

Recombination Mapping with a Two-point Test cross —

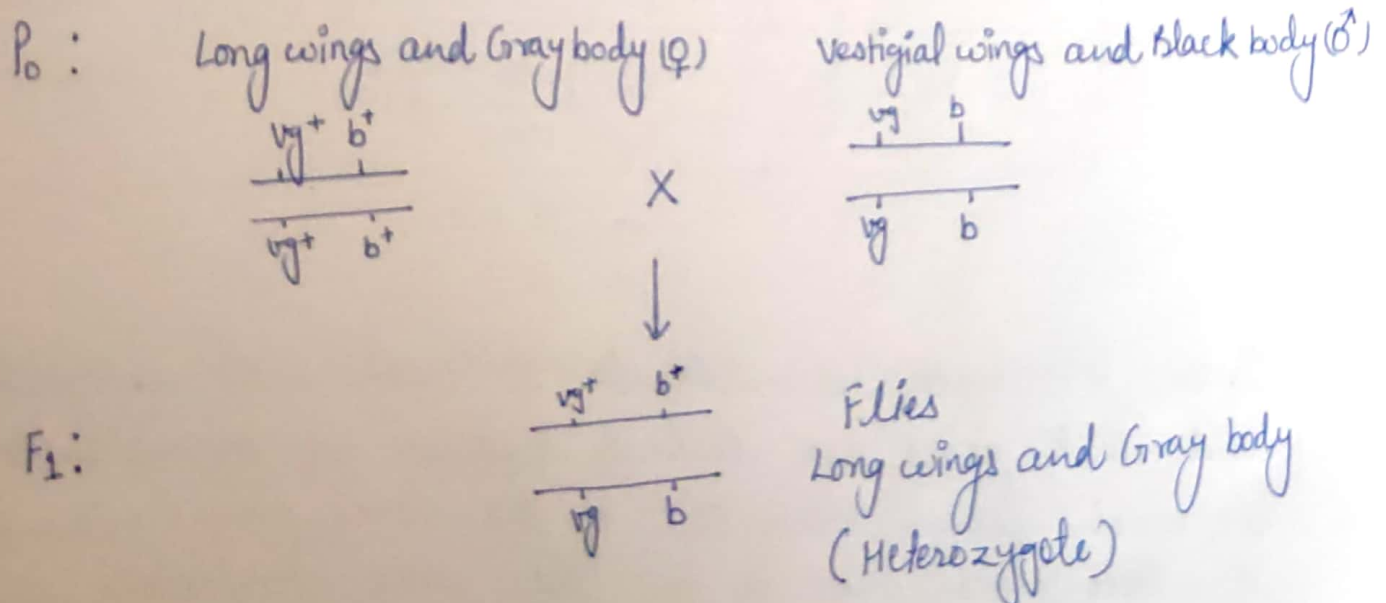
A test cross between two genes is called a two-point test cross.

- Q. In two point test cross, wild type *Drosophila* females were mated to male homozygous for two autosomal mutations — vestigial (*vg*) which produces short wings and black (*b*) which produces a black body. All the F_1 flies had long wings and gray bodies; thus the wild type alleles (vg^+ and b^+) are dominant. The F_1 females were then testcrossed to vestigial, black males and the F_2 progeny were sorted by phenotype and counted. The result was as follows:

<u>Phenotype</u>	<u>Genotype</u>	<u>no. of observed progeny</u>
Long wings, Gray body	$(vg^+ b^+)$	= 415
vestigial wings, Black body	$(vg b)$	= 405
vestigial wings, Gray body	$(vg b^+)$	= 92
Long wings, Black body	$(vg^+ b)$	= 88
		Total 1000

Is there evidence that gene for wings and the gene for body color are linked? If so, what is the map distance between the two loci?

Solution— The best approach is to begin by the diagramming the cross, using gene symbols:



Test cross: $\frac{vg^+}{vg} \frac{b^+}{b} (F_1, \text{♀}) \times \frac{vg}{vg} \frac{b}{b} (\text{♂})$

↓

Test crossed
Progeny

	<u>Genotypes</u>	<u>no. of observed Progeny</u>	
(L, G)	$vg^+ b^+$	415	Parental
(v, b)	$vg b$	405	Parental
(V, G)	$vg b^+$	92	Recombinant
(L, b)	$vg^+ b$	88	Recombinant
		Total 1000	

$$\begin{aligned}
 \text{Frequency of Recombination} &= \frac{\text{no. of recombinant progeny}}{\text{Total no. of progeny}} \times 100\% \\
 &= \frac{180}{1000} \times 100 \\
 &= 18 \text{ m.u.} \\
 &= 18\%
 \end{aligned}$$

Thus, 18% were recombinants. It means that vestigial and black genes are linked because the recombinants are much fewer than 50% of the total progeny counted. So, these genes must be on the same chromosome (coupling). a map distance between these two genes (vg and b) is 18 m.u. we can say that vg and b are 18 cM apart.

Recombination Mapping with a three point test cross

A test cross between three genes is called three point test cross.

