9.1. INTRODUCTION

It is universally accepted that genes are located in chromosomes. During cell division, each chromosome behaves as a unit. It may, therefore, be expected that genes located in the same chromosome would move together to the same pole during cell division. As a consequence, such genes would fail to show independent segregation and would tend to be inherited together. This expectation was expressed by Walter Sutton in 1903 while propounding the chromosomal theory of inheritance. A significant deviation from independent segregation was first reported by Bateson and Punnet in 1905 for flower colour and pollen shape in pea. They observed a 7 : 1 :1 : 7 ratio in a test cross in place of the normal 1 : 1 : 1 : 1 ratio expected from independent segregation. Bateson and Punnet proposed two new terms, coupling phase and repulsion phase. These terms are widely used even today. But they failed to explain the 7 : 1 : 1 : 7 ratio in the test cross.

The concept of linkage originated when Morgan demonstrated in 1910 that the *white eye (w)* gene of *Drosophila* is located in the *X* chromosome (***sex linkage***). Subsequently, he studied the inheritance of several other sex-linked genes. In 1911, he published the following conclusions from these studies. (1) Genes located in the same chromosome tend to stay together during inheritance; this tendency is called ***linkage***. (2) Genes are arranged in a linear fashion in the chromosomes. (3) The intensity of linkage between two genes is inversely related to the distance between them in the chromosome. He also proposed that (4) coupling and repulsion phases are two aspects of the same phenomenon, *i.e.*, linkage. Later studies have confirmed the above conclusions of Morgan.

9.2. LINKAGE

The tendency of two or more genes to stay together during inheritance is known as ***linkage***.

Two or more genes are linked together because they are located in the same chromosome. Linked genes do not show independent segregation. As a consequence, the dihybrid ratios obtained in F_2 *and test-cross generations are significantly different from the expected ratios of* 9 : 3 : 3 : 1 *and* 1 : 1 : 1 : 1, *respectively; this effect of linkage is more clearly noticeable in a test-cross generation. The frequencies of parental character combinations are markedly more than expected, while those of the new character combinations are considerably lower.*

9.2.1. Coupling Phase

In maize, a dominant gene *C* produces coloured seeds, while its recessive allele, *c*, determines colourless seeds. Another dominant gene *Sh* governs full seeds, whereas its recessive allele *sh* gives rise to shrunken seeds. When plants having coloured full seeds (*CC ShSh*) were crossed with those having colourless shrunken seeds (*cc shsh*), F_1 seeds were coloured full (*Cc Shsh*). The F_1 (*Cc Shsh*) was testcrossed with the double recessive strain (*cc shsh*). Out of the 8,368 seeds obtained from the test cross (*Cc Shsh* × *cc shsh*), 4,032 (48.2%) were coloured full, 4,035 (48.3%) were colourless shrunken, 149 (1.7%) were coloured shrunken and 152 (1.8%) were colourless full (Fig. 9.1). Clearly, the four phenotypic classes are not present in the expected ratio of 1 : 1 : 1 : 1. The phenotypic classes coloured full and colourless shrunken have a much higher frequency than the expected 25%. These two character combinations are referred to as ***parental combinations, parental phenotypes*** or ***parental types*** since they are the same character combinations that were present in the two parents of the F_1. The remaining two phenotypic classes, coloured shrunken and colourless full, are far less frequent than the expected 25%. These two character combinations are called ***recombinant phenotypes*** or ***recombinant types*** since they are obtained by reshuffling of the characters present in the two parents of the F_1. In the above example, it appears as if the two dominant genes *C* and *Sh* have a strong affinity for each other so that the frequencies of coloured full and colourless shrunken phenotypes are greater than expected. This situation is referred to as ***coupling phase****. It is due to the presence of genes* C *and* Sh *in the same chromosome..*

9.2.2. Repulsion Phase

Similarly, when plants having coloured shrunken seeds (*CC shsh*) were crossed with those having colourless full seeds (*cc ShSh*), the F_1 seeds were coloured full (*Cc Shsh*). But when the F_1 plants were test-crossed (*Cc Shsh* × *cc shsh*), 47.9% of the seeds so obtained were coloured shrunken, 49.1% were colourless full, 1.4% were coloured full and 1.5% were colourless shrunken. In this case also, the parental types (colourless full and coloured shrunken) were more frequent, while the recombinant types (coloured full and colourless shrunken) were less frequent than expected (Fig. 9.2). It appears as if in this cross the dominant genes *C* and *Sh* dislike each other. This situation is referred to as ***repulsion phase****. It is due to the presence of the dominant allele of one gene, e.g.,* C*, with the recessive allele of the other gene, e.g.,* sh*, in the same chromosome. Obviously, coupling and repulsion phases are only the two situations of the same phenomenon of linkage.*

9.2.3. Complete and Incomplete Linkage

Genes show linkage because they are located in the same chromosome. In Fig. 9.1, genes *C* and *Sh* are present in one chromosome, while their recessive alleles *c* and *sh* are situated in

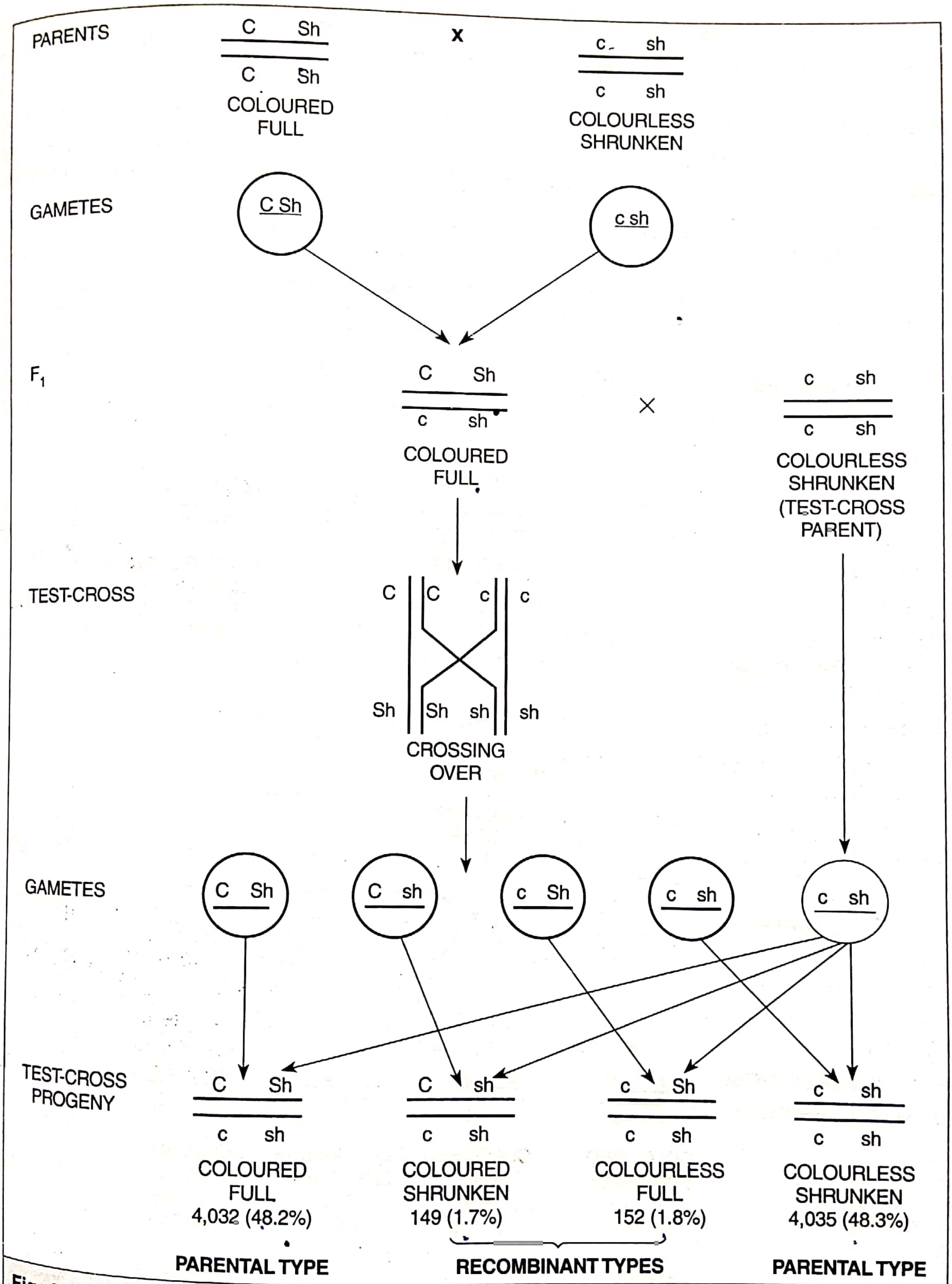


Fig. 9.1. Coupling phase linkage between genes *c* and *sh* in maize. In ***coupling phase***, dominant alleles of linked genes are located in one chromosome, and their recessive alleles are present in the homologous chromosome.

the homologous chromosome. Each chromosome behaves as a unit during cell division. Therefore, genes *C* and *Sh* would move to one pole, while *c* and *sh* would move to the opposite pole. If this always happened, the F_1 (*Cc Shsh*) would produce only two types of gametes, *viz.*, *C Sh* and *c sh*, and there would be only two phenotypic classes, *viz.*, coloured full (*Cc Shsh*) and colourless shrunken (*cc shsh*) in the test-cross progeny. When only parental character combinations are recovered in test-cross progeny, it is called **complete**

