

Experiment no. 8: Determination of linkage and crossing over Estimation (two point and three point test cross data)

Aim: To study on linkage, crossing over and gene mapping

According to chromosome theory, many genes are located linearly in each chromosome. All the genes located in a chromosome tend to go together from generation to generation. The tendency of genes to be inherited together from generation to generation is termed as linkage. The first concept of linkage was demonstrated by T. H. Morgan in 1910 while studying the inheritance pattern of white eye (w gene) of drosophila (queen of genetics).

CONSEQUENCE OF LINKAGE: -

- ❖ Genes present in the chromosome tends to stay together during linkage inheritance.
- ❖ The distances between two genes are inversely proportional to the effect of linkage.
- ❖ All the genes present in a chromosome are in a linear form.
- ❖ There are two phases of linkage.
 - a) **Coupling Phase:** the dominant alleles of the linked genes are present in the same chromosome, while the recessive alleles of the same genes are present in the other chromosome of a homologous pair. Example is AB / ab
 - b) **Repulsion Phase:** the dominant alleles of some genes are present with recessive alleles of other genes in the same chromosome of a homologous pair. Example is Ab/aB
- ❖ Linkage does not follow independent assortment, so the ratio obtained in F₂ generation and test cross progeny is deviated from 9:3:3:1 or 1:1:1:1 respectively.

Map distance / Map unit: the distance between two given genes is calculated based on the percentage of crossing over, because the frequency of crossing over is directly proportional to the distance between two genes. A map unit is the distance between two linked genes and permits one percent recombination/crossing over between the two linked genes.

Formula for calculating Crossing over Frequency (%):

$$\text{Crossing Over Frequency (\%)} = \frac{\text{number of recombinants}}{\text{Total offspring}} \times 100$$

Two point test cross

What are two point tests cross?

Two point cross is used to determine the distance between the two linked genes. Single chiasma is observed in two point test cross.

Working example: AB/ab x ab/ab

Progeny:

AB/ab = 37 } Parental Type

ab / ab = 37 } Parental Type

Ab/ab= 13 } Recombinant types

aB/ab= 13 } Recombinant types

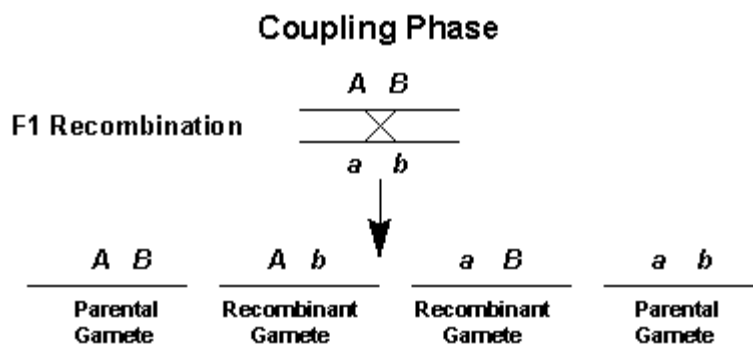
Total = 100

$$\text{Crossing Over Frequency (\%)} = \frac{\text{number of recombinants}}{\text{Total offspring}} \times 100$$

$$= 26 \times 100/100 = 26$$

Therefore the distance between the genes A & B = 26 map units.

The genes A and B are said to be linked in the coupling phase as seen below.



What is a three point cross?

Three point cross is used to determine the order and distance between the three linked genes. Two chiasma and double crossovers are observed in three point test cross.

Why are three point crosses important?

By solving a three point cross you can determine two important things:

- ❖ Order of the three genes on a chromosome.
- ❖ Determine the distance (in map units) between each pair of genes to construct linkage map.

PROCEDURE: Construction of linkage map and calculation of coefficient of coincidence and

interference can be illustrated by the following example.

Q. Draw a genetic map of these 3 genes (A, B and C) showing the distances between all pairs of genes and then calculate the value of interference

In a three point test cross, eight different phenotypic classes are obtained.

Parental types have the maximum phenotypic frequencies, double crossovers have the lowest phenotypic frequencies, and the single crossovers have phenotypic frequencies between these two classes. Suppose, ABC/abc are three linked genes located on two different chromosomes in F₁ of a cross between AABBCC and aabbcc parents.

