CHROMOSOMAL CROSSING OVER

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1. Meaning of Crossing Over:

Crossing over refers to the interchange of parts between non-sister chromatids of homologus chromosomes during meiotic prophase (pachytene). In other words, crossing over results from exchange of genetic material between non-sister chromatids involving breakage and reunion at precise point. The term crossing over was first used by Morgan and Cattell in 1912.

2. Feature of Crossing Over:

The main features of crossing over are given below:

1. Crossing over takes place during meiotic prophase, i.e., during pachytene. Each pair of chromosome has four chromatids at that time.

2. Crossing over occurs between non-sister chromatids. Thus one chromatid from each of the two homologus chromosomes is involved in crossing over.

3. It is universally accepted that crossing over takes place at four strand stage.

4. Each crossing over involves only two of the four chromatids of two homologus chromosomes. However, double or multiple crossing over may involve all four, three or two of the four chromatids, which is very rare.

5. Crossing over leads to re-combinations or new combinations between linked genes. Crossing over generally yields two recombinant types or crossover types and two parental types or non-crossover types.

6. Crossing over generally leads to exchange of equal segments or genes and recombination is always reciprocal. However, unequal crossing over has also been reported.

7. The value of crossover or recombinants may vary from 0-50%.

8. The frequency of recombinants can be worked out from the test cross progeny. It is expressed as the percentage ratio of recombinants to the total population (recombinants + parental types). Thus,

$$\text{Crossing over frequency (\%)} = \frac{\text{No. of recombinants}}{\text{Total progeny}} \times 100$$

Cases of two strand crossing over, somatic crossing over, sister strand crossing over and unequal crossing over are also known. However, frequency of such cases is extremely low, i.e. in fractions. Crossing over differs from linkage in several aspects (Table 9.1).

TABLE 9.1. Differences between crossing over and linkage

Crossing over	*Linkage*
1. It leads to separation of linked genes.	It keeps the genes together.
2. It involves non-sister chromatids of homologous chromosomes.	It involves individual chromosome.
3. Frequency of crossing over can never exceed 50%.	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.	Provides higher frequency of parental types than recombinant types in test cross progeny.

Chiasma and Crossing Over:

The point of exchange of segments between non-sister chromatids of homologous chromosomes during meiotic prophase is called chiasma (pleural chiasmata). It is thought to be the place where crossing over takes place. Chiasma was first discovered by Janssens in 1909. Depending on the position, chiasma is of two types, viz., terminal and interstitial.

When the chiasma is located at the end of the pairing chromatids, it is known as terminal chiasma and when it is located in the middle part of non-sister chromatids, it is referred to as interstitial chiasma. Later on interstitial chiasma is changed to terminal position by the process of chiasmaterminalization.

The number of chiasma per bivalent may vary from one to more than one depending upon the length of chromatids. When two chiasmata are formed, they may involve two, three or all the four chromatids.

Chiasma Terminalization:

The movement of chiasma away from the centromere and towards the end of tetrads is called terminalization. The total number of chiasmata terminalized at any given stage or time is known as coefficient of terminalization. Generally, chiasma terminalization occurs between diplotene and metaphase I.

There are three theories to explain the mechanism of chiasma terminalization, viz:

1. Electrostatic hypothesis,

2. Coiling hypothesis, and

3. Elastic chromosome repulsion theory.

These are briefly discussed below:

i. Electrostatic Hypothesis:

According to this hypothesis, terminalization takes place due to localized repulsion force in centromere and generalized repulsion force on chromosome surface during diplotene stage.

ii. Coiling Hypothesis:

According to this hypothesis, terminalization takes place by mechanical tension developed within the chromosome due to coils. Thus tension force becomes greater than the force binding the chromatids at the point of exchange resulting in terminalization.

iii. Elastic Chromosome Repulsion:

According to this theory, all bodies having a definite shape resist any change that leads to alter their shapes. Chiasma forces the chromosome out of shape by its binding force. This leads to the development of repulsion at the point of exchange resulting in terminalization of chiasma.

