LINKAGE

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LINKAGE

- The tendency of two or more genes to stay together during inheritance is known as linkage.
- Linkage is the consequence of the concerned genes being located in the same chromosome.
- Linked gen do not show independent segregation. As a result, the ratio obtained in F2 and test cross are significantly different from the expected ratio of 9 : 3 : 3 : 1 and 1 :1 :1 : 1, respectively.
- The effect of linkage is more clear in the test cross generation.

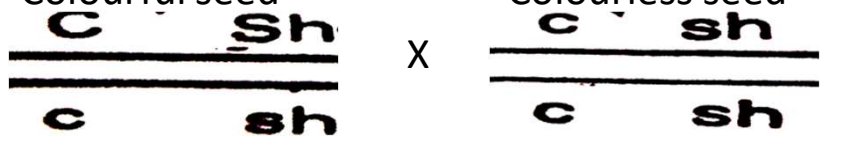
Types of linkage

Complete linkage

- Lack of crossing over is known in male Drosophila.
- The absence of recombination is due to absence of crossing over.
- Only parental character combinations are recovered in the test cross progeny.
- Complete linkage occupied in gene of chromosome IV in male Drosophila
- In maize,
- Dominant allele C is produces coloured seed,while recessive allele c produces colourless seed,
- Another Dominant allele Sh is responsible for full seed, while recessive allele sh is determined to shrunken seed.

Genes C and Sh are present in one chromosome, and their recessive alleles c and sh are located in the homologous chromosome.
Each chromosome appears to behave as an unit during cell division.
Genes C and Sh move to one pole, while c and sh move to opposite pole.
The F1 would produce parental type such as Cc ShSh and colourless shrunken cc shsh in the test cross progeny.

Parents — Heterozygous male (Colourful seed) X double recessive female (Colourless seed)



Gametes

Test progeny

Colourful seed — Colourless seed

❑ INCOMPLETE LINKAGE

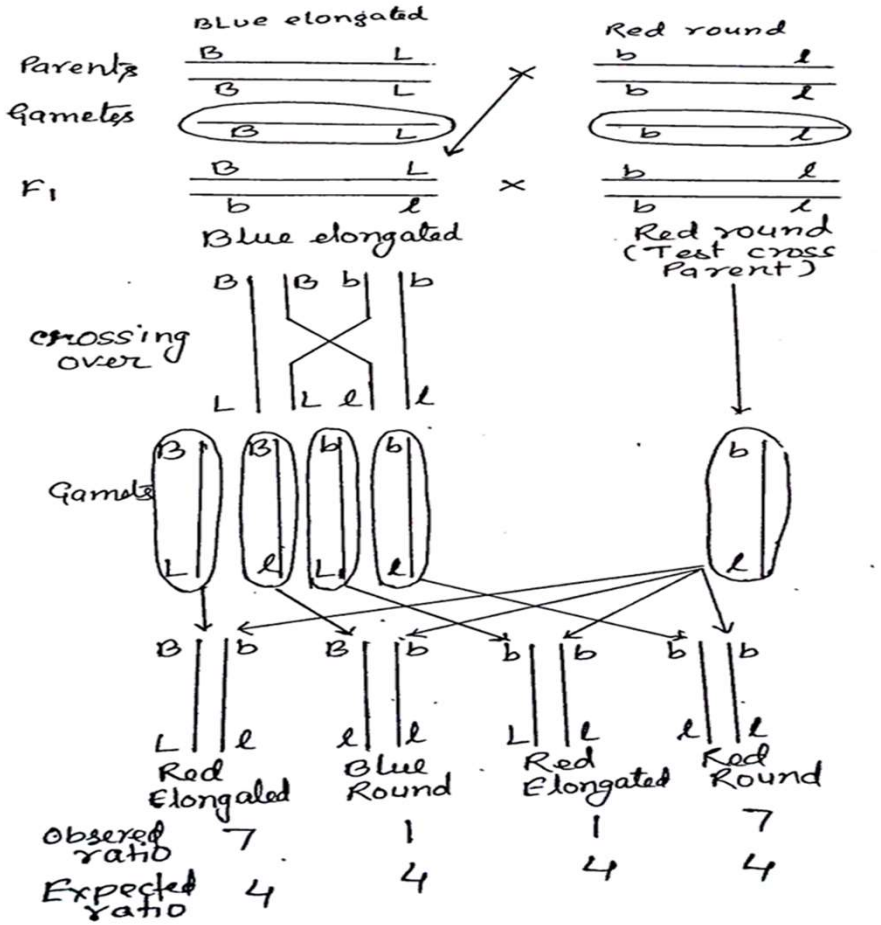
- Recombinant phenotype occur in test cross progeny.
- Some gene may be so closely linked that they may show a very low frequency of recombination and such genes are called tightly-linked.
- Crossing over or frequency of recombination occur between two linked genes.
- Two linked genes are not go to one pole, therefore, its produce recombinant phenotype. This is due to crossing over.

So incomplete linkage is again classified into two groups-1. Coupling phase, 2. Repulsion phase.

