

17

Recombinant inbred lines

(by sibling mating)



18

Equations for sib-mating

Typical mating	*Number of types*	
$AABB\times AABB$	2	$C_{n+1}=C_n+H+\frac{1}{4}(\alpha^2+\gamma^2)L+\frac{1}{4}(\beta^2+\delta^2)N+\frac{1}{8}Q+\frac{1}{8}R+\frac{1}{8}(\alpha^2+\gamma^2)$ $U+\frac{1}{8}(\beta^2+\delta^2)V+\frac{1}{16}\alpha^2\gamma^2W+\frac{1}{16}(\alpha^2\delta^2+\beta^2\gamma^2)X+\frac{1}{16}\beta^2\delta^2Y.$
$AAbb\times AAbb$	2	$D_{n+1}=D+I+\frac{1}{4}(\alpha^2+\gamma^2)M+\frac{1}{4}(\beta^2+\delta^2)P+\frac{1}{8}Q+\frac{1}{8}S+\frac{1}{8}(\beta^2+\delta^2)$ $U+\frac{1}{8}(\alpha^2+\gamma^2)V+\frac{1}{16}\beta^2\delta^2W+\frac{1}{16}(\alpha^2\delta^2+\beta^2\gamma^2)X+\frac{1}{16}\alpha^2\gamma^2Y.$
$AABB\times aabb$	2	$E_{n+1}=\frac{1}{16}\alpha^2\gamma^2W+\frac{1}{16}(\alpha^2\delta^2+\beta^2\gamma^2)X+\frac{1}{16}\beta^2\delta^2Y.$
$AAbb\times aaBB$	2	$F_{n+1}=\frac{1}{16}\beta^2\delta^2W+\frac{1}{16}(\alpha^2\delta^2+\beta^2\gamma^2)X+\frac{1}{16}\alpha^2\gamma^2Y.$
$AABB\times AAbb$	8	$G_{n+1}=\frac{1}{16}(\alpha\beta+\gamma\delta)(U+V)+\frac{1}{16}\alpha\beta\gamma\delta(W+2X+Y).$
$AABB\times AABb$	8	$H_{n+1}=\frac{1}{2}H$ $U+\frac{1}{16}$ $(\alpha\delta+\beta$
$AAbb\times AABb$	8	$I_{n+1}=\frac{1}{2}I+$ $U+\frac{1}{16}$ $(\alpha\delta+\beta$
$AABB\times Aabb$	8	$J_{n+1}=\frac{1}{16}$ $\beta\delta)(\alpha\delta$
$AAbb\times AaBB$	8	$K_{n+1}=\frac{1}{16}$ $\beta\delta)(\alpha\delta$
$AABB\times AB.ab$	4	$L_{n+1}=\frac{1}{4}(\alpha$ $\alpha^2\gamma^2W$
$AAbb\times Ab.aB$	4	$M_{n+1}=\frac{1}{2}(\alpha$ $\beta^2\delta^2W+$