3. Relationship between Crossing Over and Chiasma Formation:

There are two main theories to explain the relationship between crossing over and chiasma formation, viz., 1. classical theory and 2. chiasma type theory.

These are briefly described below:

i. Classical Theory:

This theory states that first chiasma is formed and then crossing over takes place. The genetic crossing over occurs as a result of physical strain imposed by chiasma formation. The chiasma is formed at diplotene stage of meiosis and crossing over occurs between diplotene and anaphase.

In this case, 1 : 1 relationship between chiasmata and crossing over is not observed because chiasma may not lead to breakage and subsequent genetic crossing over.

ii. Chiasma Type Theory:

This theory was proposed by lanssens and later on elaborated by Belling and Darlington. According to this theory, first crossing over occurs and then chiasma is formed. The crossing over occurs sometimes during early meiotic stages, perhaps at pachytene, when homologous chromatids are closely paired.

As the meiotic cell moves towards metaphase and reductional division, a chiasma is formed at the point where crossing over has occurred. Thus according to this theory each chiasma represents one genetic cross over. This theory remains at present the most accepted explanation for the relationship between genetic crossing over and cytological observed chiasmata.

4. Molecular Mechanism of Crossing Over:

There are two important theories viz:

1. Copy choice theory and

2. Breakage and reunion theory to explain the mechanism of crossing over.

These are briefly presented below:

i. Copy Choice Theory:

This theory was proposed by Belling. This theory states that the entire recombinant section or part arises from the newly synthesised section. The non-sister chromatids when come in close contact they copy some section of each other resulting in recombination. According to this theory, physical exchange of preformed chromatids does not take place.

The non-sister chromatids when come together during pairing, copy part of each other. Thus, recombinant chromosome or chromatids have some alleles of one chromatids and some of other. The information may be copied by one strand or both the strands. When only one strand copies, non-reciprocal recombinant is produced.

If copy process involves both strands of chromosomes, reciprocal recombinants are produced. Assume, there are two chromosomes, viz., AB and ab. When their chromatids come in close contact they copy each other and result in Ab and aB re-combinations besides parental combinations (Fig. 9.1).

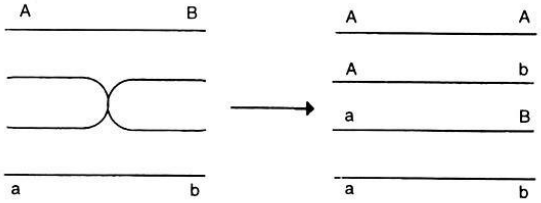

Fig. 9.2. Crossing over according to breakage and reunion theory.

This theory has two objections:

1. According to this theory breakage and reunion does not occur, while it has been observed cytological.

2. Generally crossing over takes place after DNA replication but here it takes place at the same time.

ii. Breakage and Reunion Theory:

This theory states that crossing over takes place due to breakage and reunion of non-sister chromatids. The two segments of parental chromosomes which are present in recombinants arise from physical breaks in the parental chromosomes with subsequent exchange of broken segments (Fig. 9.2).

The breakage results due to mechanical strains that result from the separation of paired homologous chromosomes and chromatids in each chromosome during pachytene stage. The broken ends of non-sister chromatids unite to produce chiasmata resulting in crossing over.

Interference:

The term interference was coined by Muller which refers to the tendency of one crossover to reduce the chance of another crossover in its adjacent region. Interference is affected by gene distance on the chromosome. Lesser the gene distance greater is the interference and vice versa. Generally, it is observed that crossing over in one region of chromosome may check the crossing over in the second region.

Sometimes, presence of recombination in one region enhances the chance of recombination in another adjacent region. This is termed as negative interference. This type of situation has been observed in some lower organisms, viz., Aspergillus and bacteriophages.

Coefficient of interference is estimated as follows:

Coefficient of interference (%) = 1 – Coefficient of coincidence x 100

Positive and negative interference differ from one another in three main aspects (Table 9.2).

