

# The genomes of recombinant inbred lines

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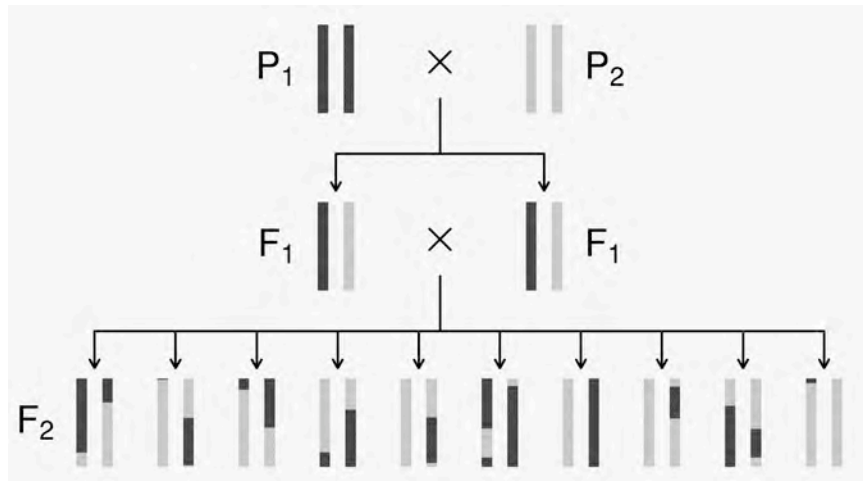
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<http://www.biostat.jhsph.edu/~kbroman>



## The intercross



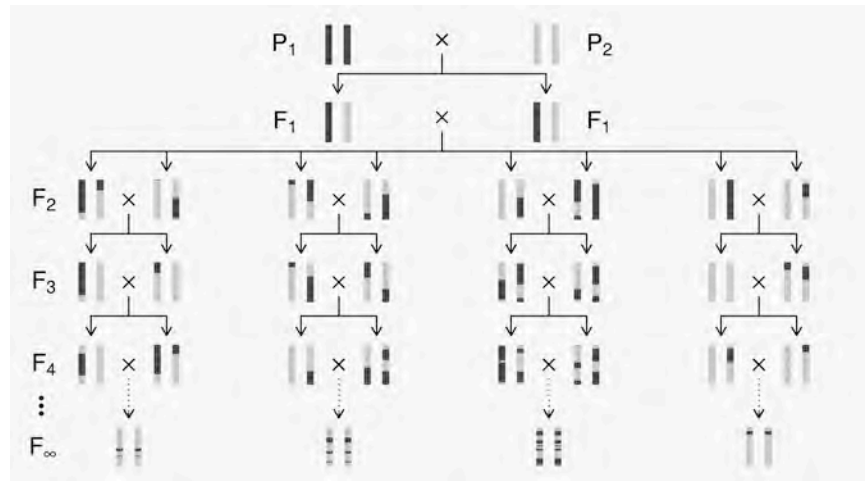
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## Opportunities for improvement

- 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.

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## Recombinant inbred lines (by sibling mating)



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## RI lines

### Advantages

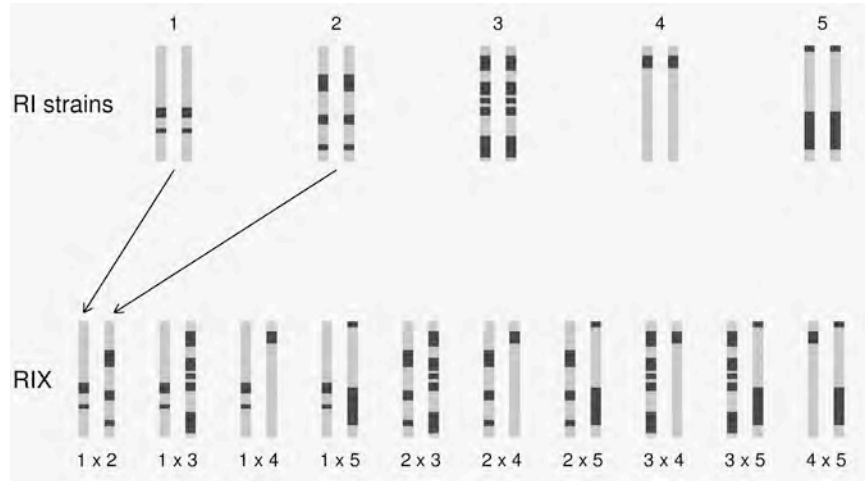
- + Each strain is an eternal resource.
  - + Only need to genotype once.
  - + Reduce individual variation by phenotyping multiple individuals from each strain.
  - + Study multiple phenotypes on the same genotype.
- + Greater mapping precision.
  - + More dense breakpoints on the RI chromosomes.

### Disadvantages

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

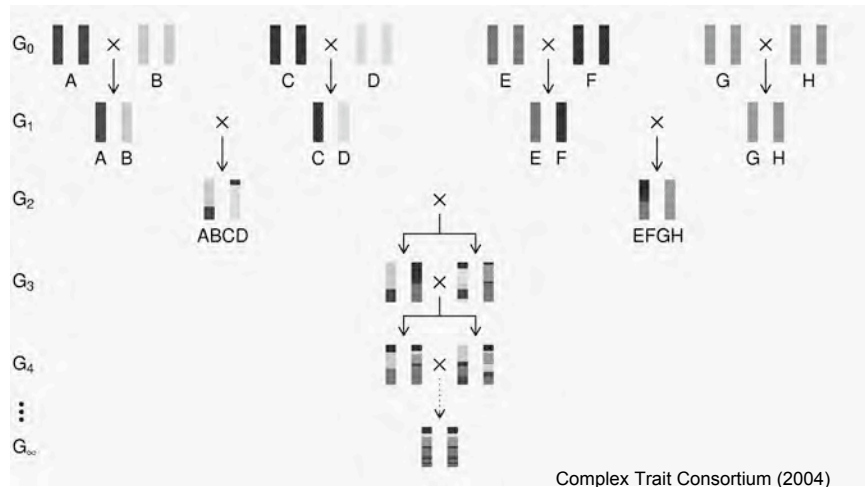
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## The RIX design



