

LINKAGE

Sutton and Boveri proposed the chromosome theory of inheritance. According to chromosome theory of inheritance, it is well established that many genes are located in each chromosome in a linear fashion. It may therefore be expected that all genes located in same chromosome would move to same pole during cell division. As a consequence, such genes will fail to show independent segregation and would tend to be inherited together. This tendency of genes to remain together in their original combination during inheritance is called linkage. Mendel's law of independent assortment is applicable only when the genes are located in different chromosomes while linkage refers to the genes located in the same chromosome.

The phenomenon of linkage was first reported by Bateson and Punnet in 1906. They studied flower colour and pollen shape in sweet pea involving two varieties / races.

Phases of linkage: In the two races experimented one parent has purple flowers with long pollen grains. The other parent has red flowers and round pollen grains. The character purple (P) is a simple monogenic dominant to red (p); while long (L) pollen is dominant to round (l) pollen, when these two plants were crossed the F₁ (PL/pl) was purple flowered with long pollen. But in F₂, the ratio of four types of plants deviated from the normal dihybrid ratio of 9:3:3:1 expected on the principle of independent assortment of flower colour and pollen shape.

PARENTS:

Purple Long x Red Round

PL/PL pl/pl

F1: Purple Long PL/pl

F ₂	Purple long	Purple round	Red long	Red round	Total
Actual numbers	4831	390	393	1338	6952
Expected numbers	3910.5	1303.5	1303.5	434.5	6952
Expected Ratio	9/16	3/16	3/16	1/16	

The plants with the character combination as in the original parents are known as parental forms or parental combinations, i.e., (i) Plants with purple flowers and long pollen and (ii) Plants with red flowers and round pollen. Plants possessing one character from one parent and another character from the second parent are known as recombined types or recombinations or new combinations or cross-overs, i.e. (i) plants with red flowers and long pollen and (ii) plants with purple flowers and round pollen.

From the above table the following features can be noted that in F₂, there are both parental forms and recombinations. The chief peculiarity of the results in the above table was that parental forms are in far excess of the expected number while the recombinations were fewer. This deviation from the expected ratio is due to linkage of the character pairs, viz., 1. Purple flowers (P) and long pollen (L) are linked because both the genes are located on the same chromosome. Similarly red flowers (p) and round pollen (l) are linked together because the genes are located in the same chromosome (which is the homologue of the previous one). The recombinations are obtained due to crossing over between the two concerned genes in some of the spore mother cells.

Another cross was made in sweet peas with the following combinations:

Purple Round X Red Long
Pl/Pl pL/pL

Purple Long Pl/pL

F ₂	Purple long	Purple round	Red long	Red round	Total
Actual numbers	225	95	97	2	419
Expected numbers	235.7	78.5	78.5	26.2	419
Expected Ratio	9/16	3/16	3/16	1/16	

Here again the parental types are more while the recombinant types are less than expected on the basis of independent assortment, viz., 9:3:3:1. This deviation is also due to linkage.

In the above two examples, it can be seen that in one cross the two dominant factors (PL) are linked in one parent and two recessive factors (pl) are linked in the other. Linkage in such crosses is said to be in coupling phase. In the second cross, dominant allele of one character pair (P) and the recessive allele of another character pair (l) are linked together in one parent, while in the second parent the other recessive (p) and dominant alleles (L) are linked. Linkage in such crosses is said to be in repulsion phase.

Later, T H Morgan put forth the theory of linkage and concluded that coupling and repulsion were two phases of single phenomenon, linkage.

Types of Linkage: Linkage is generally classified on the basis of three criteria viz., (i) Crossing over, (ii) Genes involved and (iii) Chromosomes involved

- (i) **Based on crossing over:** Linkage may be classified into (a) complete and
(b) incomplete / partial depending up on absence or presence of recombinant phenotypes in test cross progeny.
- (a) **Complete linkage:** It is known in case of males of *Drosophila* and females of silkworms, where there is complete absence of recombinant types due to absence of crossing over.
- (b) **Incomplete / partial linkage:** If some frequency of crossing over also occurs between the linked genes, it is known as incomplete / partial linkage. Recombinant types are also observed besides parental combinations in the test cross progeny. Incomplete linkage has been observed in maize, pea, *Drosophila* female and several other organisms.
- (ii) **Based on genes involved :** Depending on whether all dominant or some dominant and some recessive alleles are linked together, linkage can be categorized into (a) Coupling phase and (b) Repulsion phase
- (a) **Coupling phase:** All dominant alleles are present on the same chromosome or all recessive alleles are present on same chromosome.

TR tr
 ---- --- Coupling phase
 TR tr

(b) Repulsion phase: Dominant alleles of some genes are linked with recessive alleles of other genes on same chromosome.

Tr tR
 ---- --- Repulsion phase
 Tr tR

(iii) Based on chromosomes involved: Based on the location of genes on the chromosomes, linkage can be categorized into (a) autosomal linkage and (b) X-chromosomal linkage / allosomal linkage / sex linkage

(a) Autosomal linkage: It refers to linkage of those genes which are located in autosomes (other than sex chromosomes).

