Chapter 28

LINKAGE & CROSSING OVER

Walter Sutton, who rediscovered Mendle's work, pointed that there are many more genes than chromosomes, and these exceed the number of pairs of chromosomes, e.g., in *Drosophilla,* there are hundreds of genes as compared to only four pairs of chromosomes. This suggests that many genes are present on a chromosome and the genes in the same chromosome will not assort independently as suggested by the Mendel. In 1906 **Bateson** and **Punnett** found that when a *Sweet Pea* variety having blue flowers(B) and long pollens (L) was crossed with another variety having red flowers (b) and round pollen (l). The F_1 individuals were with blue flowers and long pollens (BbLl) showing the dominance of blue colour of flowers over red colour and that of long pollens over round ones. These F_1 individuals were crossed with plants having red flowers and round pollen (bbll)---a **Test Cross.** Instead of normal independent assortment ratio of 1:1:1:1:, a ratio of 7 (blue flowers, long pollen): 1 (blue flowers, round pollens): 1 (red flowers, long pollens): 7 (red flowers, round pollens) was obtained, showing a deviation from independent assortment. Bateson termed this deviation as **Gametic Coupling.** Similarly, it was observed that when two such dominant alleles or two recessive alleles come from different parents, they tend to remain separate. This was called **Repulsion.** In this case when parents with blue flowers and round pollens (BBll) and red flowers and long pollens (bbLL) were crossed, F_1 individuals with blue flowers and long pollens were obtained. When a testcross was made between F_1 individuals (BbLl) and double recessive plants (bbll), a test cross progeny of 1 (blue flowers, long pollens): 7 (blue flowers, round pollens): 7 (red flowers, long pollen): 1 (red flowers, round pollen) was obtained. Bateson explained the lack of independent assortment in the above experiments by means of coupling and repulsion which he called **Coupling and Repulsion Hypothesis.** Later on, it was found that coupling and repulsion are two aspects of the same phenomenon, called **Linkage.**

	Coupling				**Repulsion**			
Parents	BBLL	X	bbll		BBll	X	bbLL	
	blue flowers		red flowers		blue flowers		red flowers	
	long pollens		round pollens		round pollens		long pollens	
Gametes	BL		bl		Bl		bL	
Test Cross	BbLl	X	bbll		BbLl	X	bbll	
	blue flowers		red flowers		blue flowers		red flowers	
	long pollens		round pollens		long pollens		round pollens	
Test Cross progeny	7/16 BbLl	1/16 Bbll	1/16 bbLl	7/16 bbll	1/16 BbLl	7/16 Bbll	7/16 bbLl	1/16 bbll

T.H. Morgan in 1910 found similar situation in *Drosophilla* and supposed that the tendency of the genes to remain in their original combinations is due to their residence in the same chromosome. Such genes were termed **Linked Genes** and the phenomenon was called

Linkage. The degree or strength of linkage depends upon the distance between the linked genes in the chromosome.

The phenomenon of linkage suggests that the linked genes are not free to undergo independent assortment during meiosis. However, in many cases, this does not always occur. During the first meiotic prophase when the homologous chromosomes are paired or synapsed, a reciprocal exchange of chromosome segments may take place. This event, called **Crossing Over** results in the reshuffling of alleles between the homologues. These reshuffled alleles are known as **Recombinations.** The degree of crossing over between any two loci on a single chromosome depends upon the distance between them. If the distance between the two loci is greater, crossing over will occur more frequently than if the distance is lesser. Thus the percentage of recombinant gametes varies. This correlation (between the distance of loci and crossing over) serves as the basis for the construction of **Chromosome Maps.** Crossing over involves physical breaking and rejoining process which occurs during meiosis. The exchange of chromosome segments result in variations in gametes formed by an individual, which produces individual diversity and is also important to the process of organic evolution.

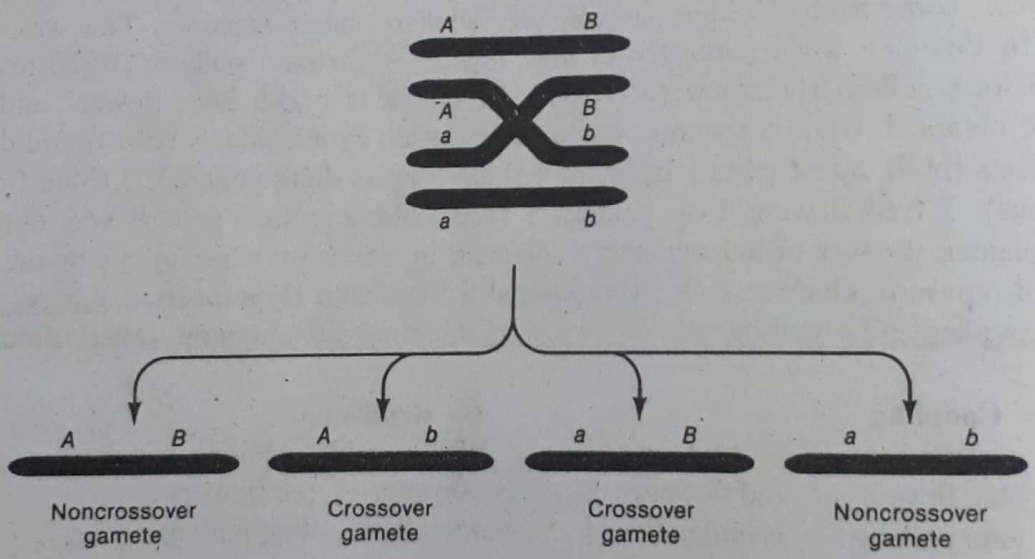


Fig. 28-1 : Gametes formed as result of Crossing Over

If no crossing over occurs between the two genes, only two genetically different gametes are formed. Each gamete receives the alleles present on one homologue or the other which has been transmitted intact as a result of seggregation. This shows **Complete Linkage** which result in production of only **Parental** or **Noncrossover Gametes.** However, if the crossing over occurs between the two linked genes, two different types of gametes are formed, each containing new combination of alleles compared with the parental gametes. This shows **Incomplete Linkage** which results in production of **Recombinants** or **Crossover Gametes.** Usually when the two genes are linked, a mixture of parental and recombinant gametes is formed, similar to the those that result from independent assortment of two unlinked genes.

However, the proportion of the gamete types varies. In linkage, the proportion of parental and recombinant gametes depends upon distance between the two genes on a chromosome. If the genes are too close only parental gametes will be produced. If a small distance separates the two genes, few recombinants and many parental gametes will be formed and if distance is much, more recombinants and few parental gemetes will be produced.

