Linkage and Crossing over

The two most important genetic laws that Mendel formulated as a result of his studies on the pea plant are (i) Law of segregation and (ii) Law of Independent Assortment. According to the *law of segregation*, two homologous chromosomes, one each contributed by the two parents, segregate in the maturation process and are included into different gametes. Segregation of homologous chromosomes brings about segregation of the two alleles of the allelic pair. *The Law of Independent Assortment* states that, while segregation of the members of the pair of homologous chromosomes must take place, each of these two homologous chromosomes can form all possible combinations with the rest of the chromosomes at the time of gamete formation. That is to say the chromosomes assort independently of one another, have no preferential associations and are included into the gametes in haploid number.

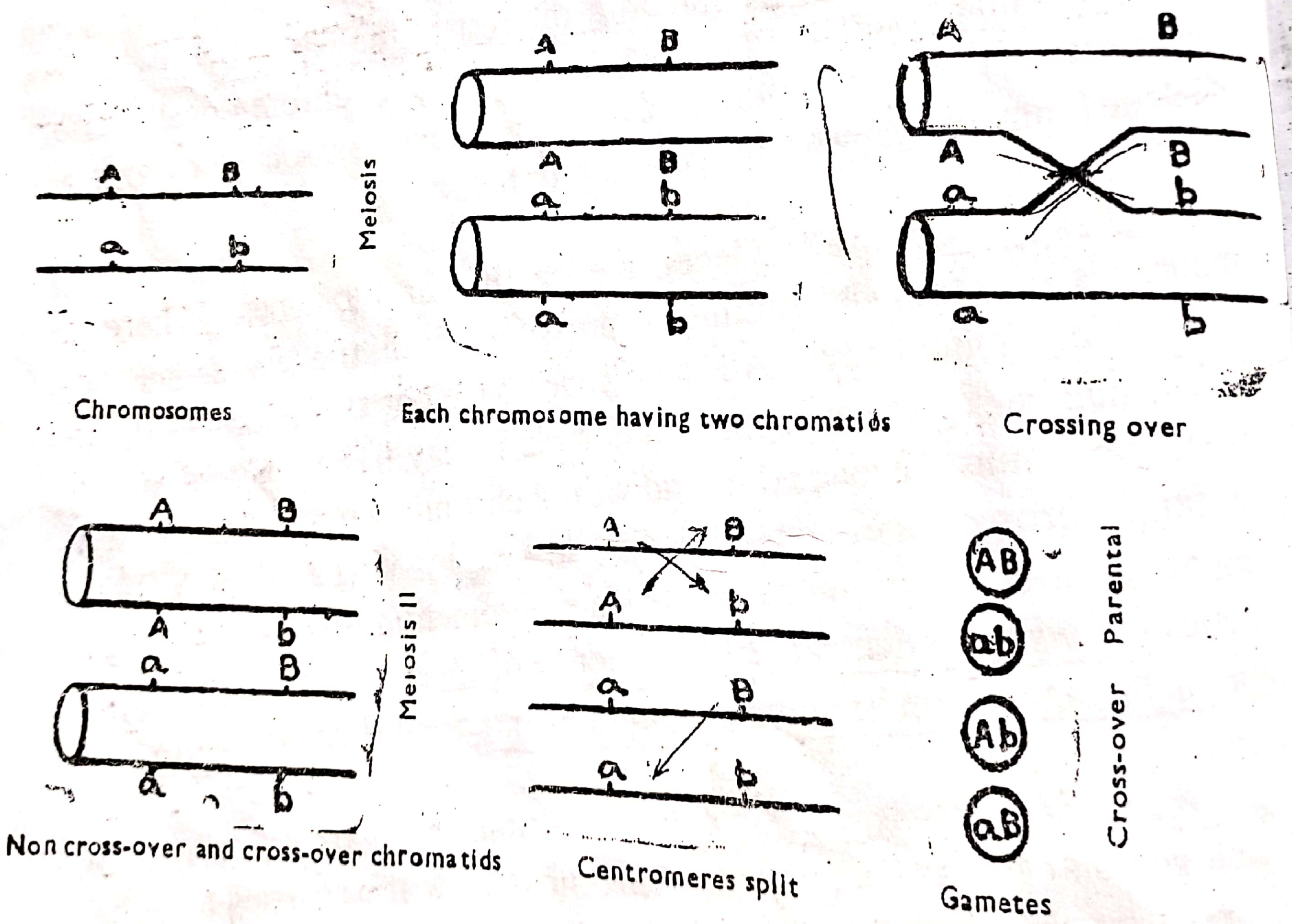
What is Linkage? Genes, are on the chromosomes. There-are thousands of genes determining expression of all the characters of a plant or any living body. All these genes have got to be located on the limited number of chromosomes that a plant may have. Consequently, several genes must be located on individual chromosomes. The genes on the same chromosomes are therefore, linked by virtue of their being present on the same chromosome. This phenomenon is called *Linkage* in genetics. *Linkage* is defined as the tendency of genes in the same chromosome or linkage group to enter the gametes in parental combinations.

