Contents:

---

1. Meaning of Mutations:

Mutation refers to sudden heritable change in the phenotype of an individual. In the molecular term, mutation is defined as the permanent and relatively rare change in the number or sequence of nucleotides. Mutation was first discovered by Wright in 1791 in male lamb which had short legs.

Later on mutation was reported by Hugo de Vries in 1900 in Oenothera, Morgan (1910) in Drosophila (white eye mutant) and several others in various organisms. The term mutation was coined by de Vries.

---

2. Characteristics of Mutations:

Mutations have several characteristic features.

Some of the important characteristics of mutations are briefly presented below:

i. Nature of Change:

Mutations are more or less permanent and heritable changes in the phenotype of an individual. Such changes occur due to alteration in number, kind or sequence of nucleotides of genetic material, i.e., DNA in most of the cases.

ii. Frequency:

Spontaneous mutations occur at a very low frequency. However, the mutation rate can be enhanced many fold by the use of physical and chemical mutagens.

The frequency of mutation for a gene is calculated as follows:

Frequency of gene mutation = M / M + N

where, M = number of individuals expressing mutation for a gene, and

N = number of normal individuals in a population.

iii. Mutation Rate:

Mutation rate varies from gene to gene. Some genes exhibit high mutation rate than others. Such genes are known as mutable genes, e.g., white eye in Drosophila. In

some genomes, some genes enhance the natural mutation rate of other genes. Such genes are termed as mutator genes.

The example of mutator gene is dotted gene in maize. In some cases, some genes decrease the frequency of spontaneous mutations of other genes in the same genome, which are referred to as anti-mutator genes. Such gene has been reported in bacteria and bacteriophages.

iv. Direction of Change:

Mutations usually occur from dominant to recessive allele or wild type to mutant allele. However, reverse mutations are also known, e.g., notch wing and bar eye in Drosophila.

v. Effects:

Mutations are generally harmful to the organism. In other words, most of the mutations have deleterious effects. Only about 0.1% of the induced mutations are useful in crop improvement. In majority of cases, mutant alleles have pleiotropic effects. Mutations give rise to multiple alleles of a gene.

vi. Site of Mutation:

Muton which is a sub-division of gene is the site of mutation. An average gene contains 500 to 1000 mutational sites. Within a gene some sites are highly mutable than others. These are generally referred to as hot spots. Mutations may occur in any tissue of an organism, i.e., somatic or gametic.

vii. Type of Event:

Mutations are random events. They may occur in any gene (nuclear or cytoplasmic), in any cell (somatic or reproductive) and at any stage of development of an individual.

viii. Recurrence:

The same type of mutation may occur repeatedly or again and again in different individuals of the same population. Thus, mutations are of recurrent nature.

3. Types of Mutation:

i. Substitution

A substitution is a mutation in which there is an exchange between two bases (i.e. a change in a single "chemical letter" such as switching a T to a C). Such a substitution could change a codon to one that encodes a different amino acid and cause a change in the protein produced. Sometimes substitutions may not effects the protein structure, such mutations are called silent mutations and sometimes they may change an amino-acid-coding codon to a single "stop" codon and cause an incomplete protein. This can seriously affect the protein structure which may completely change the organism.

Example of Substitution Mutation: Sickle Cell Anaemia is caused by substitution mutation, where in codon (**GAG mutates to --> GTG**) and leads to (**Glu --> Val**) change.

ii. Insertion

Insertions are mutations in which extra base pairs are inserted into a new place in the DNA. The number of base pairs inserted can range from one to thousands!

Example of Insertion Mutation: Huntington's disease and the **fragile X syndrome** are examples of insertion mutation wherein trinucleotide repeats are inserted into the DNA sequence leading to these diseases.

iii. Deletions

Deletions are mutations in which a section of DNA is lost, or deleted. The number of base pairs deleted can again range from one to thousands!
Insertions and Deletion mutations are often together dubbed as INDELS.