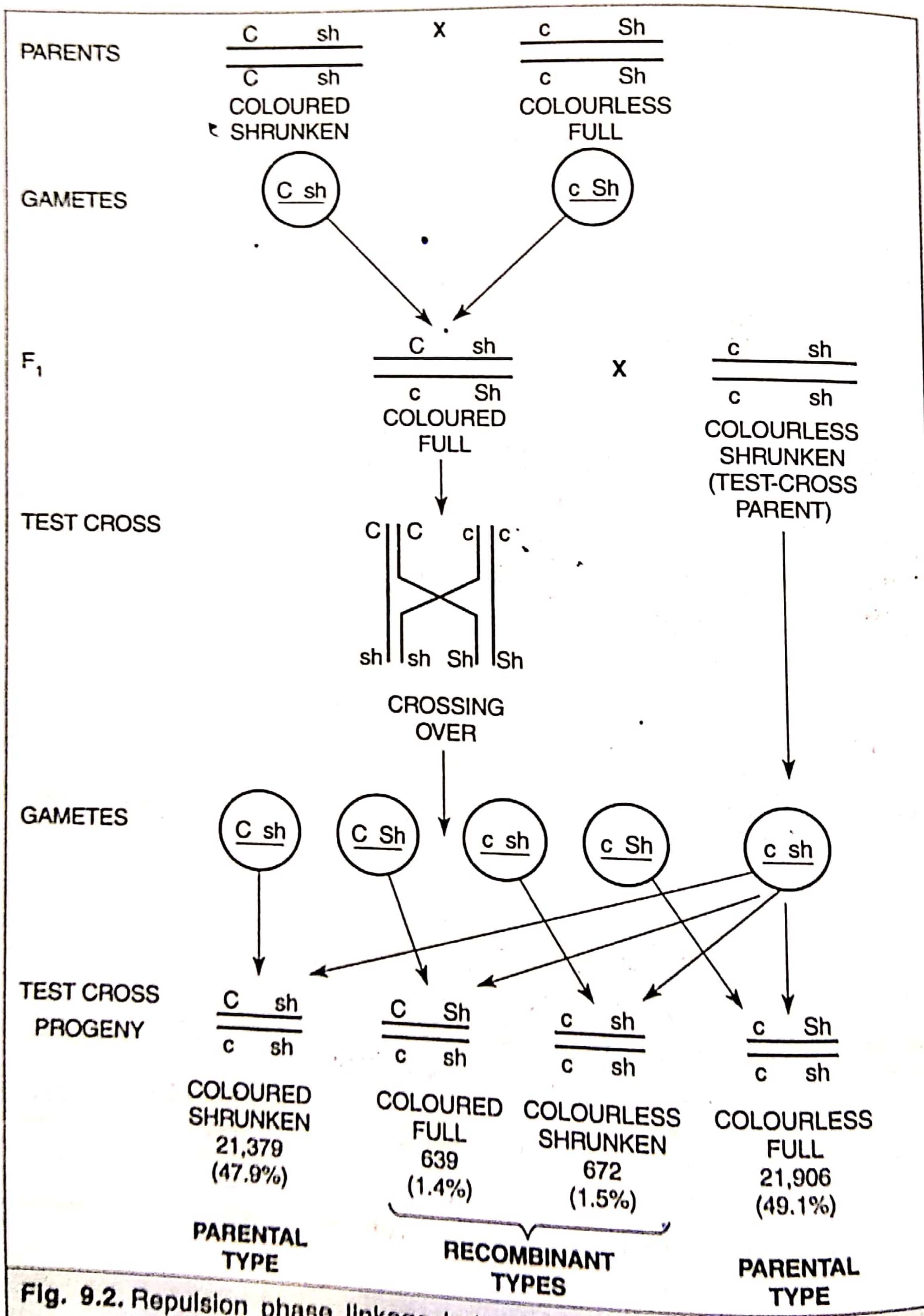


Fig. 9.2. Repulsion phase linkage between genes *c* and *sh* in maize. In ***repulsion phase***, the dominant allele of one gene and the recessive allele of the other gene are present in the same chromosome.

linkage. However, linked genes do not always move to the same pole. Sometimes their alleles recombine to produce recombinant gametes, *e.g.*, *C sh* and *c Sh*. This yields recombinant phenotypes, *e.g.*, coloured shrunken (*Cc shsh*) and colourless full (*cc Shsh*). Such cases, where recombinant types are also recovered in the test-cross progeny, are called ***incomplete linkage***.

Thus linkage may be classified as (1) complete or (2) incomplete depending upon the absence (***complete linkage***) or the presence (***incomplete linkage***) of recombinant types in the test-cross progeny. Complete linkage (lack of crossing over) is known in male *Drosophila*. Therefore, when males heterozygous for two linked autosomal genes are mated with double-recessive females, only the two parental character combinations are recovered in the progeny. In most cases, however, linkage is incomplete. But some genes may be so closely linked that they may show a very low frequency of recombination. Such genes are called ***tightly linked.*** Linkage is also classified as (1) coupling and (2) repulsion phase linkage. In ***coupling phase,*** dominant alleles of the linked genes are present in the same chromosome, *e.g.*, Fig 9.1. In contrast, in ***repulsion phase linkage*** the dominant allele of one gene is present with the recessive allele of the other gene in the same chromosome, *e.g.*, Fig. 9.2.

9.2.4. Crossing Over

Recombinant phenotypes are produced by recombinant gametes. These gametes, in turn, are produced due to crossing over. ***Crossing over*** is the exchange of strictly homologous segments between nonsister chromatids of homologous chromosomes. Since genes are located in chromosomes, any recombination among linked genes must always be associated with the exchange of corresponding segments of the homologous chromosomes. This conclusion about crossing over is based on the genetic data. But it has been shown to be correct by some ingenious experiments.

9.3. SYMBOLS

Linkage between two genes is denoted by writing genotypes in one of the following four ways.

1. The genes present in one chromosome are written together. They are separated from those present in the homologous chromosome by an oblique line (/), *e.g.*, C *Sh* / *c sh* (Fig. 9.1) or *C sh* / *c Sh* (Fig. 9.2). *This system is easiest and the most convenient. It also occupies the least space. It is the universally adopted system.* (It may be noted that in case of independent assortment, these genotypes will be represented as *Cc Shsh* in both the cases.)
2. The genotype *C Sh* / *c sh* may be written as $\dfrac{C\ Sh}{\overline{c\ sh}}$. Here the two lines represent the two chromosomes in which these genes are located.
3. The above genotype may also be written as $\dfrac{C\ Sh}{c\ sh}$. Here, only one line is drawn to represent the chromosome pair in which the genes are located.

The systems 2 and 3 are quite cumbersome and occupy the greatest space. They are rarely followed.

4. The genotype $C\ Sh\ /\ c\ sh$ may also be written as $(C\ sh)\ (c\ sh)$. Here the genes present in one chromosome are placed within one parenthesis. This system takes greater space than system 1.

9.4. DETECTION OF LINKAGE

Linkage between two dominant genes produces significant deviation from the typical dihybrid ratios of 1 : 1 : 1 : 1 (in test cross generation) and 9 : 3 : 3 : 1 (in F_2 generation). This deviation is the most easily detected in test cross data. In case of linkage, the two parental types are the most frequent; they are in excess of the expected 25% for each parental type. In contrast, the two recombinant types have markedly lower frequencies than 25% each. Thus, *it is typical of linkage that two phenotypic classes are markedly greater than 25% each, while the remaining two phenotypes are markedly lower than 25% each*. For example, in the test cross depicted in Fig. 9.1, the following data were obtained.

Coloured full	4,032	*Parental types*
Colourless shrunken	4,035	
Coloured shrunken	149	*Recombinant types*
Colourless full	152	

It may be seen that the two parental types have comparable frequencies. Similarly, the frequencies of the two recombinant types are also comparable. This type of test cross data is a sure indication of linkage. As a rule, ***the parental types have much higher frequencies than the recombinant types*** . *In fact, this relationship can be used as a safe guide to identify the two types in the test cross data whenever the identity of the parental types is not known.*

9.5. CROSSING OVER

The exchange of homologous segments between nonsister chromatids of homologous chromosomes is known as ***crossing over***. *It is responsible for recombination between linked genes. Crossing over takes place during pachytene.* In pachytene, each chromosome of a bivalent (chromosome pair) has two chromatids. Thus each bivalent contains four chromatids or strands (***four-strand stage***). Generally, one chromatid from each homologue is involved in crossing over (Fig. 9.3). In this process, a segment of one chromatid becomes attached in place of the homologous segment of the nonsister chromatid and *vice-versa*. In Fig. 9.3, it is assumed that breaks occur at precisely homologous points in the two nonsister chromatids. This is followed by reunion of the acentric segments. This produces a cross (×)-like figure at the point of exchange of the chromatid segments. This figure is called ***chiasma***.

Obviously, each event of crossing over produces two recombinant chromatids (called ***crossover chromatids***) and two original chromatids (referred to as ***noncrossover chromatids***) (Fig. 9.3). The crossover chromatids will have new combinations of the linked genes, *i.e.*, will be recombinant. Gametes carrying them will produce the recombinant phenotypes in test-cross. Therefore, these phenotypes are also called ***crossover types***. Similarly, the noncrossover chromatids will contain the parental gene combinations. The gametes carrying them will give rise to the ***parental phenotypes*** or ***noncrossover types***. Therefore, the

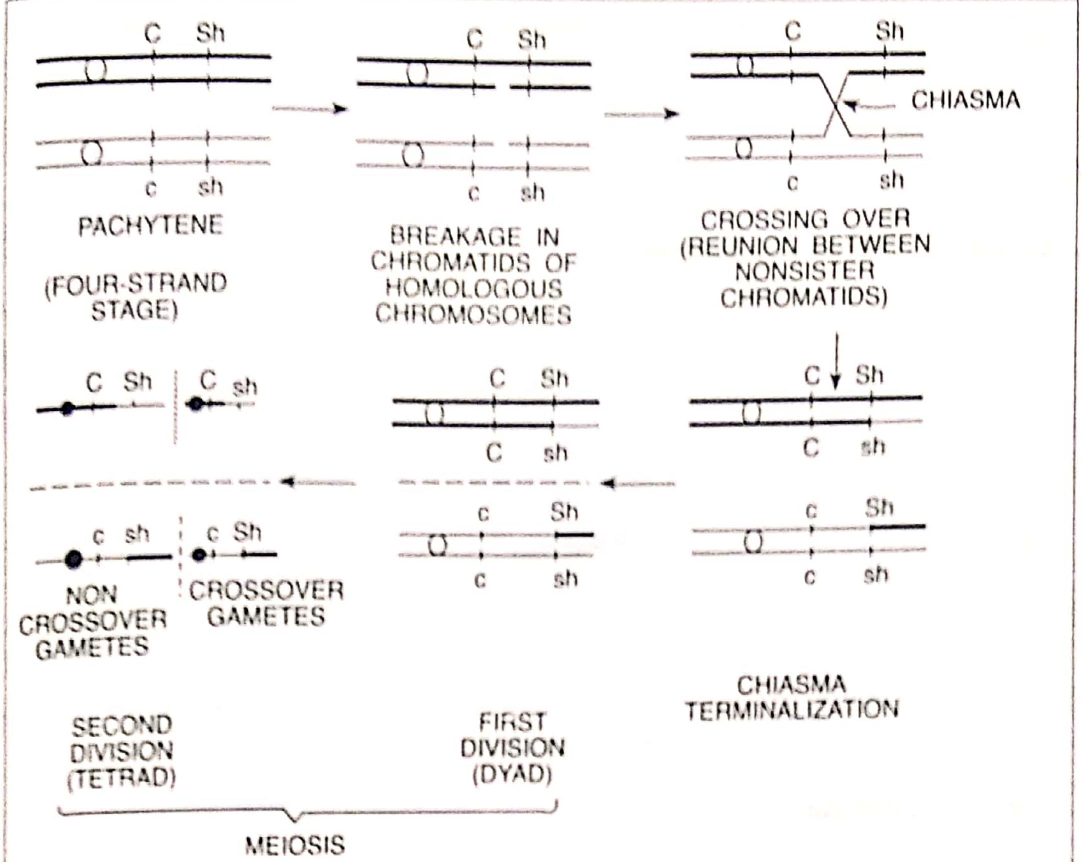


Fig. 9.3. Crossing over between nonsister chromatids of homologous chromosomes leading to recombination between linked genes. Generally, crossing over at a given point involves only two of the four chromatids of a bivalent. As a result, meiosis yields two crossover and two noncrossover gametes for one event of crossing over.

frequency of crossing over between two genes can be estimated as the frequency of recombinant progeny obtained in a test cross for these genes. This frequency is usually expressed as per cent. This per cent frequency is the frequency of crossing over between the two genes in question. Thus,

$$\text{Frequency of crossing over} = \frac{\text{Number of recombinant progeny in the test cross}}{\text{Total number of progeny in the test cross}}$$

or

$$\text{Frequency of crossing over (\%)} = \frac{\text{Number of recombinant progeny in the test cross}}{\text{Total number of progeny in the test cross}} \times 100$$

Thus the frequency of crossing over may be estimated as follows from the data depicted in Fig. 9.1.

$$\text{Frequency of crossing over} = \frac{(149 + 152)}{(4{,}032 + 4{,}035 + 149 + 152)}$$

$$= 301/8{,}368 = 0.036$$

Therefore, frequency of crossing over (%) = 0.036 × 100 = 3.6%.