1. COUPLING PHASE

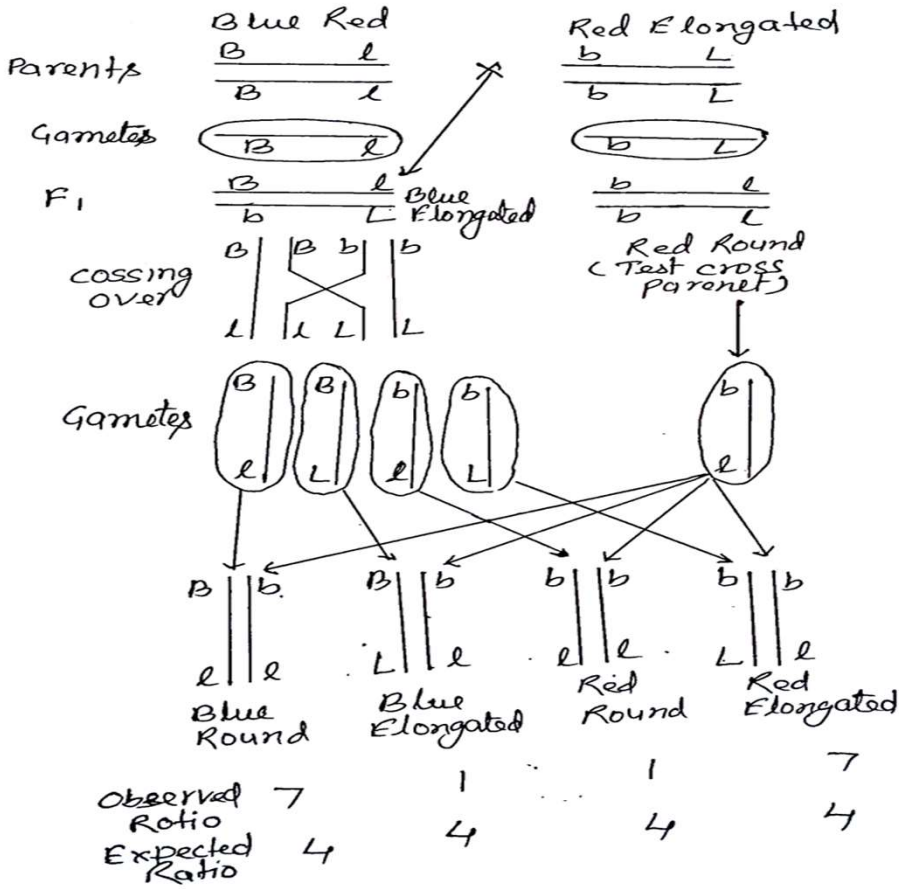
- The coupling and recombination phases of linkage was first reported by Beteson and Punnet (1905) during the study of inheritance of flower colour and pollen shape in pea.
- Dominant gene B is responsible for blue flower, while recessive gene b is responsible for red flower.
- Another dominant gene L is produces elongated pollen grain, but their recessive gene l produces normal round pollen grain.
- When blue elongated BBLL and red round bbll are crossed together, produce F1 with blue elongated BbLl.
- F1 progeny was test cross with red round bbll, the progenies produces ratio 7 blue elongated (BbLl) : 1 blue round (Bbll) : 1 red elongated (bbLl) : 7 red round (bbll) in place of expected ratio 1 : 1 : 1 : 1.
- So the two dominant allele B and L have an affinity for each other so that they tend to stay together during inheritance.

Coupling phase linkage between b and l genes



❑ REPULSON PHASE

- When blue round BBll and red elongated bbLL are crossed together, produce F1 with blue elongated BbLl.
- Due to crossing over in F1 progeny, its produces four types of gamets such as Bl, BL, bl and bL in equal frequency.
- So F1 progeny was test cross with red round bbll, the progenies produces ratio 7 blue elongated (BbLl) : 1 blue round (Bbll) : 1 red elongated (bbLl) : 7 red round (bbll) in place of expected ratio 1 : 1 : 1 : 1.
- Its appears as the two dominant genes B and L repels each other. So that they tend to stay away from each other during inheritance.

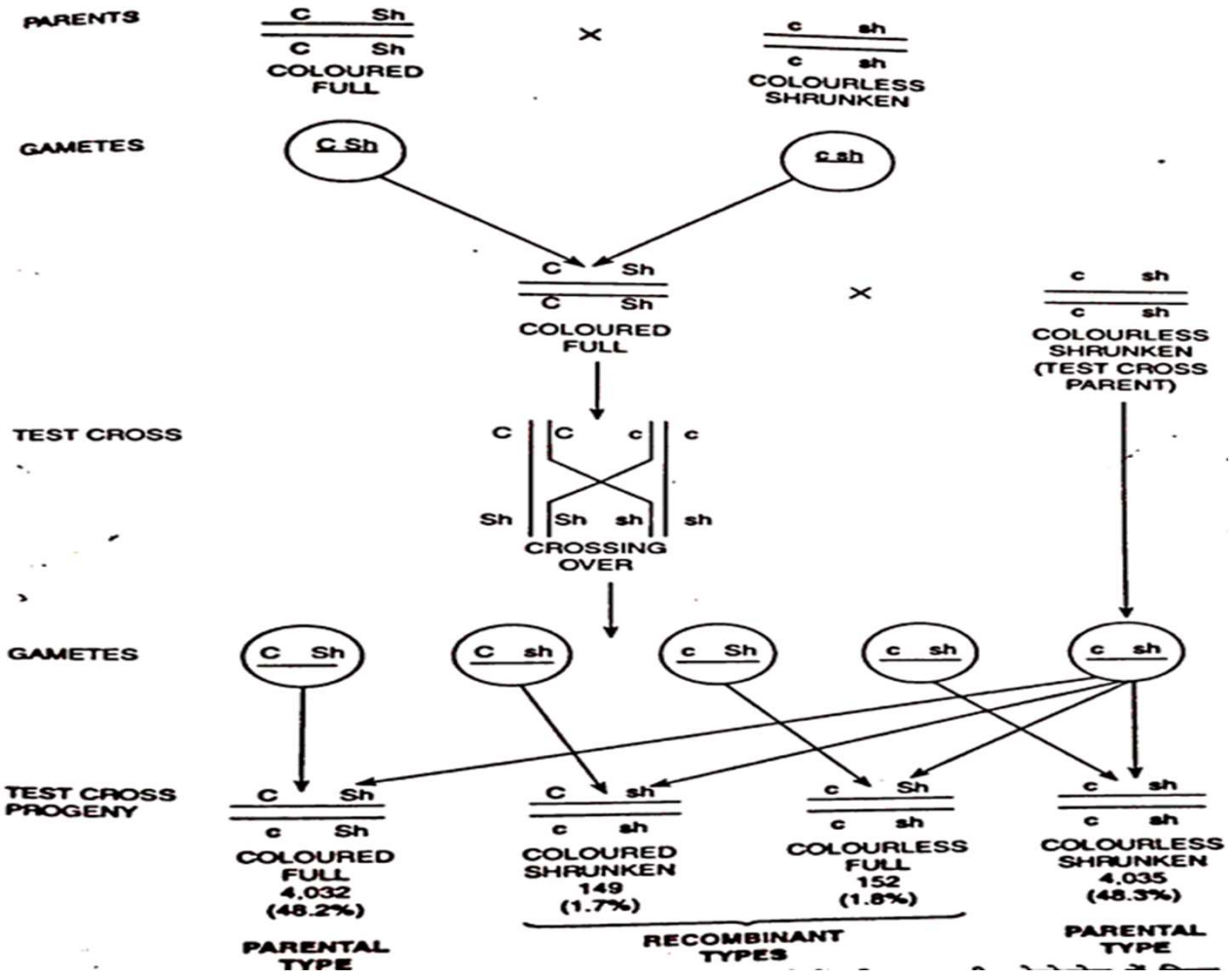


- According to Morgon study the linkage (coupling and repulson phases) in maize.