Example of Deletion Mutation: 22q11.2 deletion syndrome is caused by the deletion of some bases of *chromosome 22*. This disease is characterized by cleft palate, heart defects, autoimmune disorders etc.

iv. Frameshift

Protein-coding DNA is divided into codons which are three bases long, insertions and deletions in these codons can completely change a gene so its message cannot be decoded correctly. Such mutations are called frameshift mutations. For example, consider the sentence, "The cat ate her rat." Each word represents a codon. If we delete the first letter and read the sentence in the same way, it doesn't make sense. Similarly if the codons become jumbled up, they would no longer make any sense, in such frameshifts, a similar error occurs at the DNA level, where the codons cannot be parsed correctly. This usually gives rise to truncated proteins that are as useless as "rca tet hce tee" is uninformative.

Examples of Frameshift Mutation: Tay-Sachs Disease, Cancers of many types, Crohn's Disease, cystic fibrosis have been associated with Frameshift Mutation.

---

4. Agents of Mutations:

Mutagens:

Mutagens refer to physical or chemical agents which greatly enhance the frequency of mutations. Various radiations and chemicals are used as mutagens. Radiations come under physical mutagens. A brief description of various physical and chemical mutagens is presented below:

Physical Mutagens:

Physical mutagens include various types of radiations, viz. X-rays, gamma rays, alpha particles, beta particles, fast and thermal (slow) neutrons and ultra violet rays (Table 14.2).

A brief description of these mutagens is presented below:

TABLE 14.2. Commonly used physical mutagens (radiation), their properties and mode of action

Type of Radiation	*Main properties*	*Mode of action or changes caused*
1. X-rays	S.I., penetrating and non-particulate	Induce mutations by forming free radicals and ions. Cause addition, deletion, transitions and transversions.
2. Gamma rays	S.I., very penetrating and non-particulate	Induce mutations by ejecting atoms from the tissues. Cause all types of changes as above.
3. Alpha Particles	D.I., particulate, less penetrating and positively charged.	Act by ionization and excitation. Cause chromosomal and gene mutations.
4. Beta Rays Particles	S.I., particulate, more penetrating than alpha particles and negatively charged.	Act by ionization and excitation. Cause chromosomal and gene mutations.
5. Fast and Thermal Neutrons	D.I., particulate, neutral particles, highly penetrating	Cause chromosomal breakage and gene mutations.
6. Ultra Violet Rays	Non-ionizing, low penetrating	Cause chromosomal breakage and gene mutations.

i. X-Rays:

X-rays were first discovered by Roentgen in 1895. The wavelengths of X-rays vary from 10^{-11} to 10^{-7}. They are sparsely ionizing and highly penetrating. They are generated in X-rays machines. X-rays can break chromosomes and produce all types of mutations in nucleotides, viz., addition, deletion, inversion, transposition, transitions and trans-versions.

These changes are brought out by adding oxygen to deoxyribose, removing amino or hydroxyl group and forming peroxides. X-rays were first used by Muller in 1927 for induction of mutations in Drosophila.

In plants, Stadler in 1928 first used X-rays for induction of mutations in barley. Now X-rays are commonly used for induction of mutations in various crop plants. X-rays induce mutations by forming free radicals and ions.

ii. Gamma Rays:

Gamma rays are identical to X-rays in most of the physical properties and biological effects. But gamma rays have shorter wave length than X-rays and are more penetrating than X-rays. They are generated from radioactive decay of some elements like 14C, 60C, radium etc.

Of these, cobalt 60 is commonly used for the production of Gamma rays. Gamma rays cause chromosomal and gene mutations like X-rays by ejecting electrons from the atoms of tissues through which they pass. Now a days, gamma rays are also widely used for induction of mutations in various crop plants.

iii. Alpha Particles:

Alpha rays are composed of alpha particles. They are made of two protons and two neutrons and thus have double positive charge. They are densely ionizing, but lesser penetrating than beta rays and neutrons. Alpha particles are emitted by the isotopes of heavier elements.