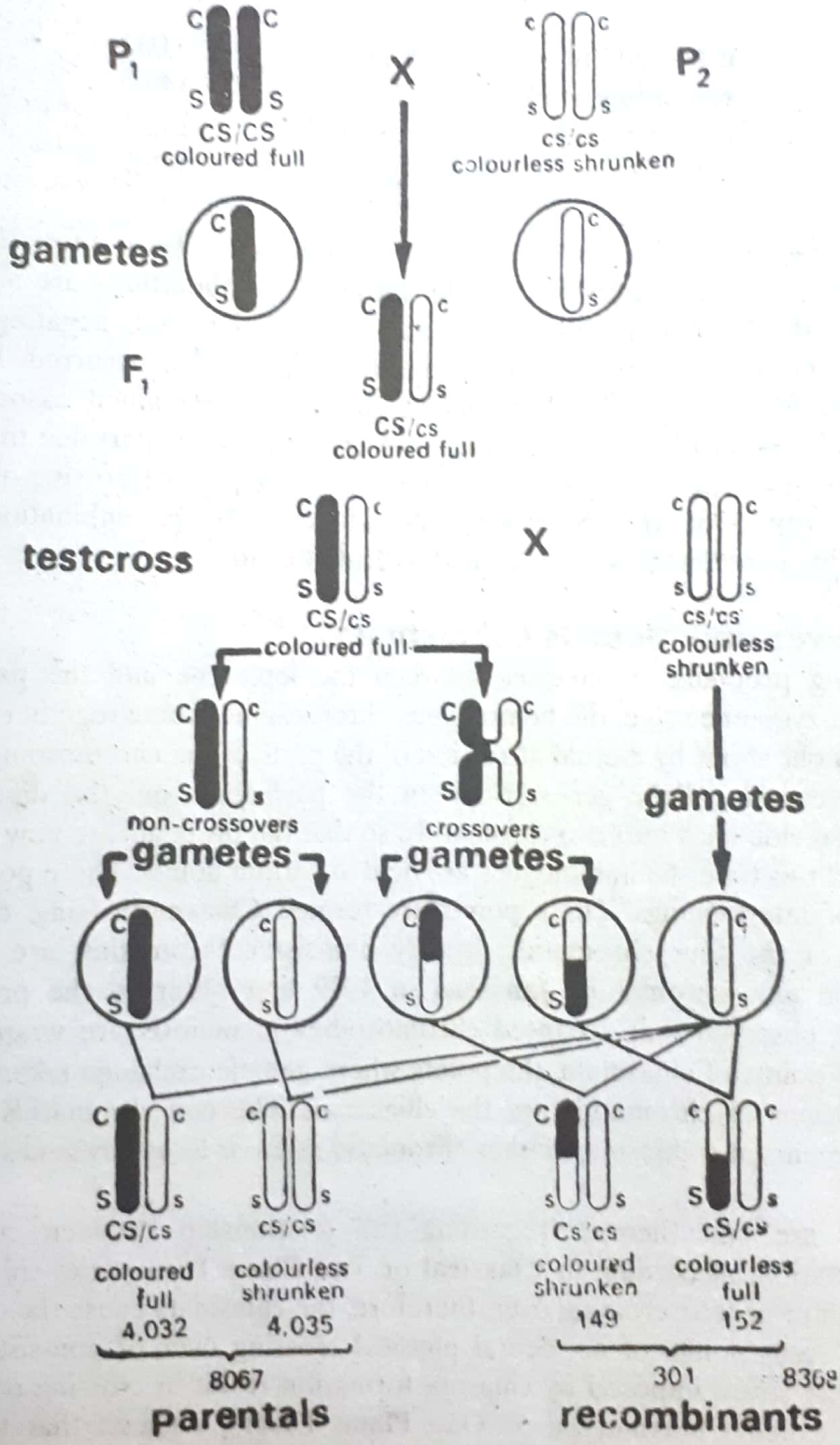


Fig. 28-2 : Figure illustrating experiment performed by Hutchinson with *Maize*

There is a variety of *Maize* having seeds which have coloured or colourless aleurone and full or shrunken endosperms. The colour gene C is dominant over colourless gene c, whereas the normal or full endosperm S is dominant over shrunken endosperm s. When CCSS and ccss parents were crossed, F_1 hybrids were CcSs (coloured and full). If the C and S assort independently, these F_1 individuals should produce CS, Cs, cS and cs gametes to produce a 1:1:1:1: test cross ratio. But when the cross was made, the expected ratio was not obtained. In a total progeny of 8,368 plants, the following results were obtained (Fig. 28.2).

coloured full	CcSs	4,032
coloured shrunken	Ccss	149
colourless full	ccSs	152
colourless shrunken	ccss	4,035

The coloured full and colourless shrunken seeds were more frequent than the coloured shrunken and colourless full. The **Parental Combinations** are about 96.4% of the total whereas the **Recombinations** are about 3.6% of the total, negating the independent assortment and suggesting that the linkage and crossing over has occurred. The genes for seed colour C and for full or shrunken endosperm S or s remained associated in parental combinations in about 97% of the gametes (linkage) but broke apart due to exchange of parts between homologous chromosomes in about 3% of the gametes (crossing over). The parental combinations represent the **Noncrossovers** whereas the recombinations represent the **Crossovers.** The experiment was performed by **Hutchinson.**

Crossing over and Meiosis (Chiasmata)

During prophase of meiosis, between the leptotene and the pachytene, and the pachytene and zygotene stage, the homologous chromosomes come together and synapse. This pairing is brought about by mutual attraction of the parts of the chromosomes that are similar because they contain allelic genes. Between the pachytene and the diplotene the paired chromosomes divide each into two chromatids, so that the bivalents are now composed of four chromatids. At this time the homologues are held at certain points. These points of contact are in the form of intertwinings. These points are termed **Chiasmata** (sing. chiasma). At each chiasma, two of the four chromatids, usually non-sister chromatids, are broken and then rejoin, to form new chromatids. **Janssens** in 1909 first observed the process of chiasma formation. He observed that synapsed chromosomes in meiosis are wrapped around each other, forming points of chiasmata, the points where genetic exchange takes place. Such cross over configurations of chromatids are the chiasmata. The two chromatids exchange exactly equivalent segments at a chiasma, neither chromatid gains or loses any genes.

There are two theories regarding the relationship between crossing over and chiasmata formation. According to **Classical** or **Two Plane Theory,** the chiasmata formation precedes the act of genetic crossing over, therefore, the chiasmata cause the crossing over. The chiasmata represent points of accidental physical crossing over of non-sister chromatids of homologues. The strain imposed by chiasma formation result in crossing over. Whereas, the second theory called **Chiasmatype** or **One Plane Theory** suggests that the crossing over precedes the chiasma formation and the true chiasmata are the result of crossing over. The segments of non-sister chromatids of homologues are broken and then rejoin. The available evidence support chiasmatype theory.

