The genomes of recombinant inbred lines

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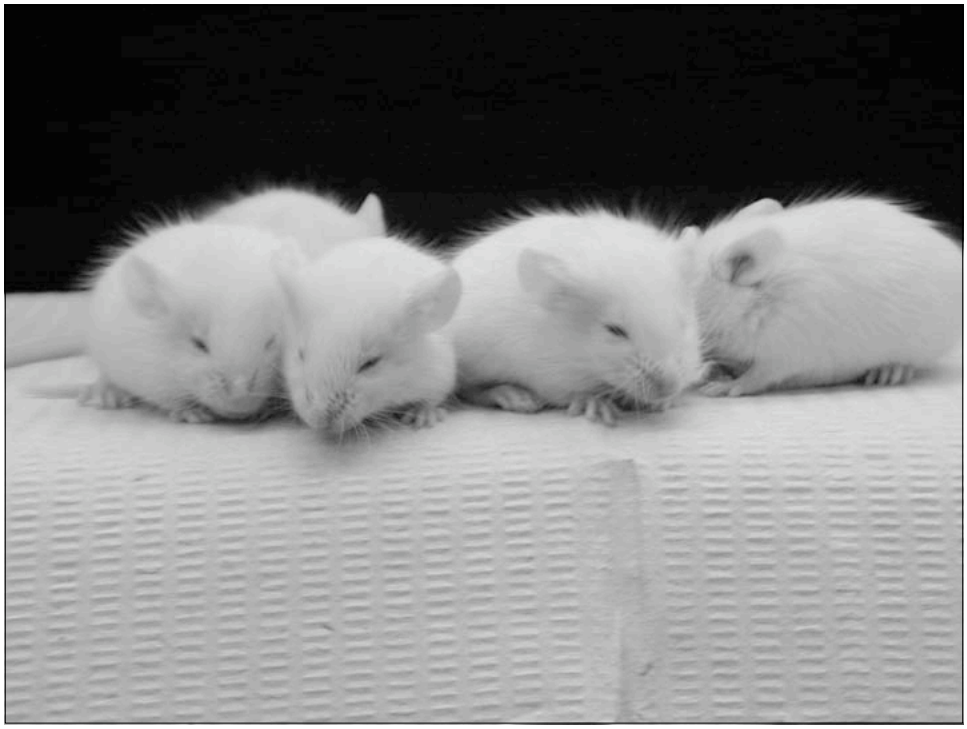
Goal

Identify genes that contribute to complex human diseases

Complex disease = one that's hard to figure out

Many genes + environment + other



Advantages of the mouse

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

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C57BL/6

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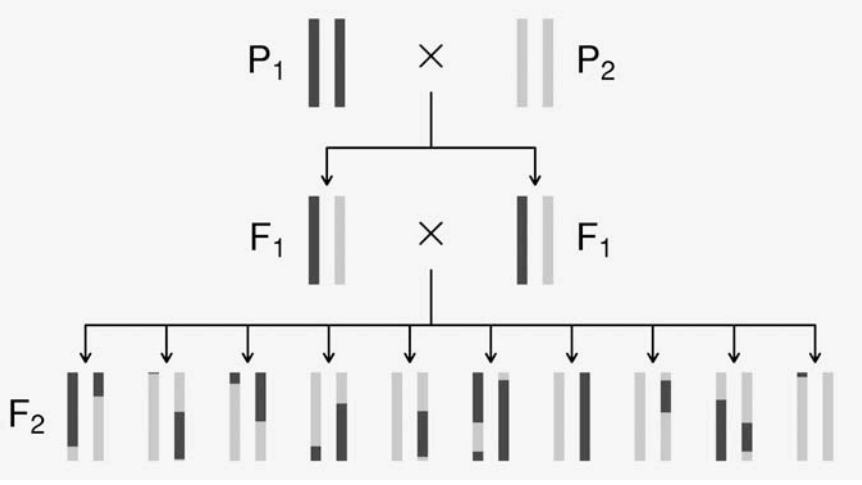
The intercross