COUPLING PHASE

- Dominant gene C produces coloured seed, while their recessive gene c produces colourless seed.
- Another dominant gene Sh governs full seed, while their recessive gene sh controlled shrunken seed.
- When colourfull (CC ShSh) were crossed with colourless shrunken (cc shsh), the F1 seeds were coloured full (Cc Shsh).
- Due to crossing over in F1 progeny, its produces four types of gamets such as C Sh, C sh, c Sh and c sh in equal frequency.
- If the F1 plant (Cc Shsh) were test cross with colourless shrunken (cc shsh) to produce test cross seeds (8368).
- Out of 8368 seeds, 4032 (48.2%) seeds coloured full, 149 (1.7%) coloured shrunken, 152 (1.8%) colourless full and 4045 (48.3%) colourless shrunken, which were not present in the expected ratio 1 : 1 : 1 : 1.
- Clearly, two phenotypic classes (coloured full and colourless shrunken) has higher frequency than expected 25%, respectively.
- The remaining two phenotypic classes (coloured shrunken and colourless full) are far less frequency than expected ratio 25%, respectively.
- So it is revealed that the two dominant genes C and Sh have a strong affinity for each other, so that the frequencies of coloured full and colourless shrunken are greater than expected.
- This is due to presence of dominant genes C and Sh in the same chromosome.

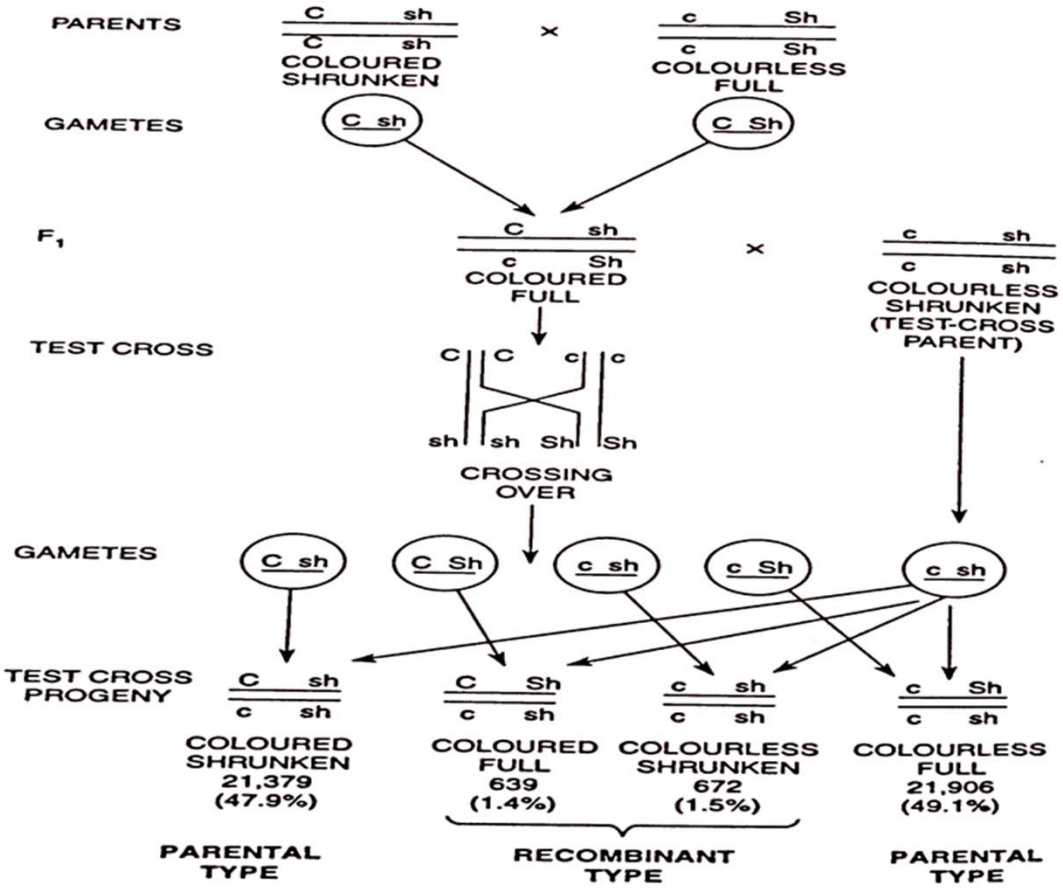
Coupling phase linkage between genes c and sh in maize