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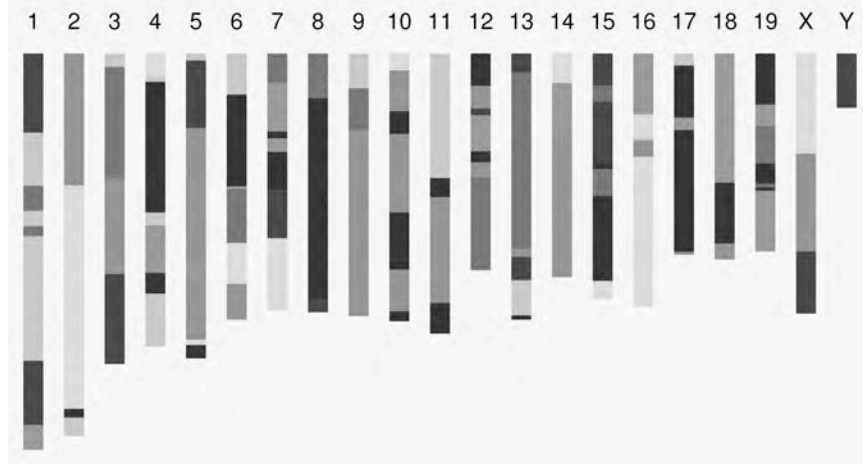
## The Collaborative Cross



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

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## Genome of an 8-way RI



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## The Collaborative Cross

### Advantages

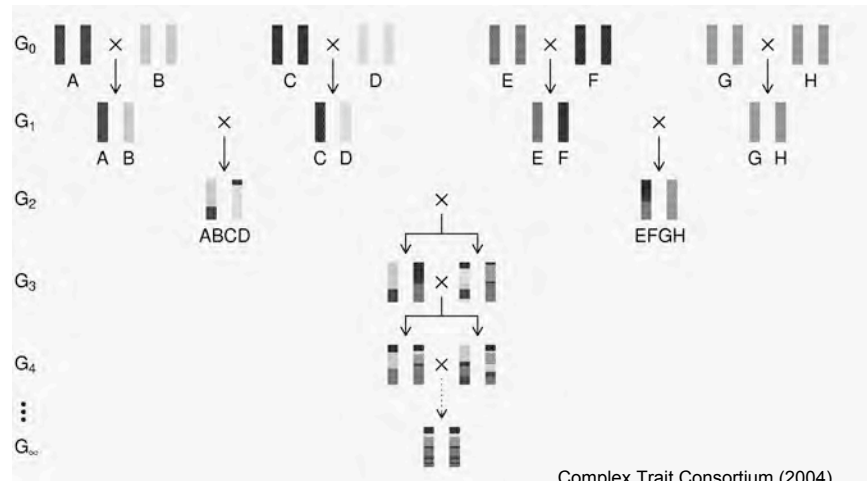
- + Great mapping precision.
- + Eternal resource.
  - + Genotype only once.
  - + Study multiple invasive phenotypes on the same genotype.

### Barriers

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

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# The Collaborative Cross



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

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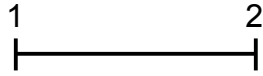
## The goal

(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.

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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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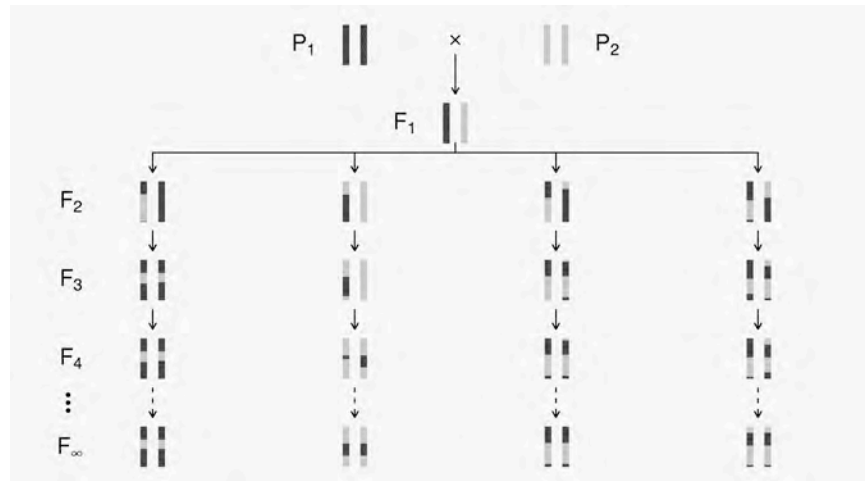
## Haldane & Waddington 1931