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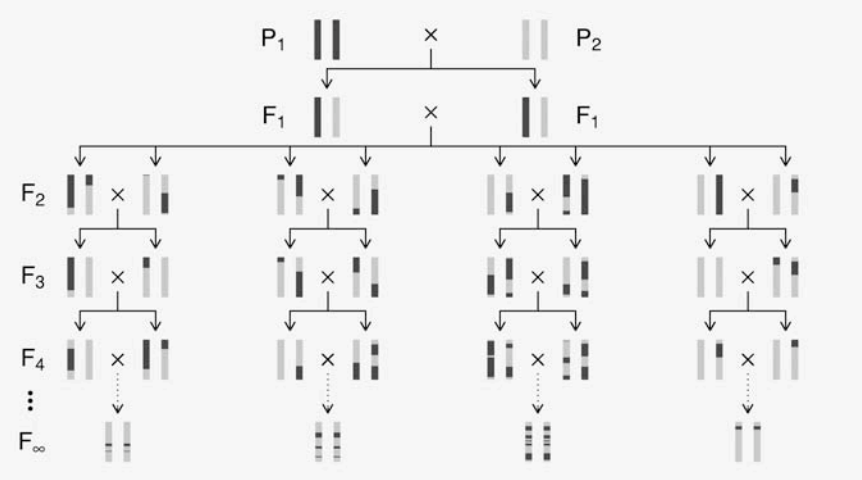
Recombinant inbred lines

(by sibling mating)



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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.

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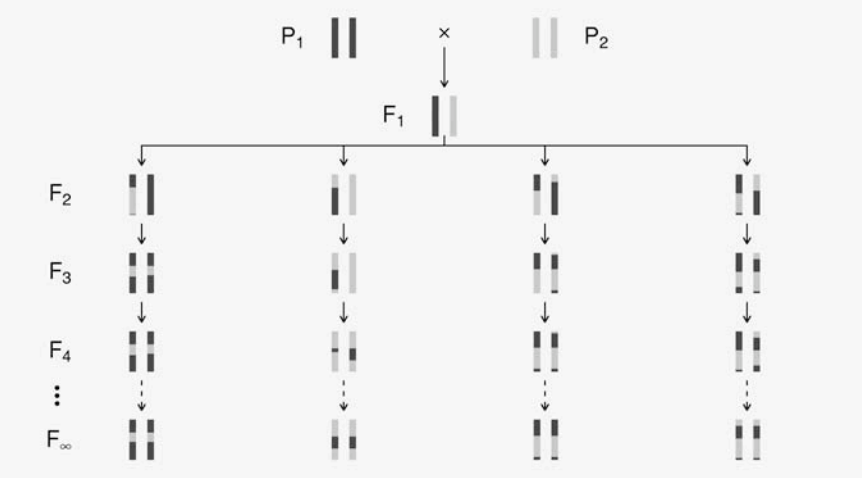
Recombinant inbred lines

(by selfing)



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Disadvantages of RI lines

- Time-consuming to create (5-6 years)
- Available panels are small (10-30 lines)
- Just 2 progenitor strains