CYTOLOGICAL BASIS OF CROSSING OVER

Discussion in preceding sections of this chapter is based on the study of progeny in BC_1 generation of the testcross progeny. In such progenies, lack of independent assortment demonstrated linkage and presence of recombinants showed crossing over. It was concluded that crossing over should be the result of exchange of chromosome segments. However, since homologous chromosomes normally exchange reciprocal segments, resulting chromosomes will exhibit no morphological differences. Therefore, except the visible chiasmata, there is no other cytological observation which will substantiate that actual exchange of chromosome segments really takes place. However, it is not necessary that chiasmata should be associated with exchange of chromosome segments. Therefore, to demonstrate that crossing over is associated with actual exchange of chromosome segments, special experiments were devised by **C. Stern** in *Drosophila* and by **H.S. Creighton** and **B. McClintock** in corn. Both these experiments were reported in 1931 and had utilized chromosomes, whose morphology was altered due to chromosomal aberrations in order to make it identifiable from its homologue.

1. Stern's Experiment in *Drosophila*

Drosophila (fruitfly) stocks carrying translocations were utilised by **C. Stern** in order to produce a female *Drosophila* having a part of Y-chromosome attached to one of the two X-chromosomes. The other X-chromosome of this female fly was also marked (identifiable due to distinct morphology) and consisted of two approximately equal fragments, each carrying its centromere. These two X-chromosomes in the female fly could be distinguished not only from each other, but also from normal X-chromosome under the microscope.

In the above female fly, one of the two fragments of an X-chromosome carried mutant alleles for *carnation* eye (*car* is recessive showing light eye colour) and *barred* eye (*B* is dominant showing narrower eyes). The other X-chromosome, having a part of Y attached, carried normal alleles of these two genes, so that the female heterozygote for both these genes (*cdr B/+ +*) had barred eyes (but normal eye colour, since *car* is recessive to +). Such females were crossed with male flies having recessive alleles for both these genes (*car,* +). In such a situation, this makes a simple testcross. If no crossing over takes place between the two genes in question, two types of gametes i.e., *car B* and ++ will be produced from the female flies. Crossing over will give two additional types of gametes i.e., *car* + and + *B* (Fig 13). Due to fertilization of two types of non-crossover and the other two types of crossover gametes by male gametes carrying X-chromosome (*car* +), four kinds of female flies will be produced. Another four kinds of male flies will be produced due to fertilization by Y carrying male gametes (Fig. 13).

The flies which are classified as crossovers on the basis of phenotype i.e., carnation (with normal eye shape) and barred (with normal eye colour) were studied cytologically. It was found that carnation flies did not have any fragmented X-chromosome, but rather had normal X-chromosome. On the other hand, barred flies had a fragmented X-chromosome with a segment of Y-chromosome attached to one of the two fragments of X-chromosome. Such cytological observations suggested that genetic crossing over

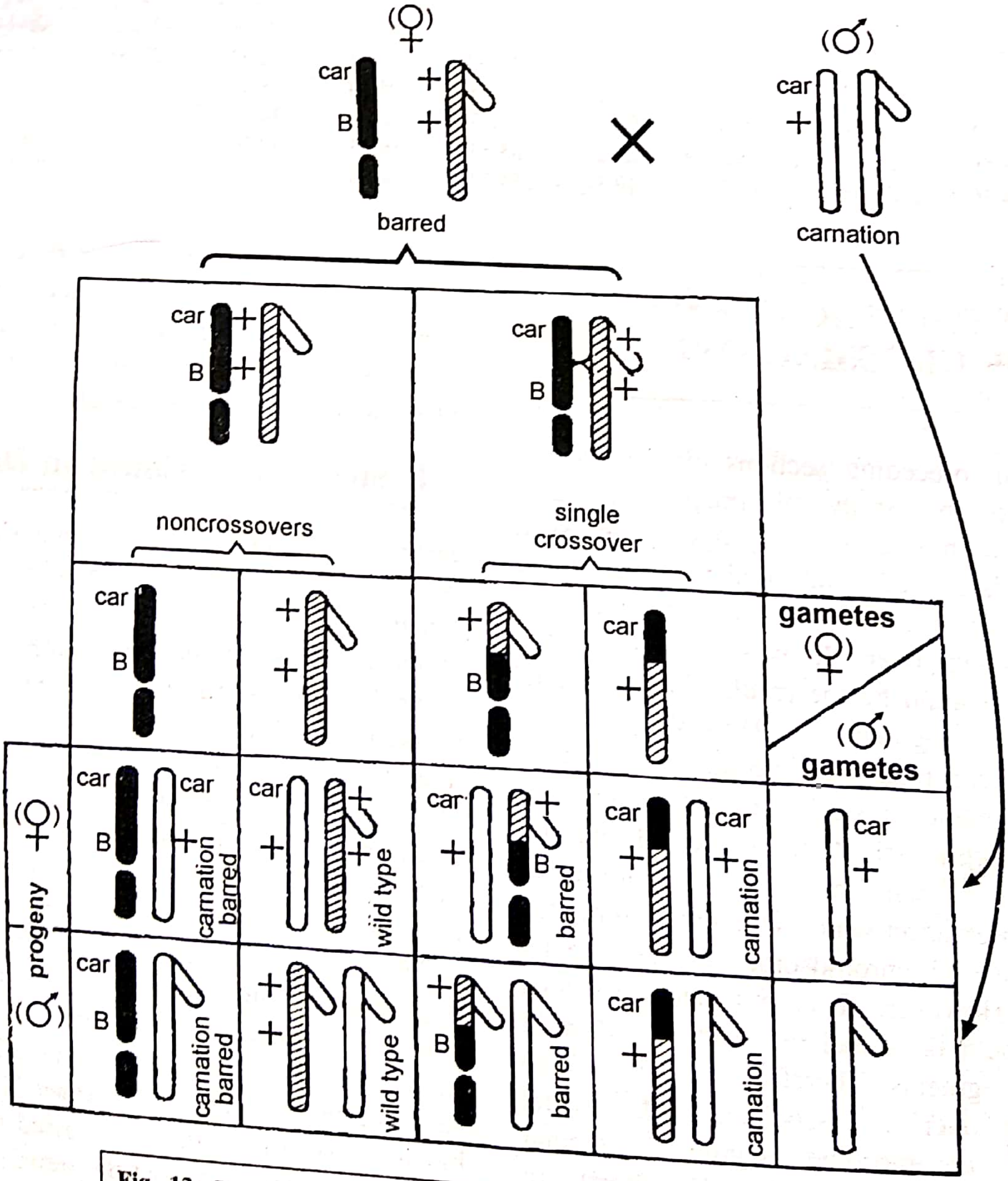


Fig. 13. Stern's experiment to demonstrate cytological crossing over.

was accompanied with an actual exchange of chromosome segments.

2. Creighton and McClintock's Experiment in Corn

Using corn as the material, **H.S. Creighton** and **Barbara McClintock** (1931) utilized the same principle which Stern utilized in case of fruitfly (*Drosophila*). They obtained a plant which had a knob on the 9th chromosome. This 9th chromosome was also involved in a reciprocal translocation with 8th chromosome (consult Chapter 11). The plant was heterozygous for coloured aleurone and waxy endosperm characters and carried these genes in repulsion phase i.e., *Cwx/cWx*. *Cwx* was carried on the knobbed chromosome and *cWx* on the knobless chromosome, (*c* = recessive for colourless seed; *wx* = recessive for waxy endosperm). Such a plant was testcrossed with plants homozygous recessive for both characters i.e., colourless and waxy (*cwx/cwx*).

If the chromosome region between the knob and *c* gene is represented as I region and that between *c* and *Wx* as II region, then one would expect two types of non-crossover gametes (*Cwx* and *cWx*) and six types of crossover gametes including single and double crossovers (Fig. 14). The progeny can be classified into eight types based on phenotypes and cytological observations.

The following observations in phenotype and cytology of progeny suggested that actual exchange of chromosome segments was involved in genetic crossing over: (i) Association of knob in chromosome with the phenotype, colourless seed (*c*) and non-waxy endosperm (*Wx*) indicated crossing over in I region, because in the parent these were located on a knobless chromosome. (ii) At meiotic metaphase I, the presence of a ring of four chromosome without a knob suggested cytological exchange of chromosome segments, because in the parent the knob was associated with 9th chromosome carrying translocation (consult Chapter 11 and note that whenever a translocation is present in heterozygous condition, a ring of four chromosomes will be formed during meiosis). The classes 4th and 6th shown in the checkerboard in Figure 14 will show this feature. (iii) Similarly, if there were no quadrivalents (ring of four) and only 10 bivalents were observed, the presence of knob in one of these bivalents could be treated as an evidence for cytological crossing over, since the knob was originally associated with translocation. This feature is exhibited by classes 3rd and 5th in Figure 14.

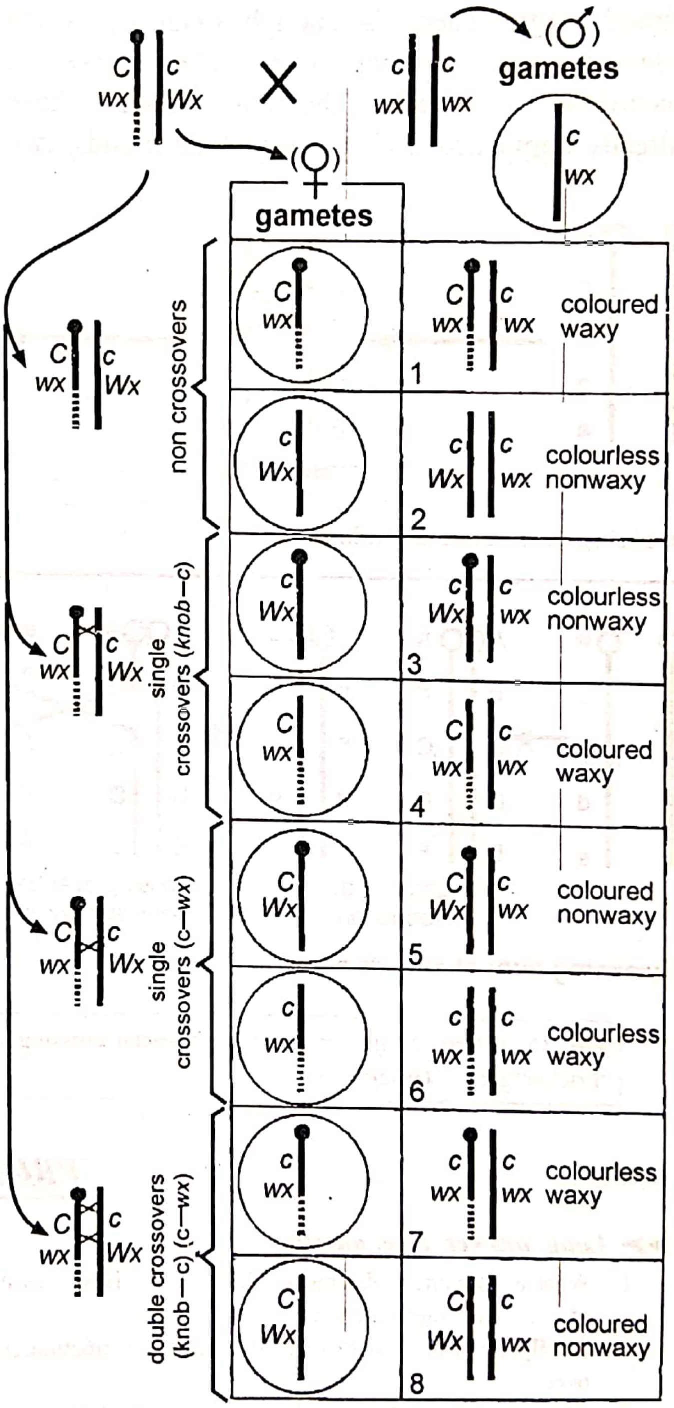


Fig. 14. Creighton and McClintock's experiment in corn to give proof for cytological crossing over.