TABLE 9.2. Differences between positive and negative interference

Positive Interference	*Negative Interference*
1. One crossover reduces the chance of another crossover in the adjacent region.	1. One crossover enhances the chance of another crossover in the adjacent region.
2. Observed in both eukaryotes and prokaryotes.	2. Found in some lower organisms like Aspergillus and bacteriophages.
3. In this case coefficient of coincidence is less than one.	3. In this case coefficient of coincidence is always more than one.

Coincidence:

This term was also coined by Muller to explain strength or degree of interference. The coefficient of coincidence is the percentage ratio of observed double crossovers to the expected double crossovers. The greater the coincidence, lesser will be the interference and vice versa. Thus,

$$\text{Coefficient of coincidence (\%)} = \frac{\text{Observed double crossovers}}{\text{Expected double crossovers}} \times 100$$

Coefficient of coincidence is a measure of the intensity of interference, because it has negative association with interference. The value of the coefficient of coincidence is less than 1 for positive interference, greater than 1 for negative interference, 1 for absence of interference and zero for complete or absolute interference.

Chromosome Mapping:

Chromosome map refers to a line diagram which depicts various genes present on a chromosome and recombination frequency between them. Such maps are also known as genetic maps or linkage maps. The process of assigning genes on the chromosomes is known as chromosomal mapping.

The mapping of chromosomes is done with the help of three point test cross. A three point test cross is a cross of a trihybrid (F_1 differing in three genes) with its homozygous recessive parent.

The three point test cross provides useful information on two important aspects, viz:

(1) About the sequence of genes, and

(2) About the recombination frequencies between genes. This information is essential for mapping of chromosomes.

5. Types of Crossing Over:

Depending upon the number of chiasmata involved, crossing over may be of three types, viz., single, double and multiple as described below:

i. Single Crossing Over:

It refers to formation of a single chiasma between non-sister chromatids of homologous chromosomes. Such cross over involves only two chromatids out of four.

ii. Double Crossing Over:

It refers to formation of two chiasmata between non-sister chromatids of homologous chromosomes. Double crossovers may involve either two strands or three or all the four strands. The ratio of recombinants and parental types under these three situations are observed as 2:2:3:1 and 4 : 0, respectively.

iii. Multiple Crossing Over:

Presence of more than two crossovers between non-sister chromatids of homologous chromosomes is referred to as multiple crossing over. Frequency of such type of crossing over is extremely low.

6. Factors Affecting Crossing Over:

The frequency of crossing over is influenced by several factors which are briefly discussed below:

i. Distance:

The distance between genes affects the frequency of crossing over. Greater the distance between genes higher is the chance of crossing over and vice versa.

ii. Age:

Generally crossing over decreases with advancement in the age in the female Drosophila.

iii. Temperature:

The rate of crossing over in Drosophila increases above and below the temperature of 22°C.

iv. Sex:

The rate of crossing over also differs according to sex. There is lack of crossing over in Drosophila male and female silk moth.

v. Nutrition:

Presence of metallic ions like calcium and magnesium in the food caused reduction in recombination in Drosophila. However, removal of such chemicals from the diet increased the rate of crossing over.

vi. Chemicals:

Treatment with mutagenic chemicals like alkylating agents was found to increase the frequency of crossing over in Drosophila female.

vii. Irradiation:

Irradiation with X-rays and gamma rays was found to enhance the frequency of crossing over in Drosophila females.

viii. Structural Changes:

Structural chromosomal changes especially inversions and translocations reduce the frequency of crossing over in the chromosomes where such changes are involved.

ix. Centromere Effect:

Generally genes that are located adjacent to the centromere show reduced frequency of crossing over.

x. Cytoplasmic Genes:

In some species cytoplasmic genes also lead to reduction in crossing over. For example, Tifton male sterile cytoplasm in pearl millet.

7. 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 conducted with Drosophila. He used cytological markers in his studies. He selected a female fly in which one X-chromosome was broken into two segments.

Out of these two segments, one behaved as X-chromosome. The other X-chromosome had small portion of Y-chromosome attached to its one end. Thus, both the X-chromosomes in the female had distinct morphology and could be easily identified under microscope. In female fly, the broken X-chromosome had one mutant allele (carnation) for eye colour and another dominant allele (B) for bar eye shape.