Crossing Over and Recombinations

It is difficult to explain how exchange of segments or recombination of gene clusters is brought about. Different theories and views are available to explain the exchange. According to **Precocity Theory** suggested by **C.D. Darlington,** which was originally proposed to explain chromosome pairing at meiosis, but later on Darlington extended this theory to explain recombination also. According to this theory DNA synthesis or chromosome duplication takes place in pachytene or diplotene and then separation of homologues take place. But recent informations that DNA synthesis takes place before the onset of prophase I of meiosis during S-phase leads to the rejection of the theory. On the basis of this theory Darlington explained that the crossing over takes place as a result of tension or strain produced due to coiling of homologous chromosomes and sister chromatids. **J. Belling** proposed that crossing over is brought about due to attachements formed between newly synthesized genes. This theory is called **Belling's Theory.** According to this theory, chromosome duplication involves two stages, (i) formation of new genes; and (ii) formation of new connections between these genes. This results in the formation of recombinants. **J. Lederberg** in 1955, suggested a modified version of Belling's theory after sudying recombinations in microbes. According to this theory known, as **Copy Choice Mechanism of Recombination**, a newly synthesized daughter chromatid is derived due to copying of one chromosome upto a certain distance and then switching on to the other homologous chromosome for copying the remaining distance, or region of the chromosome. This theory is also inadequate as it is based upon conservative replication of DNA duplexes. According to this theory a chromosome with DNA duplex is synthesized de novo by copying the parent chromosome. But recent investigations show that the chromosomes replicate in semi-conservative manner. In 1963. **H.L.K. Whitehouse** and in 1964 **R. Holliday** proposed **Hybrid DNA Models**, suggesting that only one strand in each of the two DNA duplexes belonging to non-sister chromatids from homologues, breaks and pair with unbroken strands crosswise by complementary base pairing. This results in the formation of hybrid DNA segments. According to Whitehouse, the breaks occur in single strands having opposite polarity whereas Holliday believes that the breaks would occur in strands having same polarity. **Yasuo Hotta** and **Herbert Stern** have demonstrated that in *Lily oocytes,* a small amount of DNA synthesis occurs during the pachytene stage of the first division, this synthesis may serve as the molecular basis of the rejoining of the non-sister chromatids.

As the science of molecular biology has expanded, many models have been proposed to explain crossing over in molecular terms. The presence of many enzymes which has DNA strands as their substrates are the basis of these models, e.g. certain endonucleases are capable of nicking one of the two strands of the double helix. Ligases are known to seal small openings along a DNA strand. Thus, these enzymes may cause the breaks and seal the opposite strands in the breaking and rejoining steps proposed for crossing over.

The ultrastructural organelle, the **Synaptonemal Complex** (SC), is also considered to be involved in the process of crossing over. The SC is found between synapsed homologues during the first meiotic prophase. In male *Drosophilla* where no crossing over occurs, no SC are found when spermatocytes were observed under electron microscope. In silk moth *Bombyx* no crossing over occurs in females, and no SC are observed in oocytes. Because of constant association of SC and the crossing over, it is possible that the SC may be involved in the crossing over.

In 1939, **Curt Stern** demonstrated that exchanges similar to crossing over occur during mitosis in *Drosophilla.* This is unusual as the homologues do not normally pair during

mitosis in most organisms. However, such synapsis is apparently the rule in *Drosophilla* and is known as **Mitotic Recombination.** In 1958 **George Pontecorvo** and others described a similar phenomenon in the fungus *Aspergillus*. The vegetative phase in the fungus is normally haploid but some cells and their nuclei fuse, producing diploid cells which divide mitotically. Occasionally, crossing over occurs between linked genes during mitosis in this diploid stage so that the resultant cells are recombinants. Pontecorvo referred to these events which repoduce genetic variability as the **Parasexual Cycle.** However, the mitotic recombinations exhibit much lower frequency of occurrence than meiotic recombinations.

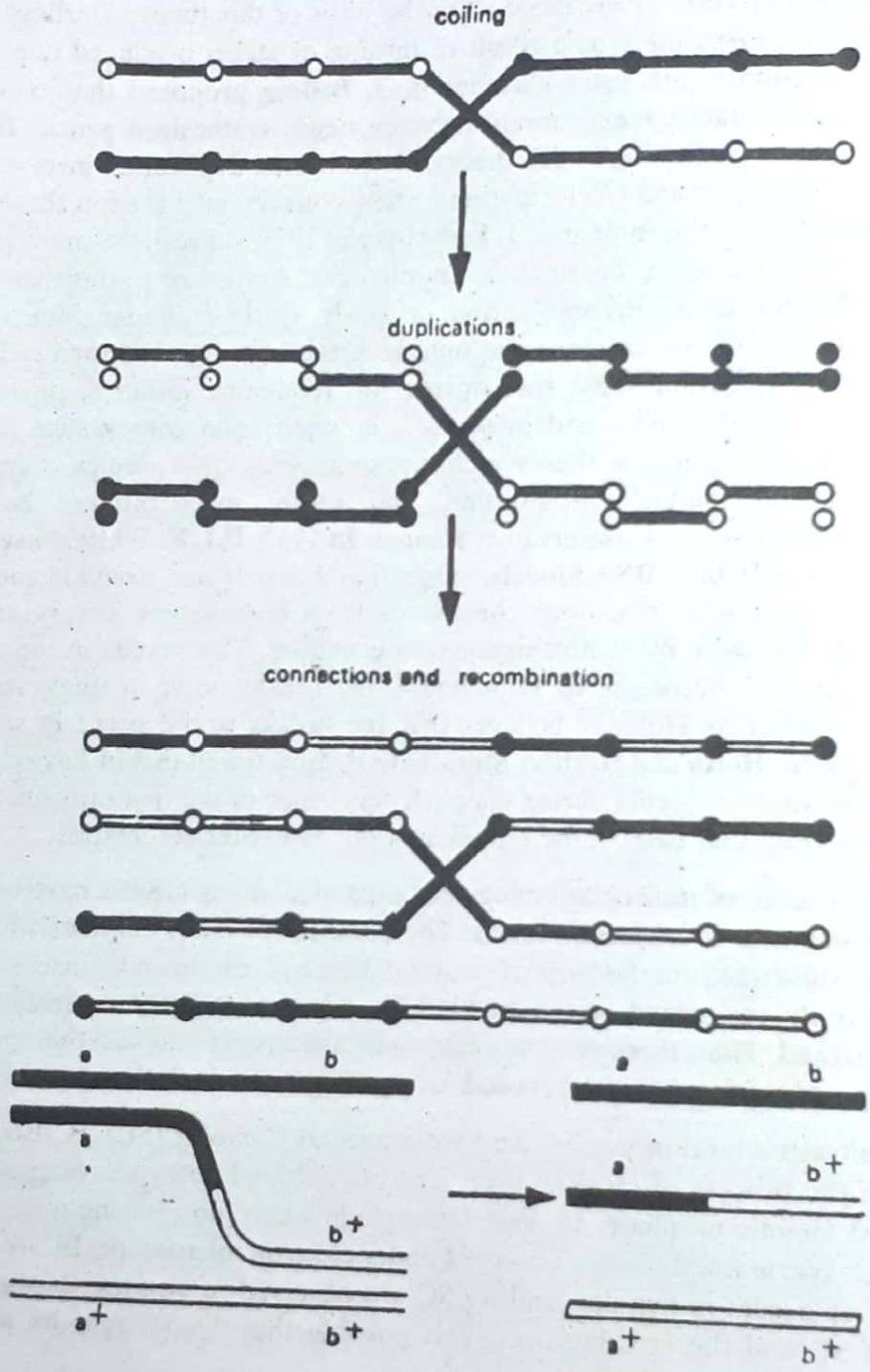


Fig. 28-3 : A - Mechanism of recombination as suggested by Belling; B - Mechanism of recombination based on Copy Choice Mechanism Hypothesis.

