

GENETICS
6**CONSTRUCTION OF
GENETIC MAPS**

6.1 INTRODUCTION

The discovery of the phenomenon of linkage and crossing over by T.H. Morgan established the following facts.

1. The number of genes usually exceeds the number of chromosomes in a species, so many genes will be located in the same chromosome.
2. The genes are arranged in a linear order in a chromosome.
3. All the genes present in a chromosome inherit together and form one linkage group.
4. The number of linkage groups correspond to the number of chromosome pairs i.e. gametic number e.g. 4 in *Drosophila*.
5. The linked genes are occasionally separated from the members of their linkage group by crossing over.
6. The frequency of recombination or crossing over between two genes depends upon the distance between them. Closely placed genes will have less chances of crossing over.
7. Each gene has a specific order and location in a linkage group of chromosome.

Based upon the above observations STURTEVANT (1913), a student of MORGAN developed the idea that the percentage of crossing over could be used as a tool to determine the relative positions of genes in chromosomes. Working on this concept, he emerged with the first chromosome map of *Drosophila*. This showed the positions of five genes on the X - chromosome of *Drosophila*. These five genes were for yellow body (Y), white eyes (W), vermilion eyes (V), miniature wings (m) and forked bristles (f).

This graphic representation of the genes in a chromosome is now known as *chromosome map* or *genetic map*.