They have positive charge and hence they are slowed down by negative charge of tissues resulting in low penetrating power. Alpha particles lead to both ionization and excitation resulting in chromosomal mutations.

iv. Beta Particles:

Beta rays are composed of beta particles. They are sparsely ionizing but more penetrating than alpha rays. Beta particles are generated from radioactive decay of heavier elements such as 3H, 32P, 35S etc. They are negatively charged, therefore, their action is reduced by positive charge of tissues. Beta particles also act by way of ionization and excitation like alpha particles and result in both chromosomal and gene mutations.

v. Fast and Thermal Neutrons:

These are densely ionizing and highly penetrating particles. Since they are electrically neutral particles, their action is not slowed down by charged (negative or positive) particles of tissues. They are generated from radioactive decay of heavier elements in atomic reactors or cyclotrons. Because of high velocity, these particles are called as fast neutrons.

Their velocity can be reduced by the use of graphite or heavy water to produce slow neutrons or thermal neutrons. Fast and thermal neutrons result in both chromosomal breakage and gene mutation. Since they are heavy particles, they move

in straight line. Fast and thermal neutrons are effectively used for induction of mutations especially in asexually reproducing crop species.

vi. Ultraviolet Rays:

UV rays are non-ionizing radiations, which are produced from mercury vapour lamps or tubes. They are also present in solar radiation. UV rays can penetrate one or two cell layers. Because of low penetrating capacity, they are commonly used for radiation of micro-organisms like bacteria and viruses.

In higher organisms, their use is generally limited to irradiation of pollen in plants and eggs in Drosophila UV rays can also break chromosomes. They have two main chemical effects on pyrimidine's.

The first effect is the addition of a water molecule which weakens the H bonding with its purine complement and permits localized separation of DNA strands. The second effect is to join pyrimidines to make a pyrimidine dimer.

This dimerization can produce TT, CC, UU and mixed pyrimidine dimers like CT. Dimerization interferes with DNA and RNA synthesis. Inter-strand dimers cross link nucleic acid chains, inhibiting strand separation and distribution.

Chemical Mutagens:

There is a long list of chemicals which are used as mutagens. Detailed treatment of such chemicals is beyond the scope of this discussion.

The chemical mutagens can be divided into four groups, viz:

(a) Alkylating agents,

(b) Base analogues,

(c) Acridine dyes, and

(d) Others (Table 14.3).

A brief description of some commonly used chemicals of these groups is presented below.

TABLE 14.3. Some commonly used chemical mutagens and their mode of action

Group of mutagen	*Name of chemical*	*Mode of action*
1. Alkylating Agents	Ethyl methane Sulphonate	*AT* ↔ *GC* Transitions
	Methyl Methane Sulphonate	Transitions
	Ethyl Ethane Sulphonate	*GC* ↔ *AT* Transitions
	Ethylene Imines	Transitions.
2. Base Analogues	5 Bromo Uracil	*AT* ↔ *GC* Transitions
	2 Amino Purine	*AT* ↔ *GC* Transitions
3. Acridine Dyes	Acriflavin, Proflavin	Deletion, addition and frameshifts.
4. Others	Nitrous Acid	*AT* ↔ *GC* Transitions
	Hydroxylamine	*GC* ↔ *AT* Transitions
	Sodium Azide	Transitions

a. Alkylating Agents:

This is the most powerful group of mutagens. They induce mutations especially transitions and transversions by adding an alkyl group (either ethyl or methyl) at various positions in DNA. Alkylation produces mutation by changing hydrogen bonding in various ways.

The alkylating agents include ethyl methane sulphonate (EMS), methyl methane sulphonate (MMS), ethylene imines (EI), sulphur mustard, nitrogen mustard, etc.

Out of these, the first three are in common use. Since the effect of alkylating agents resembles those of ionizing radiations, they are also known as radiomimetic chemicals. Alkylating agents can cause various large and small deformations of base structure resulting in base pair transitions and transversions.