9.9. LINKAGE MAPS AND LINKAGE GROUPS

All the genes that are linked together form a ***linkage group.*** Genes of a linkage group can be represented 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 neighbouring genes is proportional to the frequency of recombination (%) between them. These values are also depicted in the drawing (Fig. 9.6). Such a line drawing depicting the linked genes and the recombination frequencies between them is known as ***linkage map, genetic map*** or *chromosome map*. A simple linkage map of the genes *c* and *sh* in maize is shown in Fig. 9.5. The map unit has been derived form Fig. 9.1.

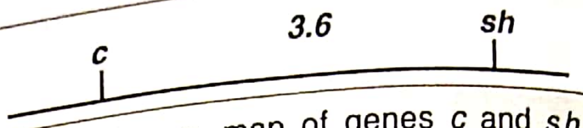


Fig. 9.5. A linkage map of genes *c* and *sh* of maize. The frequency of recombination (%) between genes serves as a measure of the distance between them in the linkage map and is shown between two neighbouring genes.

For preparing a chromosome map, the following two informations are essential: (1) the frequencies of recombination between linked genes, and (2) the order or sequence of these genes in the chromosome. ***Recombination frequencies between linked genes*** are determined from appropriate test crosses. These per cent frequencies are used as map units for preparing linkage maps. A ***map unit*** is that distance in a chromosome, which permits one per cent recombination between two linked genes. A map unit is also called a ***centi-Morgan*** (*cM*). It may be noted that *map unit is an imaginary distance and it does not represent the actual physical distance between two linked genes in the chromosome*. The actual length of chromosome represented by a map unit varies considerably mainly depending on the organism in question (Table 9.2).

The sequence of linked genes is determined by studying test crosses for three linked genes at a time. The data from such a test cross provide information on the order of the three genes in the chromosome as well as the frequency of recombination among them. To begin with, three linked genes are mapped. Subsequently, a three-point test cross involving any two of the three already mapped linked genes and a new gene expected to be linked with them is studied to map the new gene. In this manner, each new gene is mapped. It is desirable to include only those genes that show less than 20% recombination with each other.

TABLE 9.2. The Physical Distance in Chromosomes Represented by 1 Map Unit

Organism	*Physical distance represented by 1 map unit*
Man	1,000 kb*
Mouse	1,500 kb

* kb, kilobase pairs.

The number of different linkage groups in a species is, as a rule, equal to its gametic chromosome number (n). For example, the number of linkage groups in *D.* melanogaster is four, it is 7 in barley, and 10 in maize. 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* (Fig. 9.6).

Linkage maps provide information on the genes that are linked together and the frequencies of recombination that may be expected between them. The total map distance between two genes present in a linkage group may exceed 50 or even 100 (Fig. 9.6). But it does not mean that such two genes would show more than 50% recombination. ***The frequency of recombination between two linked genes cannot exceed 50%, which is the frequency in case of independent segregation. Usually two linked genes would show independent segregation if they are more than about 80 map units apart.***

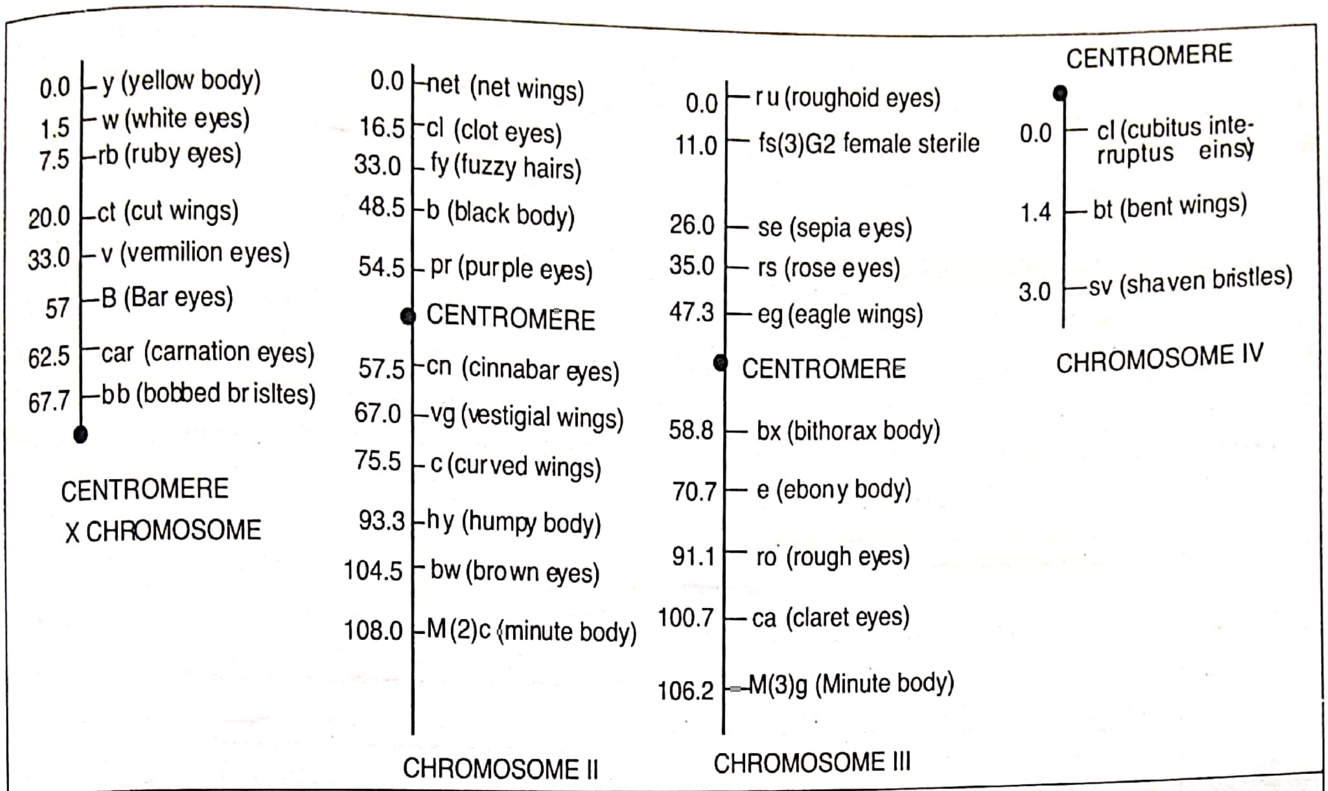


Fig. 9.6. The four linkage groups of *Drosophila melanogaster* (only selected genes are shown)

9.10. CROSSING OVER AMONG THREE LINKED GENES

A test cross for three linked genes, *e.g.*, *c*, *sh*, and *wx* in maize, yields eight types of gametes and phenotypes (Table 9.3.). Two of these types (*e.g.*, *C Wx Sh* and *c wx sh*) are the most frequent and represent the parental or nonrecombinant types. Two others (*e.g.*, *CWx sh* and *cwx Sh*) are the least frequent and are double crossovers. The remaining four types (*e.g.*, *C wx*

TABLE 9.3. The Different Types of Progeny Obtained from a Test-cross for Three Genes (*c, sh, wx*) in Maize

Genotype	*Phenotype**	*Number*	*Per cent frequency***
C Wx Sh/ c wx sh	Coloured, nonwaxy, full	2777	39.7
c wx sh/ c wx sh	Colourless, waxy, shrunken	2708	38.7
C wx sh/ c wx sh	Coloured, waxy, shrunken	116	1.7
c Wx Sh/ c wx sh	Colourless, nonwaxy, full	123	1.8
C wx Sh/ c wx sh	Coloured, waxy, full	643	9.2
c Wx sh/ c wx sh	Colourless, nonwaxy, shrunken	626	8.9
C Wx sh/ c wx sh	Coloured, nonwaxy, shrunken	4	0.06
c wx Sh/ c wx sh	Colourless, waxy, full	3	0.04
Total		7000	100

*Since the alleles *c wx sh,* uniformly contributed by the triple recessive test cross parent, have no effect on the phenotype, and are common to all the genotypes, (1) they may be ignored and (2) the phenotypes may be represented as '*C Wx Sh*' etc. in the place of 'coloured, nonwaxy, shrunken' etc., for brevity and simplicity.

** $\text{Per cent frequency} = \frac{\text{Number of progeny in a phenotypic class}}{\text{Total number of progeny}} \times 100$

Sh, c Wx sh, c Wx Sh and C wx sh) are produced by single crossing over between the three linked genes. ***As a rule, the parental types are the most frequent, the double cross-overs are the least frequent (or, sometimes, even absent), while the single crossovers are intermediate between these two.***

9.10.1. Gene Sequence

The sequence of three genes involved in a test cross can be readily determined by comparing the genotypes of parental and double crossover types. In ***a double crossing over***, simultaneous crossing over occurs on both the sides of the gene located in the middle. In Fig. 9.7, the gene *sh* is located between the genes *c* and *wx*. In this case, the double crossovers will