*Coefficient of Coincidence — one crossover inhibits to the occurrence of another nearby, a phenomenon called Interference (I). The extent of the interference is measured by the coefficient of coincidence (c) which is the ratio of observed frequency of double crossovers to expected frequency of double crossovers.

$$\text{Coefficient of Coincidence (C)} = \frac{\text{observed frequency of D.C.O}}{\text{Expected frequency of D.C.O}}$$

Question — The data obtained from a three factor test-cross is as follows:

<u>Genotype</u>		<u>Number of Progeny</u>
wildtype	$st^+ ss^+ e^+$	283
Scarlet, ebony, spineless	$st ss e$	278
spineless, ebony	$st^+ ss e$	50
Scarlet	$st ss^+ e^+$	52
Ebony	$st^+ ss^+ e$	43
Scarlet, spineless	$st ss e^+$	41
spineless	$st^+ ss e^+$	05
Scarlet, ebony	$st ss^+ e$	03
		Total = 755

Based on the data —

- (1) Determine the order of genes
- (2) Draw a linkage map and calculate the map distance between the genes.
- (3) Calculate the coefficient of coincidence and Interference.

Solution — Use the following steps to determine the gene order.

- ① Assign classes —

1.	$st^+ ss^+ e^+$	283	} Parental (Non-Recombinant)
2.	$st ss e$	278	
3.	$st ss^+ e^+$	52	} I S.C.O (single crossover)
4.	$st^+ ss e$	50	
5.	$st^+ ss^+ e$	43	} II S.C.O
6.	$st ss e^+$	41	
7.	$st^+ ss e^+$	05	} D.C.O (Double crossover)
8.	$st ss^+ e$	03	
		Total 755	

② First of all, we compare the non-recombinant progeny with double crossover progeny, they should differ only in alleles of the middle locus. Comparing Double crossover classes (7 and 8) to parental classes (1 and 2), we see that the normal bristles allele has been switched with respect to Red eyes and Gray body color. So, the normal bristles gene must lie in the middle. Therefore the correct gene order is:

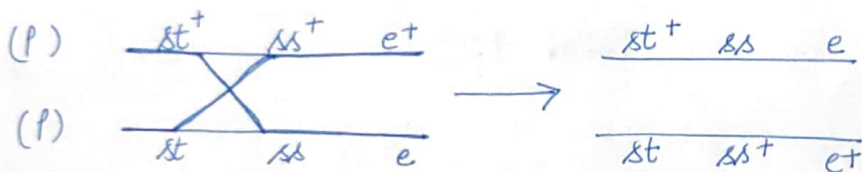
$st - ss - e$
 OR
 $e - ss - st$

- ③ calculating the Distances between genes —
we can determine the map distances which are based on the frequency of recombination.

$$\text{Recombination Frequency} = \frac{\text{No. of Recombinant Progeny}}{\text{Total no. of Progeny}} \times 100$$

(a) Distances between st — ss :-

Ist S.C.O between st — ss



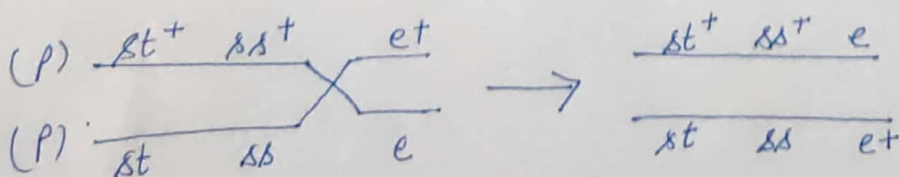
Two non-sister chromatids

$$\begin{aligned} \text{Recombination Frequency between } st - ss &= \frac{52 + 50 + 5 + 3}{755} \times 100 \\ &= 14.6\% \end{aligned}$$

The distance between st and ss loci can be expressed as 14.6 m.u.

⑥ Distance between ss — e :-

IInd S.C.O between ss — e



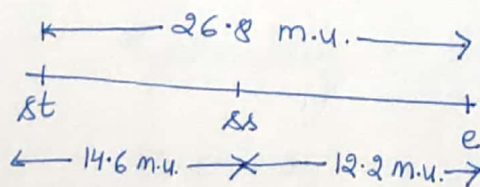
$$\begin{aligned} \text{b/w ss} \text{---} e \quad \text{recombination frequency} &= \frac{43 + 41 + 5 + 3}{755} \times 100 \\ &= 12.2\% \end{aligned}$$

Thus, map distance between ss—e is 12.2 m.u.

Finally, To calculate the map distance between the outer two loci, st and e, it is obtained by summing the map distance between st—ss and ss—e.

$$\begin{aligned} \text{Map Distance between st—e} &= 14.6 + 12.2 \\ &= 26.8 \text{ m.u.} \end{aligned}$$

Now, we can use the map distances to draw a map of the three genes on the chromosome:



A Linkage map or Genetic map of *D. melanogaster*

④ To calculate coefficient of coincidence (C)

$$C = \frac{\text{observed frequency of D.C.O}}{\text{Expected frequency of D.C.O}}$$

$$\text{Observed frequency of D.C.O} = \frac{\text{No. of progeny of D.C.O}}{\text{Total no. of progeny}}$$

$$= \frac{05 + 03}{755}$$

$$= 0.010$$

$$\text{Expected frequency of D.C.O} = \text{Recombination frequency of 1st crossover} \times \text{Recombination frequency of 2nd crossover}$$

$$= 14.6 \times 12.2$$

$$= 0.146 \times 0.122$$

$$= 0.017$$

$$(\text{Coefficient of Coincidence}) \quad C = \frac{0.010}{0.017}$$

$$= 0.58$$

$$= 0.6$$

$$\text{Interference} = 1 - C$$

$$= 1 - 0.6$$

$$= 0.4$$

Thus, 40% D.C.O expected progeny will not be observed because of Interference.