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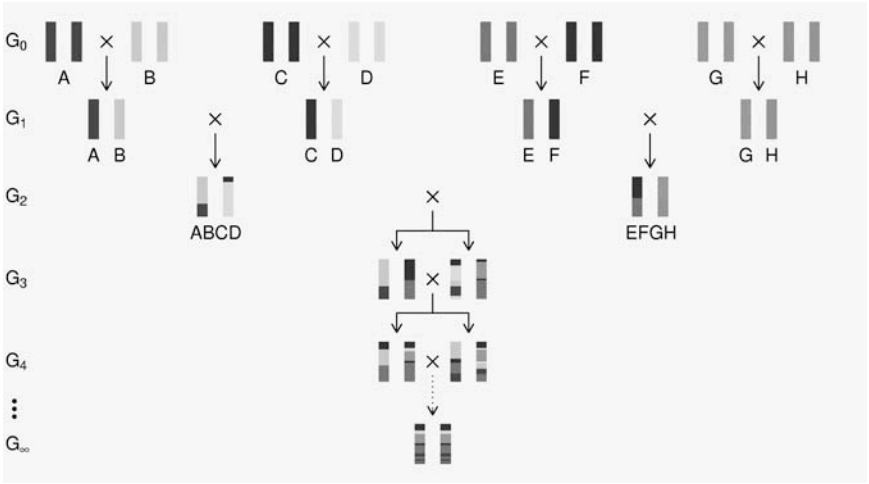
The "Collaborative Cross"



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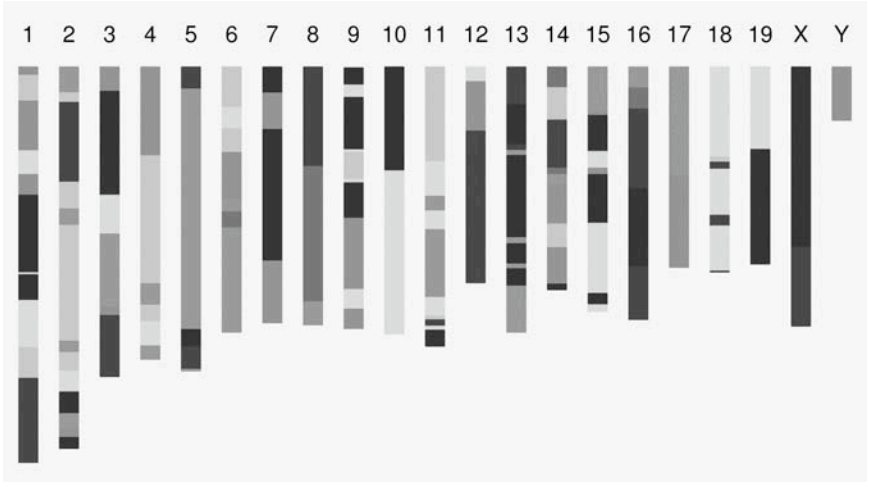
Genome of an 8-way RI



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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.

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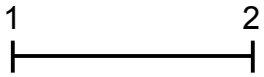
2 points in an RIL



- 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.

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Haldane & Waddington 1931

INBREEDING AND LINKAGE*

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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 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.

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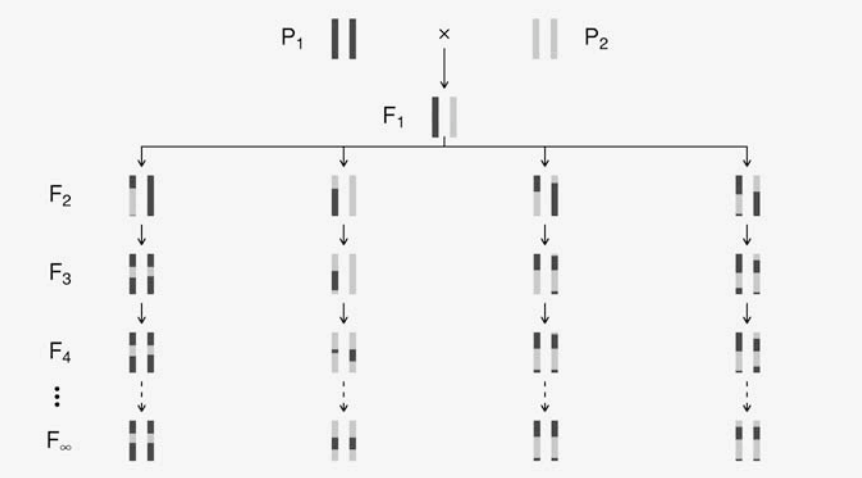
Recombinant inbred lines

(by selfing)



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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
  $$\Pr(X_n \to j \mid X_0)$$

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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)

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

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

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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.