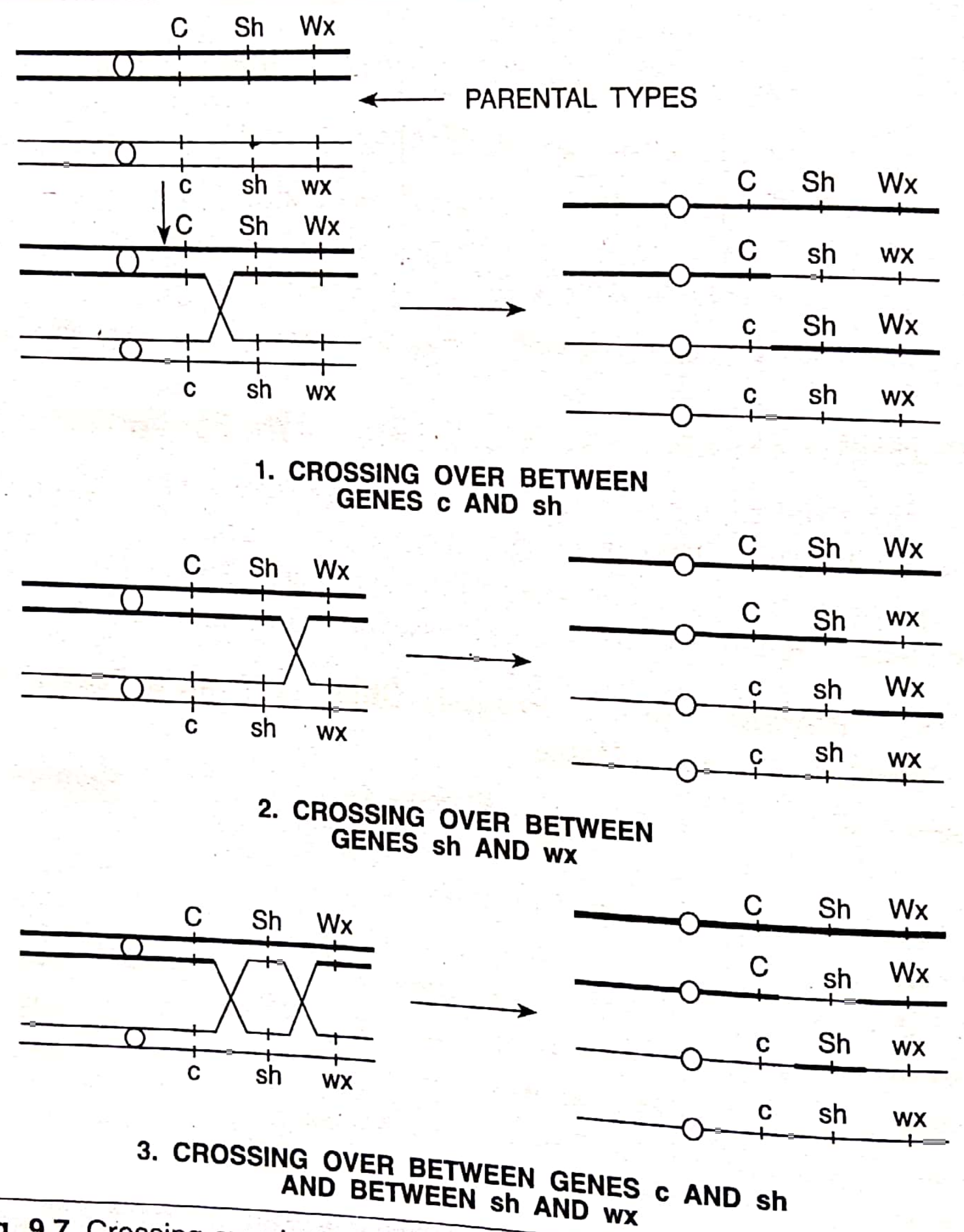


Fig. 9.7. Crossing over in the different regions between three linked genes *c*, *sh* and *wx*, and their consequences: (1) crossing over between genes *c* and *sh*, (2) crossing over between *sh* and *wx*, and (3) simultaneous crossing over between genes *c* and *sh*, and between *sh* and *wx* (double crossing over). Note that in double crossing over the position of only the gene located in the middle changes.

be produced due to the simultaneous occurrence of crossing over between genes *c* and *sh* and between *sh* and *wx*. In this figure, the parental type chromosomes (or gametes or phenotypes) are *C Sh Wx* and *c sh wx*, while the double crossover chromosomes are *C sh Wx* and *c Sh wx*. A comparison of the parental and the double crossover types shows that *in the double crossovers only the gene located in the middle* (sh) *has switched its position*. This property of the double crossovers is used for determining the gene located in the middle. In table 9.3, the parental types are *C Wx Sh* and *c wx sh*, whereas the double crossovers are *C Wx sh* and *c wx Sh*. Here the alleles of gene *sh* have switched their position in the double crossovers as compared to the parental types. Therefore, the gene *sh* must be located between the genes *c* and *wx*. This is the sequence of these three genes in the chromosome.

9.10.2. Recombination Frequencies

In Table 9.3, the parental types are *C Wx Sh* and *c wx sh*. The gene *sh* is located in the middle. Therefore, we estimate the recombination frequencies between the genes *c* and *sh*, and between *sh* and *wx*. These two estimates will allow us to prepare the genetic map using the gene sequence deduced above. Recombination between genes *c* and *sh* would yield the types *C wx sh*, *c Wx Sh* and the double crossovers. In these classes, *C* is present with *sh* and *c* is present with *Sh* as compared to the parental combinations *C-Sh* and *c-sh*. Hence,

Recombination (%) between *c* and *sh* = Sum of the frequencies (%) of *C wx sh*, *c Wx Sh*, and the double crossovers
= 1.7 + 1.8 + 0.06 + 0.04 per cent
= 3.6 per cent

Similarly, recombination between the genes *wx* and *sh* will give the types *C wx Sh*, *c Wx sh* and the double crossovers. In these classes, the combinations *wx Sh* and *Wx sh* are present as against the parental combinations *Wx Sh* and *wx sh*. Therefore,

Recombination (%) between *sh* and *wx* = Sum of the frequencies (%) of *C wx Sh*, *cWx sh* and the double crossovers
= 9.2 + 8.9 + 0.06 + 0.04 per cent
= 18.2 per cent.

9.10.3. Linkage Map

We can now map the genes *c*, *sh* and *wx* in the order *c-sh-wx* at the distance of 3.6 and 18.2 map units, respectively (Fig. 9.8). A new gene expected to be linked with these genes can be mapped by ordering the new gene with either genes *c* and *sh* or *sh* and *wx* in a three point test cross.

Fig. 9.8. A linkage map of the genes *c*, *sh* and *wx* of maize.

9.10.4. Coefficient of Coincidence

Double crossovers are produced by two simultaneous crossing overs. These occur one each on the either side of the gene located in the centre. If the occurrence of crossing over in the two regions (*e.g.*, between the genes *c* and *sh*, and between *sh* and *wx*) were independent of each other, the frequency of double crossovers will be the product of the frequencies of crossing overs, which occur 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 chances of its occurrence in the other region*. Therefore, the expected frequency of double crossovers may be estimated from the data of table 9.3 as follows.

Frequency (%) of crossing over between *c* and *sh* $(= \alpha) = 3.6$

and, frequency (%) of crossing over between *sh* and *wx* $(= \beta) = 18.2$

The expected frequency (%) of double crossovers = The product of the frequencies of crossing overs between *c* and *sh*, and that between *sh* and *wx*

$$= \alpha \times \beta/100$$

$$= (3.6 \times 18.2)/100$$

$$= 0.66\%$$

The product $\alpha\beta$ $(= 3.6 \times 18.2)$ is divided by 100 since both these values (α and β) are already in per cent. Thus the estimated frequency of double crossovers is 0.66%. In comparison, the frequency of observed double crossovers is only 0.1% (= sum of the frequencies of double crossover types *C Wx sh* and *c wx Sh* = 0.06 + 0.04 per cent). This observed frequency is only 15.1 per cent [(= (0.1/0.66) ×100)] of the expected frequency. The ratio between the observed and the expected frequencies of double crossovers is called ***coefficient of coincidence***. Thus,

$$\text{Coefficient of Coincidence} = \frac{\text{Observed frequency of double crossovers}}{\text{Expected frequency of double crossovers}}$$

$$= 0.10/0.66 = 0.151$$

and, Coefficient of coincidence (%) $= 0.151 \times 100 = 15.1\%$.

The estimate of coefficient of coincidence indicates the degree of agreement between the observed and the expected frequencies of double crossovers.

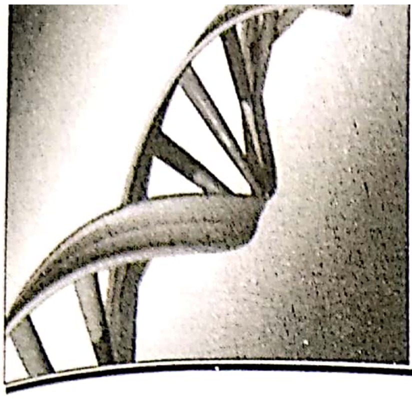
9.10.5. Coefficient of Interference

As a rule, the observed frequencies of double crossovers are lower than their expected values. This is interpreted as follows: *the occurrence of crossing over in one region of a chromosome interferes with its occurrence in the neighboring segments; this is called* ***interference***. It may be expected that the intensity of interference would progressively decrease as the point of second crossing over becomes farther from that of the first one. Therefore, the coefficient of coincidence would be lower when the concerned genes are located close to each other than when they are located farther apart. The intensity of interference may be estimated as ***coefficient of interference***, which is equal to one minus the coefficient of coincidence. Thus, Coefficient of interference = 1 – coefficient of coincidence = 1 – 0.151 = 0.849

and, Coefficient of interference (%) = 0.849 × 100

= 84.9%.

The coefficient of interference indicates the degree of interference by a crossing over with the occurrence of another crossing over in its neighbourhood. Generally, it is a positive value, but in some prokaryotes, e.g., *bacteriophages and in* Aspergillus, *negative interference has been observed. Negative interference means that the occurrence of a crossing over in a chromosome region promotes the chances of occurrence of another crossing over in the neighbouring regions.*



Sex Linkage

10.1. INTRODUCTION

Ordinarily, F_1 and F_2 generations from reciprocal crosses yield identical results, and it does not matter if the female or the male parent had the recessive character. Further, both males and females in the progeny show identical ratios. But a notable exception to this general rule is produced due to the phenomenon of ***sex linkage***. *This is an association during inheritance of a character with sex of the progeny. Sex linkage is the consequence of a gene being located in the* X *or sex chromosome*. The *X* chromosome occurs in different numbers in the two sexes (*XX* in one and only one *X* in the other). The earliest known case of sex linkage is that of haemophilia in human beings. Persons afflicted by hemophilia bleed profusely even from smallest cuts since their blood does not clot on exposure to air. Jews had recognised even before 600 A.D. that haemophilia was a hereditary defect and that it was transmitted through females, although ordinarily only males were haemophilic. Other similar examples have also been described. But a clear-cut explanation for this phenomenon was presented by Morgan in 1910 for the *white* eye (*w*) gene of *Drosophila*.

10.2. INHERITANCE OF *WHITE* EYE IN *DROSOPHILA*

In a pedigree culture of normal dull red-eyed *Drosophila*, Morgan observed a single white-eyed male. When this white-eyed male was mated with red-eyed females, F_1 flies had red eyes. In F_2, on an average, 3 flies had red eyes and 1 had white eyes (Fig. 10.1). These results reveal that white eye is due to a single recessive gene. *But when the* F_2 *individuals were classified on the basis of their sex as well as eye colour, a peculiar picture emerged. All the* F_2 *females had red eyes, but half of the males had red and the other half had white eyes. It appears that the eye colour of a fly in* F_2 *depended on its sex.*

But when white-eyed female flies were mated with red-eyed males, *half the flies in* F_1 *were red-eyed and the remaining half had white eyes. In* F_2 *from this reciprocal cross, red-eyed and white-eyed flies were obtained in a 1 : 1 ratio, which is the same as in the* F_1 *generation*. These findings are very puzzling since *segregation for eye colour was obtained in* F_1 *and a 1 : 1 ratio was found in* F_2. Further, the classification of F_1 and F_2 flies on the basis of their sex as well revealed that *all the* F_1 *males were white-eyed, while the females were red eyed*. However, in F_2 both male and female flies showed the ratio 1 red : 1 white (Fig. 10.1). Morgan correctly reasoned that the *white* eye gene was located in the *X* chromosome of *Drosophila*. Therefore, the inheritance pattern of this gene closely parallels that of the *X* chromosome (Fig. 10.2). It was also assumed that the *Y* chromosome does not carry an allele for this gene. Accordingly, female files will have two copies of *w* gene (*WW*, *Ww* or *ww*). But males will have only one copy (*W* or *w*). Thus male *Drosophila* will be ***hemizygous*** (having only one copy of a gene) for *w* gene. Therefore, a single copy of the recessive allele *w* will express itself in the males.

