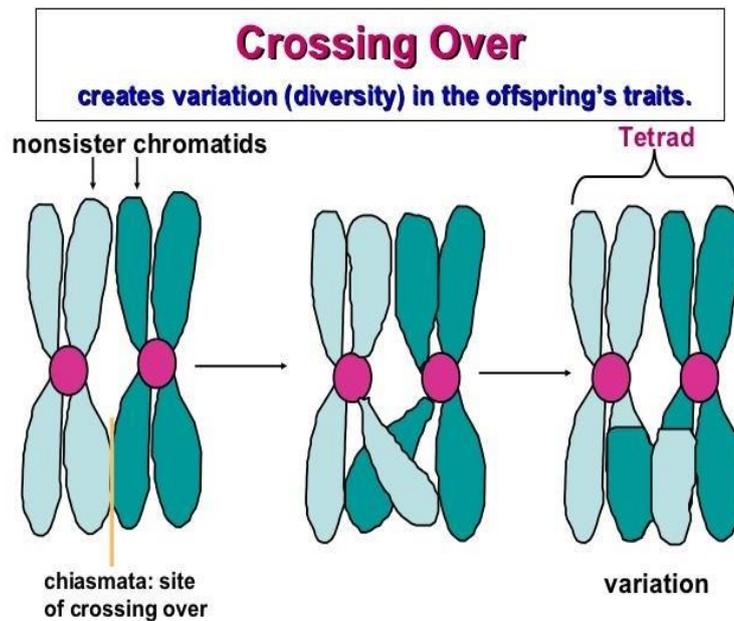


9. CROSSING OVER

The term crossing over was first used by Morgan and Cattell in 1912. The exchange of precisely homologous segments between non-sister chromatids of homologous chromosomes is called crossing over.

Mechanism of crossing over: It is responsible for recombination between linked genes and takes place during pachytene stage of meiosis i.e. after the homologous chromosomes have undergone pairing and before they begin to separate. It occurs through the process of breakage and reunion of chromatids. During pachytene, each chromosome of a bivalent (chromosome pair) has two chromatids so that each bivalent has four chromatids or strands (four-strand stage). Generally, one chromatid from each of the two homologues of a bivalent is involved in crossing over. In this process, a segment of one of the chromatids becomes attached in place of the homologous segment of the nonsister chromatid and vice-versa. It is assumed that breaks occur at precisely homologous points in the two nonsister chromatids involved in crossing over; this is followed by reunion of the acentric segments. This produces a cross (x) like figure at the point of exchange of the chromatid segments. This figure is called chiasma (which is seen in diplotene stage of meiosis) (plural-chiasmata).



Obviously, each event of crossing over produces two recombinant chromatids (involved in the crossing over) called cross over chromatids and two original chromatids (not involved in crossing over) referred to as noncrossover chromatids. The crossover chromatids will have new combinations of the linked genes, i.e. will be recombinant; gametes carrying them will produce the recombinant phenotypes in test-crosses, which are called crossover types. Similarly, the noncrossover chromatids will contain the parental gene combinations and the gametes carrying them will give rise to the parental phenotypes or noncrossover types. Therefore, the frequency of crossing over between two genes can be estimated as the frequency of recombinant progeny from a test-cross for these genes. This frequency is usually expressed as percent. Thus, the frequency of crossing over (%) can be calculated using the formula;

$$\text{Frequency of crossing over (\%)} = \frac{\text{No. of recombinant progeny from a test cross}}{\text{Total number of progeny}} \times 100$$

Types of crossing over: Depending upon the number of chiasmata involved, crossing over is of three types.

- 1. Single crossing over:** It refers to the formation of single chiasma between non-sister chromatids of homologous chromosomes. It involves two linked genes (Two-point test cross).
- 2. Double crossing over:** It refers to the formation of two chiasmata between non-sister chromatids of homologous chromosomes. It involves three linked genes (Three-point test cross).

3. Multiple crossingover: Occurrence of more than two crossing overs between non-sister chromatids of homologous chromosomes is known as multiple crossing over. However, the frequency of such type of crossing over is extremely low.

Factors affecting crossing over: The frequency of crossing over between the linked genes is affected by several factors.