The other X-chromosome with attached portion of Y chromosome had alleles for normal eye colour (red eye) and normal eye shape (oval eye). Thus, phenotype of female was barred. A cross of such females was made with carnation male (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 crossover types (car+ and B+).The male flies produce only two types of gametes (car + and Y), because crossing over does not take place 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, means there will be four females and four males (Fig. 9.4).

Stern examined the chromosomes of recombinant types, viz., red bar and carnation normal under microscope. He observed that in carnation normal females both the X-chromosomes were of equal length. In red bar flies, one X-chromosome was normal and other was fragmented.

The fragmented X-chromosome also had attached part of Y-chromosome. Such chromosome combination in red bar is possible only through exchange of segments between non-sister chromatids of homologous chromosomes. This has proved that genetic crossing over is the result of cytological crossing over. Similar proof of cytological crossing over was provided by Creighton and McClintock in maize.

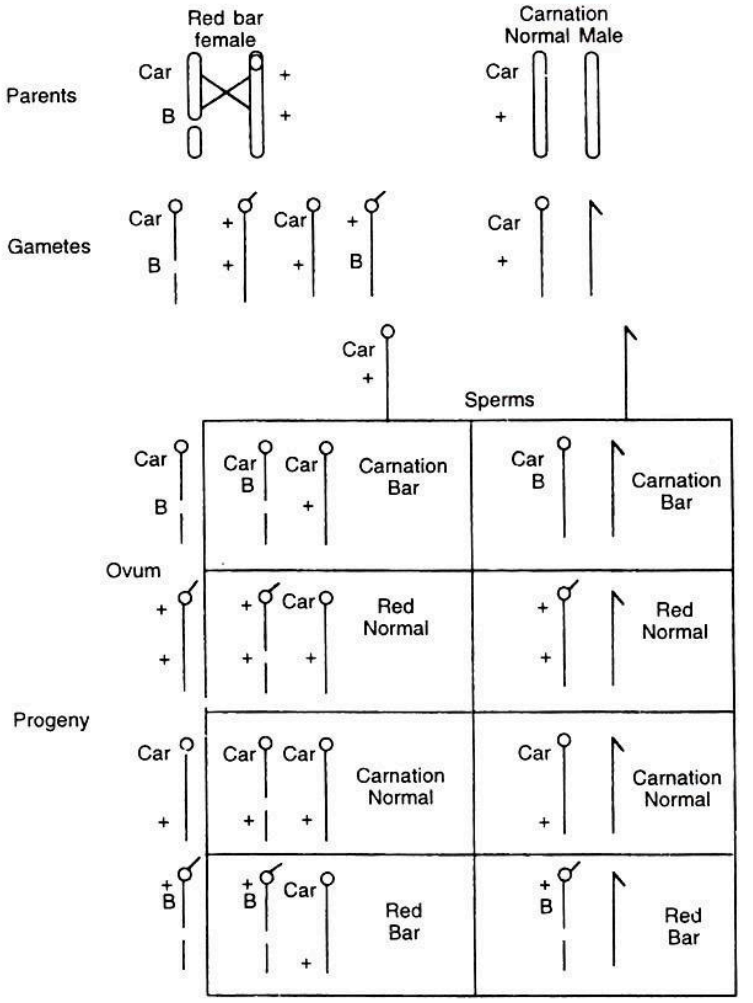

Fig. 9.4. Cytological proof of crossing over in **Drosophila.**

8. Significance of Crossing Over:

Crossing over is useful in three principal ways, viz:

(1) Creation of variability,

(2) Locating genes on the chromosomes, and

(3) Preparing linkage maps as described below:

i. Creation of Variability:

Crossing over leads to recombination or new combination and thus is a potential genetic mechanism for creating variability which is essential for improvement of genotypes through selection.

ii. Locating Genes:

Crossing over is a useful tool for locating genes in the chromosomes.

iii. Linkage Maps:

Crossing over plays an important role in the preparation of chromosome maps or linkage maps. It provides information about frequency of recombination's and sequence of genes which are required for preparation of linkage maps.

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