Typical mating	*Number of types*	
$AABB\times Ab.aB$	4	$N_{n+1}=\frac{1}{2}R+\frac{1}{4}(\alpha\beta+\gamma\delta)(U+V)+\frac{1}{4}\alpha\beta\gamma\delta(W+2X+Y).$
$AAbb\times AB.ab$	4	$P_{n+1}=\frac{1}{2}S+\frac{1}{4}(\alpha\beta+\gamma\delta)(U+V)+\frac{1}{4}\alpha\beta\gamma\delta(W+2X+Y).$
$AABb\times AABb$	4	$Q_{n+1}=2G+\frac{1}{2}(H+I+J+K)+\frac{1}{4}(\alpha^2+\gamma^2)(L+M)+\frac{1}{4}(\beta^2+\delta^2)$ $(N+P)+\frac{1}{8}Q+\frac{1}{8}(R+S+T)+\frac{1}{8}(\alpha^2+\alpha\beta+\beta^2+\gamma^2+\gamma\delta+\delta^2)$ $(U+V)+\frac{1}{16}(\alpha\delta+\beta\gamma)^2(W+Y)+\frac{1}{8}(\alpha\gamma+\beta\delta)^2X.$
$AABb\times AaBB$	4	$R_{n+1}=\frac{1}{4}(\beta^2+\delta^2)L+\frac{1}{4}(\alpha^2+\gamma^2)N+\frac{1}{8}R+\frac{1}{8}(\beta+\delta)U+\frac{1}{8}(\alpha+\gamma)V+$ $\frac{1}{16}(\alpha\delta+\beta\gamma)^2(W+Y)+\frac{1}{8}(\alpha\gamma+\beta\delta)^2X.$
$AABb\times Aabb$	4	$S_{n+1}=\frac{1}{4}(\beta^2+\delta^2)M+\frac{1}{4}(\alpha^2+\gamma^2)P+\frac{1}{8}S+\frac{1}{8}(\alpha+\gamma)U+\frac{1}{8}(\beta+\delta)V+\frac{1}{16}$ $(\alpha\delta+\beta\gamma)^2(W+Y)+\frac{1}{8}(\alpha\gamma+\beta\delta)^2X.$
$AABb\times aaBb$	4	$T_{n+1}=\frac{1}{8}(\alpha\beta+\gamma\delta)(U+V)+\frac{1}{16}(\alpha\delta+\beta\gamma)^2(W+Y)+\frac{1}{8}(\alpha\gamma+\beta\delta)^2X.$
$AABb\times AB.ab$	8	$U_{n+1}=\frac{1}{2}J+\frac{1}{4}(\alpha\beta+\gamma\delta)(L+N)+\frac{1}{8}(S+T)+\frac{1}{8}(\alpha+\gamma)U+\frac{1}{8}(\beta+\delta)$ $V+\frac{1}{8}\alpha\gamma(\beta\gamma+\alpha\delta)W+\frac{1}{8}(\alpha\gamma+\beta\delta)(\alpha\delta+\beta\gamma)X+\frac{1}{8}\beta\delta(\beta\gamma+\alpha\delta)Y.$
$AABb\times Ab.aB$	8	$V_{n+1}=\frac{1}{2}K+\frac{1}{4}(\alpha\beta+\gamma\delta)(M+P)+\frac{1}{8}(R+T)+\frac{1}{8}(\beta+\delta)U+\frac{1}{8}(\alpha+\gamma)$ $V+\frac{1}{8}\beta\delta(\beta\gamma+\alpha\delta)W+\frac{1}{8}(\alpha\gamma+\beta\delta)(\alpha\delta+\beta\gamma)X+\frac{1}{8}\alpha\gamma(\beta\gamma+\alpha\delta)Y.$
$AB.ab\times AB.ab$	1	$W_{n+1}=2(E+J)+\frac{1}{2}(\alpha^2+\gamma^2)L+\frac{1}{2}(\beta^2+\delta^2)N+\frac{1}{4}(S+T)+\frac{1}{4}(\alpha^2+\gamma^2)$ $U+\frac{1}{4}(\beta^2+\delta^2)V+\frac{1}{4}\alpha^2\gamma^2W+\frac{1}{4}(\alpha^2\delta^2+\beta^2\gamma^2)X+\frac{1}{4}\beta^2\delta^2Y.$
$AB.ab\times Ab.aB$	2	$X_{n+1}=\frac{1}{2}T+\frac{1}{2}(\alpha\beta+\gamma\delta)(U+V)+\frac{1}{2}\alpha\beta\gamma\delta(W+2X+Y).$
$Ab.aB\times Ab.aB$	1	$Y_{n+1}=2(F+K)+\frac{1}{2}(\alpha^2+\gamma^2)M+\frac{1}{2}(\beta^2+\delta^2)P+\frac{1}{2}(R+T)+\frac{1}{2}(\beta^2+$ $\delta^2)U+\frac{1}{2}(\alpha^2+\gamma^2)V+\frac{1}{2}\beta^2\delta^2W+\frac{1}{2}(\alpha^2\delta^2+\beta^2\gamma^2)X+\frac{1}{2}\alpha^2\gamma^2Y.$

19

Result for sib-mating

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

$$\zeta = \frac{q}{2-3q}, \quad \theta = \frac{2q}{2-3q}, \quad \kappa = \frac{1}{2-3q},$$

$$\lambda = \frac{1-2q}{2-3q}, \quad \mu = \frac{1-2q}{2-3q}, \quad \nu = \frac{2q}{2-3q}$$

as may easily be verified.