❑ Repulsion phase

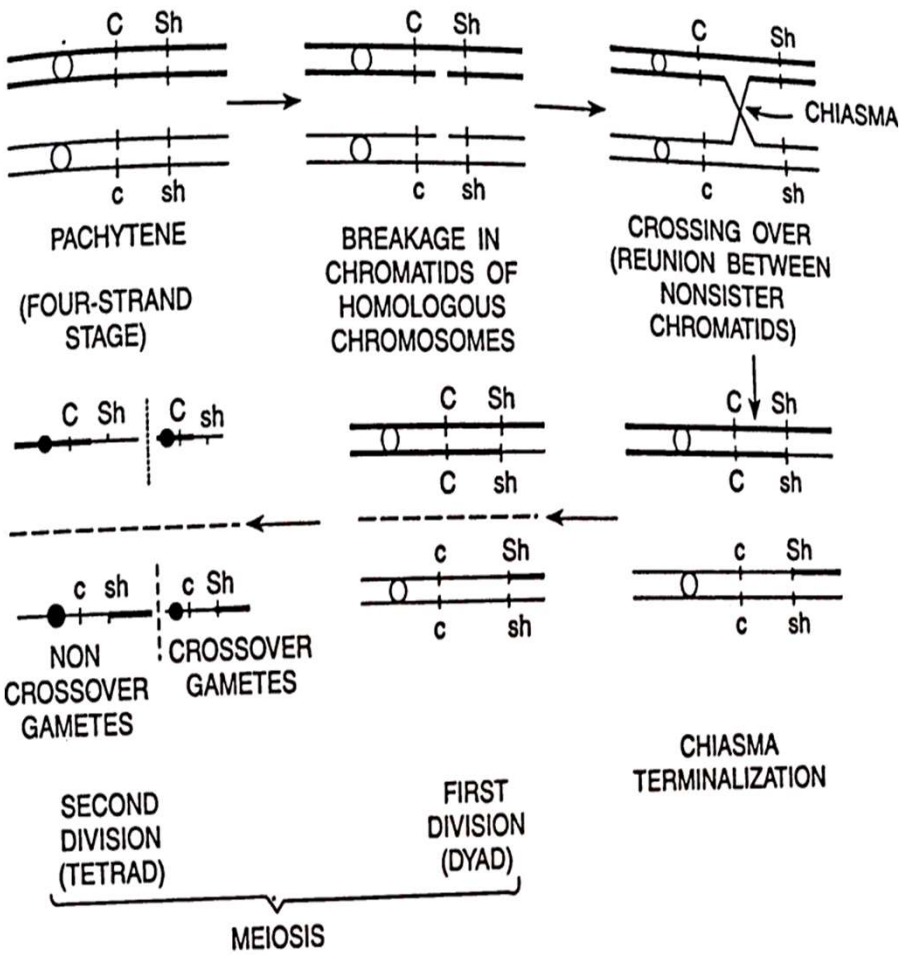
- Dominant gene C produces coloured seed, while their recessive gene c produces colourless seed.
- Another dominant gene Sh governs full seed, while their recessive gene sh controlled shrunken seed.
- When colourfull (CC shsh) were crossed with colourless shrunken (cc ShSh), the F1 seeds were coloured full (Cc shSh).
- Due to crossing over in F1 progeny, its produces four types of gamets such as C sh, C Sh, c sh and c Sh in equal frequency.
- If the F1 plant (Cc shSh) were test cross with colourless shrunken (cc shsh) to produce test cross seeds (44596).
- Out of 44596 seeds, 21379 (47.9%) seeds coloured shrunken, 639 (1.4%) coloured full, 672 (1.5%) colourless shrunken and 21906 (49.1%) colourless full, which were not present in the expected ratio 1 : 1 : 1 : 1.
- Clearly, two phenotypic classes (coloured shrunken and colourless full) has higher frequency than expected 25%, respectively.
- The remaining two phenotypic classes (coloured full and colourless shrunken) are far less frequency than expected ratio 25%, respectively.
- So it is showed that the two dominant genes C and Sh were dislike to each other, so that the frequencies of coloured shrunken and colourless full are greater than expected ratio 25%.
- This is due to presence of one dominant allele C of one gene with the recessive allele sh of other gene.

Repulsion phase linkage between genes c and sh in maize



CROSSING OVER

- Recombinant phenotypes in linkage are produced by recombinant gametes which is due to crossing over.
- Crossing over is defined as " the exchange of homologous segments between non-sister chromatids of homologous chromosomes".
- Genes are located in chromosome. Genes are transfer from one chromosome to other with the exchange of corresponding segments of homologous chromosome.
- Crossing over takes place in pachytene stage during meiosis division, when homologous chromosome have undergone pairing. In this stage, each chromosome of a bivalent has two chromatids. So that each bivalent has four strands.
- Generally, one chromatid from each of the two homologues of a bivalent is involved in crossing over which produces two recombinant chromatids, called cross over chromatids, while two original chromatids are not involved in the crossing over, is known as non-crossover chromatids.
- A segment of a chromatids is attached with the homologous segment of the non-sister chromatid and vice-versa.
- It produces a cross(X) like figure at the point of exchange of the chromatid segment, is called chiasma.
- Breaks occur at place of homologous segment of the non-sister chromatids involved in the crossing over.



FREQUENCY OF CROSSING OVER

- The frequency of crossing over between two genes can be estimated as the frequency of recombinant progeny from a test cross.
- The frequency of crossing over is usually expressed as per cent.
- The frequency of crossing over may be estimated as follows from the data deviated in coupling phase.

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

$$= \frac{152+149}{4032+4035+152+149} \times 100$$

$$= \frac{301}{8368} \times 100 = 3.6\ \%$$

FACTORS AFFECTING THE FREQUENCY OF RECOMBINATION

DISTANCE BETWEEN GENE: Crossing over increase with an increase in distance between the genes.

SEX: Recombinant frequencies show lower in heterogametic sex than homogametic sex of the same species.

AGE OF FEMALE: The crossing over frequency show less in female Drosophila with increase in their age.

TEMPERATURE: In Drosophila, the rate of crossing over is less at 22 degree Celsius, and tend to increase with lower and higher temperature than 22 degree Celsius.

NUTRITION: The frequency of recombination at larvae stage of drosophila is also affected by the presence of metallic ions such as Ca and Mg ions. High Ca ion diet reduce recombination frequency, but decrease with low ca ion diet.

RADIATION: In female Drosophila, irradiation with X-rays and gama rays is increase the recombinant frequency. Crossing over occur also in male Drosophila when irradiated with X-rays.

CHEMICAL: If the mature female Drosophila are injected with some antibiotic such as mitomycin C and mitomycin D to promote recombination frequency. Some alkylating agents like EMS (Ethyl methane sulphonate) has also promote recombination.

GENOTYPE: In Drosophila, Some gene affect the chromosome pairing and formation of synaptonemal complex.

Crossing over takes place after synapsis but asynapsis reduce the chromosome pairing.

Desynapsis allow chromosome pairing but they reduce recombination frequency.