INBREEDING AND LINKAGE*	
J. B. S. HALDANE AND C. H. WADDINGTON	
<i>John Innes Horticultural Institution, London, England</i>	
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  $\{X_0, X_1, X_2, \dots\}$  satisfying
 
$$\Pr(X_{n+1} | X_0, X_1, \dots, X_n) = \Pr(X_{n+1} | X_n)$$
- 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

$$\Pr(X_n \rightarrow j | X_0)$$

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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  $D_\infty$  is the final proportion of crossover zygotes. Then considering the results of selfing each generation, we have:

$$\begin{cases}
 C_{n+1} = C_n + \frac{1}{2}E_n + \frac{1}{4}(1 - \beta - \delta + \beta\delta)F_n + \frac{1}{4}\beta\delta G_n \\
 D_{n+1} = D_n + \frac{1}{2}E_n + \frac{1}{4}\beta\delta F_n + \frac{1}{4}(1 - \beta - \delta + \beta\delta)G_n \\
 E_{n+1} = \frac{1}{2}E_n + \frac{1}{4}(\beta + \delta - 2\beta\delta)(F_n + G_n) \\
 F_{n+1} = \frac{1}{2}(1 - \beta - \delta + \beta\delta)F_n + \frac{1}{2}\beta\delta G_n \\
 G_{n+1} = \frac{1}{2}\beta\delta F_n + \frac{1}{2}(1 - \beta - \delta + \beta\delta)G_n
 \end{cases} \quad (1.1)$$

for  $C_{n+1}$ ,  $D_{n+1}$ , and  $F_{n+1}$ ,  $G_{n+1}$ ,

$$\left. \begin{matrix} d_n \\ \end{matrix} \right\} \quad (1.2)$$

for all values of  $n$ .

$$- 2x)d_n$$

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

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

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



$$\therefore y = \frac{2x}{1 + 2x}$$

$$(1.3)$$

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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*.

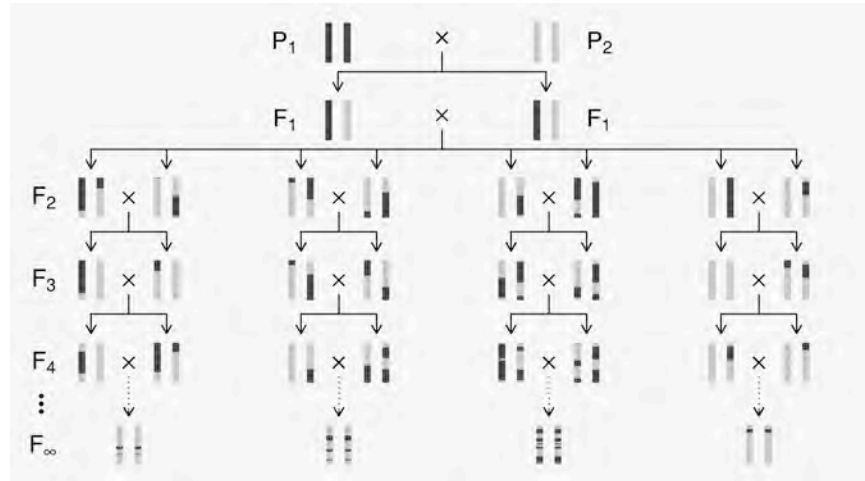
Then  $h_{AA|AA} = 1$  and  $h_{AB|AB} = 0$ .

