The genomes of recombinant inbred lines

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C57BL/6 mouse

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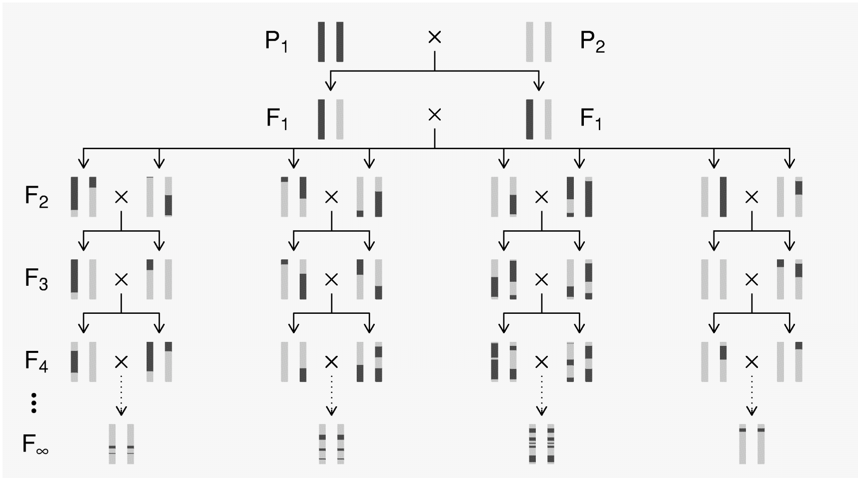
Recombinant inbred lines

(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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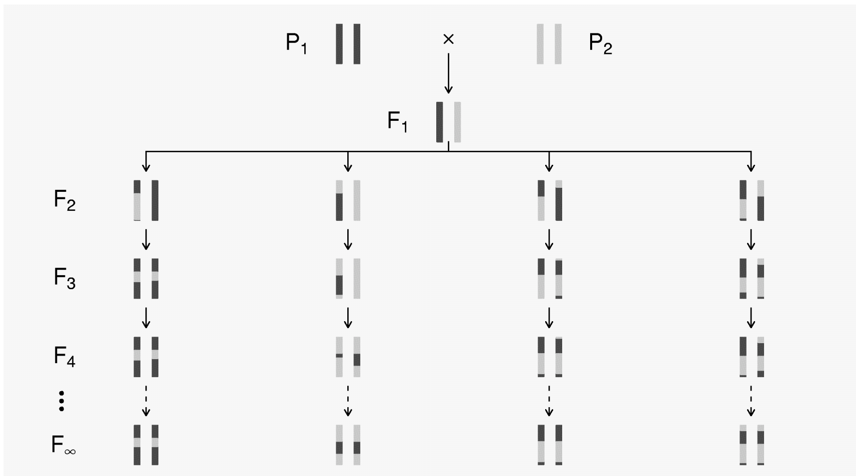
Recombinant inbred lines

(by selfing)



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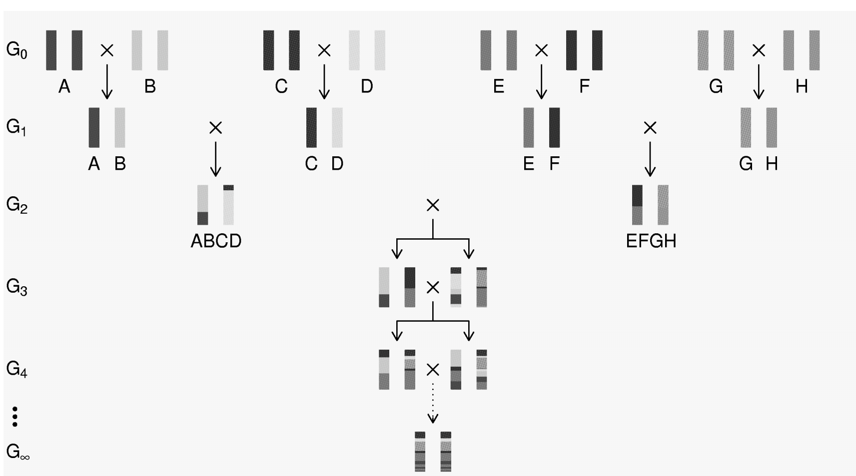
The "Collaborative Cross"