- 1. Distance between the genes:** The frequency of crossing over between the two genes is positively associated with the distance between their location in the chromosome. Crossing over between the two genes would increase with an increase in distance between them.
- 2. sex:** The frequency of recombination is markedly influenced by the sex of heterozygotes for linked genes. In general, the heterogametic sex shows relatively lower recombination frequencies than the homogametic sex of the same species. Eg: No crossing over occurs between linked genes in *Drosophila* males and females of silkworm.
- 3. Age of female:** The frequency of crossing over shows a progressive decline with the advancing age of *Drosophila* females.
- 4. Temperature:** In *Drosophila*, the lowest frequency of crossing over is observed when females are cultured at 22°C. The frequency of recombination tends to increase both at the lower and higher temperatures than 22°C.
- 5. Nutrition:** The frequency of crossing over in *Drosophila* is affected by the presence of metallic ions Eg: Ca⁺² and Mg⁺² in its food. Higher the amount, lower will be the crossing over frequency and vice-versa.
- 6. Chemicals:** Treatment of *Drosophila* females with certain antibiotics like mitomycin D and actinomycin D and certain alkylating agents such as ethylmethane sulphonate promotes crossing over.
- 7. Radiations:** An increase in frequency of crossing over is observed when *Drosophila* females are irradiated with x-rays and γ -rays.
- 8. Plasmagenes:** In some species, plasma genes reduce the frequency of crossing over. Eg: The Tifton male sterile cytoplasm reduces the frequency of crossing over in bajra.
- 9. Genotype:** Many genes are known to affect the occurrence as well as the rate of crossing over. For example, C₃G gene of *Drosophila* located in chromosome 3 prevents crossing over when present in homozygous state while it promotes crossing over in the heterozygous state.
- 10. Chromosomal aberrations:** In *Drosophila*, some chromosomal aberrations Eg: paracentric inversions reduce recombination between the genes located within the

inverted segment.

11. Distance from centromere: Centromere tends to suppress recombination. Therefore genes located in the vicinity of centromeres show a relatively lower frequency of crossing over than those located away from them.

Significance of crossing over in Plant Breeding:

1. It increases variability
2. It helps to break linkages
3. It makes possible to construct chromosome maps

Cytological proof of crossing over: The first cytological evidence in support of genetic crossing over was provided by Curt Stern in 1931 on the basis of his experiments with *Drosophila* by using cytological markers. He used a *Drosophila* female fly in which one X-chromosome was broken into two segments and out of these two segments, one behaved as X-chromosome. This chromosome had one recessive mutant allele *car* (carnation) for eye colour and another dominant allele *B* (Bar) for eye shape. The other X-chromosome had small portion of Y-chromosome attached to its one end. This chromosome had the dominant allele + (wild type allele of *car*) producing dull red eye colour and a recessive allele + (wild type allele of *B*) producing normal ovate eye shape. Thus, the phenotype of female is barred (since *B* is dominant to +) with normal eye colour (since *car* is recessive to +) and both the X-chromosomes in the female had distinct morphology and could be easily identified under microscope. Such females were crossed with male flies having recessive alleles for both genes (*car* +). As a result of crossing over female flies produce four types of gametes viz., two parental types or non-crossover types (*car B* and + +) and two recombinant types or cross over types (*car* + and + *B*). The male flies produce only two types of gametes (*car* + and Y), because crossing over does not occur in *Drosophila* male. A random union of two types of male gametes with four types of female gametes will produce males and females in equal number of four each.

Stern cytologically examined the chromosomes of recombinant types i. e. carnation with normal eye shape and barred with normal eye colour. It was found that carnation flies did not have any fragmented X-chromosome but rather had normal X-chromosome. On the other hand, barred flies had a fragmented X-chromosome with a segment of Y-chromosome attached to one of the two fragments of X-chromosome.

Such chromosome combination in barred is possible only through exchange of segments between non-sister chromatids of homologous chromosomes. This has proved that genetic crossing over was accompanied with an actual exchange of chromosome segments.

Similar proof of cytological crossing over was provided by Creighton and McClintock in maize.

Coincidence: It refers to the occurrence of two or more distinct crossing overs at the same time in the same region of a pair of homologous chromosomes and as a result, a double cross over product is obtained. Coefficient of coincidence is estimated by using the formula:

$$\text{Coefficient of coincidence} = \frac{\text{Observed frequency of double cross over}}{\text{Expected frequency of double cross over}}$$

(The ratio between the observed and the expected frequencies of double crossovers is called coefficient of coincidence)

Interference: The occurrence of crossing over in one region of a chromosome interferes with its occurrence in the neighbouring segment. This is known as interference. The term interference was coined by Muller. It may also be defined as the tendency of one crossing over to prevent another crossing over from occurring in its vicinity. This is called positive interference. Sometimes, one crossing over enhances the chance of another crossing over in the adjacent region. This is termed as negative interference. Eg: *Aspergillus*, bacteriophages. The effect of interference reduces as the distance from the first crossing over increases. The intensity of interference may be estimated as coefficient of interference.