Transversions can occur either because a purine has been so reduced in size that it can accept another purine for its complement, or because a pyrimidine has been so increased in size that it can accept another pyrimidine for its complement. In both cases, diameter of the mutant base pair is close to that of a normal base pair.

b. Base Analogues:

Base analogues refer to chemical compounds which are very similar to DNA bases. Such chemicals sometimes are incorporated in DNA in place of normal base during replication. Thus, they can cause mutation by wrong base pairing. An incorrect base pairing results in transitions or transversions after DNA replication. The most commonly used base analogues are 5 bromo uracil (5BU) and 2 amino purine (2AP).

5 bromo uracil is similar to thymine, but it has bromine at the C5 position, whereas thymine has CH_3 group at C5 position. The presence of bromine in 5BU enhances its tautomeric shift from keto form to the enol form. The keto form is a usual and more stable form, while enol form is a rare and less stable or short lived form. Tautomeric change takes place in all the four DNA bases, but at a very low frequency.

The change or shift of hydrogen atoms from one position to another either in a purine or in a pyrimidine base is known as tautomeric shift and such process is known as tautomerization.

The base which is produced as a result of tautomerization is known as tautomeric form or tautomer. As a result of tautomerization, the amino group ($-NH_2$) of cytosine and adenine is converted into imino group (-NH). Similarly keto group (C = 0) of thymine and guanine is changed to enol group (-OH).
5BU is similar to thymine, therefore, it pairs with adenine (in place of thymine). A tautomer of 5BU will pair with guanine rather than with adenine. Since the tautomeric form is short-lived, it will change to keto form at the time of DNA replication which will pair with adenine in place of guanine.

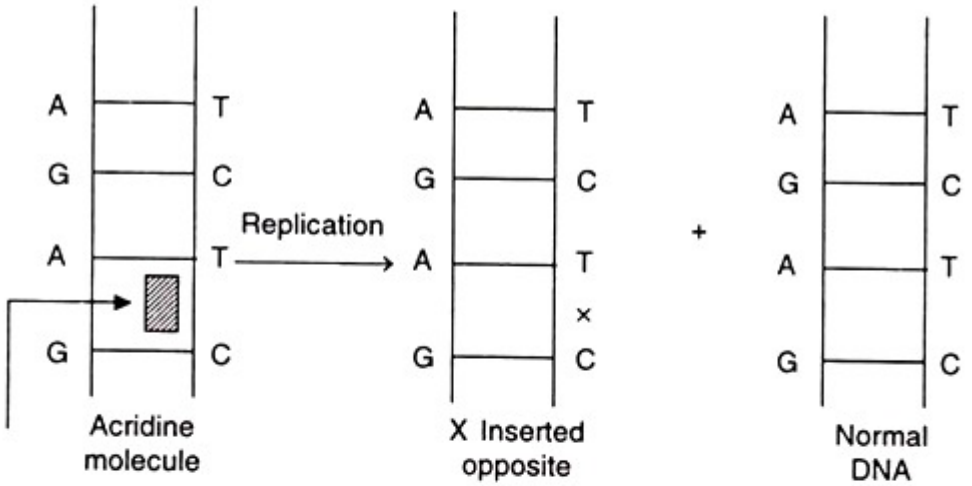
In this way it results in AT GC and GC —> AT transitions. The mutagen 2AP acts in a similar way and causes AT <-> GC transitions. This is an analogue of adenine.

c. Acridine Dyes:
Acridine dyes are very effective mutagens. Acridine dyes include, pro-flavin, acridine orange, acridine yellow, acriflavin and ethidium bromide. Out of these, pro-flavin and acriflavin are in common use for induction of mutation. Acridine dyes get inserted between two base pairs of DNA and lead to addition or deletion of single or few base pairs when DNA replicates (Fig. 14.1).