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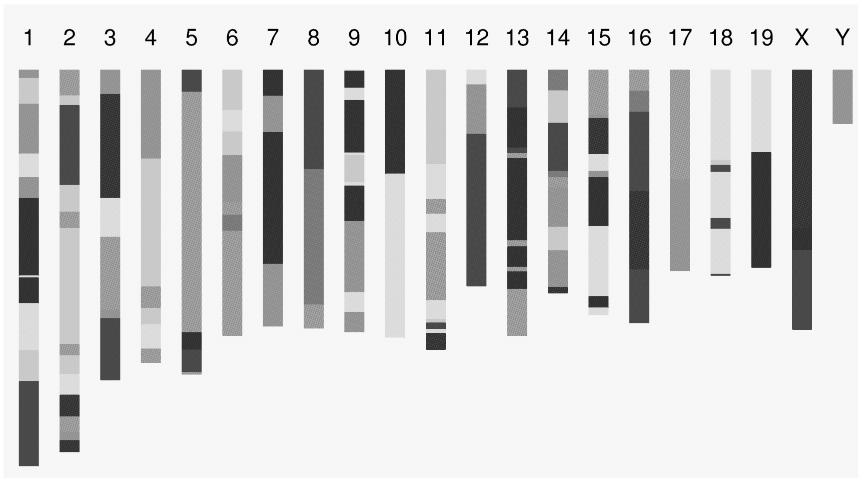
Genome of an 8-way RI



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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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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.

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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*

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.

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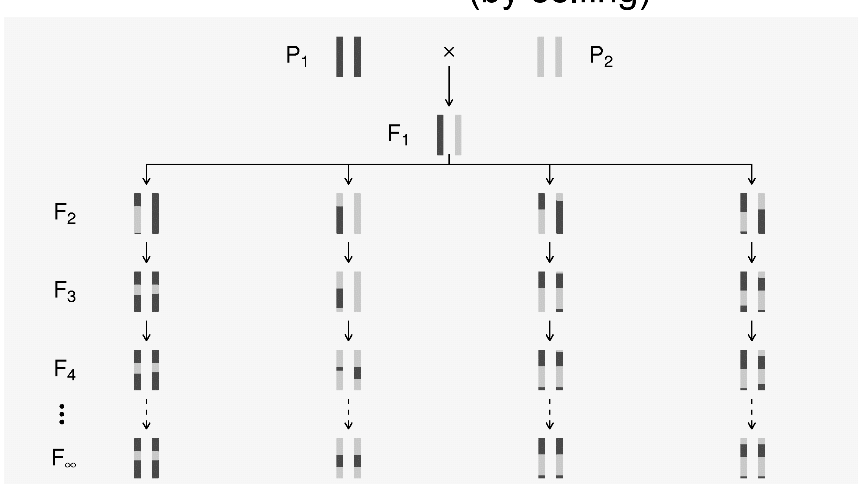
Recombinant inbred lines

(by selfing)



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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.

...- $2x)d_n$

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

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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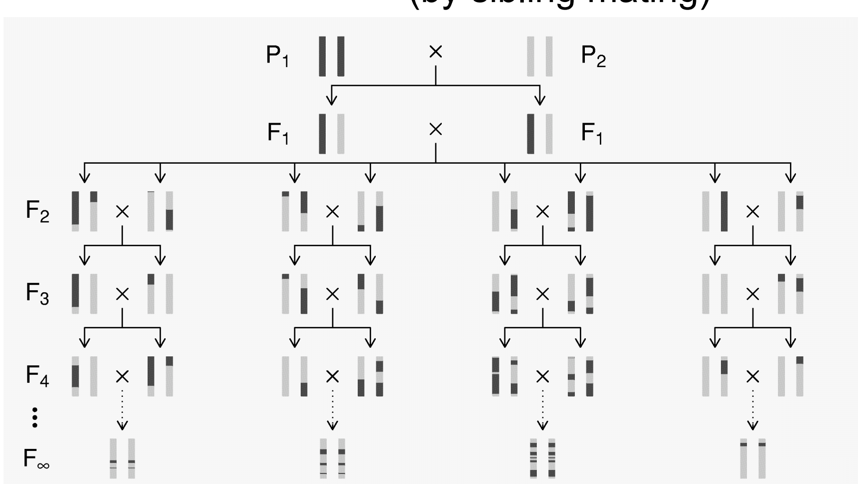
Recombinant inbred lines