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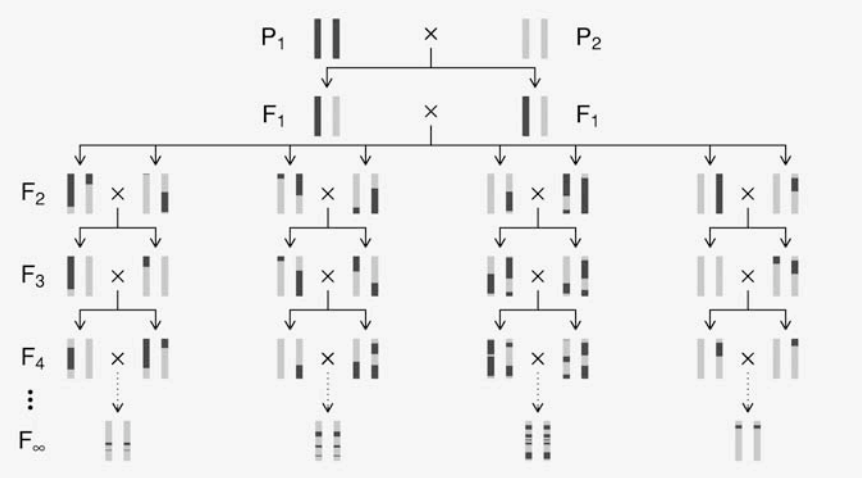
Recombinant inbred lines

(by sibling mating)



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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	$H_{n+1}=\frac{1}{2}H+\frac{1}{16}(\alpha\beta+\gamma\delta)(U+V)+\frac{1}{16}\alpha\beta\gamma\delta(W+2X+Y).$
$AAbb\times AABb$	8	$I_{n+1}=\frac{1}{2}I+\frac{1}{16}(\alpha\beta+\gamma\delta)(U+V)+\frac{1}{16}\alpha\beta\gamma\delta(W+2X+Y).$
$AABB\times Aabb$	8	$J_{n+1}=\frac{1}{16}(\alpha\delta+\beta\gamma)(U+V)+\frac{1}{16}\alpha\beta\gamma\delta(W+2X+Y).$
$AAbb\times AaBB$	8	$K_{n+1}=\frac{1}{16}(\alpha\delta+\beta\gamma)(U+V)+\frac{1}{16}\alpha\beta\gamma\delta(W+2X+Y).$
$AABB\times AB.ab$	4	$L_{n+1}=\frac{1}{4}(\alpha\beta+\gamma\delta)(U+V)+\frac{1}{16}\alpha\beta\gamma\delta(W+2X+Y).$
$AAbb\times Ab.aB$	4	$M_{n+1}=\frac{1}{4}(\alpha\beta+\gamma\delta)(U+V)+\frac{1}{16}\alpha\beta\gamma\delta(W+2X+Y).$