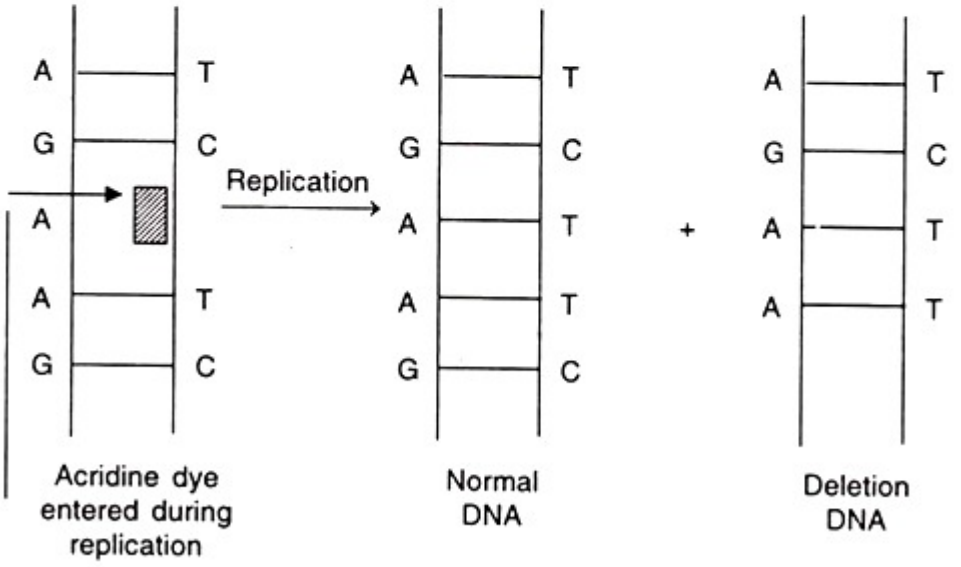
Thus, they cause frameshift mutations and for this reason acridine dyes are also known as frameshift mutagens. Proflavin is generally used for induction of mutation in bacteriophages and acriflavin in bacteria and higher organisms.

d. Other Mutagens:
Other important chemical mutagens are nitrous acid and hydroxy amine. Their role in induction of mutation is briefly described here. Nitrous acid is a powerful mutagen which reacts with C6 amino groups of cytosine and adenine. It replaces the amino group with oxygen (+ to – H bond). As a result, cytosine acts like thymine and adenine like guanine.

Thus, transversions from GC —> AT and AT —> GC are induced. Hydroxylamine is a very useful mutagen because it appears to be very specific and produces only one kind of change, namely, the GC —> AT transition. All the chemical mutagens except base analogues are known as DNA modifiers.



(a) Insertion



(b) Deletion

Fig. 14.1. Mode of action of acridine dyes : (*a*) insertion, (*b*) deletion.

5. Detection of Mutation:

Detection of mutations depends on their types. Morphological mutations are detected either by change in the phenotype of an individual or by change in the segregation ratio in a cross between normal (with marker) and irradiated individuals. The molecular mutations are detected by a change in the nucleotide, and a biochemical mutation can be detected by alteration in a biochemical reaction.

The methods of detection of morphological mutants have been developed mainly with Drosophila. Four methods, viz., (1) CIB method, (2) Muller's 5 method, (3) attached X-chromosome method, and (4) curly lobe plum method are in common use for detection of mutations in Drosophila.

A brief description of each method is presented below:

i. CIB Method:

This method was developed by Muller for detection of induced sex linked recessive lethal mutations in Drosophila male.

This method was invented by Muller and used for the unequivocal demonstration of mutagenic action of X rays. In this method, females containing one normal X-chromosome and another X-chromosome (CIB) containing extra 3 genes are used for the analysis. Out of the 3 extra genes, one gene suppresses crossover (c), the other is a recessive lethal (L) in heterozygous condition, and the last gene is semidominant marker, Bar (B) gene. Females containing CIB chromosome are called as CIB stock drosophila. The normal males are exposed to mutagenic source for a fixed period and then mated to the CIB stock drosophila. Males containing CIB chromosome will die due to the effect of lethal genes, whereas normal males and females both normal and with CIB will survive. Females with CIB chromosomes and identified by barred phenotype are selected and crossed to normal males. In this next generation 50% of males (which have received the CIB gene) will die. If mutation has occurred in normal X chromosome then even the normal male (without CIB gene) will die. If no mutation has occurred all the other 50% of males will survive. The frequency of lethal mutations can be accurately scored in large samples. This technique is simple, rapid and there is little chance of an error in scoring. However, it is suitable for the scoring of sex linked recessive lethal only.