(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	$D_{n+1} = D + I + \frac{1}{4}(\alpha^2+\gamma^2)M + \frac{1}{4}(\beta^2+\delta^2)P + \frac{1}{8}Q + \frac{1}{8}S + \frac{1}{8}(\beta^2+\delta^2)U + \frac{1}{8}(\alpha^2+\gamma^2)V + \frac{1}{16}\beta^2\delta^2W + \frac{1}{16}(\alpha^2\delta^2+\beta^2\gamma^2)X + \frac{1}{16}\alpha^2\gamma^1Y.$
$AABB \times aabb$	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	$M_{n+1} = \frac{1}{4}($ … $\beta^2\delta^2W +$ …

Typical mating	Number of types	
$AABB \times Ab.aB$	4	$N_{n+1} = \frac{1}{8}R + \frac{1}{8}(\alpha\beta+\gamma\delta)(U+V) + \frac{1}{8}\alpha\beta\gamma\delta(W+2X+Y).$
$AAbb \times AB.ab$	4	$P_{n+1} = \frac{3}{8}S + \frac{1}{8}(\alpha\beta+\gamma\delta)(U+V) + \frac{1}{8}\alpha\beta\gamma\delta(W+2X+Y).$
$AABb \times AABb$	4	$Q_{n+1} = 2G + \frac{1}{2}(H+I+J+K) + \frac{1}{4}(\alpha^2+\gamma^2)(L+M) + \frac{1}{4}(\beta^2+\delta^2)(N+P) + \frac{1}{4}Q + \frac{1}{8}(R+S+T) + \frac{1}{8}(\alpha^2+\alpha\beta+\beta^2+\gamma^2+\gamma\delta+\delta^2)(U+V) + \frac{1}{16}(\alpha\delta+\beta\gamma)^2(W+Y) + \frac{1}{8}(\alpha\gamma+\beta\delta)^2X.$
$AABb \times AaBB$	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	$Y_{n+1} = 2(F+K) + \frac{1}{2}(\alpha^2+\gamma^2)M + \frac{1}{2}(\beta^2+\delta^2)P + \frac{1}{4}(R+T) + \frac{1}{4}(\beta^2+\delta^2)U + \frac{1}{4}(\alpha^2+\gamma^2)V + \frac{1}{4}\beta^2\delta^2W + \frac{1}{4}(\alpha^2\delta^2+\beta^2\gamma^2)X + \frac{1}{4}\alpha^2\gamma^2Y.$

Result for sib-mating

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

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

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

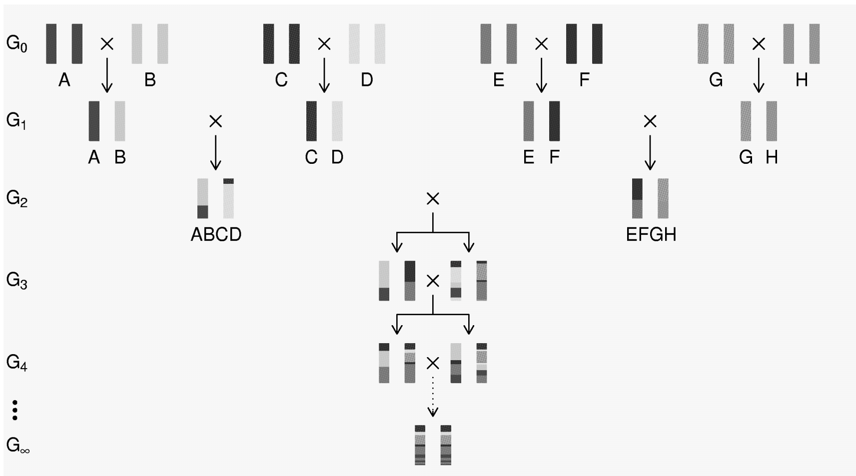
as may easily be verified.