In experiments with the roots of *Vicia faba* to study the mode of replication in eukaryotic cells, it was found that sometimes exchanges occur between the sister chromatids of mitotic figures. These exchanges are known as **Sister Chromatid Exchanges.** In mitosis, the sister chromatids represent one chromosome that has duplicated itself, and they are, therefore, genetically identical. The significance of sister chromatid exchanges is still uncertain, however, it is revealed that the agents that induce chromosome damages (viruses, X-rays, ultraviolet rays, chemical mutagens) increase the frequency of sister chromatid exchange. **Bloom Syndrome,** an autosomal recessive disorder of humans, characterised by retardation of growth, a great sensitivity of the facial skin to the sun, abnormal immunological function, and a predispostion to cancer, is caused due to excessive amounts of sister chromatid exchanges.

Cytological Evidence for Crossing Over

During crossing over homologues exchange reciprocal segments, therefore the resultant chromosomes exhibit no morphological differences, i.e., visual proof of crossing over is absent. **Curt Stern**, and **Harriet Creighton** and **Barbara McClintock** demonstrated that crossing over is associated with actual exchange of chromosome segements in their independent experiments with *Drosphilla* and *Maize* respectively. In both experiments such chromosomes were utilized whose morphology was altered due to chromosomal aberrations in order to make it identifiable from its homologues.

Creighton and **McClintock** obtained *Corn* plants, heterozygous for coloured aleurone (Cc) and waxy endosperm (Wx/wx) characters. The genes are carried on chromosome 9. Chromosome 9 has a knob and is involved in a reciprocal translocation with chromosome 8. The plant was heterozygous for coloured aleurone and waxy endosperms characters. Such a plant (Cwx/cWx) was crossed with a homozygous recessive plant (cwx/cwx) to obtain a testcross. If the chomosome region between the knob and c genes is represented as region I and that between the c and Wx as region II, then one would expect two types of non-crossover gametes, cWx and Cwx and six types of crossover gametes including single and double crossovers, as shown in the figure 28.4. The progeny can be classified into eight types based on phenotypes and cytological observations.

The following observations in the phenotype and cytology of the progeny suggest that actual exchange of chromosome segments was involved in genetic crossing over:

1- Association of knob in chromosome with the phenotype, colourless seed and non-waxy endosperm, indicating that crossing over in region I has taken place because in the parents these were located on knobless chromosome.

2- At mitotic prophase the presence of a ring of four chromosomes without a knob suggests cytological exchange of chromosome segment because in parents the knob was associated with chromosome 9 carrying the translocation.

3- If there was no ring of four chromosomes (quadrivalents) and only ten bivalents are observed, the presence of knob in one of these bivalents could be treated as evidence for cytological crossing over, since knob was originally associated with translocation.

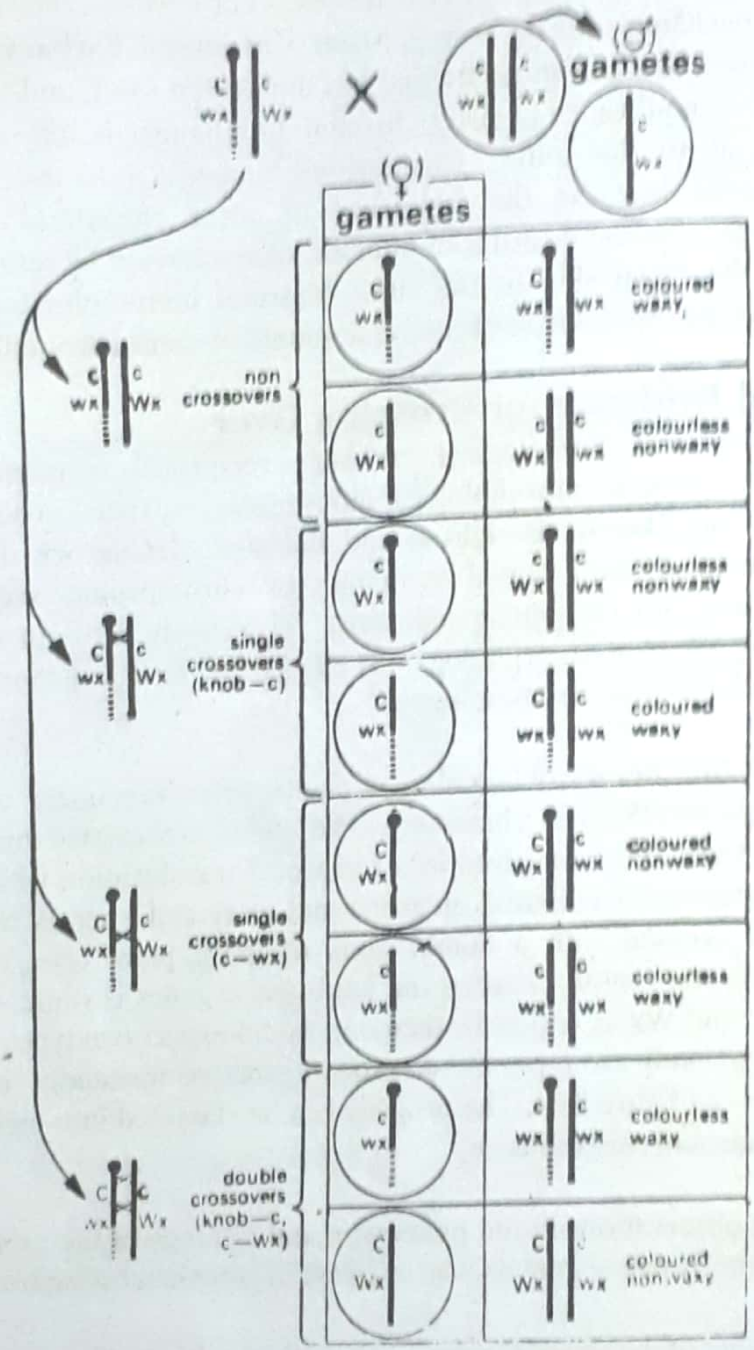


Fig. 28-4 : Creighton and McClintock's experiment in *Corn* - Cytological Evidence for Crossing Over

Crossing Over in the Four-Strand Stage

The question that when crossing over takes place during meiosis is quite critical. The crossing over takes place at two times, first before or early in the meiosis in **Two-Strand Stage** and secondly in **Four-Strand Stage** after the duplication of chromosomes into the sister chromatids as suggested by the **Jenssen**. If crossing over occurs at the two-strand stage, all four products of a single meiotic event will be recombinant gametes because each pair of sister chromatid in the tetrad is derived from one of the members of the two-strand stage. Whereas, if the crossing over takes place at four-strand stage between two nonsister chromatids, two parental gametes (noncrossovers) and two recombinants (crossovers) will be formed.