The gene c3G of third chromosome in Drosophila is present in homologous state which reduce the crossing over, but in heterozygous state it promote the crossing over.

CHROMOSOMAL ABERRATION: In Drosophila, centromeric inversion and translocation reduce the recombination frequency, that is called crossover suppressor.

DISTANCE FROM CENTROMERE: If genes located near the centromere, it show lower recombination frequency than those located away from the centromere.

CYTOLOGICAL BASIS OF CROSSING OVER

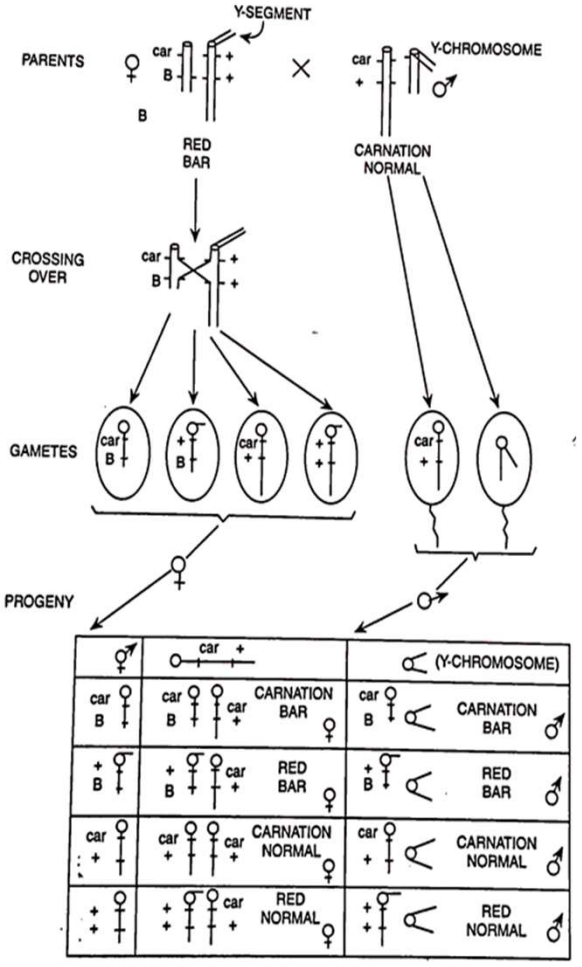
- The first experimental evidence of cytological basis of crossing over was presented by Curt Stern (1931) in Drosophila.
- He use a female Drosophila in his experiments.
- In female, One X-chromosome of female was shorter than normal on which recessive gene Car is responsible for carnation eye color, other dominant gene B is responsible for bar eye shape.
- Another X-chromosome was normal length (but a segment of Y-chromosome was translocated onto it short arm)

on which dominant gene Car+ is responsible for dull red, other recessive gene B+ is responsible for normal oval eye shape.

- In male, Y-chromosome was shorter than normal (but a segment of Y-chromosome was translocated onto it short arm)

Another X-chromosome was normal on which recessive gene Car is responsible for carnation eye color, other gene B+ is responsible for normal oval eye shape.

- Tern were test cross between female red bar and carnation eye Drosophila which produce four types of flies such as
  1. Carnation Bar (Car B)
  2. Red Bar (Car+ B)
  3. Carnation Normal (Car B+)
  4. Red Normal (Car+ B+)
- Two of four phenotypes, carnation bar and red normal are non cross over. Carnation bar individual carry one short X-chromosome, while red normal have long X-chromosome with Y-segment.
- Another two phenotype, red bar and carnation normal are cross over. These are involved an exchange of homologous segment between homologous chromosomes. Therefore, red bar individual have one short X-chromosome with attached Y-segment, while carnation normal flies have normal X-chromosome without attached Y-segment.
- Tern was concluded as follows:
- During meiosis, there is exchange of precisely homologous segment between homologous chromosomes (crossing over)
- Crossing over is responsible for the combination between linked genes.

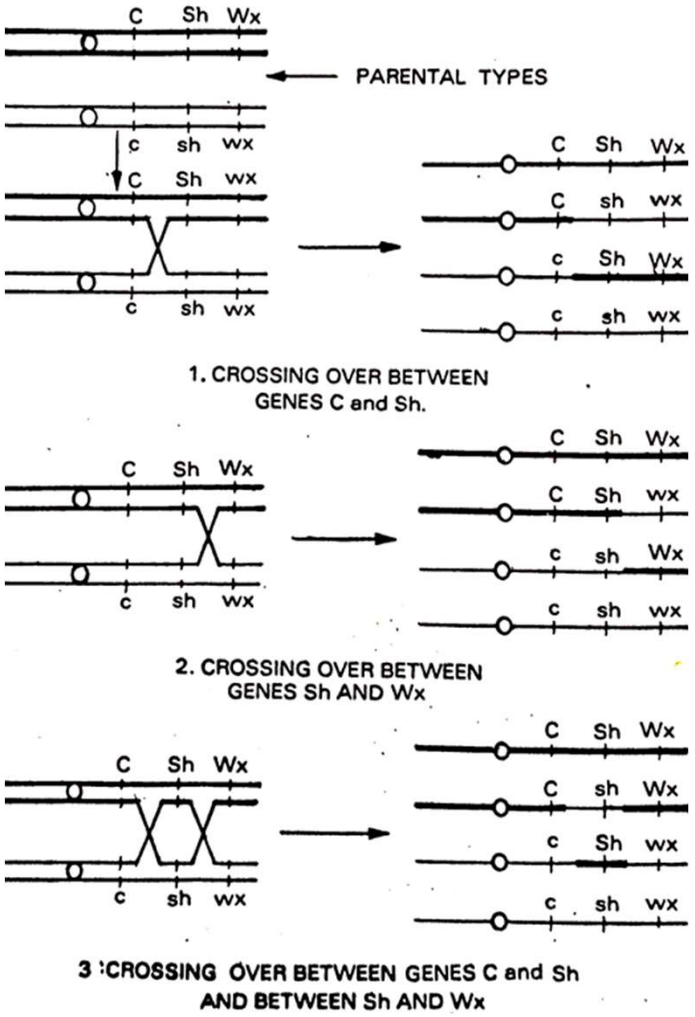


CROSSING OVER AMONG THREE LINKED GENES

- C= colourled, c= colourless, Sh= full, sh= shrunken, Wx=non-waxy and wx= waxy.
- A test cross for three genes (c, sh and wx) in maize which produce eight types of gametes. In a test cross, the phenotype of a progeny is not affected by the test cross parent as it contributes only the recessive alleles of the genes.
- Two of the eight type of progeny C Sh Wx and c sh wx are more frequent and represent the parental or non-recombinant type.
- Two other C sh Wx and c Sh wx are least frequent and are double cross over.
- Remaining four types, C Sh wx, c sh Wx, C sh wx c and c Sh Wx are produced by single cross over and are intermediately frequent between three linked genes.