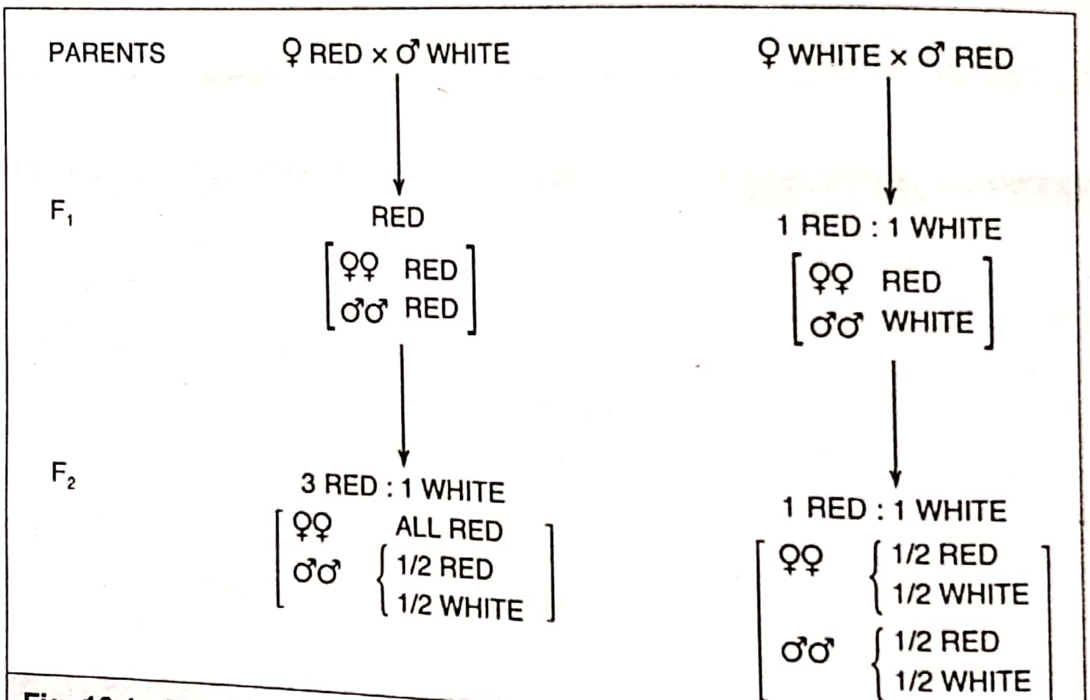


Fig. 10.1. The peculiar pattern of inheritance of white eye in *Drosophila*. Note that only F_2 males have white eyes in the first cross, while a 1 : 1 ratio is obtained in F_1 as well as F_2 in the reciprocal cross.

The genotypes of red-eyed females and white-eyed males may be written as $X^W X^W$ and $X^w Y$, respectively. The location of *w* gene in the *X* chromosome is indicated by writing it as a superscript of *X*. All the eggs of the red-eyed female will be X^W. But half of the sperms of the white-eyed male will be X^w and the other half will be *Y*. A random union between male and female gametes will produce $X^W X^w$ and $X^W Y$ zygotes. These will develop into red-eyed female and red-eyed male flies, respectively. Segregation in the F_1 females ($X^W X^w$) will produce two types of eggs, X^W and X^w, in equal proportion. Similarly, the F_1 males ($X^W Y$) will also produce two types of sperms X^W and *Y*, in equal frequency. A random union between male and female gametes will produce the following four types of zygotes: $X^W X^W$, $X^W X^w$, $X^W Y$ and $X^w Y$. The first two of these would produce red-eyed females, the third red-eyed male and the fourth white-eyed male (Fig. 10.2). This explanation agrees closely with the results obtained from the experiment (Fig. 10.1).

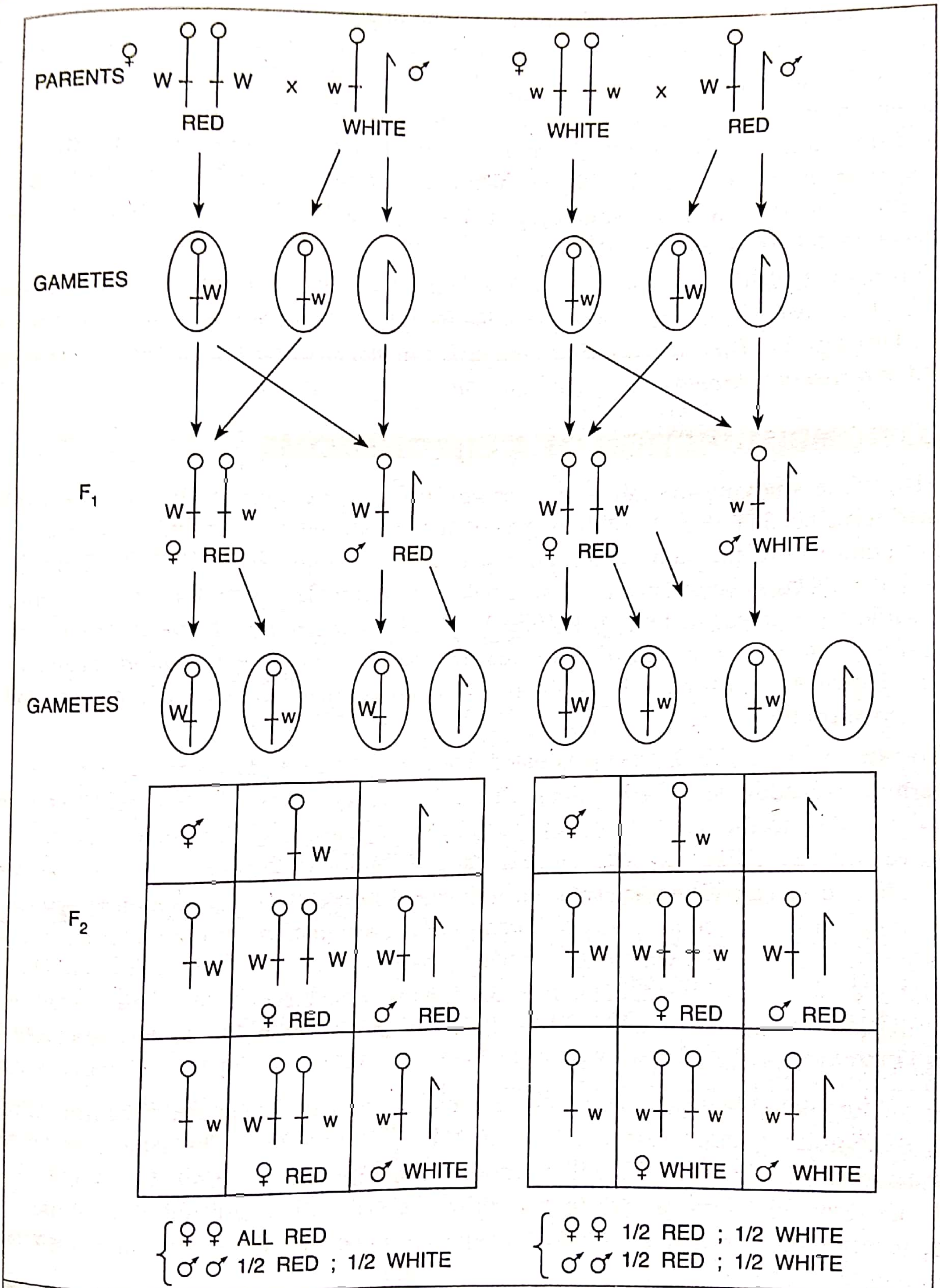


Fig. 10.2. Explanation of the inheritance of white eye in *Drosophila* by assuming that the *white*-eye gene is located in the *X* chromosome

In the reciprocal cross, a white-eyed female ($X^w X^w$) was mated with a red eyed male ($X^W Y$). All the eggs of this female will be X^w. The male will produce X^W and Y sperms in equal proportion. In F_1, two types of zygotes will be produced: (1) $X^W X^w$ zygotes will give rise to red-eyed females, and (2) $X^w Y$ zygotes will develop into white-eyed males. The F_1 females ($X^W X^w$) will produce X^W and X^w eggs. F_1 males ($X^w Y$) will produce X^w and Y sperms. A random union between eggs and sperms from F_1 individuals will generate 4 types of zygotic combinations: $X^W X^w$, $X^w X^w$, $X^W Y$ and $X^w Y$. The first combination ($X^W X^w$) will give rise to red-eyed females, the second ($X^w X^w$) to white-eyed females, the third ($X^W Y$) to red-eyed males and the fourth ($X^w Y$) to white-eyed males (Fig. 10.2). Clearly, these are the same as the results actually obtained (Fig. 10.1).

Thus *the hypothesis that* w *gene is located in the* X *chromosome and that it has no allele in the* Y *chromosome is adequate to explain the peculiar inheritance pattern of the white eye trait in* Drosophila. *This was the first conclusive demonstration that a specific gene was located in a specific chromosome of an organism.*

10.3. NONDISJUNCTION OF *X* CHROMOSOME

In 1916, while studying the inheritance of *vermilion* eye colour in *Drosophila*, Bridges obtained some peculiar data. Vermilion eye colour is produced by a sex-linked recessive gene *v*. This gene shows the same inheritance pattern as *w* (Figs. 10.1, 10.2). Among the F_1 progeny from a cross between vermilion females and red males, some females had vermilion eyes, while some males had red eyes (Fig. 10.3). *The appearance of vermilion females and red males in the* F_1 *generation of this cross was contrary to the expectations based on sex linkage*. However, a vast majority of the progeny females had, as expected, red eyes and the males had vermilion eyes.

To explain these data, Bridge postulated that during oogenesis, in some of the oocytes of the vermilion-eyed females ($X^v X^v$), the X chromosomes failed to separate at AI and they both moved together to the same pole. As a result, the opposite pole in such oocytes did not recessive any X chromosome. Such an irregular distribution of the homologous chromosomes at AI is known as ***nondisjunction***. Since in this case irregular distribution of X chromosome is postulated, it is referred to as ***nondisjunction of X chromosome***. From those oocytes of the vermilion females ($X^v X^v$), which show nondisjunction of the X chromosome, two types of unusual eggs will be obtained: (1) half of the eggs will have two X chromosome and will be $X^v X^v$, and (2) the other half will be O as they would receive no X chromosome. Red-eyed males ($X^V Y$) will produce X^V and Y sperms.

A random union between these male and female gametes will produce the following four types of zygotic combinations: $X^v X^v X^v$, $X^v X^v Y$, $X^V O$ and OY. The first and the fourth combinations ($X^v X^v X^v$ and OY) will normally die. The second combination ($X^v X^v Y$) will produce vermilion female, while the third ($X^V O$) will yield red-eyed male. Since nondisjunction of X chromosome may be expected to occur only occasionally, such unusual males and females will be recovered in low frequencies. Bridges *concluded that the unusual vermilion-eyed females and the red-eyed males obtained in this study were* XXY *and* XO *respectively. Subsequent cytological analyses confirmed that they were really so. This clearly demonstrated that the gene* v *was located in the* X *chromosome of* Drosophila.