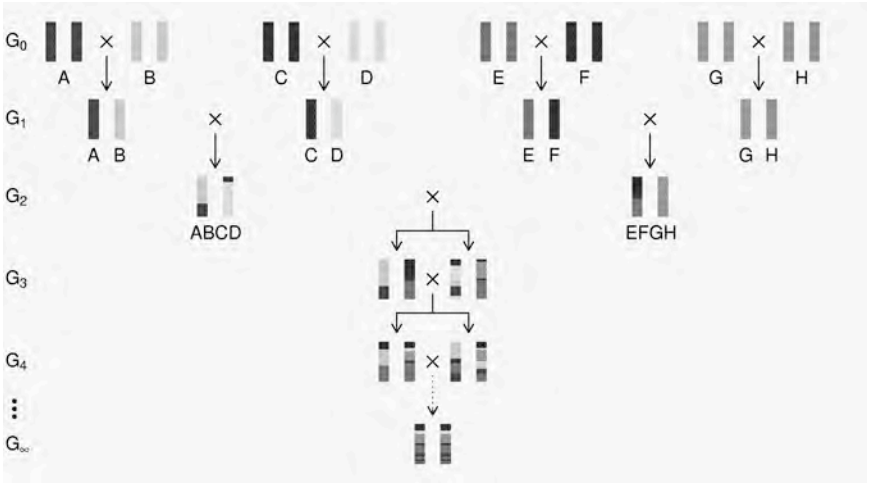
$$\therefore\ c_\infty = c_n + 2e_n + \frac{1}{1+6x}[(1-2x)(d_n + 2f_n + 2j_n + \tfrac{1}{2}k_n) + 2g_n + 4x(h_n + i_n)] \qquad (3.4)$$

and $y=\frac{1}{2}(1-c_\infty)$.

In the case considered, $d_0=1, \therefore c_\infty=\zeta d_0=1-2x/1+6x$. Hence the proportion of crossover zygotes, $y=4x/1+6x$ (3.5).

20

The "Collaborative Cross"