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	Equation
$AABB \times AABB$	2	$C_{n+1} = C_n + H + \frac{1}{2}(\alpha^2 + \gamma^2)L + \frac{1}{4}(\beta^2 + \delta^2)N + \frac{1}{2}Q + \frac{1}{2}R + \frac{1}{4}(\alpha^2 + \gamma^2)U + \frac{1}{4}(\beta^2 + \delta^2)V + \frac{1}{4}\alpha\gamma^2W + \frac{1}{4}\alpha^2\beta^2X + \frac{1}{4}\alpha^2\delta^2Y$
$Aabb \times AAbb$	2	$D_{n+1} = D + I + \frac{1}{2}(\alpha^2 + \gamma^2)M + \frac{1}{4}(\beta^2 + \delta^2)P + \frac{1}{2}Q + \frac{1}{2}S + \frac{1}{4}(\beta^2 + \delta^2)U + \frac{1}{4}(\alpha^2 + \gamma^2)V + \frac{1}{4}\alpha\beta^2W + \frac{1}{4}\alpha^2(\beta^2 + \delta^2)X + \frac{1}{4}\alpha^2\gamma^2Y$
$AABB \times aabb$	2	$E_{n+1} = \frac{1}{2}\alpha\gamma^2W + \frac{1}{4}(\alpha^2\beta^2 + \beta^2\gamma^2)X + \frac{1}{4}\alpha\delta^2Y$
$Aabb \times aaBB$	2	$F_{n+1} = \frac{1}{2}\alpha\beta^2W + \frac{1}{4}(\alpha^2\delta^2 + \delta^2\gamma^2)X + \frac{1}{4}\alpha\gamma^2Y$
$AABB \times AAbb$	8	$G_{n+1} = \frac{1}{2}(\alpha\beta + \gamma\delta)(U + V) + \frac{1}{4}\alpha\beta\gamma\delta(W + 2X + Y)$
$AABB \times AaBb$	8	$H_{n+1} = \frac{1}{2}(\alpha\beta + \gamma\delta)(U + V) + \frac{1}{4}(\alpha\delta + \beta\gamma)(W + 2X + Y) + \frac{1}{4}(\alpha\delta + \beta\gamma)(U + V) + \frac{1}{4}(\alpha\delta + \beta\gamma)(W + 2X + Y)$
$Aabb \times AaBb$	8	$I_{n+1} = \frac{1}{2}(\alpha\delta + \beta\gamma)(U + V) + \frac{1}{4}(\alpha\delta + \beta\gamma)(W + 2X + Y)$
$AABB \times Aaabb$	8	$J_{n+1} = \frac{1}{2}(\alpha\delta + \beta\gamma)(U + V) + \frac{1}{4}(\alpha\delta + \beta\gamma)(W + 2X + Y)$
$AAbb \times AaBB$	8	$K_{n+1} = \frac{1}{2}(\alpha\delta + \beta\gamma)(U + V) + \frac{1}{4}(\alpha\delta + \beta\gamma)(W + 2X + Y)$
$AABB \times AB.ab$	4	$L_{n+1} = \frac{1}{2}(\alpha\delta + \beta\gamma)W + \frac{1}{4}(\alpha\delta + \beta\gamma)(U + V) + \frac{1}{4}(\alpha\delta + \beta\gamma)(W + 2X + Y)$
$Aabb \times Ab.aB$	4	$M_{n+1} = \frac{1}{2}(\alpha\delta + \beta\gamma)W + \frac{1}{4}(\alpha\delta + \beta\gamma)(U + V) + \frac{1}{4}(\alpha\delta + \beta\gamma)(W + 2X + Y)$
$AABB \times Ab.aB$	4	$N_{n+1} = \frac{1}{2}R + \frac{1}{4}(\alpha\delta + \gamma\delta)(U + V) + \frac{1}{4}\alpha\beta\gamma\delta(W + 2X + Y)$
$AAbb \times AB.ab$	4	$P_{n+1} = \frac{1}{2}S + \frac{1}{4}(\alpha\delta + \gamma\delta)(U + V) + \frac{1}{4}\alpha\beta\gamma\delta(W + 2X + Y)$
$AAbb \times AaBb$	4	$Q_{n+1} = 2G + \frac{1}{2}(H + I + J + K) + \frac{1}{4}(\alpha^2 + \gamma^2)(L + M) + \frac{1}{2}(\beta^2 + \delta^2)(N + P) + \frac{1}{2}Q + \frac{1}{4}(R + S + T) + \frac{1}{4}(\alpha^2 + \alpha\beta + \beta^2 + \gamma^2 + \gamma\delta + \delta^2)(U + V) + \frac{1}{4}(\alpha\delta + \beta\gamma)(W + Y) + \frac{1}{4}(\alpha\gamma + \beta\delta)X$
$AABB \times AaBB$	4	$R_{n+1} = \frac{1}{2}(\beta^2 + \delta^2)L + \frac{1}{4}(\alpha^2 + \gamma^2)N + \frac{1}{4}R + \frac{1}{4}(\beta^2 + \delta^2)U + \frac{1}{4}(\alpha + \gamma)V + \frac{1}{4}(\alpha\delta + \beta\gamma)(W + Y) + \frac{1}{4}(\alpha\gamma + \beta\delta)X$
$AAbb \times Aaabb$	4	$S_{n+1} = \frac{1}{2}(\beta^2 + \delta^2)M + \frac{1}{4}(\alpha^2 + \gamma^2)P + \frac{1}{4}S + \frac{1}{4}(\alpha + \gamma)U + \frac{1}{4}(\beta + \delta)V + \frac{1}{4}(\alpha\delta + \beta\gamma)(W + Y) + \frac{1}{4}(\alpha\gamma + \beta\delta)X$
$AABB \times aaBb$	4	$T_{n+1} = \frac{1}{2}(\alpha\delta + \gamma\delta)(U + V) + \frac{1}{4}(\alpha\delta + \beta\gamma)(W + Y) + \frac{1}{4}(\alpha\gamma + \beta\delta)X$
$AAbb \times AB.ab$	8	$U_{n+1} = \frac{1}{2}J + \frac{1}{4}(\alpha\delta + \gamma\delta)(L + N) + \frac{1}{4}(S + T) + \frac{1}{4}(\alpha + \gamma)U + \frac{1}{4}(\beta + \delta)V + \frac{1}{2}\alpha\gamma(\beta\gamma + \alpha\delta)W + \frac{1}{4}(\alpha\gamma + \beta\delta)(\alpha\delta + \beta\gamma)X + \frac{1}{4}\beta\delta(\beta\gamma + \alpha\delta)Y$
$AABB \times Ab.aB$	8	$V_{n+1} = \frac{1}{2}K + \frac{1}{4}(\alpha\delta + \gamma\delta)(M + P) + \frac{1}{4}(R + T) + \frac{1}{4}(\beta + \delta)U + \frac{1}{4}(\alpha + \gamma)V + \frac{1}{2}\beta\delta(\beta\gamma + \alpha\delta)W + \frac{1}{4}(\alpha\gamma + \beta\delta)(\alpha\delta + \beta\gamma)X + \frac{1}{4}\alpha\gamma(\beta\gamma + \alpha\delta)Y$
$AB.ab \times AB.ab$	1	$W_{n+1} = 2(E + J) + \frac{1}{4}(\alpha^2 + \gamma^2)L + \frac{1}{4}(\beta^2 + \delta^2)N + \frac{1}{4}(S + T) + \frac{1}{4}(\alpha^2 + \gamma^2)U + \frac{1}{4}(\beta^2 + \delta^2)V + \frac{1}{4}\alpha\gamma^2W + \frac{1}{4}\alpha^2\beta^2X + \frac{1}{4}\alpha^2\delta^2Y$
$AB.ab \times Ab.aB$	2	$X_{n+1} = \frac{1}{2}T + \frac{1}{4}(\alpha\delta + \gamma\delta)(U + V) + \frac{1}{4}\alpha\beta\gamma\delta(W + 2X + Y)$
$Ab.aB \times Ab.aB$	1	$Y_{n+1} = 2(F + K) + \frac{1}{4}(\alpha^2 + \gamma^2)M + \frac{1}{4}(\beta^2 + \delta^2)P + \frac{1}{4}(R + T) + \frac{1}{4}(\beta^2 + \delta^2)U + \frac{1}{4}(\alpha^2 + \gamma^2)V + \frac{1}{4}\alpha\beta^2W + \frac{1}{4}(\alpha^2\delta^2 + \delta^2\gamma^2)X + \frac{1}{4}\alpha\gamma^2Y$

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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.