What is crossing over? At the time of gamete formation (gametogenesis) genes on a particular chromosome will be carried together wherever that chromosome goes. But, this does not happen all the time and all of the genes on a chromosome do not go together due to the breakage of chromosomes and the resultant exchange of the homologous parts of the chromosomes during synapsis. *Crossing over* may be defined as the tendency of genes to enter the gametes in combinations other than parental.

The exchange or crossing over in the homologous chromosomes occurs in almost all animals and plants with a few exceptions. In male drosophila and female silk moth crossing-over does not occur and linkage is complete.

The crossing-over occurs at the four-strand stage during prophase of meiosis. At the point of chiasma (cross-shaped configuration) the broken ends of the nonsister chromatids reunite and thereby the exchange of genes takes place. These chromatids separate at the time of second meiotic division and the cross-over chromatids separate from the parental combinations and enter into different gametes.

A crossing-over in an organism heterozygous for two gene pairs is shown diagramatically.



The cross-over gametes will be less frequent because:

1. Only 1/2 of the chromatids take part in the crossing-over.
2. All the chiasmata do not represent a crossing-over.
3. Chiasma is not formed in all the cells undergoing meiotic division but their frequency between two adjacent genes is usually very low.

Factors affecting crossing-over

1. *Environments:* High temperature usually increases the rate of crossing over.
2. *Age:* Aged individuals may show more crossing-over.
3. *Distance from the centromere:* Genes in the immediate vicinity of the centromere do not show crossing-over.
4. *Distance between the genes:* The closer the distance between the genes, the stronger the linkage and less the crossing-over.

Linkage group

A linkage group represents all the genes situated on a chromosome. There are as many linkage groups as the number of haploid chromosomes in a particular organism.

Linkage studies in the pea

Bateson and Punnett (1906) discovered the linkage of some characters in sweet peas. They, however, could not present a satisfactory explanation of this phenomenon, as they did not believe that genes are on the chromosomes. They crossed two varieties of the pea, i.e. one with a purple flower colour and long pollen (PPLL) and the other with red flower and round pollen (ppll). In an earlier experiment they had noted that the flower colour and the pollen size were controlled by different genes. The F_1 was purple and long (PpLl), while the F_2 did not conform to the law of independent assortment as the ratio of 9:3:3:1 was not obtained. The parental types were more frequent and the two classes (recombinations) occurred less frequently. The two gene combinations PL and pl which stayed together more frequently came from the parent, and their separation occurred less frequently to produce new combinations.

From these results, they developed the theory of coupling and repulsion. As understood today, when the two dominant characters and, for that matter, the two dominant genes are on the same chromosome and their corresponding recessive alleles on the other homologue, the phenomenon is called *coupling* and when the two dominant genes are located on two different chromosomes, the phenomenon is termed repulsion. Suppose A and B are linked, then a cross between AABB x aabb will show the coupling and a cross between AAbb x aaBB will show the repulsion phase.

Linkage studies in drosophila

*Morgan (1910) came across a similar situation in his studies on drosophila. He was able to explain the tendency of the two characters to remain together in crosses by demonstrating that these characters were controlled by the genes on the same chromosome and thus replaced the Batesonian theory of coupling and repulsion with that of linkage and crossing over.

Detection of linkage

* Detection of linkage is possible when two or more genes are considered simultaneously. In the event of the two genes being located on two different chromosomes, independent assortment of these genes will take place and a plant of AaBb genotype will form four types of gametes AB, Ab, aB and ab in equal numbers. If the two genes A and B are linked, i.e., they are on the same chromosome, the AaBb plant will still form the same four types of gametes, because of the crossing-over, but however, the two linked genes will tend to remain together and be included in the same gamete, and consequently, AB and ab gametes will be more numerous than the Ab and aB gametes.