Genotype	Phenotype	Number	Frequency (%)
C Sh Wx/c sh wx	Coloured, full, non waxy	2777	2777/7000X100=39.7
C sh wx/ c sh wx	Colourless, shrunken, waxy	2708	2708/7000X100=38.7
C sh wx/ c sh wx	Coloured, shrunken, waxy	116	116/7000X100 =1.7
C Sh Wx/c sh wx	Colourless, full, non-waxy	123	123/7000X100 =1.8
C Sh wx/c sh wx	Coloured, full, waxy	643	643/7000X100 =9.2
c sh Wx/c sh wx	Colourless, shrunken, non-waxy	626	626/7000X100 =8.9
C sh Wx/c sh wx	Coloured, shrunken, non-waxy	4	4/7000X100 =0.06
c Sh wx/c sh wx	Colourless, full, waxy	3	3/7000X100 =0.04
Total		7000	100

$$\text{Frequency (\%)} = \frac{\text{No of progeny in a phenotypic class}}{\text{Total number of progeny}} \times 100$$



COFFICIENT OF COINCIDENCE

- Double cross over are produced by two simultaneous cross overs one each on either side of gene located between two genes.
- Example- gene sh located between c and wx.
- If the occurrence of crossing over in the two region (between caand sh, and between sh and wx) were independent of each other.
- The frequency of double cross overs will be the product of the frequencies of crossing overs in the two regions.
- 'The occurrence of crossing over in one region does not affect the change of it occurrence in the other region'.
- Therefore, calculation according to double cross over data:

$$\text{Expected Frequency (\%)} = \frac{\text{The product of the frequency of crossing overs between c and sh, and between sh and wx}}{100}$$

$$= \frac{1.7 + 1.8 + 0.06 + 0.04 \times 9.2 + 8.9 + 0.06 + 0.04}{100}$$

$$= 0.66\ \%$$

Obsered frequency of double cross over type C sh Wx and c Sh wx= 0.04 + 0.06 = 0.10 %

$$\text{Coefficient of coincedence (\%)} = \frac{\text{Observed frequency of double cross}}{\text{Expected frequency of double cross}} \times 100$$

$$= \frac{0.10}{0.66}$$

$$= 0.151 \times 100 = 15.1\%$$

COFFICIENT OF INTERFERENCE

- The observed frequencies of double cross over are lower than expected values.
- This is interpreted as follows: 'the occurrence of crossing over in one region of a chromosome interferes with its occurrence in the neighbouring segments, called coefficient of interference'.
- The intensity of interference would decrease at the point of second crossing over than that of first one.
- Therefore, coefficient of coincidence would be lower, when the concerned genes are located close to each other than they are located further apart.

Coefficient of interference (%)= 1 - coefficient of coincidence X 100

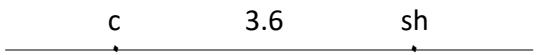
= 1 - 0.151 X 100

= 0.849 X 100

= 84.9%

LINKAGE MAP OR LINKAGE GROUPS

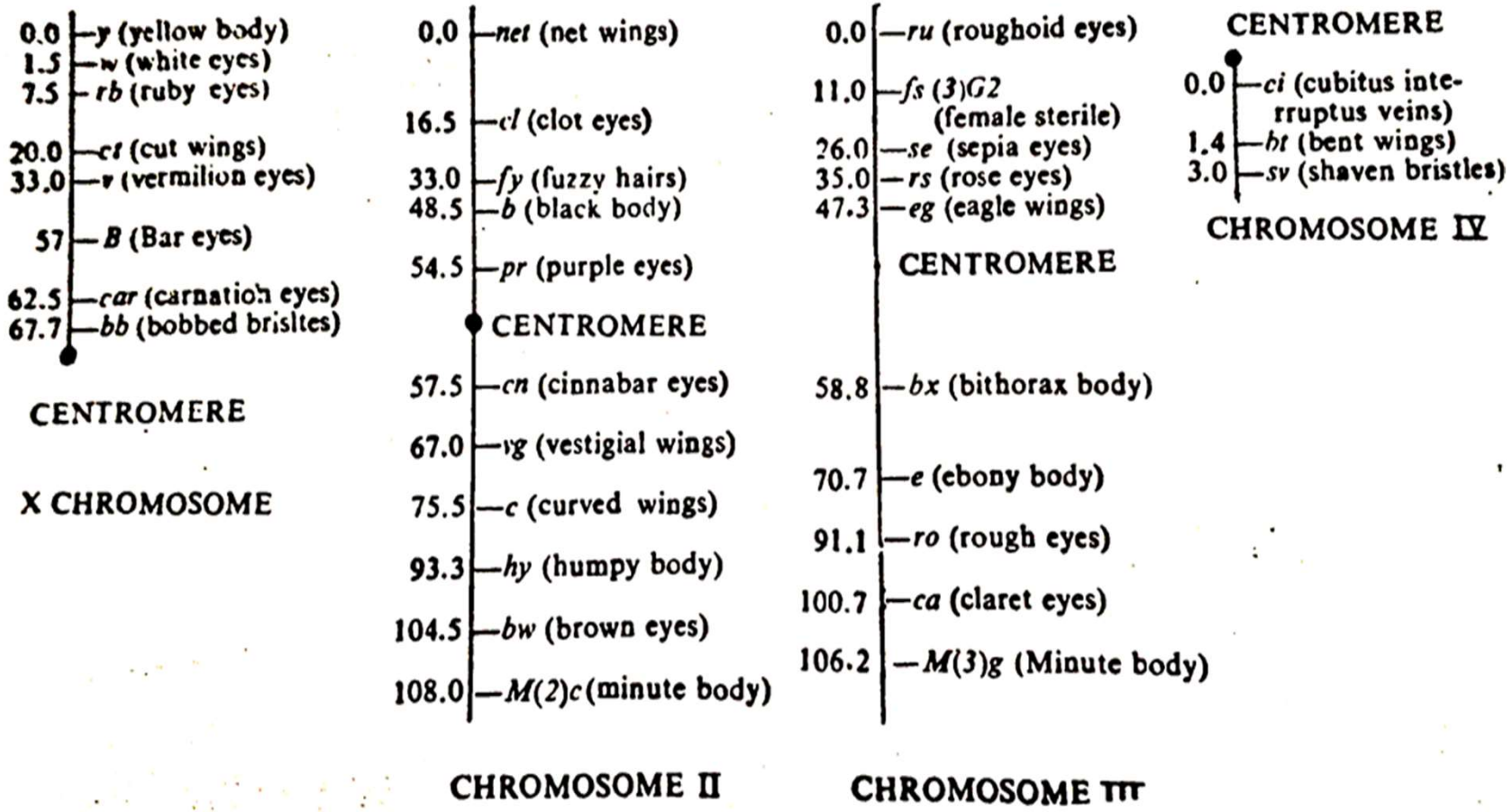
- A linear map of the genes showing linkage with each other, it depicts the sequences in which genes are located in the chromosome as well as the frequency of recombination between the adjacent genes, it is known as linkage map, genetic map or chromosome map.
- Recombination frequencies between linked genes are determined from appropriate test crosses, these per cent frequencies are used as map units for linkage map.
- A map unit is that distance in a chromosome which permits 1 per cent recombination between two linked genes.
- Map unit is an imaginary distance and it does not represent the actual distance between the two linked genes in the chromosome. Therefore, map unit does not have a unit of measurement, e.g., cm, mm, µm, Å, etc.
- The recombinant frequency 3.6% observed in the test cross progeny which obtained by linkage between c and sh gene. A single linkage map of the genes c and sh is depicted in the drawing.