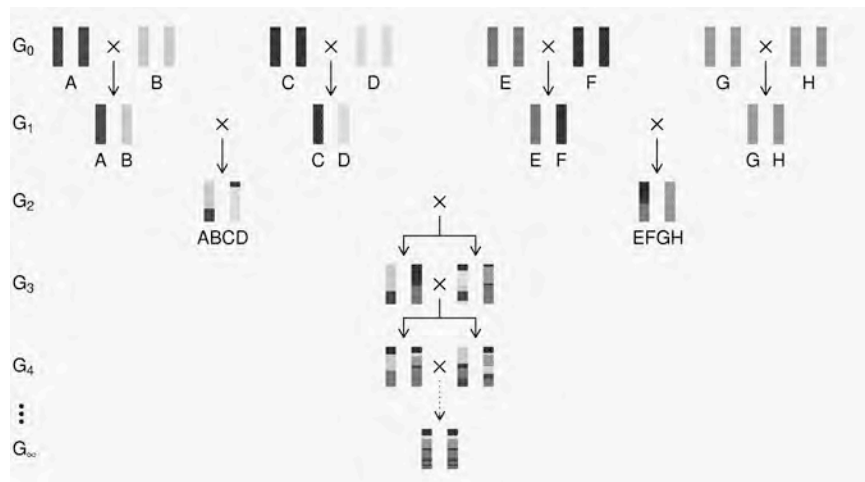
$$\therefore c_{\infty} = c_n + 2e_n + \frac{1}{1+6x} [(1-2x)(d_n + 2f_n + 2j_n + \frac{1}{2}k_n) + 2g_n + 4x(h_n + i_n)] \quad (3.4)$$

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

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

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## The "Collaborative Cross"



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## 8-way RILs

### Autosomes

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

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

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

### X chromosome

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

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

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

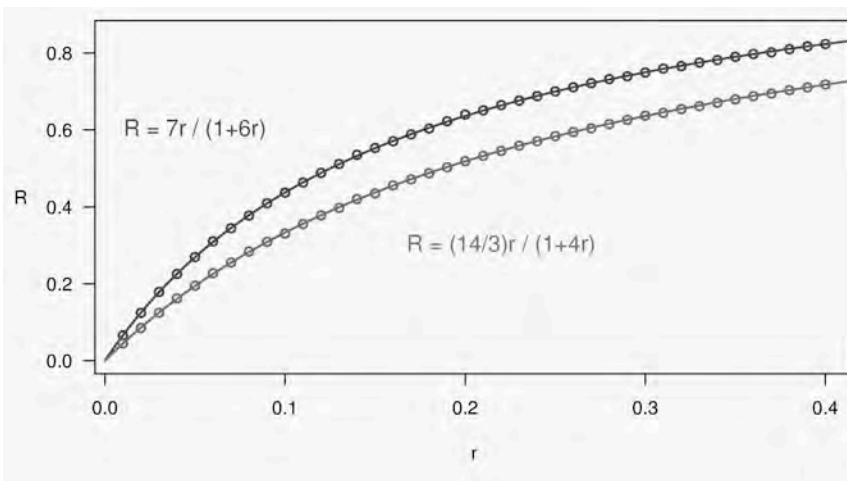
$$\Pr(G_2=C \mid G_1=A) = 2r / (1+4r)$$

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

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

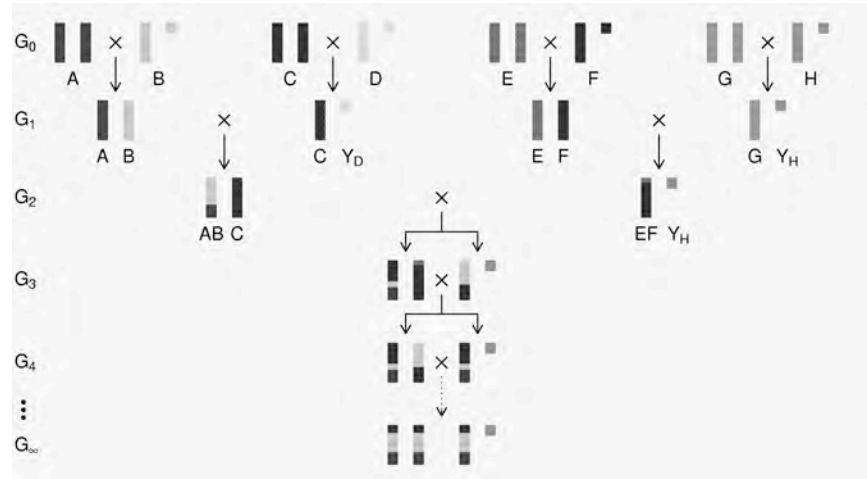
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## Computer simulations



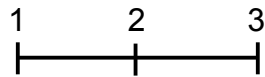
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## The X chromosome



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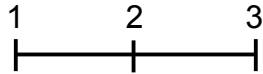
## 3-point coincidence



- $r_{ij}$  = recombination fraction for interval  $i,j$ ;  
assume  $r_{12} = r_{23} = r$
- Coincidence =  $c = \text{Pr}(\text{double recombinant}) / r^2$   
=  $\text{Pr}(\text{rec'n in } 23 \mid \text{rec'n in } 12) / \text{Pr}(\text{rec'n in } 23)$
- 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