Let us now consider a cross that Hutchison made in maize, using one variety whose seed was coloured and normally filled (coloured, full) and the other variety having colourless and shrunken seed (colourless, shrunken). The colour gene was dominant over the colourless and the full endosperm was dominant over the shrunken. Using genetic formula, we can describe his results as follows:

	Coloured full	x	Colourless shrunken
Parents	CCFF $\left(\frac{CF}{CF}\right)$		ccff $\left(\frac{cf}{cf}\right)$
F_1	CcFf $\left(\frac{CF}{cf}\right)$ (coloured, full)		

He test-crossed CcFf with ccff and got the following results:

(1) CcFf (CF/cf) = 4032 coloured full

(2) Ccff (Cf/cf) = 149 coloured shrunken

(3) ccFf (cf/cF) = 152 colourless full

(4) ccff (cf/cf) = 4035 colourless shrunken

It will be observed that parental combinations in the progeny of this test-cross are far in excess of the new combinations, i.e. the coloured full and the colourless, shrunken being the parental combination, are far more numerous. This proves that, of the four types of the gametes formed by CcFf (F_1), the parental gametes CF and cf are very many compared to a few recombinations, Cf, cF. If assortment were independent then all of these four gametes would have been equally frequent. This reveals that the two genes C and F are on the same chromosome (they are linked). In such a test cross where parental combinations occur in the progeny more frequently than the new combinations, it is indicative of linkage.

How is linkage calculated?

1. *Testcross method*: The most convenient and efficient method to determine strength of linkage is to make a test cross (cross F_1 with double recessive). The cross will yield four phenotypes if dominance is involved. If there is no linkage, all the four phenotypes i.e., the parental as well as the new combinations will be equal in number. If the two genes are linked the four types will be unequal, the parental ones being more frequent.

If the number of parental combinations and of new combinations are separately added the percentage of each can be worked out. For instance, in the above testcross of Hutchison, the total parental combinations are 4032+4035=8067, and the total of new combinations is 149+152=301. The percentage of parental combinations will represent the strength of linkage and the percentage of new or recombinations will indicate the cross-over value or recombination value.

$$\text{Recombination or cross-over value} = \frac{301}{8368} \times 100 = 3.6\%$$

$$\text{Linkage strength} = \frac{8067}{8368} \times 100 = 94.4\%$$

If one value is known, the other is obtained by subtracting it from one hundred. The two genes are 3.6 units apart on the chromosome.

(2.) *F_2 analysis to calculate the crossing-over value of the two linked genes.* F_2 data from dihybrid individuals can also be subjected to analysis to determine the strength of linkage or the cross-over value. The key to the F_2 analysis is to look for the double recessive individuals in the F_2 population, count them and compute their relative frequency (fraction). It will be realised that a double recessive individual is the product of the double recessive male and female gametes. So the frequency of the double recessive individuals is equal to the product of the frequencies of the male and female gametes. Also because the male and female gametes are equally frequent in the population, the frequency (fraction) of the double recessive gamete (male or female) can be obtained by taking the square root of the frequency (fraction) of the double recessive individuals. The fraction of the double recessive gamete thus obtained is also the fraction of the double dominant gamete because obviously the two dominant alleles are carried on the other homologous chromosome. When the frequency of ab and AB gametes are known, the frequencies of their recombinational or cross-over types can be calculated by subtracting the sum of AB and ab from one. The following example, where A&B are linked, may be considered.

$$\text{Parents} = \frac{AB}{AB} \times \frac{ab}{ab}$$

$$F_1 = \frac{AB}{ab}$$

F_2 =
A—B— = 160
A—b b = 40
a a B— = 40
a a b b = 60

If the entire F_2 individuals are added up, the frequency (fraction) of aabb, the double recessive individuals, can be calculated.

Frequency of aabb = 60/300 = 1/5 = 0.2
Frequency of ab gametes = 0.2 = 0.44
0.44 will also be the frequency of AB gamete; their sum = 0.44+0.44 = 0.88

The frequency of Ab and aB gametes will be 1—0.88=0.12. Since Ab and aB are the cross-over types, their frequency in the gametic population is the cross-over value of the A and B genes. So the cross-over value = 0.12 or 12% and linkage strength = 0.88 or 88%.

PROBLEMS