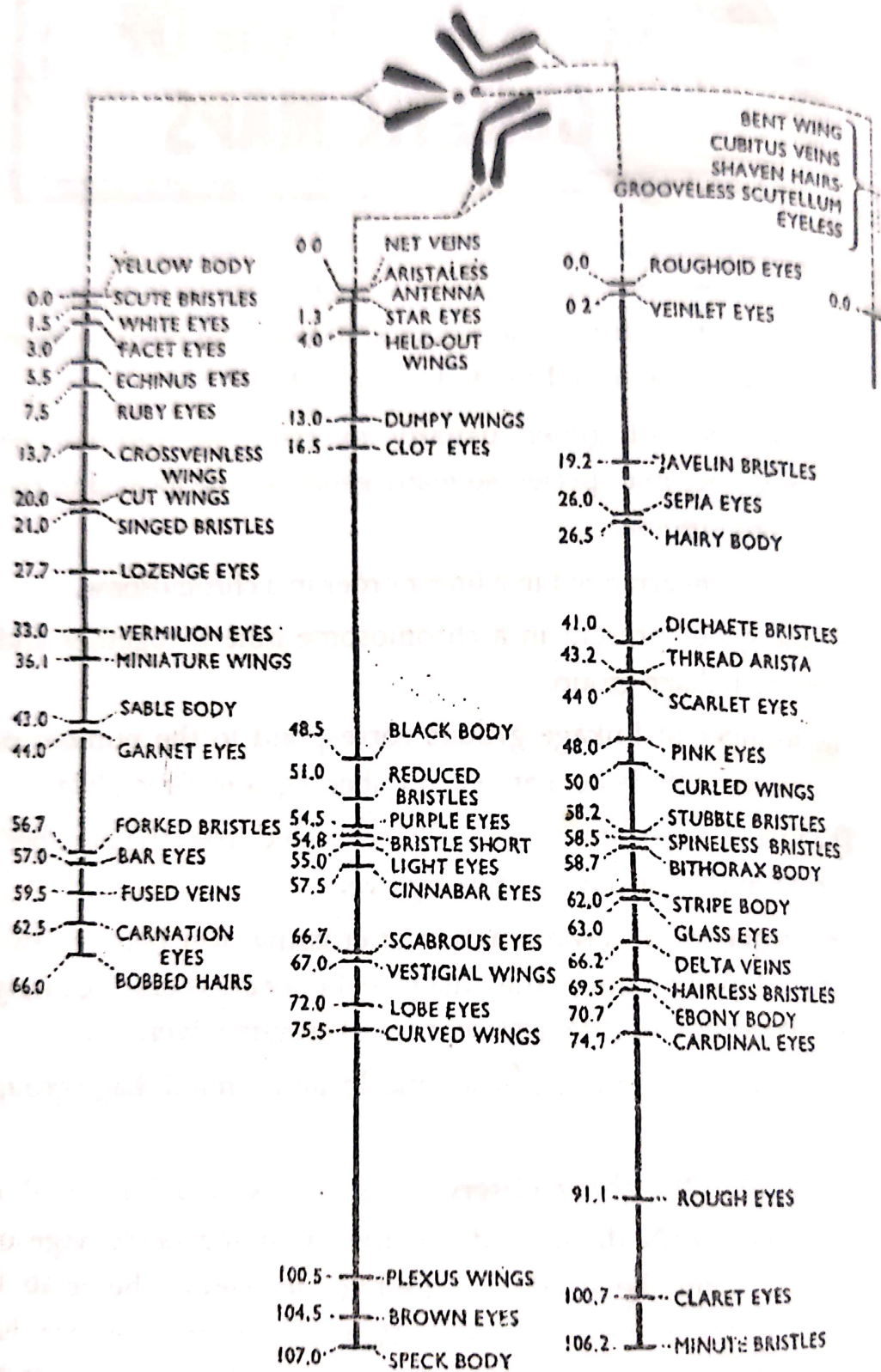


Fig. 6.1 : Linkage maps of four different chromosomes of *Drosophila* showing alignment of different genes on the four chromosomes

6-2 DEFINITION

The graphic representation of relative distance between linked genes of a chromosome is called *linkage* or *genetic map* of chromosome. The genes are represented by points on a single straight line in the same order in which they are normally present in the chromosome. In such a representation, the distance between two neighboring genes is proportional to the frequency of recombination (%) between them. The units used in the mapping are called *map units* or *Centi Morgan units* (CM). A map unit is that distance in a chromosome which permits one percent of recombination among two linked genes. The map unit is an imaginary distance and it does not represent the actual distance between linked genes in the chromosome. Therefore, a map unit does not have a unit of measurement e.g. A^0 , μ etc.

6-3 CONSTRUCTION OF CHROMOSOME MAPS

The entire process of chromosome mapping includes the following steps.

A Determination of Linkage groups :

Before starting the genetic mapping of a chromosome of a species, we have to know the exact number of chromosomes present in that species. Then, we have to determine the total number of genes of that species by making hybridization experiments between wild (original) and mutant strains. Linkage groups of the species have to be worked out by studying the percent cross over.

The number of different linkage groups in a species, is as a rule, equal to its gametic chromosome number. For example, the number of linkage groups in *Drosophila melanogaster* is 4, in barley it is 7, in maize it is 10, while in man it is 23.

B Determination of Map distance :

After knowing the total number of genes in each linkage group, the relative distances between each linked gene have to be calculated. This distance is calculated according to the percentage of crossing over, because cross over frequency is directly proportional to distances between the genes. For example, if the percentage crossing over is 10%, the map distance between two linked genes is 10 units.

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. The frequency of recombination between two linked genes can not exceed 50%, which is the frequency in case of independent segregation. There is a 1 : 1 correspondence between map distance and the observed recombination frequency upto 20 map units. But, there is a progressive decline in the frequency of observed recombination for every additional map unit distance beyond 20 map units.

Two genes belonging to the same linkage group are called *syntemic*. Such genes may show linkage or independent segregation depending on the distance between them.

C Determination of gene order :

After determining the relative distances between the genes of a linkage group, it becomes easy to place genes in their proper linear