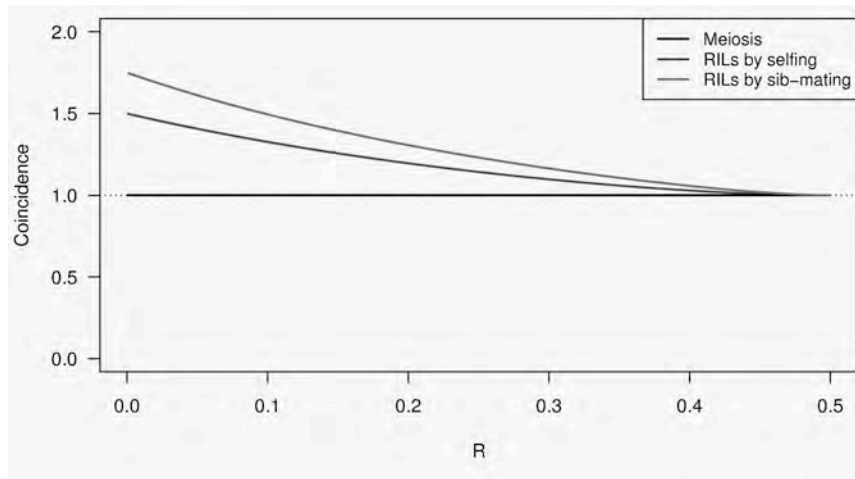


- $r_{13} = 2 r (1 - c r)$
- $R = f(r); \quad R_{13} = f(r_{13})$
- $\text{Pr}(\text{double recombinant in RIL}) = \{ R + R - R_{13} \} / 2$
- $\text{Coincidence (in 2-way RIL)} = \{ 2 R - R_{13} \} / \{ 2 R^2 \}$

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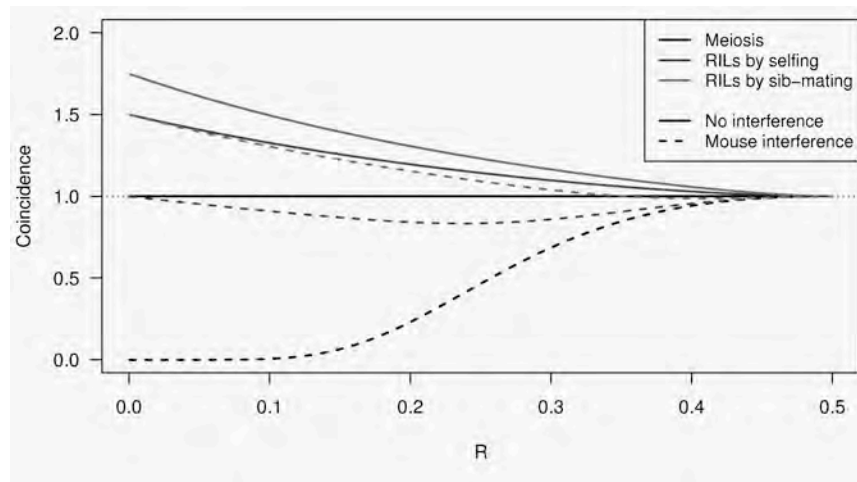
## Coincidence

No interference



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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.

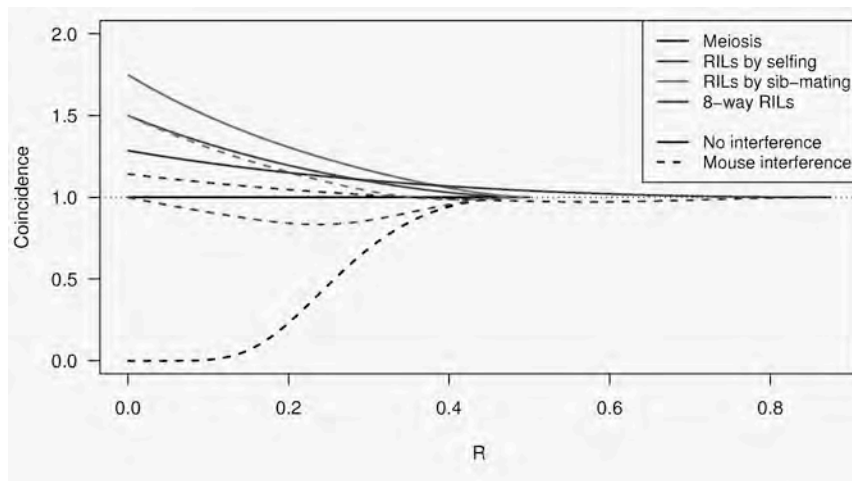
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## Coincidence in 8-way RILs

- 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 → 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.

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## Coincidence



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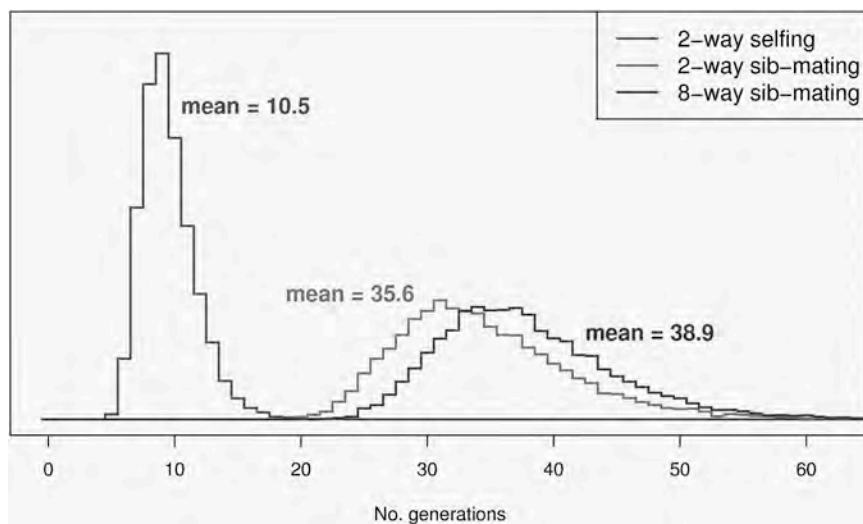