A linkage map of genes c and sh of maize

- So if all the genes that are linked together form a linkage group. Genes of a linkage group may be represented on a single straight line in the same order in which they are normally present in the concerned chromosome.
- In such studies, it is desirable to include only those genes that show less than 20%, preferably 10% or less, recombination with each other to avoid confusion due to double and triple cross overs.
- If crossing over between the genes included in the test cross is more than 20%, the linkage map would not be very reliable.
- In addition, the number of test cross progeny should be sufficiently large to yield reliable recombination frequencies.
- The number of different linkage groups in a species is, as rule, equal to its gametic chromosome number (n). For example, the number of linkage in d. melanogaster is 4, in barley it is 7, in maize it is 10, in wheat it is 21 and in man it is 23.
- Each linkage group of a species is assigned to a specific chromosome of that species with the help of chromosomal aberrations. In general, the relative lengths of different linkage groups of a species correspond closely with the relative lengths of the chromosomes in which they are located.
- The total map distance between two genes of a linkage group may exceed 50 or even 100, but it does not mean that they would show more than 50% recombination., which is the frequency in case of independent segregation.

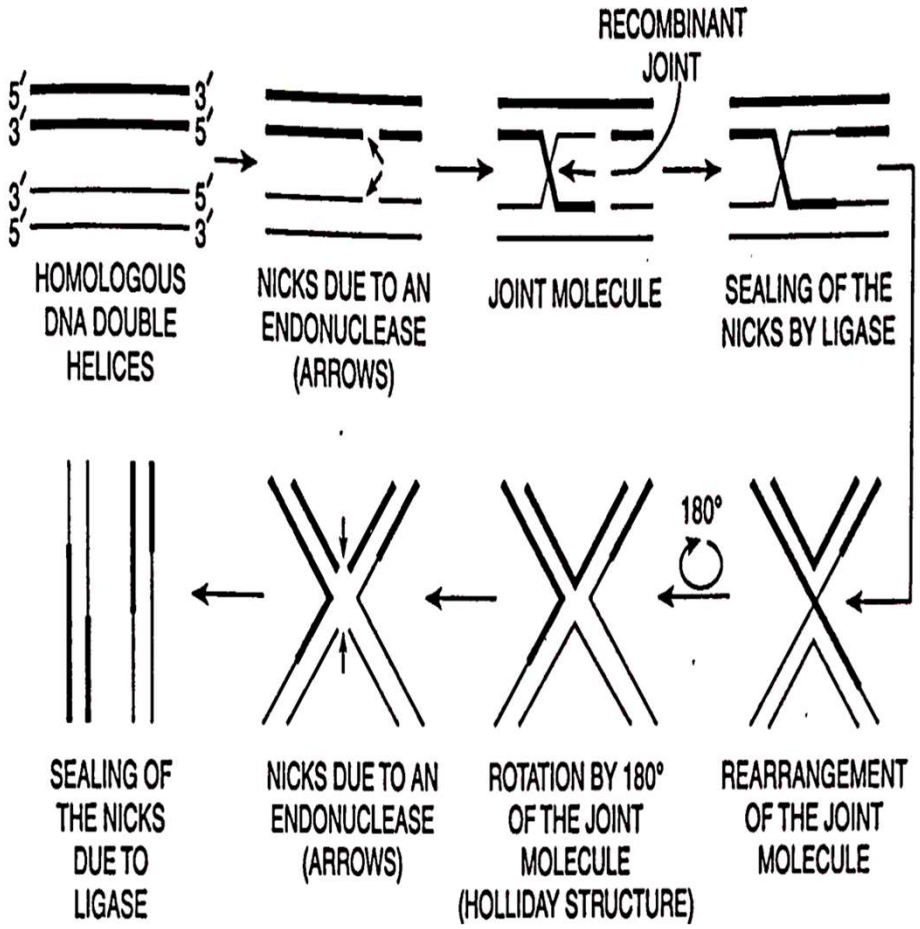
THE FOUR LINKAGE GROUPS OF Drosophila melanogaster