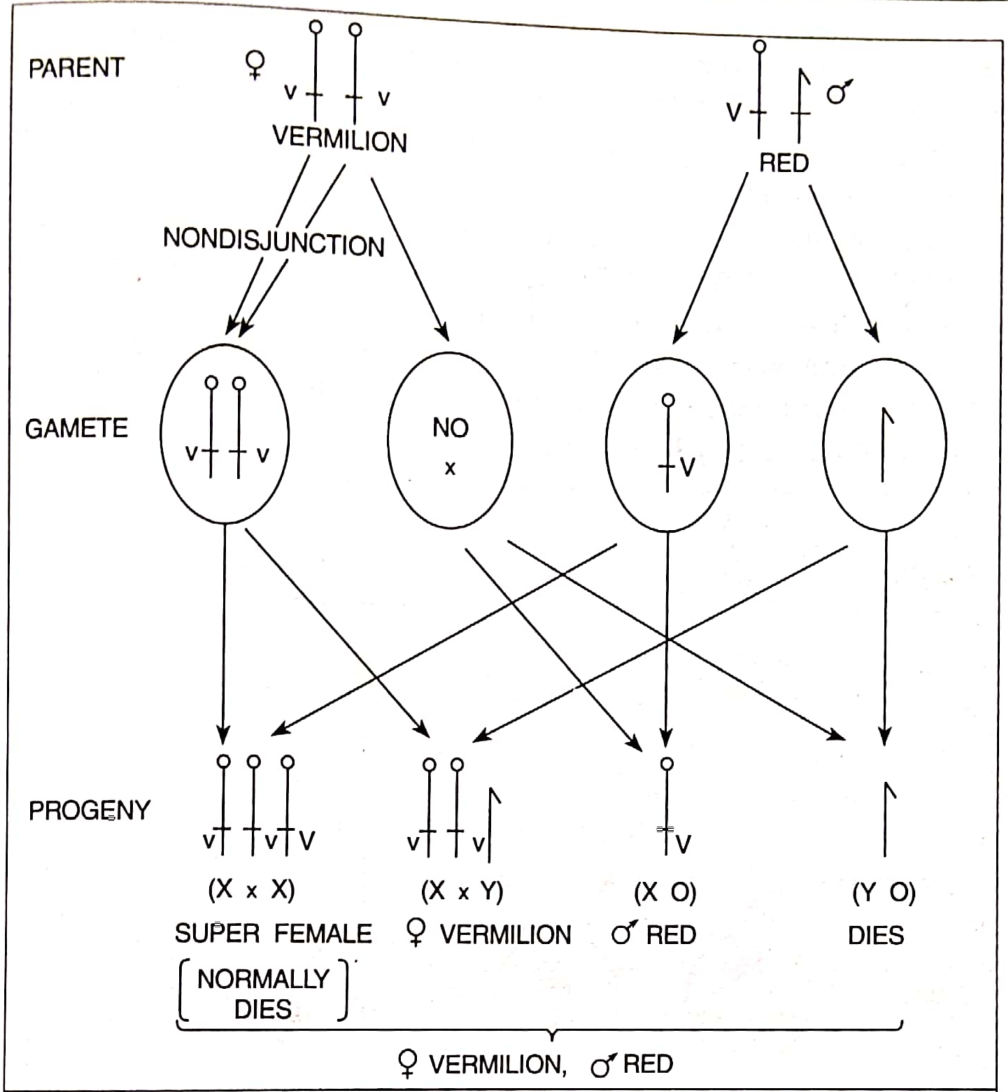


Fig. 10.3. Occasional unusual transmission of *vermilion* eye gene in *Drosophila* due to the non-disjunction of *X* chromosome. Note the transmission of *v* allele from male to male

10.4. ATTACHED-X CHROMOSOME IN *DROSOPHILA*

In 1922, L.V. Morgan obtained a female *Drosophila* with yellow body colour. This female, when mated with a gray body male, produced all females with yellow body and all males with gray body colour. Yellow body is recessive to normal gray body. It is determined by the gene *Y* located in the *X* chromosome. Clearly, this yellow body female was producing only unusual females and males in its progeny. In comparison, Bridges had obtained the unusual individuals only in a low frequency (one fly per 2-3 thousand flies). L.V. Morgan reasoned that nondisjunction of the *X* chromosome was regularly taking place in all the oocytes of this female (X^yX^y). Therefore, it produced only the two unusual egg types: X^yX^y and *O* (without an *X* chromosome). Such a highly regular nondisjunction is only possible if the two *X* chromosomes behaved as a single chromosome during meiosis, that is, both shared the same centromere. In other words, the two *X* chromosomes should have fused to form a single chromosome, the ***attached-X chromosome*** (Fig. 10.4). A cytological analysis confirmed that the *X* chromosomes of this female were actually fused into one. Such an attached-*X* chromosome may be represented as X-X. X-X regularly passes as a unit to the same pole during AI, while the opposite pole does not receive any *X* chromosome.

The yellow body female with an attached-*X* (X^y-X^y) would produce only the two types of unusual eggs: X^y-X^y and *O*. The gray body male (X^YY) will produce two types of sperms, X^Y and *Y*. A union between these male and female gametes will produce the following four types of zygotes: X^y-X^yX^Y, X^y-X^yY, X^YO and *OY*. The first combination will produce a superfemale, which normally dies. The fourth combination (*OY*) does not survive. The X^y-X^yY zygotes receive both their *X* chromosomes from their female parent. Therefore, they will develop into yellow-body females. The third combination (X^YO) receives its *X* chromosome from the male parent. Hence it will produce gray-body males.

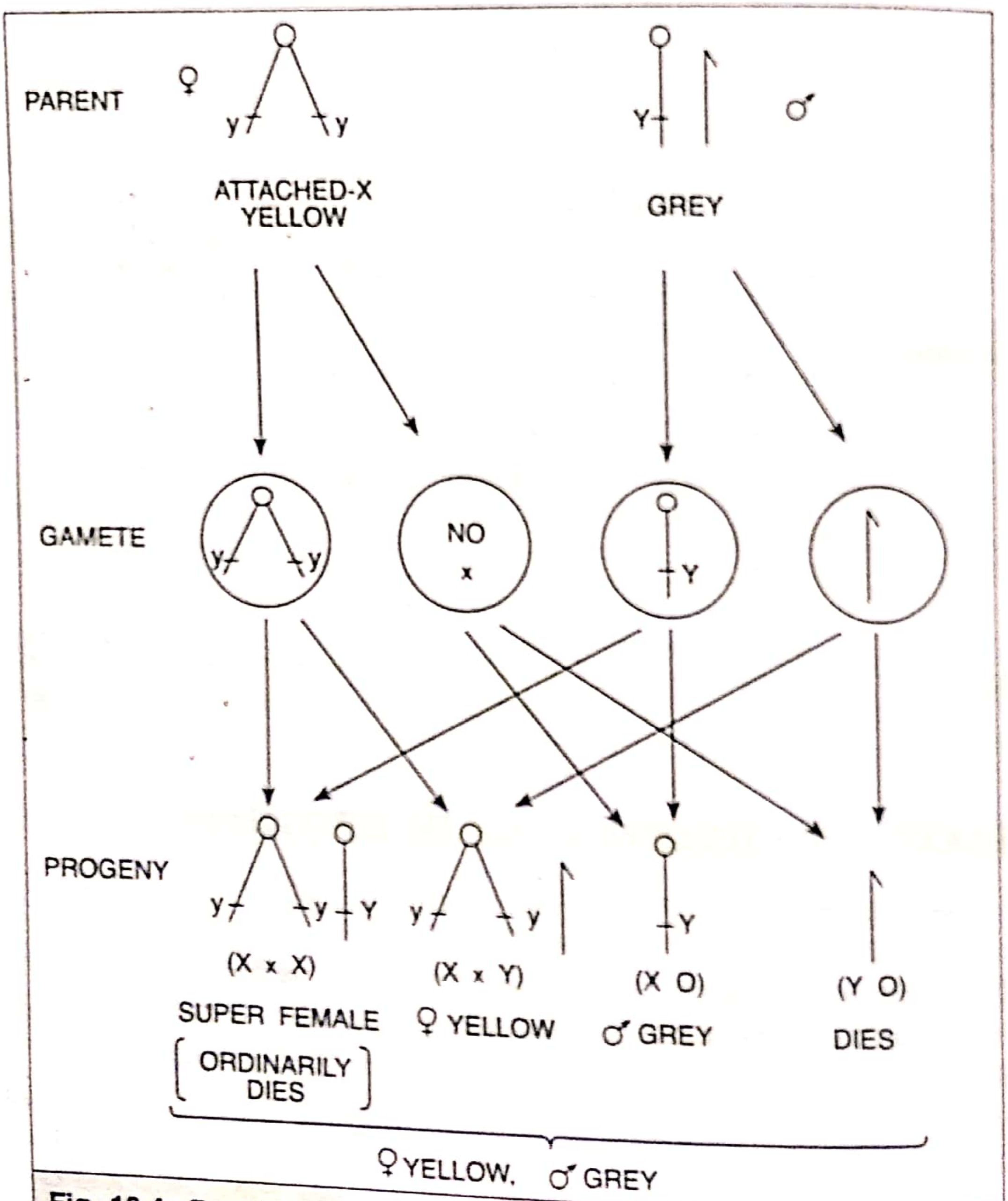


Fig. 10.4. Regular male-to-male transmission of the dominant allele of *yellow* body gene (*Y*) due to the attached-*X* in *Drosophila*.

Thus, unusual transmission of the X *chromosome and, consequently, of* X*-linked genes from male parent to male progeny could be explained by the nondisjunction of* X *chromosome in the female*. There was a complete correspondence between the expectations from this hypothesis and the actual cytological data on the unusual progeny in the studies of Bridges and L.V. Morgan. These studies, therefore, provided a conclusive proof that sex-linked genes are located in the *X* chromosome of *Drosophila*. *Thus these findings established the chromosomal basis of heredity beyond any reasonable doubt.*

10.5. CHARACTERISTICS OF SEX-LINKED INHERITANCE

The characteristic features of inheritance of a sex-linked trait may be summarised as follows.

1. The frequency of individuals showing a recessive sex-linked trait is markedly higher in the heterogametic sex (*e.g.*, male *Drosophila* and humans, and female birds) than that in the homogametic sex (*e.g.*, female *Drosophila* and humans, and male birds).
2. Ordinarily, genes governing sex-linked traits in man and *Drosophila* are not transmitted from male parents directly to their male progeny. For example, *white* eye gene (*w*) is not transmitted from male *Drosophila* to its male progeny.
3. A male human or *Drosophila* transmits its sex linked genes to all its daughters. These daughters transmit this gene to half of their male progeny. As a result, *a sex-linked recessive gene is transmitted from a male to its female progeny and then to half the male progeny of such females. Thus* ***a sex-linked gene passes from male to female then back to male;*** *such an inheritance pattern is known as* ***criss-cross inheritance.***
4. Sex-linked genes are not present in the *Y* chromosome. Consequently, the heterogametic sex (male humans, and *Drosophila,* and female birds) is hemizygous for such genes, *i.e.*, it has only one allele of the sex-linked genes.
5. It was noted that a region of the *Y* chromosome is homologous to a region in the *X* chromosome. For example, the long arm of human *Y* chromosome is homologous to the short arm of human *X* chromosome. Similarly, one-third of the *Drosophila X* chromosome (the region close to the centromere) is homologous to a region of the *Y* chromosome. *Genes located in such regions of* X *chromosome do not show sex-linked inheritance*. (These genes show normal disomic inheritance pattern as exhibited by autosomal genes.)