## Whole genome simulations

- 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

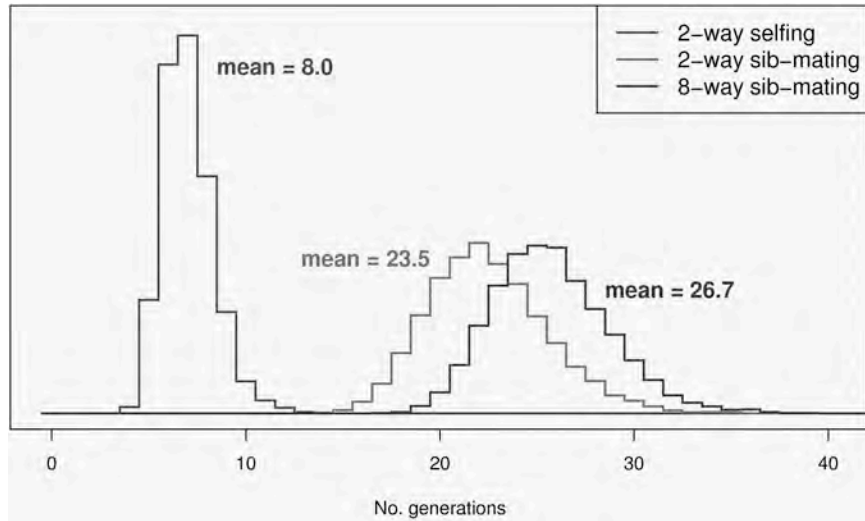
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## No. generations to fixation

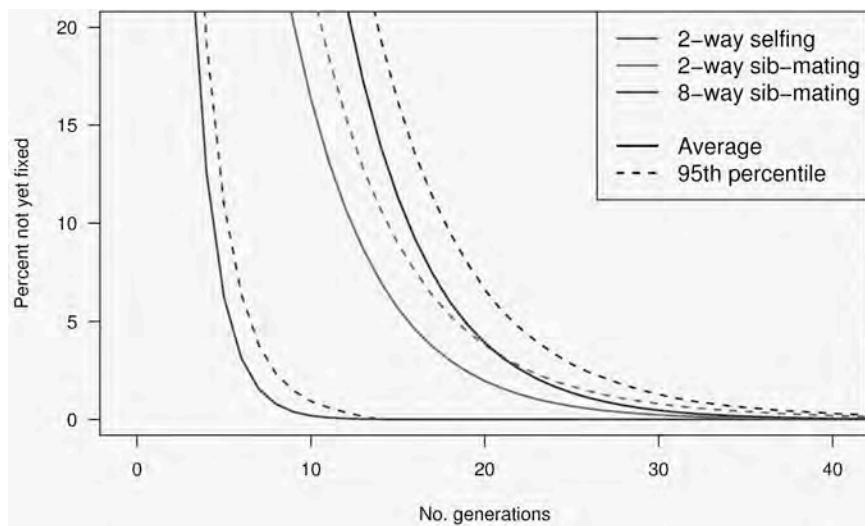


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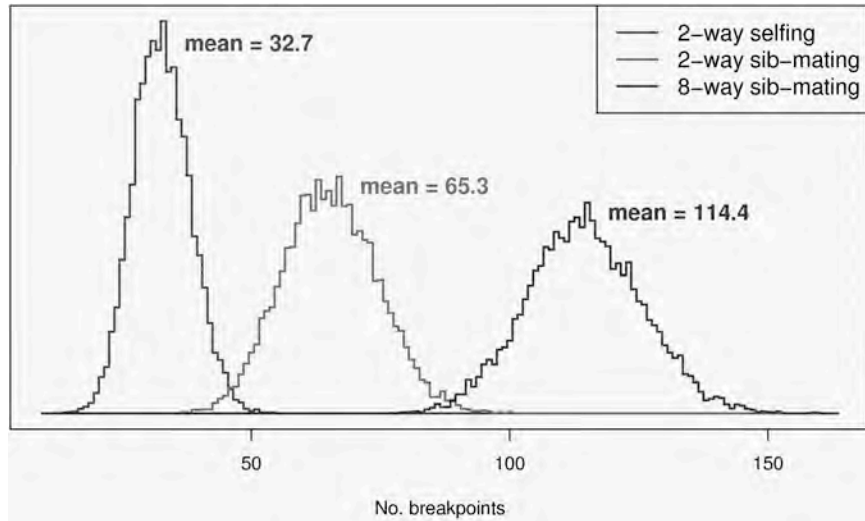
## No. gen's to 99% fixation