21

8-way RILs

Autosomes

$Pr(G_1 = i) = 1/8$

$Pr(G_2 = j \mid G_1 = i) = r / (1+6r)$ for $i \neq j$

$Pr(G_2 \neq G_1) = 7r / (1+6r)$

X chromosome

$Pr(G_1=A) = Pr(G_1=B) = Pr(G_1=E) = Pr(G_1=F) = 1/6$

$Pr(G_1=C) = 1/3$

$Pr(G_2=B \mid G_1=A) = r / (1+4r)$

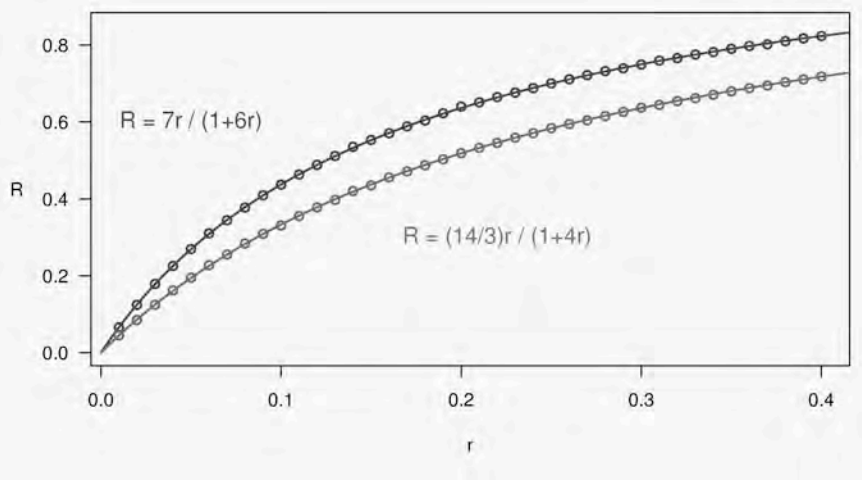
$Pr(G_2=C \mid G_1=A) = 2r / (1+4r)$

$Pr(G_2=A \mid G_1=C) = r / (1+4r)$

$Pr(G_2 \neq G_1) = (14/3)\, r / (1+4r)$

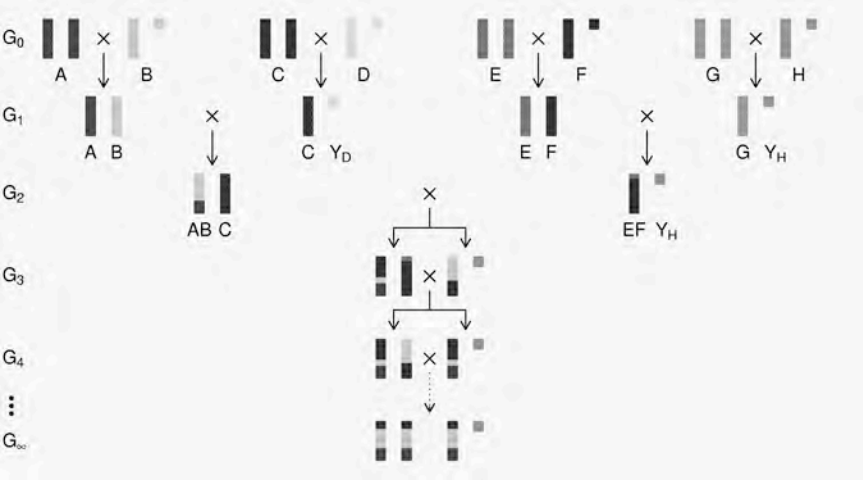
22

Computer simulations



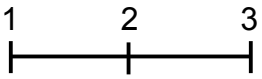
23

The X chromosome



24

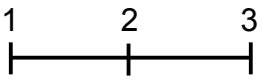
3-point coincidence



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

25

3-points in 2-way RILs

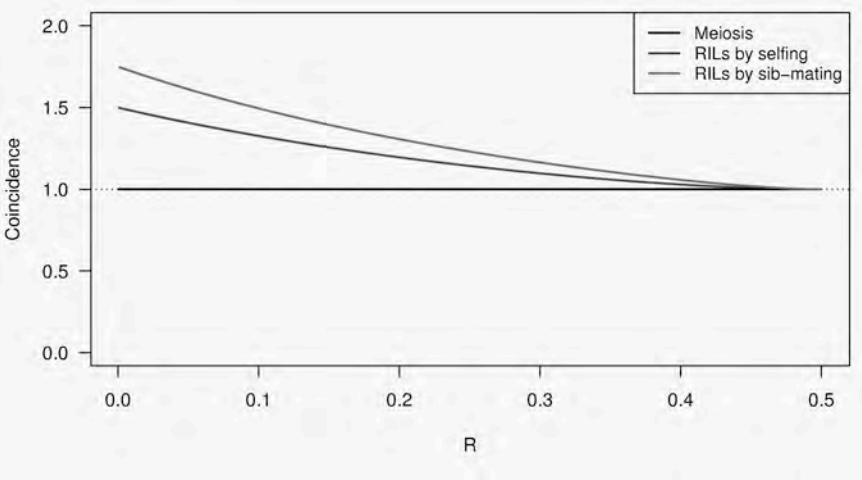


- $r_{13} = 2\ r\ (1 - c\ r)$
- $R = f(r)$; $R_{13} = f(r_{13})$
- Pr(double recombinant in RIL) = $\{ R + R - R_{13} \} / 2$
- Coincidence (in 2-way RIL) = $\{ 2\ R - R_{13} \} / \{ 2\ R^2 \}$