If crossing over occurs at two-strand stage and if the two genes involved in the cross are located far apart from each other. A 100% recombinant (crossovers) gametes should be expected. But, in fact, the maximum number of offspring recovered as a result of crossing over, i.e., recombinants, is 50%. This observation strongly favours, but does not prove, that exchange occurs at four-strand stage.

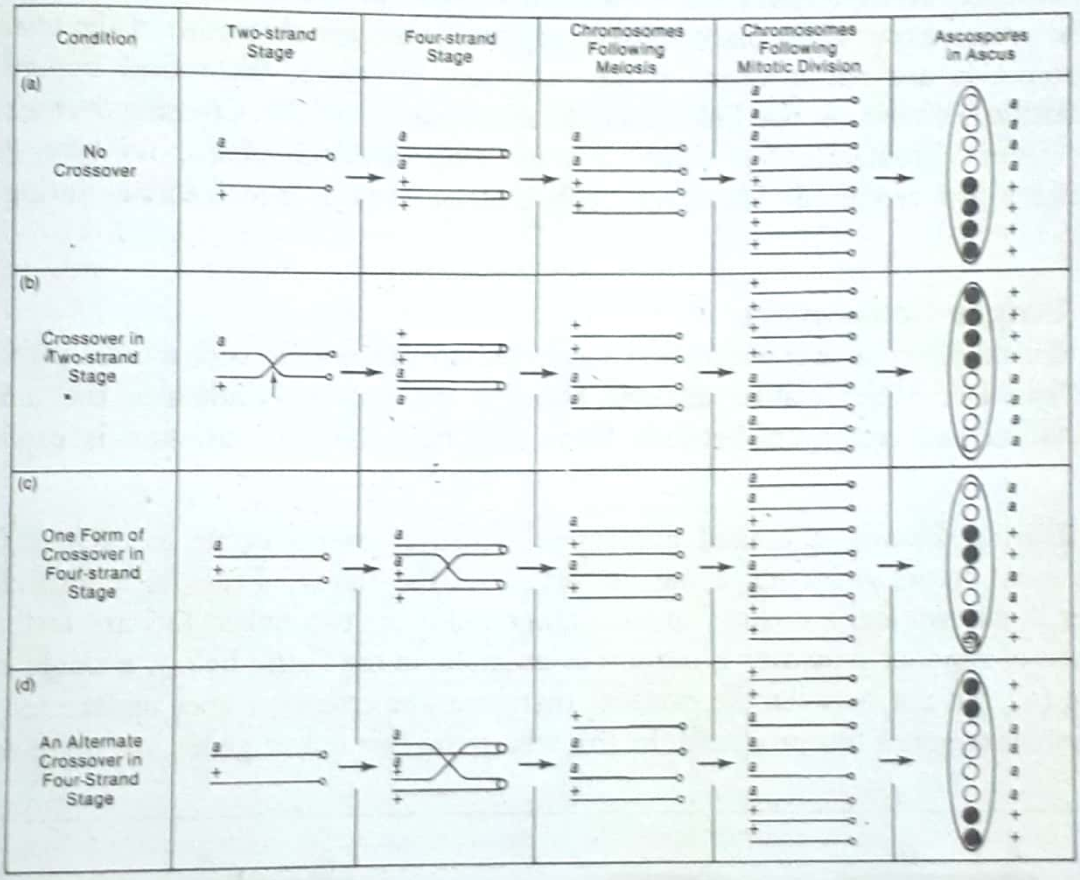


Fig. 28-5 : Ascospore formation patterns in asci of *Neurospora* showing that crossing over occurs in four- strand stage.

Experimental evidence in favour of crossing over taking place at four-strand stage was provided by **Carl D. Lindergren.** He experimented with pink bread mold, *Neurospora crass,* in which meiosis results in the formation of a four-celled tetrad. A mitotic division of each tetrad results in the production of eight haploid **Ascospores.** These spores are produced in a specialised fruiting body, the **Ascus,** in which the spores are arranged in order in which they are formed. Lindergren examined various possiblities of ascopore formation resulting from a cross between an **albino mutant strain (a)** and one with **normal pigmentation (+).** He considered a crossover that occurred in the region between the mutant albino locus and the centromere. The figure 28.5 illustrates the theoratical results of crossovers in this region for both two-strand and four-strand stage. In case (a) no exchange occurs and in case (b) results of exchange at two-strand stage are predicted. In both cases the resulting ascus always contains four pigmented and four unpigmented ascospores. Therefore, the asci produced during case (a) and (b) cannot be distinguished from each other. However, if crossover takes place