## Percent genome not fixed

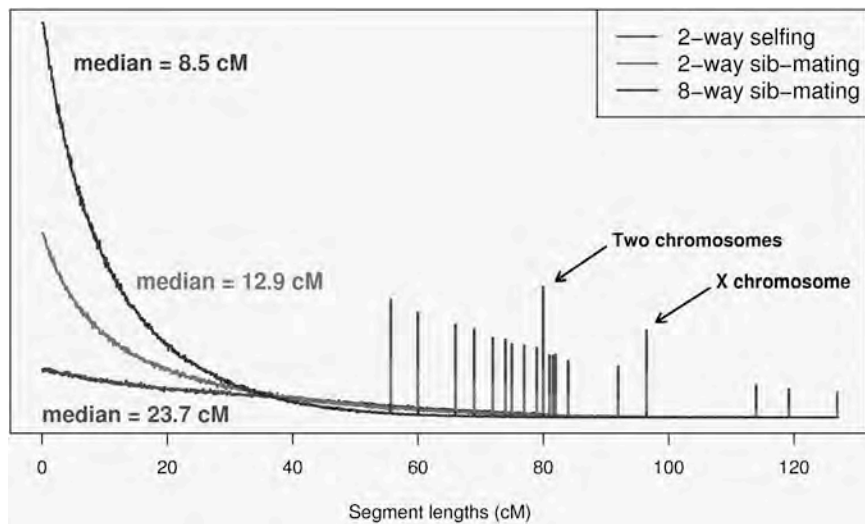


## Number of breakpoints



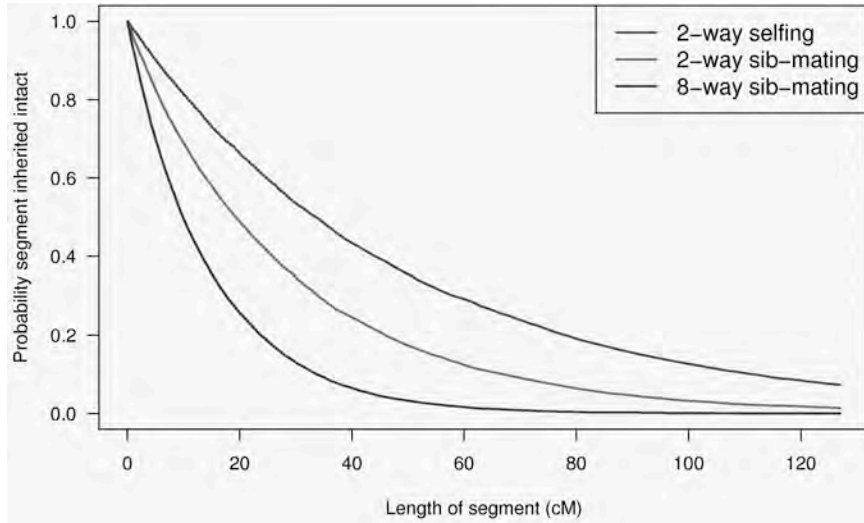
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## Segment lengths



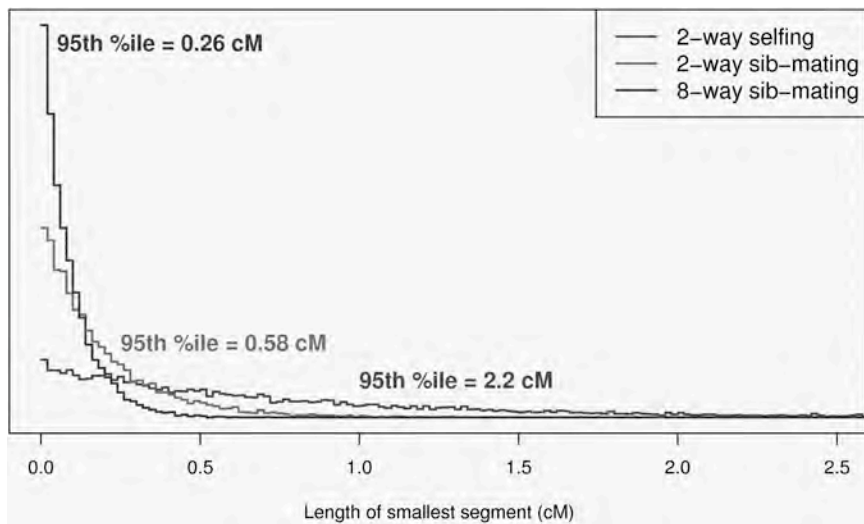
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## Probability a segment is inherited intact



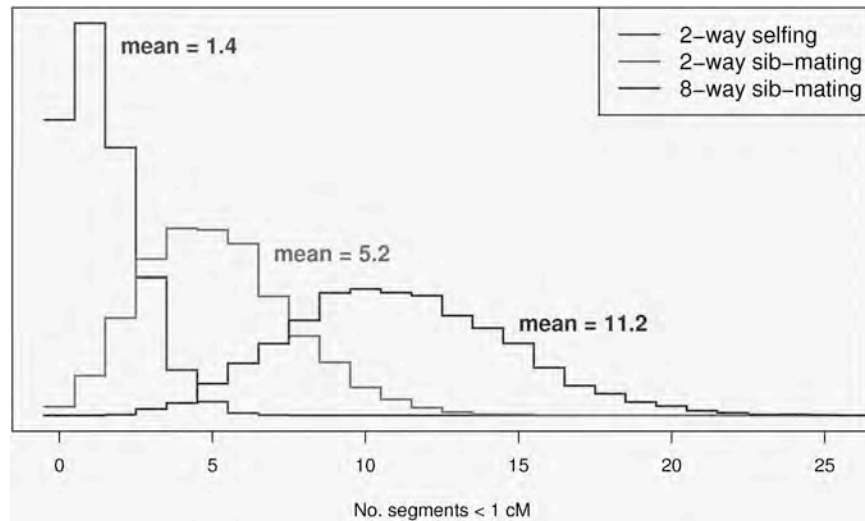
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## Length of smallest segment



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## No. segments < 1 cM



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

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