Typical mating	*Number of types*	
$AABB\times Ab.aB$	4	$N_{n+1}=\frac{1}{2}R+\frac{1}{8}(\alpha\beta+\gamma\delta)(U+V)+\frac{1}{8}\alpha\beta\gamma\delta(W+2X+Y).$
$AAbb\times AB.ab$	4	$P_{n+1}=\frac{1}{2}S+\frac{1}{8}(\alpha\beta+\gamma\delta)(U+V)+\frac{1}{8}\alpha\beta\gamma\delta(W+2X+Y).$
$AABb\times AABb$	4	$Q_{n+1}=2G+\frac{1}{2}(H+I+J+K)+\frac{1}{4}(\alpha^2+\gamma^2)(L+M)+\frac{1}{4}(\beta^2+\delta^2)$ $(N+P)+\frac{1}{8}Q+\frac{1}{8}(R+S+T)+\frac{1}{32}(\alpha^2+\alpha\beta+\beta^2+\gamma^2+\gamma\delta+\delta^2)$ $(U+V)+\frac{1}{16}(\alpha\delta+\beta\gamma)^2(W+Y)+\frac{1}{8}(\alpha\gamma+\beta\delta)^2X.$
$AABb\times AaBB$	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	$U_{n+1}=\frac{1}{2}J+\frac{1}{8}(\alpha\beta+\gamma\delta)(L+N)+\frac{1}{8}(S+T)+\frac{1}{8}(\alpha+\gamma)U+\frac{1}{8}(\beta+\delta)$ $V+\frac{1}{8}\alpha\gamma(\beta\gamma+\alpha\delta)W+\frac{1}{8}(\alpha\gamma+\beta\delta)(\alpha\delta+\beta\gamma)X+\frac{1}{8}\beta\delta(\beta\gamma+\alpha\delta)Y.$
$AABb\times Ab.aB$	8	$V_{n+1}=\frac{1}{2}K+\frac{1}{8}(\alpha\beta+\gamma\delta)(M+P)+\frac{1}{8}(R+T)+\frac{1}{8}(\beta+\delta)U+\frac{1}{8}(\alpha+\gamma)$ $V+\frac{1}{8}\beta\delta(\beta\gamma+\alpha\delta)W+\frac{1}{8}(\alpha\gamma+\beta\delta)(\alpha\delta+\beta\gamma)X+\frac{1}{8}\alpha\gamma(\beta\gamma+\alpha\delta)Y.$
$AB.ab\times AB.ab$	1	$W_{n+1}=2(E+J)+\frac{1}{2}(\alpha^2+\gamma^2)L+\frac{1}{2}(\beta^2+\delta^2)N+\frac{1}{2}(S+T)+\frac{1}{2}(\alpha^2+\gamma^2)$ $U+\frac{1}{2}(\beta^2+\delta^2)V+\frac{1}{4}\alpha^2\gamma^2W+\frac{1}{2}(\alpha^2\delta^2+\beta^2\gamma^2)X+\frac{1}{2}\beta^2\delta^2Y.$
$AB.ab\times Ab.aB$	2	$X_{n+1}=\frac{1}{2}T+\frac{1}{2}(\alpha\beta+\gamma\delta)(U+V)+\frac{1}{8}\alpha\beta\gamma\delta(W+2X+Y).$
$Ab.aB\times Ab.aB$	1	$Y_{n+1}=2(F+K)+\frac{1}{2}(\alpha^2+\gamma^2)M+\frac{1}{2}(\beta^2+\delta^2)P+\frac{1}{2}(R+T)+\frac{1}{2}(\beta^2+$ $\delta^2)U+\frac{1}{2}(\alpha^2+\gamma^2)V+\frac{1}{8}\beta^2\delta^2W+\frac{1}{2}(\alpha^2\delta^2+\beta^2\gamma^2)X+\frac{1}{2}\alpha^2\gamma^2Y.$

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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).

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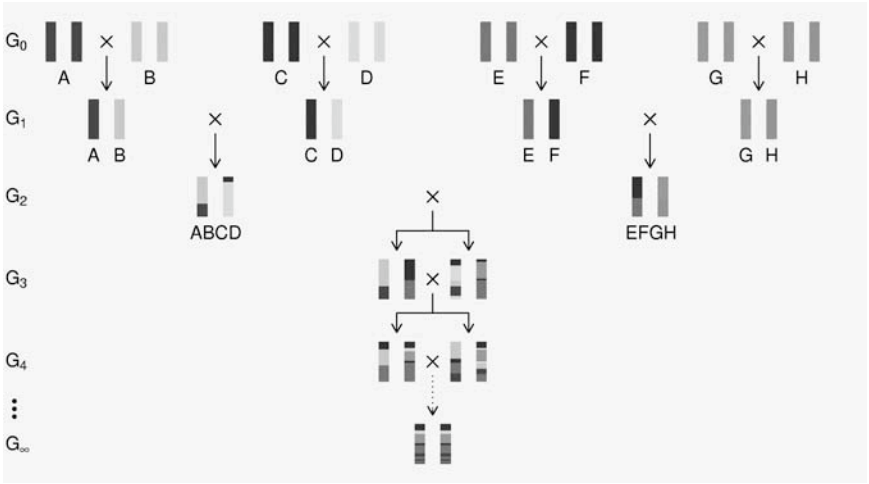
The "Collaborative Cross"