26

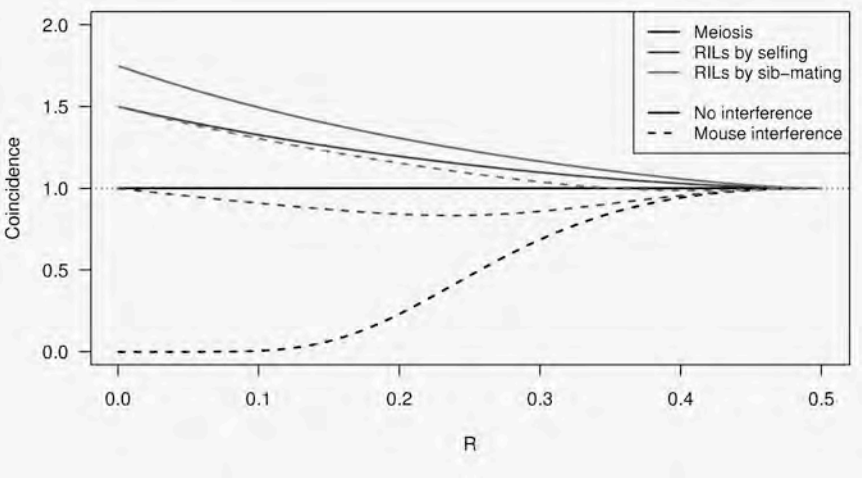
Coincidence

No interference



27

Coincidence



28

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.

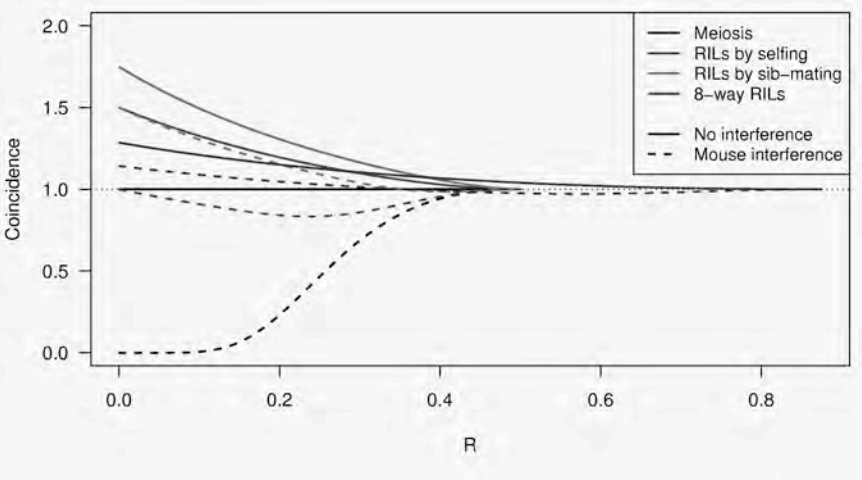
29

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.