10.6. THE CAUSE OF SEX-LINKAGE

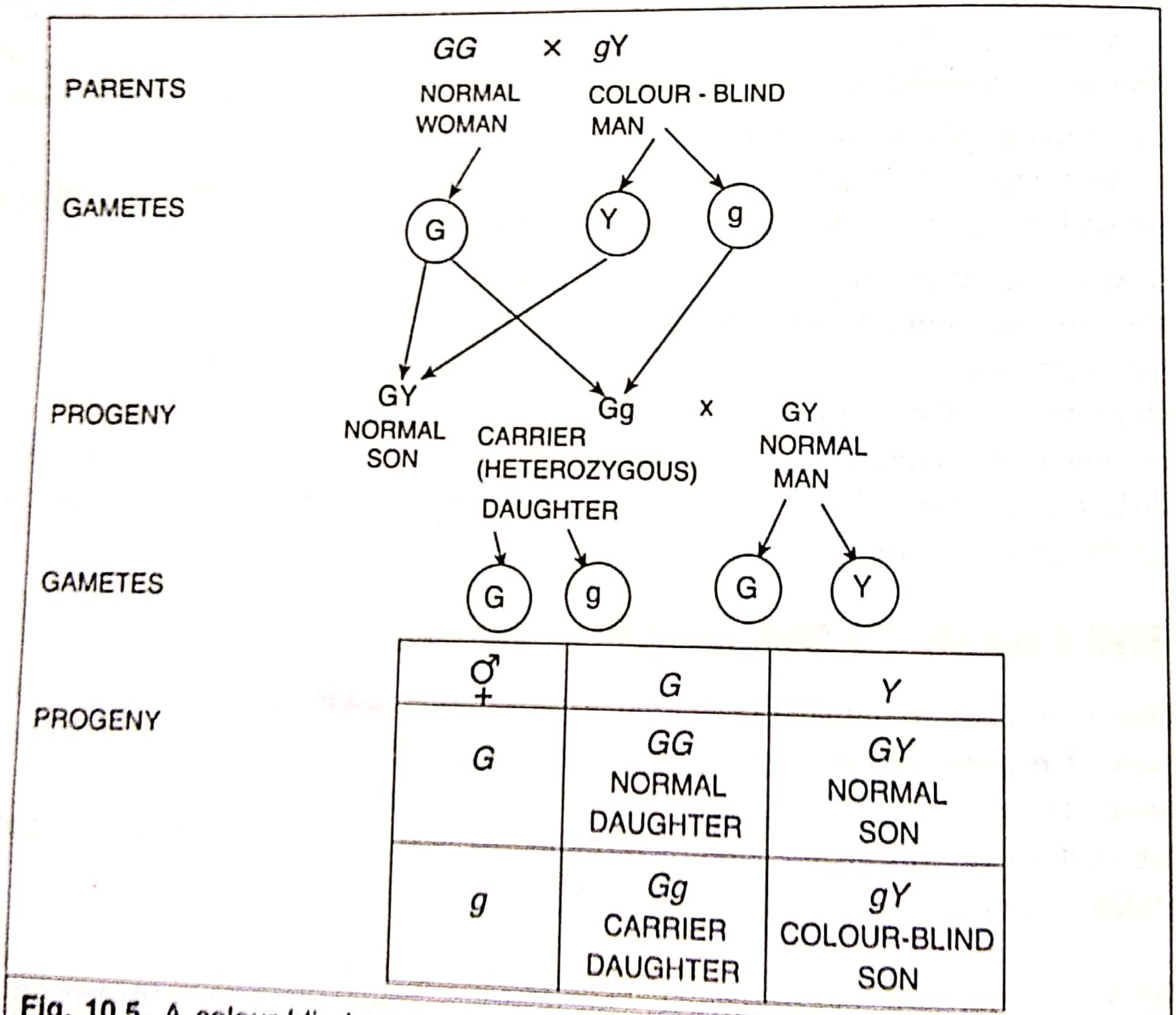
The peculiar inheritance pattern of sex-linked traits is due to the following two reasons: (1) ***the location of a gene in the X chromosome, and*** (2) ***the absence of its allele in the Y chromosome.*** These two features were postulated by Morgan in 1910 on the basis of the inheritance pattern of *white* eye gene of *Drosophila*. Experimental evidence in support of these postulates came from the studies of Bridges and L.V. Morgan.

10.7. SEX LINKAGE IN MAN AND OTHER ORGANISMS

It is safe to conclude that every species exhibiting differences in either the number or the kind of sex chromosomes of its males and females will show sex-linked inheritance. This will be true for those traits genes for which are located in the sex chromosome. Sex-linked inheritance is known in *Drosophila,* man, mice, cat, insects, poultry, cattle, guinea pigs etc. In

Drosophila, more than 150 genes are sex-linked. In humans, over 200 genes exhibit sex linkage; most of these genes cause genetic diseases. Some of the well-known examples of sex-linked human traits are, haemophilia (inability of blood to clot on exposure to air), colour blindness (inability to see one or the other colour, *e.g.*, green colour), optic atrophy (degeneration of the optic nerve), juvenile glaucoma (hardening of the eye ball), myopia (near-sightedness), defective iris, epidermal cysts, distichiasis (double eyelashes), white occipital lock of hair, mitral stenosis (abnormality of mitral valve in the heart), a form of mental retardation, Lesch-Nyhan disease, cystic fibrosis, etc.

In humans, three proteins (one protein each for red, green and blue lights) present in specialized cone cells of retina mediate colour perception. The protein absorbing red light is structurally very similar to that absorbing green light, and the genes encoding them are located in the X chromosome. (The gene encoding blue light receptor is located in an autosome.) Colour blindness may be caused by an abnormality in any of these three receptor proteins. The classic type of colour blindness involves a faulty perception of red and green lights. About 5-10% of human males are red-green colour blind, while only less than 1% of the females are afflicted; this trait shows sex-linked inheritance. We may use G as the symbol for the gene responsible for perception of green colour, and g for the mutant allele leading to



⚥	G	Y
G	GG NORMAL DAUGHTER	GY NORMAL SON
g	Gg CARRIER DAUGHTER	gY COLOUR-BLIND SON

Fig. 10.5. A colour-blind man transmits the defective gene to all his daughters. These daughters are normal, but are carriers for the gene g. When the daughters marry normal men, 1/2 of their sons are colour-blind. Thus the sex-linked gene g passes from father-to-daughter-to-grandson.

the inability to see green colour. If such a colour blind man (*gY*) is married to a normal homozygous female (*GG*), all their sons will have normal colour vision (*GY*). However, all the daughters of this couple will be carriers (heterozygous) for this gene (*Gg*), but they will have normal vision. When such carrier females (*Gg*) are married to normal males (*GY*), 50% of their sons will have normal vision (*GY*), but the remaining 50% will be colour blind (*gY*). However, all the daughters from such marriages will have normal colour vision (Fig. 10.5).

10.8. PARTIAL SEX LINKAGE

The human X and Y chromosomes are morphologically distinct. But they pair during meiosis in male cells; the pairing occurs in the two telomeric regions, which are called ***pseudoautosomal regions* (*PAR*)**. The two pseudoautosomal regions are called *PAR1* and *PAR2* (Fig. 10.6).

1. ***PAR1*** is the *major pseudoautosomal region;* it is 2.6 Mb long (Mb = mega base pairs). It is located at the tip of the short arms of the X and Y chromosomes, and is the site of obligate crossing over during male meiosis. The frequency of recombination in *PAR1* is 70 times the normal recombination frequency, and it approaches 50%. *PAR1* region has around a dozen genes, *e.g.*, *SHOX, XE7, ANT3, Tramp, MIC2*, etc.
2. ***PAR2*** is the *minor pseudoautosomal region* of 320 Kb (Kb = kilo base pairs). It is located at the tips of the long arms of X and Y chromosomes, and contains at least two genes (*IL9R* and *SYBL1*). Crossing over in *PAR2* is not so frequent as in *PAR1*.

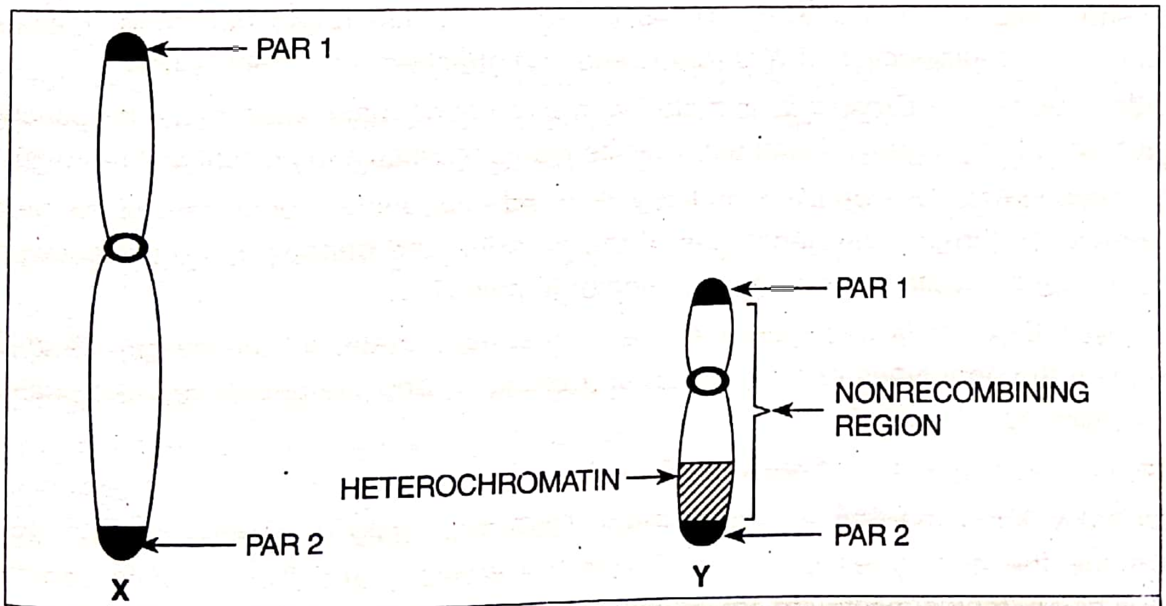


Fig. 10.6. The human X and Y chromosomes have two main regions of homology, referred to as *PAR1* and *PAR2* (*PAR* = pseudoautosomal region), at their ends.

The genes located in *PAR1* and *PAR2* are also present in the X chromosome. But these genes do not show the typical inheritance pattern for sex-linkage. This is because these genes have alleles in the Y chromosome as well. As a result, their inheritance pattern resembles that of autosomal genes. This phenomenon is called ***partial sex-linkage***, and the chromosome regions involved in it are referred to as ***pseudoautosomal regions***. *In partial sex linkage, a* *gene located in X chromosome shows autosomal inheritance pattern*. But such a gene will show linkage with those genes that show typical sex-linked inheritance; this has been clearly shown for barbed bristles of *Drosophila*.

10.9. THE CAUSE OF SEX-LINKAGE

The peculiar inheritance pattern of sex-linked traits is due to the following two reasons: (1) ***the location of a gene in the X chromosome, and*** (2) ***the absence of its allele in the Y chromosome***. These two features were postulated by Morgan in 1910 on the basis of the inheritance pattern of *white* eye gene of *Drosophila*. Experimental evidence in support of these postulates came from the studies of Bridges and L.V. Morgan.