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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

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

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

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

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

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

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

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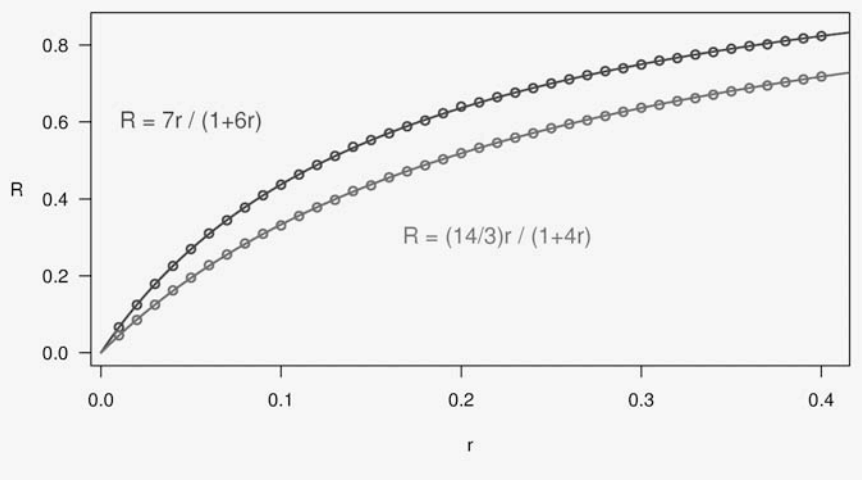
Computer simulations



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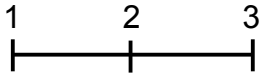
3-point coincidence



- 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 2-3 | rec'n in 1-2) / Pr(rec'n in 2-3)
- No interference → = 1
  Positive interference → < 1
  Negative interference → > 1
- Generally c is a function of r.

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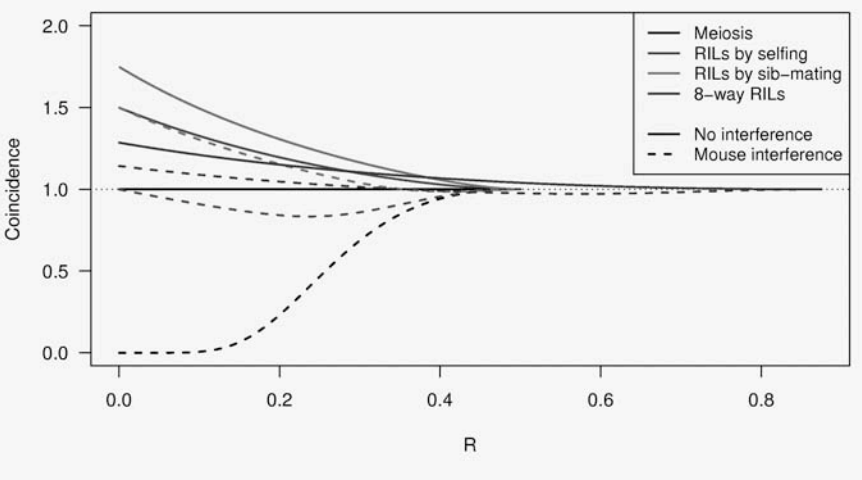
Coincidence on RIL chromosome



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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.

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

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