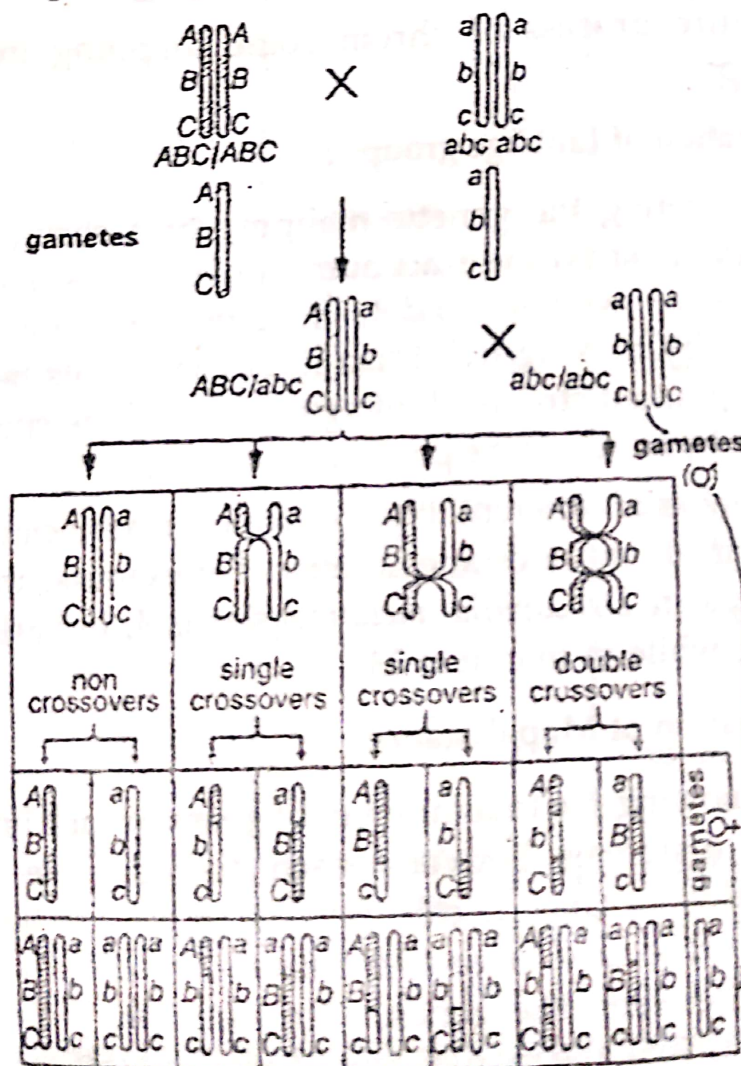


Fig. 6-2 : A three - point test cross involving three hypothetical genes A, B and C

order. For example, if there are three genes A, B and C present in the same chromosome (i.e. these are linked), then these three genes may be present in any one of the following orders. These are A - B - C, A - C - B or B - A - C. It is obvious that in one case B is present in the middle, while in the other cases C and A are present in the middle. Therefore, in finding out the linear order, we have to find out the gene which is present in the centre. For this purpose, a three point test - cross is made, which involves crossing of a trihybrid ABC/abc (obtained from a cross ABC/ABC $\times$ abc/abc) with triple homozygous recessive parent abc/abc. The progeny obtained will represent the gametes formed by the hybrid.

Presuming A - B - C as the order of genes, results expected can be diagrammatically represented as in Figure 6.2. The hypothetical frequencies of eight types of progenies are listed in the Table below.

Table 6.1 : Hypothetical frequencies of different gene types and phenotypes obtained in a cross

State of Crossing over	Genotype	Phenotype	Frequency
1. Non - cross over	ABC/abc	ABC	37
	abc/abc	abc	38
2. Crossing over (A-B)	Abc/abc	Abc	12
	aBC/abc	aBC	8
3. Crossing over (B - C)	ABc/abc	ABc	2.8
	abC/abc	abC	1.2
4. Double crossing over (A - B) & (B - C)	AbC/abc	AbC	0.6
	aBc/abc	aBc	0.4
		Total	100

$$\text{Crossing over (A - B)} = 12 + 8 + 0.6 + 0.4 = 21\%$$

$$\text{Crossing over (B - C)} = 2.8 + 1.2 + 0.6 + 0.4 = 5\%$$

$$\text{Crossing over (A - C)} = 12 + 8 + 2.8 + 1.2 = 24\%$$

In this case, recombination value A - C (24%) is close to (A - B) + (B - C) = 21 + 5 = 26%. Therefore, B should be located between A and B. The slight difference between the two total individuals and the third value is due to the fact that in the third value (A - C) double cross - overs are not included.

D Linkage Map :

We can now map the genes A, B and C in the order A - B - C at the distance of 21 and 5 map units, respectively.

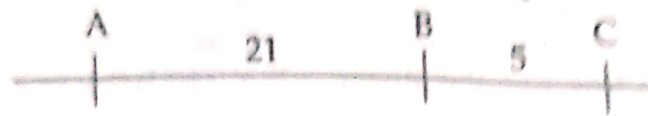


Fig. 6-3 : A linkage map of the genes A, B and C

E Coefficient of Coincidence :

Double cross overs are produced by two simultaneous crossing overs one each on either side of the gene (B) located between two genes (e.g. Between A and B and between B and C). If the occurrence of crossing over in the two regions (e.g. between the genes A and B and between B and C genes) 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 independence of crossing over in the two regions means that, the occurrence of crossing over in one region does not affect the chance of its occurrence in the other region. Therefore, the expected frequency of double cross over may be estimated from the data of Table - 6.1 as follows

Frequency (%) of crossing over between A and B ($= \alpha$) = 2

Frequency (%) of crossing over between B and C ($= \beta$) = 5

The Expected frequency (%) of double cross overs = $\alpha \times \beta / 100$

$$= (21 \times 5) / 100$$

$$= 1.05\%$$

The actual frequency of double cross overs is = 1%. The ratio between the observed and the expected frequencies of double cross over is called *coefficient of coincidence*. Thus,

Coefficient of Coincidence

$$= \frac{\text{Observed frequency of double cross over}}{\text{Expected frequency of double cross over}}$$

$$= \frac{1.0}{1.05}$$

$$= 0.95$$

This value indicates the degree of agreement between the observed and the expected frequencies of double cross overs. It also indicates the strength of interference. When the interference is complete (1.0) no double cross over will be observed, and coincidence becomes zero. On the otherhand when interference decreases (becomes less than 1) coincidence increases. Coincidence values ordinarily vary between 0 and 1.