30

Coincidence



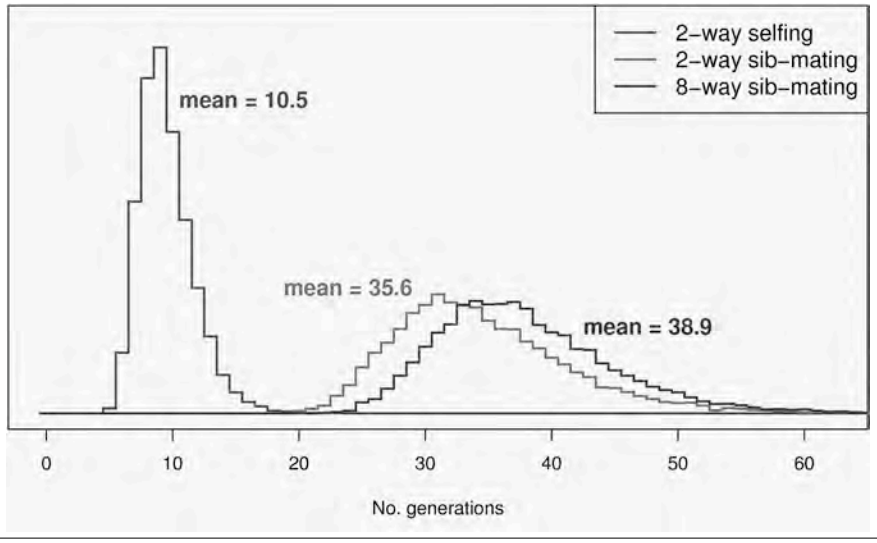
31

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

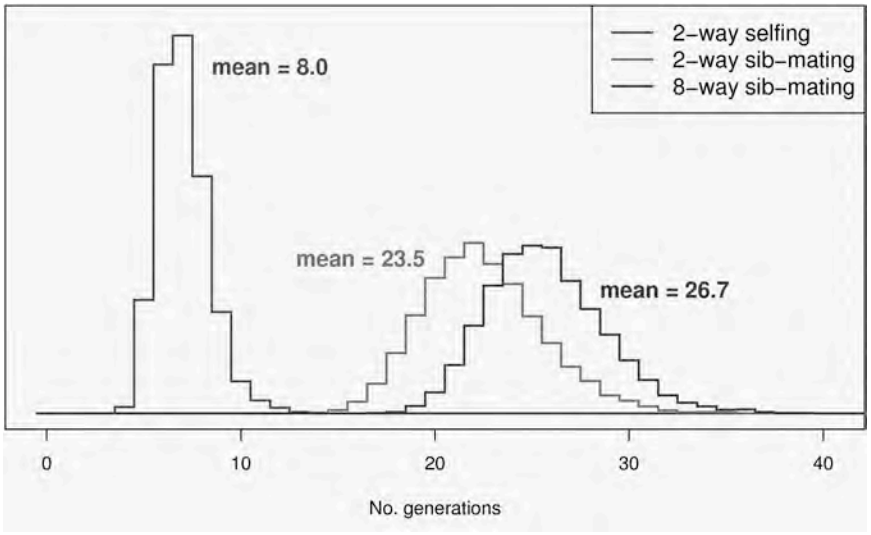
32

No. generations to fixation



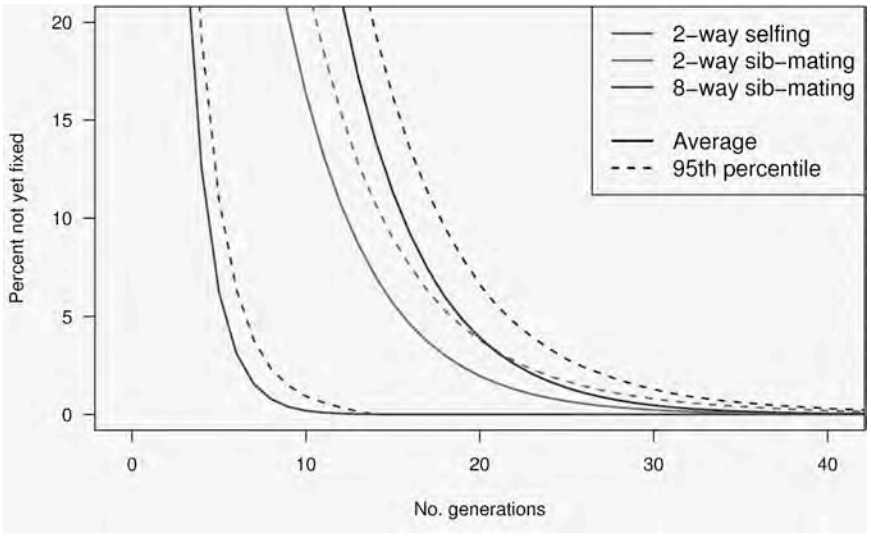
33

No. gen's to 99% fixation



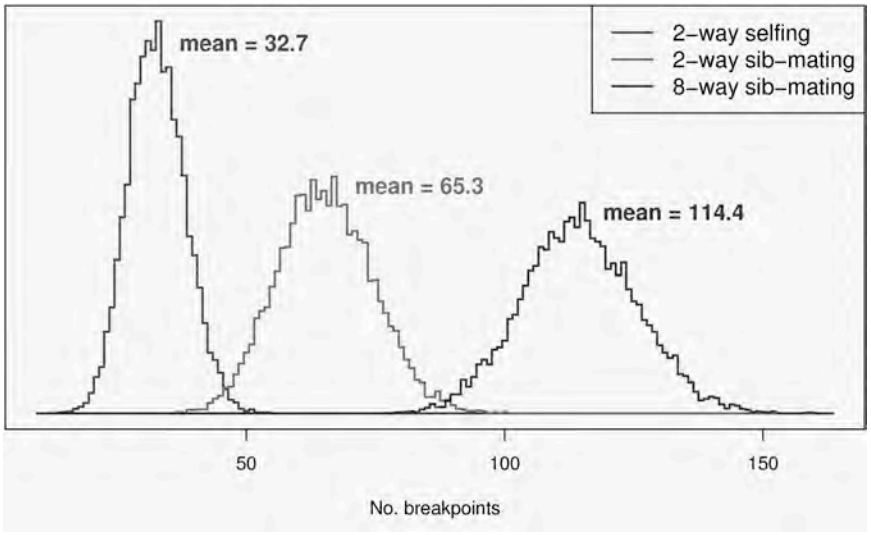
34