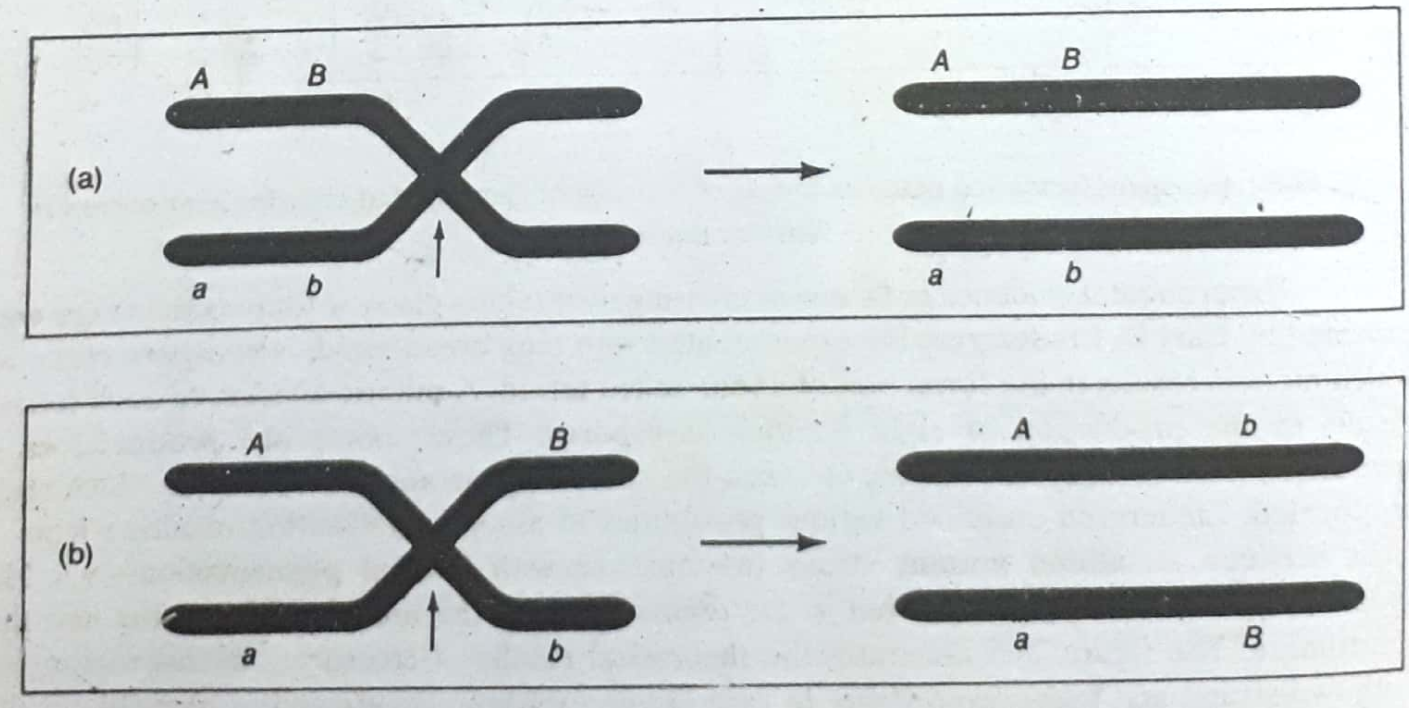
between this region during four strand stage, an alternate arrangement of ascospores in the ascus is predicted, as illustrated in case (c). Case (d) shows another arrangement that occurs as a result of a slightly different exchange during four-strand stage. Lindergren observed asci with arrangements found in case (a), (b), (c) and (d) and concluded that crossing over takes place in the four-strand stage because the crossing over at two-strand stage cannot produce arrangements (c) and (d).

To date, no experiment has been reported that dispute crossing over in four-strand stage. The crossing over takes place between any two non-sister chromtids of the tetrad stage. Two chromatids are not involved in the exchange, therefore, theoretical upper limit of recombination between any two linked loci, distantly located, is 50%. Crossing over also occurs between sister chromatids, but since each contains identical alleles, no new genotypic combinations are produced. Therefore, such crossing over is undetectable during genetic analysis.

Single Crossovers

if a single croos over occurs between non-sister chromatids, such a cross over is called **Single Crossover**. The relative distance between the two loci influence the amount of recombination and crossovers between them. The basis for this variation is explained as follows:

During meiosis, a limited number of crossover events occur in each tetrad. The crossing over occurs randomly along the length of the tetrad. There is a less chance of crossover if the two loci are closer to each other and if the two linked loci are farther apart, the chance of random crossover is more. For example, in the figure below, a single crossover occurs in (a), but not between the two loci, therefore, the crossover goes undetected because no recombinant genes are produced. In (b), where the two linked genes are quite apart, the

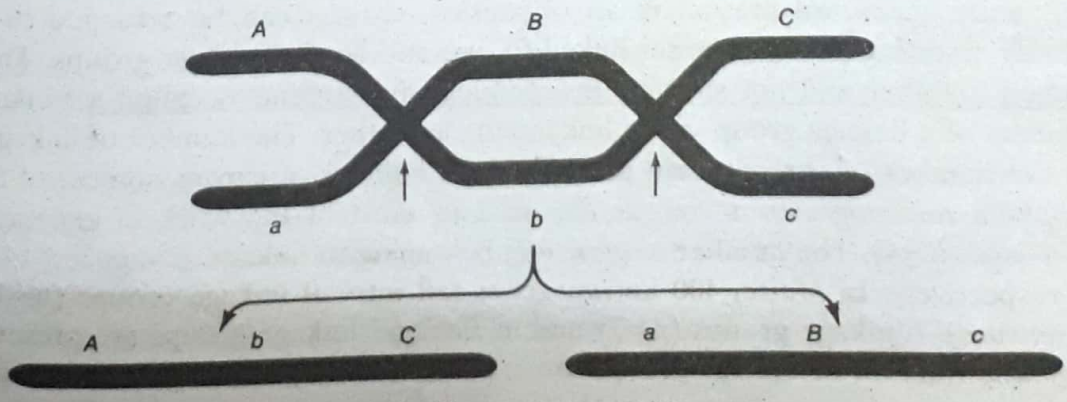


crossover between them yield recombinant gametes. When a single crossover occurs between two non-sister chromatids, the other two chromatids not involved in the crossover enter a gamete unchanged, thus even if the crossover occurs 100% of the time between the two linked genes, the recombinants will be observed only in 50% of the gametes formed. For example, when 20% recombinant gametes are observed, crossing over actually occurred between these two loci, in 40% of the tetrads. Therefore, upper limit of the observed crossing over is 50%. The general rule is that twice the percentage of recombinant gametes of tetrads are actually involved in a genetic exchange.

Multiple Crossovers

It is possible that in a single tetrad, two, three or more exchanges will occur between nonsister chromatids as a result of several crossing over events. If two exchanges take place, such a crossover is termed **Double Crossover.** To study a double crossover, three genes must be considered, each heterozygous for two alleles. For example, three genes A, B, and C are residing on the same chromosome. The probability of a single exchange between A and B or the B and C genes is directly related to the distance between each locus. The closer A is to B and B is to C, the less likely it is that a single exchange will occur between either of the two sets of loci. In a double crossover, two separate and independent exchanges occur simultaneously.

The mathematical probability of two independent events occurring simultaneously is equal to the product of the individual probabilities. Suppose that crossover gametes resulting from single exchange between A and B are recovered 20% of the time and between B and C 30% of the time. The probability of recovering a double-crossover gametes arising from two exchanges, between A and B and B and C, is predicated to be 0.2 X 0.3 = 0.6 or 6%. This shows that frequency of double crossover gametes is always expected to be much lower than that of either single crossover class of gametes. If the three genes are relatively close together along one chromosome, the expected frequency of double crossover gametes is extremely low. Therefore, a very large number of offspring is required to detect double-crossover events. This also shows that a very few triple-and quadruple-crossovers can be expected to occur than double-crossovers.



Double-crossover gametes

Interference and Coincidence

The phenomenon of **Interference** suggests that crossing over in one region interferes with the crossing over in the adjacent regions. This phenomenon explains the occurrence of less double-crossovers than expected, i.e., occurrence of crossing over at one point in the chromosome decreases the probability of its occurrence elsewhere in the same chromosome. The degree of interference may vary in different regions. If double crossovers are absent altogether, we would say that interference is 100%, while if it equals the expected value, we would say that there is no interference.