Molecular mechanism of crossing over

BREAKAGE AND REUNION THEORY (HYBRID DNA MODELS):

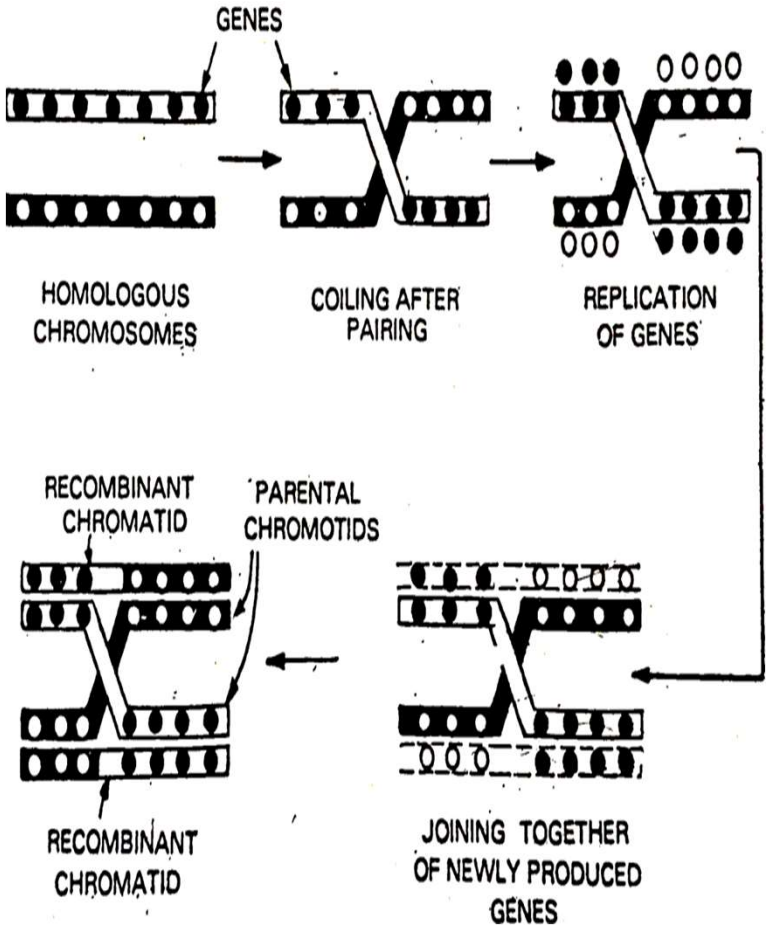
- The breakage and reunion among non-sister chromatids must essentially be based on breakage and reunion of DNA double helices. These theory are also called hybrid DNA models.
- There are two models differ in one important aspect.
- Whitehouse (1963) proposed that single strand breaks occur in the strands having opposite polarity, while
- Holliday (1964) proposed that single strand breaks occur in the strands having same polarity. This model is relatively similar and more attractive.
- An endonuclease produces single strand nick at identical point in the two homologous DNA molecules in strands having the same polarity.
- The two strands of each DNA molecules separate from each other up to same distance from the point of nicks; the free strands now pair with the intact strand of the homologous DNA molecule.
- The two nicks present in these molecules are then sealed by DNA ligase.
- The hybrid DNA molecules undergoes reorientation to form a X-shaped figure. One end of this X now rotate by 180 degree.
- An endonuclease now induces nicks in the two intact (which not cut earlier) strands of the hybrid molecule.
- This yield two recombinant DNA molecules. Each recombinant molecules has a nick, which is finally sealed by DNA ligase to produces four types of recombinant hybrid gametes.



COPY-CHOICE THEORY

Copy choice theory was proposed by Belling (1933). According to this theory:

- Gene present in the chromosome are the first to be replicated
- They are subsequently connected with each other through the synthesis of the remaining parts of chromosomes.
- The homologous chromosomes are likely to be coiled with each other, so that the newly produce copies of genes present in a segment of one chromosome would be adjacent to those of the neighbouring segment of the homologous chromosome.
- As a result, the new copy of genes present in a segment of an chromosome may sometimes become joined with those of the neighbouring of the homologous chromosome giving rise to cross over or recombinant chromatids.
- According to this theory, chromosome replication or at least replication of segments involved in crossing over, must occur after synopsis, which is contrary to the known facts. Hence this postulate appears to be unrealistic.
- Lederberg (1955) was proposed in a modification of Belling theory to explain some unusual features of recombination in bacteria. This modification is also known as copy-choice theory.
- This theory also requires that (1) chromosome replication take place after synopsis, and (20 DNA replication be conservation.
- This is contrary to the known facts as in eukaryotes chromosome replication occurs before synopsis and DNA replication is universally semi-conservation.
- But some DNA replication in prokaryotes appear to be of the copy-choice theory.



CROSSING OVER IN FOUR STRAND

- After 1955, it is well estimated that, chromosome replication during premeiotic prophase and bivalents formed due to chromosome pairing during zygotene are four stranded.
- Before 1955, the studies with microscope showed that- till pachytene, they were seen to be four stranded. This leads to interesting question that crossing over takes place before or after the bivalents become four stranded.
- This question was answered through genetic studies in Neurospora (Ascospore colour).
- The dominant gene Al produce black ascospores, while the recessive gene al produce albino ascospores.
- Each zygote undergo meiosis to produce four haploid nuclei. These nuclei divide mitotically to produce eight haploid nuclei which are arranged in linear order and each nuclei give rise to one ascospore.
- The regular arrangement of ascospores make it possible to predict the consequences of crossing over between gene and it centromere on the spore arrangement.
- If the crossing over take place during two strand stage, the first meiotic division would produce two nuclei having Al and al.
- The four haploid nuclei obtained after second meiotic division would be arranged in the order Al, Al, al and al.
- The eight nuclei generated after the mitotic division would lie in the order A, Al, Al, Al, al, al, and al, and they would show 4 black : 4 albino spore arrangement.
- If the crossing over occurred during the four strand stage, the spores would show 2 black : 2 albino : 2 black : 2 albino arrangement.