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

Differences between crossing over and linkage

SL NO	Crossing over	Linkage
1	It leads to separation of linked genes	It keeps the genes together
2	It involves exchange of segments between non-sister chromatids of homologous chromosomes	It involves individual chromosomes
3	The frequency of crossing over can never exceed 50 %	The number of linkage groups can never be more than haploid chromosome number
4	It increases variability by forming new gene combinations	It reduces variability
5	It provides equal frequency of parental and recombinant types in test cross progeny	It produces higher frequency of parental types than recombinant types in test cross progeny

CHROMOSOME MAPS

Chromosome maps can be prepared by genetical or cytological methods

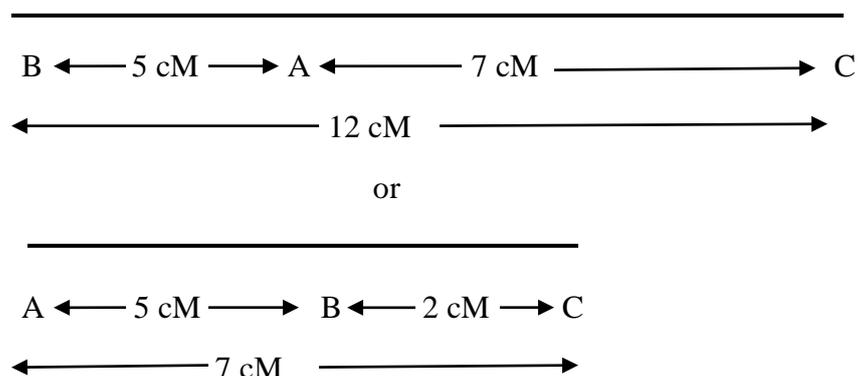
1. Genetical method: This is the general method and is based upon cross over data. The resulting map is the linkage map. Linkage map (cross over map or genetical map) map be defined as a line on which the relative positions of genes proportional to the amount of crossing over between them is represented.

A rule widely followed in plotting genes is that if genes A and B are known to be linked and if a particular gene is found by experiment to be linked with gene A it must also be linked with gene B. This principle follows from the fact that two linked genes are on the same chromosome. The genes, which are linked together on the same chromosomes are called syntenic genes.

Genetic mapping of chromosomes is based on the following assumptions:

- The genes are arranged in a linear order.
- Crossing over is due to breaks in the chromatids
- Crossing over occurs by chance and is at random
- The percentage of crossing over between the genes is an index of their distance apart.

Map distance: Recombination frequencies between the linked genes are determined from appropriate testcrosses. These percent frequencies are used as map units for preparing linkage maps. A map unit is that distance in a chromosome, which permits one percent recombination (crossing over) between two linked genes. A map unit is also called a centi-Morgan, after the name of the scientist Morgan, who first constructed the linkage map in *Drosophila*. Thus 5 % crossing over between genes A and B is taken to mean that they are situated 5 map units of distance apart on the same chromosome. If a third gene C with 7 % crossing over between A and C is included the relationship of linkage between the three genes A, B and C is indicated as below:



To choose the correct one between these two alternatives, one more information i.e. either the order of arrangement of the three genes or the cross over value between B and C is required. Eg: If the crossover value between B and C is found to be 2 % by actual experiments, the second arrangement is the correct one. Therefore, for preparing a chromosome map of three genes either the map distances (cross over frequencies) between all three gene pairs must be known or the cross over frequencies between any two gene pairs plus the order or sequence of these three genes in the chromosome must be known. In obtaining cross over value care should be taken about the occurrence of double crossing over between the concerned genes. If two genes A and B are rather far apart in a chromosome and if two crossing overs (i.e. double cross over) occur between A and B, the chromatids involved do not show recombination of marker genes. If double crossing over occurs frequently, the recombination value will be less and gives a false impression that the distance between the concerned two genes is less. To overcome this difficulty, data for chromosome mapping should be taken from linked gene pairs that are quite close together. Usually double crossing over does not occur within distances less than 5 map units or for certain chromosome segments within distances upto 15 or 20 map units.

2. Cytological maps: By cytological studies of chromosomal aberrations and by their behaviour in genetical experiments, it is possible to construct map of chromosome showing the actual physical location of gene in a chromosome. Such maps are called cytological maps of chromosomes. The work on cytological maps also confirm the theory of linear arrangement of genes in chromosomes.

Comparison between linkage maps and cytological maps: The relative distances between the genes on linkage map and cytological map do not always correspond. The discrepancies are greatest in the vicinity of the centromere where one cross over unit corresponds to a relatively much greater physical distance on the chromosome than in other regions of the same chromosome.

These discrepancies may be explained on the basis that different chromosomes and various regions in the same chromosome may also show variations in frequency of crossing over. Eg: In *Drosophila*, frequency of crossing over seems to be affected by temperature of the mother flies and by environmental factors.

Importance of linkage and chromosome maps in plant breeding:

1. They give an idea whether particular genes are linked or segregate independently.
2. Linkage intensities can be known and the probability of obtaining a given combination of genes can be assessed. If linkage between two genes is close, it is difficult to obtain recombination. In such cases, linkage can be broken artificially by irradiating with x-rays etc. and the desired combinations may be obtained. However, close linkage is useful to preserve desirable gene combinations.
3. Help the geneticist to plan how large the experimental population should be to obtain plants with the desired gene combination.
4. If an easily identifiable qualitative character is found to be linked with the quantitative character, the qualitative character can be used to easily identify the recombinants. This means that when a particular qualitative character is observed in a recombinant plant, it can be understood that the associated linked quantitative character is also present. Eg: Anthocyanin pigment and yield in rice
5. Linkage limits the variability among the individuals