F Coefficiency of Interference :

As a rule, the observed frequencies of double cross overs are always less than the expected values. This is because of interference. The tendency of one cross over to reduce the probability of another cross over in the vicinity is called *interference*. This phenomenon was discovered by Muller in 1911.

The degree of interference may vary in different regions. It is greatest over a distance and decreases as the distance increases *i.e.*, interference is inversely proportional to the distance between the neighbouring points of crossing over. The intensity of interference may be estimated as coefficient of interference, which is equal to one minus the coefficient of coincidence.

$$\begin{aligned}\text{Coefficient of interference} &= \text{Coefficient of Coincidence} \\ &= 1 - 0.95 = 0.05\end{aligned}$$

$$\text{And coefficient of interference (\%)} = 0.05 \times 100 = 5\%$$

6-4 TWO - POINT TEST CROSS

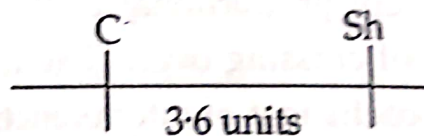
The percentage of crossing over between two linked genes is calculated by test cross, in which an F_1 dihybrid is crossed with a double recessive parent ($Aa Bb \times aa bb$). Such a test cross is called two point test cross, because it involves crossing over at two points.

The distance between two genes can be calculated by taking the example of Maize. When a maize plant with seeds having colour and full endosperm (CSh/CSh) with another plant having recessive alleles for shrunken colourless and seeds (csh/csh), all the F_1 hybrids produced coloured and full seeds (CSh/csh). When F_1

female hybrids are test crossed with double recessive parent (csh/csh), four types of seeds are produced. These are -

Coloured, full (CSh/csh)	— 4032	} 96.4% of the total offspring
Colourless, shrunken (csh/csh)	— 4035	
Coloured, shrunken (Csh/csh)	— 149	} 3.6% of the total offspring
Colourless, full (cSh/csh)	— 152	
Total 8368		

From the test cross data, it becomes clear that the genes C and Sh were located in the same chromosome and are linked together. Only 3.6% individuals were obtained due to crossing over between the genes C and Sh . Since, the percentage of crossing over indicates the relative distance between the linked genes, we can determine the distance between the genes C and Sh to be 36 centi Morgans or map units. Thus, we may draw a genetic map of that part of chromosome in which these genes are located as -



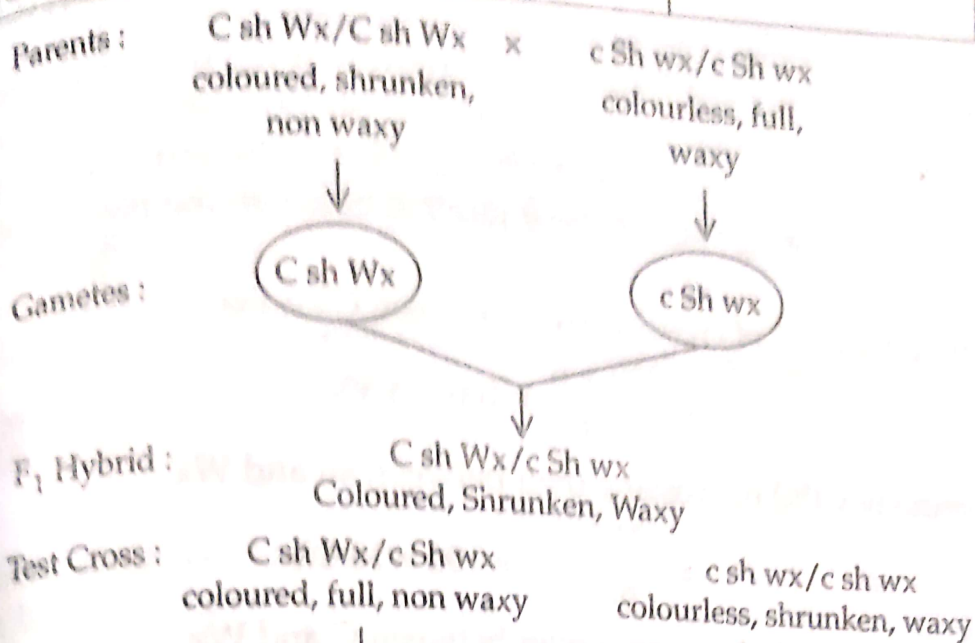
6-5 THREE - POINT TEST CROSS

A three point test cross or trihybrid test cross gives us information regarding the relative distances between three linked genes. It also shows in the linear order in which these genes are present in the chromosome.