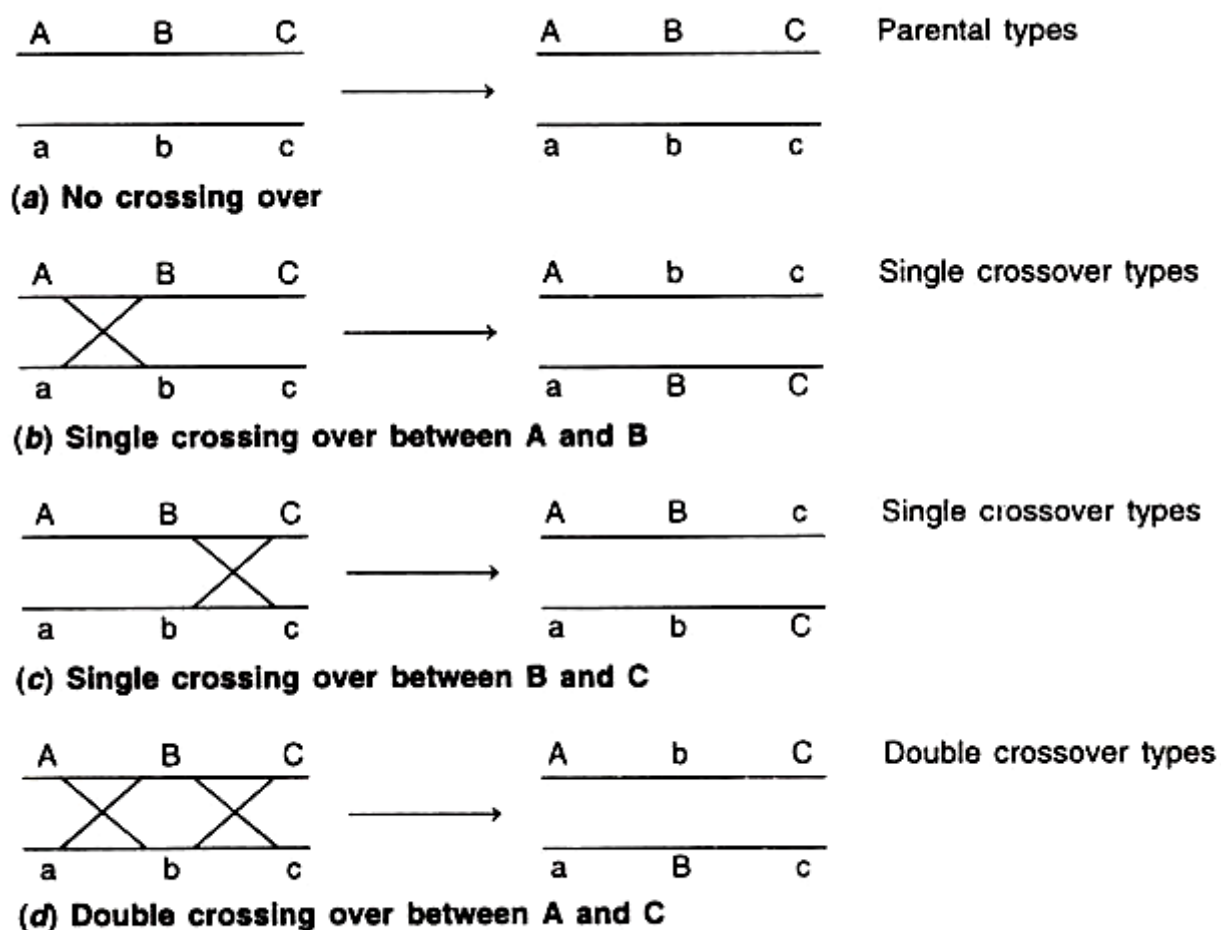


Fig. 9.3. Single and double crossing over between three linked genes.

1. Single crossover between A and B will alter the position of two genes, viz., B and C (Fig. 9.3).
2. Single crossover between B and C will alter the position of only one gene, i.e., C (Fig. 9.3).
3. Double crossover between A and C will alter the position of only middle gene, i.e., B (Fig. 9.3).

Thus eight types of gametes are produced by F₁ and only one type of gamete is produced by homozygous recessive parent. Union of male and female gametes will produce eight different phenotypic classes (Table 9.3).

TABLE 9.3. Summary of the results obtained from a three point test cross between $ABC/abc \times abc/abc$

| <i>Genotypic classes</i> | <i>Phenotypic classes</i> | <i>Assumed frequencies</i> | <i>Remarks</i> |
|--------------------------|---------------------------|----------------------------|-------------------------------------|
| ABC/abc | ABC | 349 | } Parental types. |
| abc/abc | abc | 360 | |
| Abc/abc | Abc | 114 | } Single crossover between A and B. |
| aBC/abc | aBC | 116 | |
| ABc/abc | ABc | 128 | } Single crossover between B and C. |
| abC/abc | abC | 124 | |
| AbC/abc | AbC | 5 | } Double crossover between A and C. |
| aBc/abc | aBc | 4 | |
| Total | | 1200 | |

2. Recombination (%) of Three point Test Cross:

Crossing over percentage between genes A and B = $(114+116)+(5+4)/1200 \times 100 = 230 + 9/1200 \times 100 = 19.92\%$

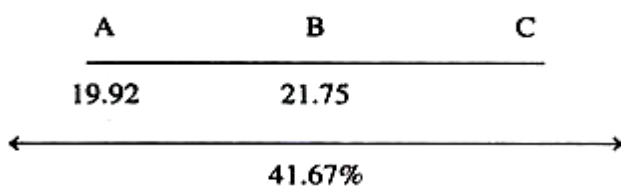
Crossing over percentage between genes B and C = $(128 + 124)+ (5 + 4)/1200 \times 100 = 252 + 9/1200 \times 100 = 21.75\%$

3. Gene Sequence of Three Point Test Cross:

Hence, distance between genes A & B = 19.92 map units

Distance between genes B& C= 21.75 map units

Distance between genes A & C= $21.75 + 19.92 = 41.67$ map units



4. Coefficient of Coincidence in Three point Test Cross:

Coefficient of coincidence =

Observed no. of double crossovers/Expected no. of double crossovers

1. Observed double crossovers = $9/1200 \times 100 = 0.75$

2. Expected double crossovers = Product of two single crossovers between AB & BC values/100

= $19.92 \times 21.75/100 = 4.33$

Coefficient of coincidence = $0.75/4.33 = 0.17$

Coefficient of interference = $1 - \text{Coefficient of coincidence} = 1 - 0.17 = 0.83$