An inverse measure of interference is called **Coincidence.** It is the ratio of the observed number of double crossovers to the expected number of double crossovers. The coefficient of coincidence can be calculated as follows:

$$\text{Coefficient of Coincidence} = \frac{\text{\% age of double crossovers observed}}{\text{\% age of double crossovers expected}}$$

If double crossovers equal the expected value, there is 100% coincidence, while if no double crossovers are found, the coincidence is zero.

The interference is greatest over short distance in the chromosome, so that within a certain minimal distance there is no double crossing over (coincidence = o). Farther apart, interference dimnishes and at a certain distance disappears entirely (coincidence = 1). The interference is important in placing the genes in the genetic chromosome maps.

In flowering plants **Positive Interference** occurs but in bacteriophages and the fungus *Aspergillus,* **Negative Interference** was observed, i.e., the recombination at a particular region enhances rather decreasing recombinations in the adjoining regions. This is partly due to different method of repoduction in these organsisms. It is beleived that in these organisms effective pairing sites with high recombination frequencies exist.

Linkage Groups

If a certain gene A, is linked to two other genes, B and C then B and C as well are linked. If many genes are present in an organisms, crosses can be arranged to determine indepedently assorting genes or genes linked to one another in pairs or groups. The group of genes linked together and not showing independent assortment, is called a **Linkage Group.** The members of a linkage group shows linkage to each other. The number of linkage groups is equal to the number of chromosome pairs (haploid number of chromosomes) of the species. In *Drosophilla melanogaster,* a vinegar fly, used in most of the work in genetics, has four linkage groups (n = 4). The number of gene loci belonging to linkage groups are 141, 228, 156, and 12, respectively. In *Maize,* 400 known genes fall into 10 linkage groups (n = 10). In Pea *(Pisum sativum)* 7 linkage groups (n = 7) and in *Barley* 7 linkage groups are present. In some organisms the number of linkage groups are less than the haploid number, e.g., in *Mouse* 16 linkage groups are present as compared to 20 pairs of chromosomes and in *Tomato* 10 linkage groups are found as compared to 12 paris of chromosomes.

Complete Linkage in *Drosophilla* Males (Absence of Crossing Over)

In most of organisms used for genetic studies, e.g., *Maize, Pea, Mice, Poultry, Man,* etc., recombination of linked genes takes place both in males and females. However, in *Drosophilla,* the situation is different in the two sexes. In *Drosophilla,* the crossing over rarely or never takes place in the male.

If a grey-bodied, vestigeal winged fly is crossed to a black-bodied, long-winged one, the F_1 generation consists of grey, long-winged flies (normal). This means that the gene for grey body colour B is dominant over its allele b which causes black body colour; and the gene for long wings V is dominant over its vestigeal allele v. Now if the F_1 male hybrids are crossed to double recessive females, i.e., black-bodied, vestigeal-winged females, only two kinds of offspring are produced: grey vestigeal and black long. If, however, an F_1 female fly is crossed with a black vestigeal male, the four expected types are produced, i.e., grey vestigeal, black long, black vestigeal and grey long. Recombinations were found in about 17% of the gametes showing that the genes for black and vestigeal are distantly located and the absence of crossover is not due to closeness of the genes rather due to non-occurrence of chiasmata in spermatogenesis.

Later on it was discovered that the crossing over is also absent in silkworm moths.

The sex-linked inheritance was first studied by **Morgan** and later on **Strutevant** and **Calvin Bridges** showed that the phenomenon of linkage and crossing over is not restricted to sex-linked genes. They found linked genes on autosomes and observed crossing over between them. The absence of crossing over in *Drosophilla* males help in ascertaining that which genes are linked and which seggregate independently.

Crossing Over and Linkage Maps

Morgan's student, **Alfred H. Sturtevant,** was first to relaize that Morgan's proposal, "that recombination frequencies are directly proportional to the distances between the genes", can be used **to map the sequence and distance between linked genes**. Sturtevant was of the view that if the frequency of crossing over between the genes is related to the distance between them, the recombination frequencies between a series of linked genes must be additive. Also experiments of **Bridges** and **Olbrycht** with *Drosophilla melanogaster* helped in suggesting the theory that genes are arranged in chromosomes in a **single linear series.** This theory also helped in drawing **Genetic Maps** of chromosomes.

For example, **Sturtevant** compiled data on recombinations between the genes represented by the yellow, white and miniature mutants initially studied by Morgan. Frequencies of crossing over between each pair of these three genes were observed to be:

(1) Yellow, White	0.5%
(2) White, Miniature	34.5%
(3) Yellow, Miniature	35.4%

Since the sum of (1) and (2) is approximately equal to (3), Sturtevant suggested that the order of genes on the X-chromosome was yellow-white miniature. The yellow and white genes are apparently close to each other because the recombination frequency is low. However, both of these genes are quite far apart from miniature because the white, miniature

and yellow miniature combinations show large recombination frequencies. Since miniature shows more recombination with yellow than with white, it follows that white is between the other two genes.

Sturtevant also suggested that the frequency of exchange could be taken as a direct measure of the distance between two genes or loci along the chromosome. Using this information, he constructed a map of the three genes on the X-chromosome. The distance between yellow and white would be 0.5 map unit, and between yellow and miniature 35.4 map units. It followed close to that observed. One **Map Unit** is directly equated with one percent recombination between two genes. In honour of Morgan's work, geneticists have suggested that one map unit be called a **Centimorgan.**

Three Point Mapping

Three criteria must be met for a successful mapping cross:

1- The organisms producing crossover gametes must be heterozygous at all loci under consideration.
2- The cross must be constructed so that the genotypes of all gametes can be accurately determined by observing the phenotypes of the resulting offspring.
3- A sufficient number of offspring must be produced in mapping experiment to recover a representative sample of all crossover classes.

A three point testcross will give us information regarding relative distances between these genes. Also it will tell us about the linear order in which the genes are present on the chromosome.

Let us assume that there are three linked genes A, B and C. There could be three possible linear orders in which these three genes may be present on a chromosome.

I- A-B-C (B in midde)
II- A-C-B (C in middle)
III- B-A-C (A in middle)

In order to find out linear order, one has to find out which gene is present in the centre. For this prupose a testcross is arranged which involves crossing of trihybrid ABC/abc, obtained from a cross ABC/ABC x abc/abc, with a triple homozygous recessive abc/abc. The phenotypes obtained will represent the gametes formed by the hybrid (genotype). Assuming A-B-C as the order of genes, the expected results are given in table below.