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

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).

The "Collaborative Cross"



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

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

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

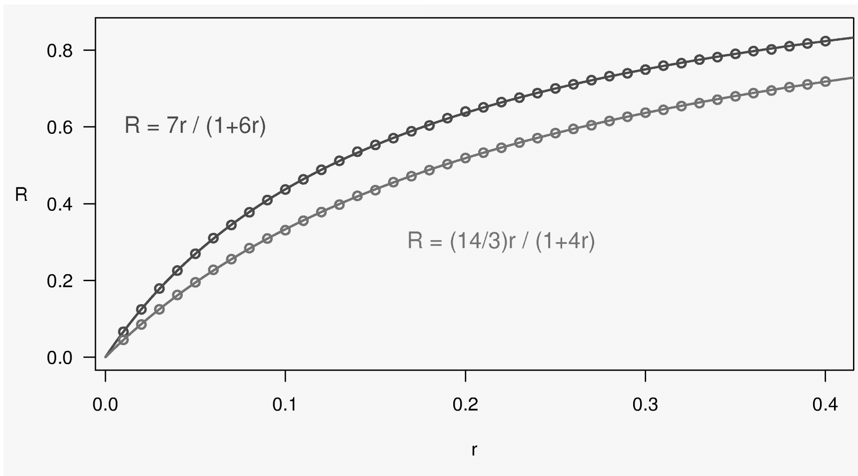
$\Pr(G_2=B \mid G_1=A) = r / (1+4r)$

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

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

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

Computer simulations



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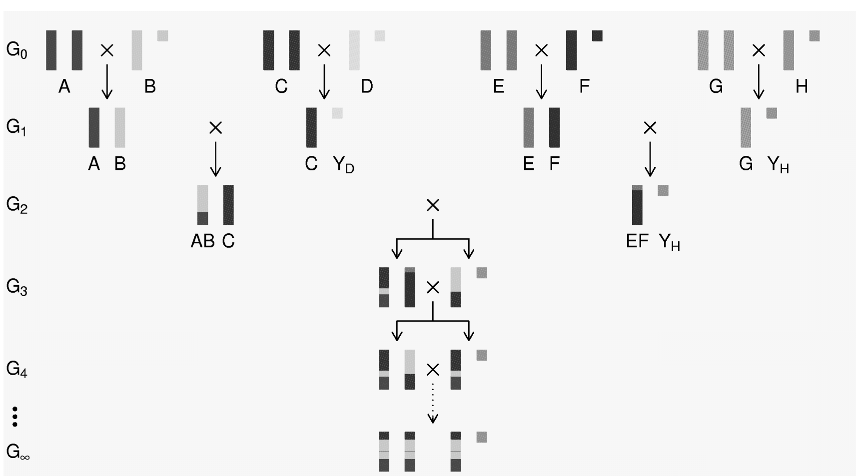
The X chromosome



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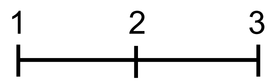
3-point coincidence



- 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 23 | rec'n in 12) / Pr(rec'n in 23)
- No interference $\rightarrow$ = 1
  Positive interference $\rightarrow$ < 1
  Negative interference $\rightarrow$ > 1
- Generally c is a function of r.