Percent genome not fixed



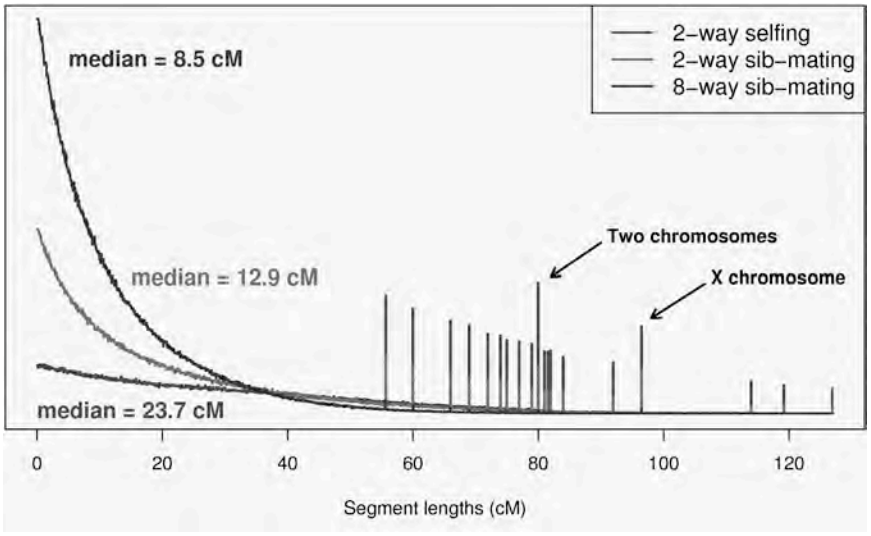
35

Number of breakpoints



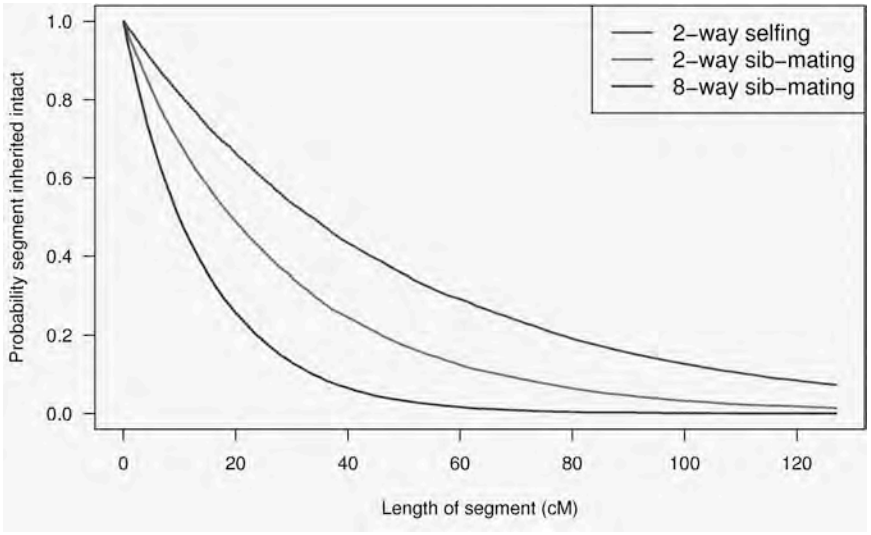
36

Segment lengths



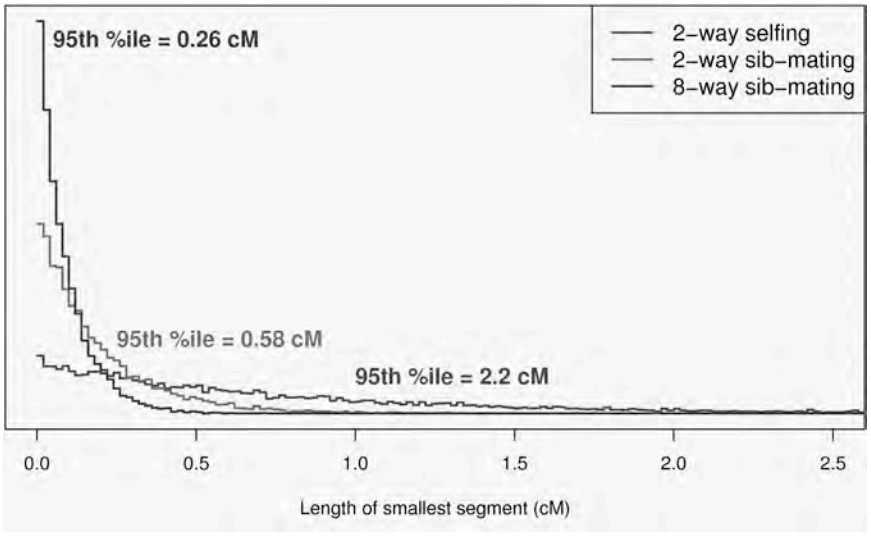
37

Probability a segment is inherited intact



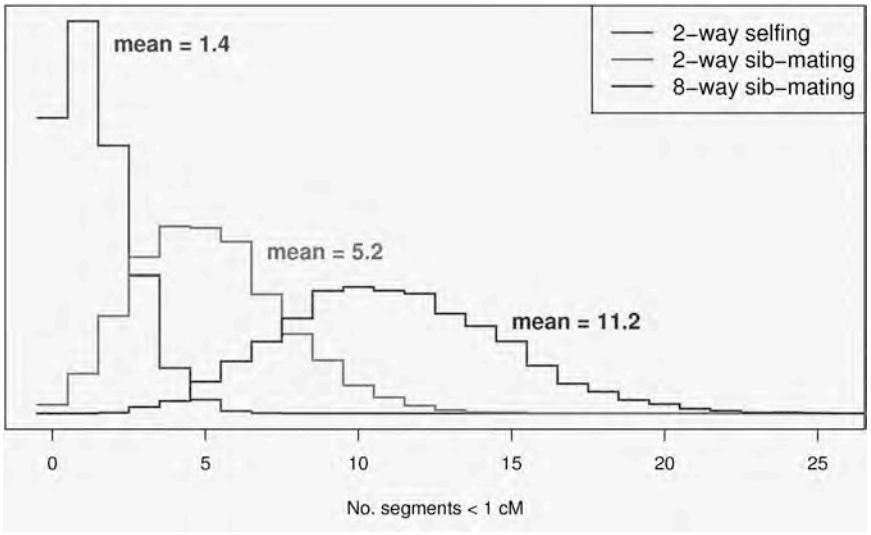
38

Length of smallest segment



39

No. segments < 1 cM



40

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: $R = 7\,r / (1 + 6\,r)$.
- We've shown clustering of breakpoints in RILs by sib-mating, even in the presence of strong crossover interference.
- Broman KW (2005) The genomes of recombinant inbred lines. *Genetics* 169:1133-1146

41