(b) X-chromosomal linkage / allosomal linkage / sex linkage: It refers to linkage of genes which are located in sex chromosomes i.e. either 'X' or 'Y' (generally 'X')

Characteristic features of Linkage:

1. Linkage involves two or more genes which are located in same chromosome in a linear fashion.
2. Linkage reduces variability.
3. Linkage may involve either dominant or recessive alleles (coupling phase) or some dominant and some recessive alleles (repulsion phase).
4. It may involve either all desirable traits or all undesirable traits or some desirable and some undesirable traits.
5. It is observed for oligo-genic traits as well as polygenic traits.
6. Linkage usually involves those genes which are located close to each other.
7. The strength of linkage depends on the distance between the linked genes. Lesser the distance, higher the strength and vice versa.
8. Presence of linkage leads to higher frequency of parental types than recombinants in test cross. When two genes are linked the segregation ratio of dihybrid test cross progeny deviates significantly from 1:1:1:1 ratio.
9. Linkage can be determined from test cross progeny data.
10. If crossing over does not occur, all genes located on one chromosome are expected to be inherited together. Thus the maximum number of linkage groups possible in an organism is equal to the haploid chromosome number.

Eg. Onion $2n = 16$ $n = 8$ maximum linkage groups possible = 8 Maize $2n = 20$ $n = 10$ maximum linkage groups possible = 10

11. Linkage can be broken by repeated intermating of randomly selected plants in segregating

population for several generations or by mutagenic treatment.

12. Besides pleiotropy, linkage is an important cause of genetic correlation between various plant characters.

Linkage and pleiotropy: A close association between two or more characters may result either due to linkage or pleiotropy or both. Pleiotropy refers to the control of two or more characters by a single gene. A tight linkage between two loci can be often confused with pleiotropy. The only way to distinguish between linkage and pleiotropy is to find out a crossover product between linked characters. Intermating in segregating populations may break a tight linkage, but a huge population has to be raised to find out the crossover product. If a cross over product is not found in spite of repeated intermatings, there seems to be the case of pleiotropy rather than linkage.

Linkage groups : Linkage group refers to a group of genes which are present in one chromosome. In other words, all those genes which are located in one chromosome constitute one linkage group. The number of linkage groups is limited in each individual. The maximum number of linkage groups is equal to the haploid chromosome number of an organism. For example there are ten linkage groups in corn ($2n = 20$), seven in garden pea ($2n = 14$), seven in barley ($2n = 14$), four in *Drosophila melanogaster* ($2n = 8$) and 23 in man ($2n = 46$).

Detection of linkage: Test cross is the most common method of detecting the linkage. In this method, the F₁ heterozygous at two loci (AB/ab) is crossed to a double recessive parent (ab/ab) and the phenotypic ratio of test cross progeny is examined. If the phenotypic ratio of test cross progeny shows 1:1:1:1 ratio of parental and recombinant genotypes, it indicates absence of linkage. If the frequency of parental types and recombinant types deviate significantly from the normal dihybrid test cross ratio of 1:1:1:1, it reveals presence of linkage between two genes under study.

Another way to detect the presence or absence of linkage is to self pollinate the individual heterozygous at two loci. If there is complete dominance at each locus and no epistasis, the segregation ratio of the progeny will be 9:3:3:1. Presence of linkage either in coupling or repulsion phase will lead to significant deviation from 9:3:3:1 ratio. The deviation of observed values from the expected ratio is tested with the help of χ^2 test.

Significance of Linkage in Plant Breeding :

1. Linkage limits the variability among the individuals.
2. Linkage between two or more loci controlling different desirable characters is advantageous for a plant breeder. A linkage between genes controlling two different desirable characters will help in simultaneous improvement of both the characters.
3. Linkage is undesirable when desirable and undesirable genes are linked together.
4. The estimates of genetic variances for quantitative characters are greatly influenced by the presence of linkage

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 crossing over:** 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 C_3G 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 with in 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

Differences between crossing over and linkage

Crossing over	Linkage
1. It leads to separation of linked genes	1. It keeps the genes together
It involves exchange of segments between non-sister chromatids of homologous chromosomes	2. It involves individual chromosomes
3. The frequency of crossing over can never exceed 50 %	3. The number of linkage groups can never be more than haploid chromosome number
It increases variability by forming new gene combinations	4. It reduces variability
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

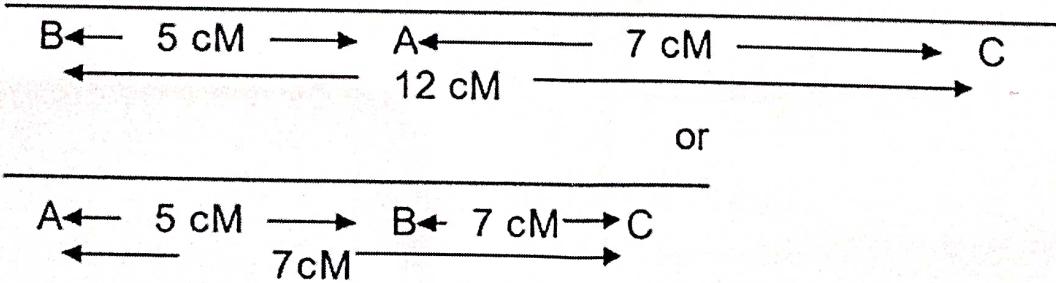
1. **Genetical method:** This is the general method and is based upon cross over data. The resulting map is the linkage map. Linkage map (cross over map or genetical map) may be defined as a line on which the relative positions of genes proportional to the amount of crossing over between them is represented.

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 chromosomes 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
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