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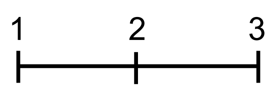
3-points in 2-way RILs



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

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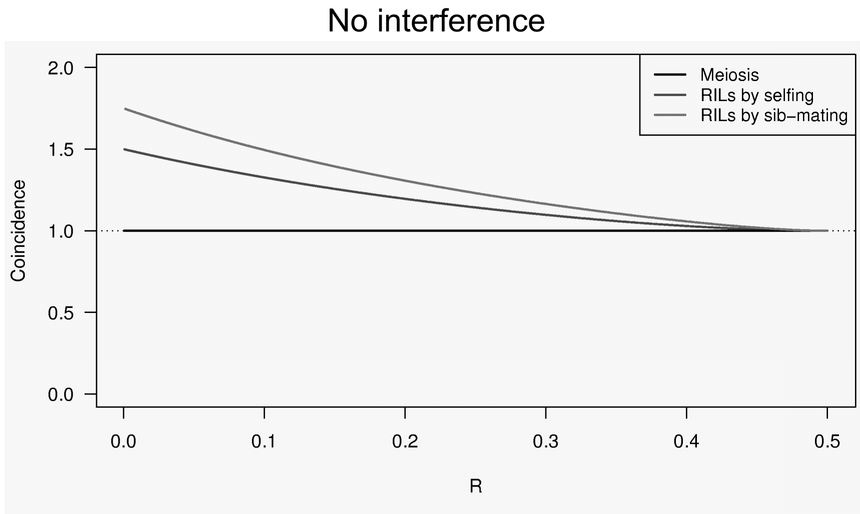
Coincidence



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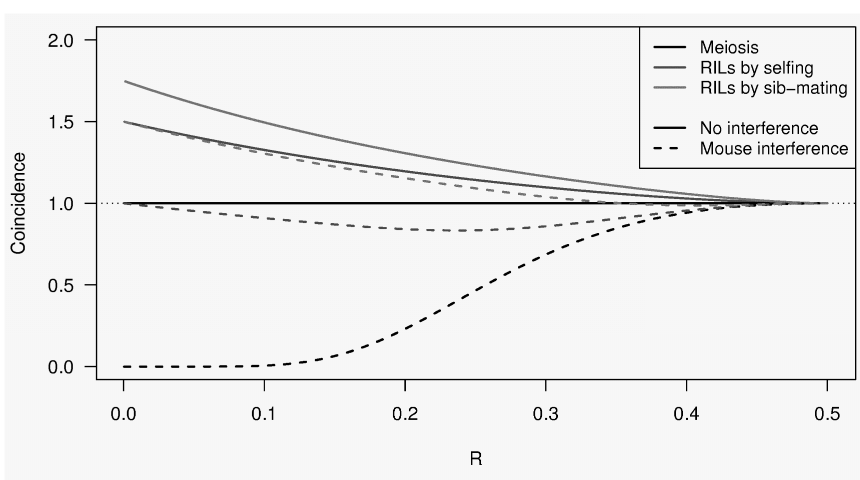
Coincidence



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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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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.

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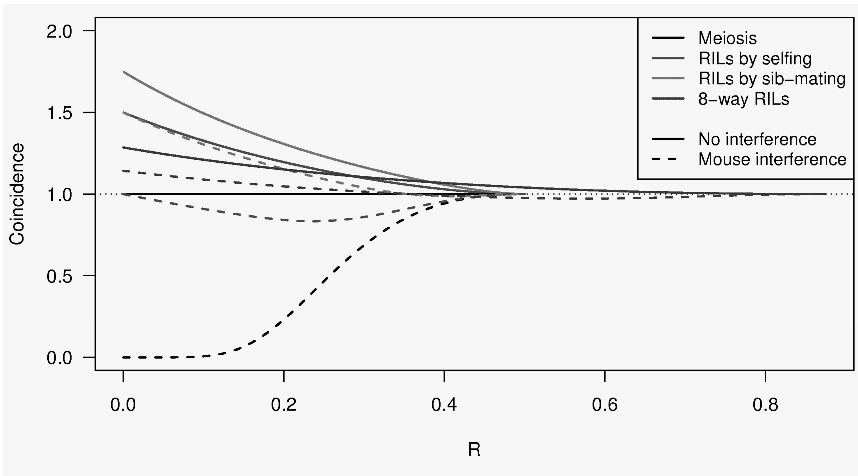
Coincidence



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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.
- 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.

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The key points

- R = 7 r / (1 + 6 r)
- 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.

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