The preparation of a linkage map can be explained by taking an example from maize involving three endosperm characters

Dominant		Recessive
(i) Coloured aleurone (C)		Colourless aleurone (c)
(ii) Full endosperm (Sh)		Shrunken endosperm (sh)
(iii) Starch endosperm (Wx)		Waxy endosperm (wx)

The cross made by Hutchinsm (1922) is shown below :



	♀ gametes	♂ gametes $c\ sh\ wx$	Progeny
→ non crossing over	$C\ sh\ Wx$	$C\ sh\ Wx / c\ sh\ wx$ coloured, shrunken, non Waxy	2538
	$c\ Sh\ wx$	$c\ Sh\ wx / c\ sh\ wx$ Colourless, full, Waxy	2798
→ single cross over (C - sh)	$C\ Sh\ wx$	$C\ Sh\ wx / c\ sh\ wx$ coloured, full, waxy	116
	$c\ sh\ Wx$	$c\ sh\ Wx / c\ Sh\ wx$ colourless, shrunken, non waxy	113
→ Single cross over (sh - Wx)	$C\ sh\ Wx$	$C\ sh\ Wx / c\ sh\ wx$ coloured, shrunken, waxy	601
	$c\ Sh\ Wx$	$c\ Sh\ Wx / c\ sh\ Wx$ Colourless, full, non waxy	626
→ Double cross over (C - sh), (c - Wx)	$C\ sh\ Wx$	$C\ sh\ Wx / c\ sh\ wx$ coloured, full non waxy	4
	$c\ sh\ wx$	$c\ sh\ wx / c\ sh\ wx$ colourless, shrunken waxy	2
Total			6,708

A test - cross for the three linked genes e.g. c , sh and wx in maize yield eight types of gametes and phenotypes. The triple recessive parent ($c\ sh\ wx / c\ sh\ wx$) contributes gametes only with the recessive alleles $c\ sh$ and wx , and show no effect on the phenotypes of test cross progeny. Two of the eight types of progeny (e.g. $C\ sh\ Wx$

and $c\ Sh\ wx$) are the most frequent and represent the parental or non recombinant types; while two others (e.g. $C\ Sh\ Wx$ and $c\ sh\ wx$) are the least frequent and are double cross overs. The remaining four types are produced by single crossing overs between the three linked genes. Therefore,

Frequency (%) of crossing over between C and sh

$$= \frac{229 + 6}{6708} \times 100 = 3.5\%$$

Frequency (%) of crossing over between sh and Wx

$$= \frac{1227 + 6}{6708} \times 100 = 18.4\%$$

Frequency (%) of crossing over between C and Wx

$$= \frac{229 + 1227}{6708} \times 100 = 21.7\%$$

In this case, recombination value of $C - Wx$ (21.7%) is close to the recombination value of $(C - sh) + (sh - Wx) = 3.5 + 18.4 = 21.9\%$. This shows that 'sh' gene is present between C and Wx . On the basis of the above values, the linkage map of genes C , sh and Wx can be shown as below.

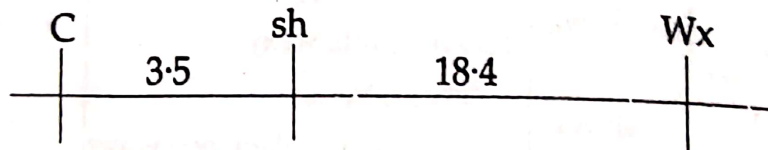


Fig. 6.4 : A linkage map of the genes C , sh and Wx of maize

Questions

1. Give a brief account of the procedure used in preparing a chromosome map with the help of three point test cross.
2. In a three - points test cross ($ABC/abc \times abc/abc$) the following progeny in obtained.

ABC	abc	aBc	AbC	ABc	abC	aBc	Abc	
230	240	96	104	138	142	12	8	= 970

Find out the correct linear order of genes and calculate the map distance values.

3. Write short notes on :
 - (a) Chromosome or genetic maps
 - (b) Two - point test cross
 - (c) Interference and Coincidence.