Hypothetical frequencies of eight types of phenotypes will be as follows below:

State of Crossing Over	Genotype	Phenotype	Supposed Frequency
1- Non-crossovers	ABC/abc	ABC	a
	abc/abc	abc	b
2- Crossing over (A-B)	Abc/abc	Abc	c
	aBc/abc	aBC	d
3- Crossing over (B-C)	ABc/abc	ABc	e
	abC/abc	Abc	f
4- Crossing over (A-B & B-C)	Abc/abc	Abc	g
	aBc/abc	aBc	h
		Total:	T

If crossing over recombination value (%age) between the A and B is called X, that between B and C is called Y, and that between A and C is called Z, then:

(a) Crossing over (A-B) (where A and B is separated) = X = c+d+g+h T x 100

(b) Crossing over (B-C) (where B and C is separated) = Y = e+f+g+h T x 100

(c) Crossing over (A-C) (where A and C is separated) = Z = c+d+e+f T x 100

From the above values of X,Y and Z, order of genes can be worked out and linkage maps can be prepared using the following cirteria.

1- if Z= X+Y, the order of the gnes is A-B-C.
2- if Z=X - Y, the order of the genes is A-C-B.
3- if Z=Y - X, the order of the genes is B-A-C.

Three-point Mapping in *Maize*

In *Maize*, endosperm involves three characters. These are the coloured aleurone (C) versus colourless aleurone (c), full endosperm (Sh) versus shrunken endosperm (sh) and non-waxy endosperm (Wx) versus waxy endosperm (wx).

C.B. Hutchinson in 1922 reported the following data as shown in Fig 28-6:

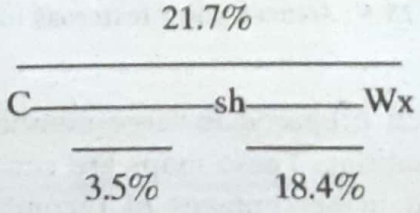
Three recombination values between C-sh, sh-Wx and C-Wx can be worked out in order to find out the linear order of the three genes C, Sh and Wx.

Recombination C-sh = 229+6/6708 x 100 = 3.5%

Recombination sh-Wx = 1227+6/6708 x 100 = 18.4%

Recombination C-Wx = 229/6708 x 100 = 21.7%

The recombination value C-Wx (21.7%) is close to C-sh + sh=Wx (3.5 + 18.4 = 21.9%). Therefore, sh should be located between C and Wx



The slight difference between the total of two individual values and the third value is due to the fact that in the third value (C-Wx), double cross-over are not included. However, if C-Wx were close to (C-sh)-(sh-Wx), then Wx would be between C and sh.

More genes can be added by performing other three-point tetcrosses to the linkage maps.

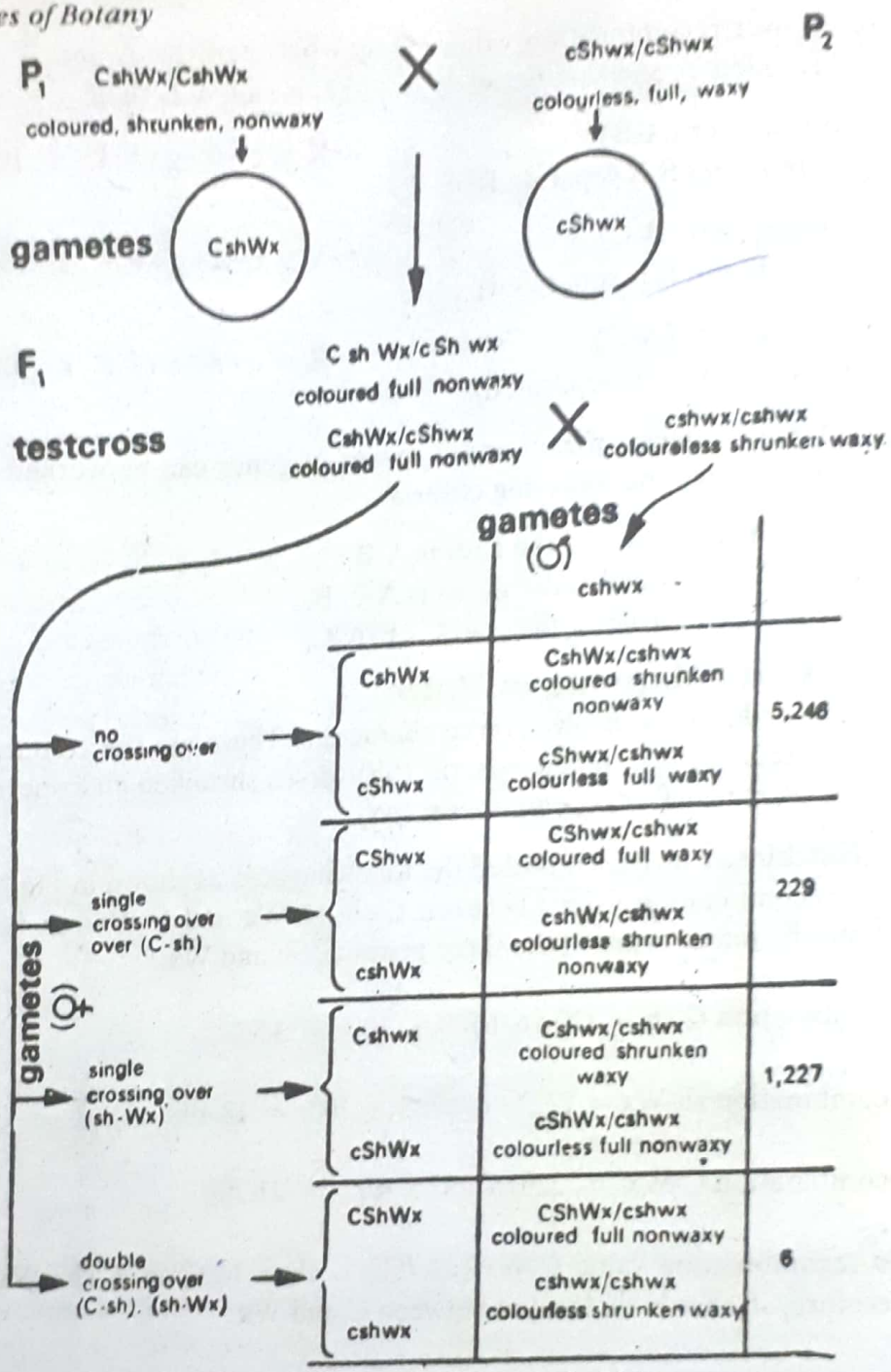


Fig. 28-6 : A three point testcross in *Maize*

Linkage Maps

Linkage maps have been prepared in large number of animals and plants with the help of frequencies of recombinations. These maps are condensed graphic representations of the relative distances, expressed in percentages of recombination among the genes in one linkage group, consequently located in a single chromosome.

C.B. Bridges led the group of investigators who constructed linkage maps of *Drosophilla melanogaster.* The genetic length of the four chromosomes, measured in terms of percentage frequencies of crossing over between genes, are 66 units for X chromosomes, 107.3 for the second, 106.2 for the third and only 0.2 units for the fourth chromosome.

R.A. Emerson, led the work of many geneticists and plant breeders in *Maize* plant and plotted linkage maps for maize. Later on construction of linkage maps started in man particularly in X chromosome.