The important steps of this method are as follows:

(a) A cross is made between CIB female and mutagen treated male. In F_1 half of the males having normal X-chromosome will survive and those carrying CIB chromosome will die. Among the females, half have CIB chromosome and half normal chromosome (Fig. 14.2). From F_1, females with CIB chromosome and male with normal chromosome are selected for further crossing.

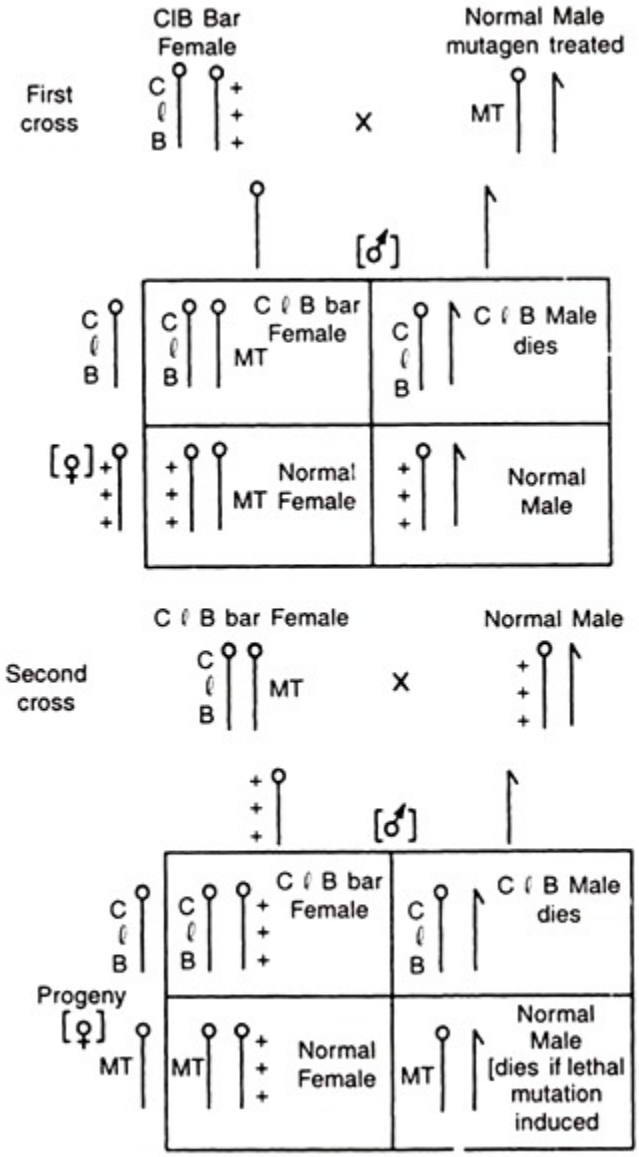


Fig. 14.2. Muller's CIB method for detection of lethal induced mutations in X-chromosome of **Drosophila**. Mt denotes mutagen treated X-chromsome.

(b) Now a cross is made between CIB female and normal male. This time the CIB female has one CIB chromosome and one mutagen treated chromosome received from the male in earlier cross.

This will produce two types of females, viz., half with CIB chromosome and half with mutagen treated chromosome (with normal phenotype). Both the progeny will survive. In case of males, half with CIB will die and other half have mutagen treated chromosome.

If a lethal mutation was induced in mutagen treated X-chromosome, the remaining half males will also die, resulting in absence of male progeny in the above cross. Absence of male progeny in F_2 confirms the induction of sex linked recessive lethal mutation in the mutagen treated Drosophila male.

ii. Muller 5 Method:

This method was also developed by Muller to detect sex linked mutation in Drosophila. This method is an improved version of CIB method. This method differs from CIB method in two important aspects. First, this method utilizes apricot recessive gene in place of recessive lethal in CIB method. Second, the female is homozygous for bar apricot genes, whereas it is heterozygous for IB genes in CIB method.

In this method, the mutation is detected by the absence of wild males in F_2 progeny. This method consists of following important steps (Fig. 14.3).

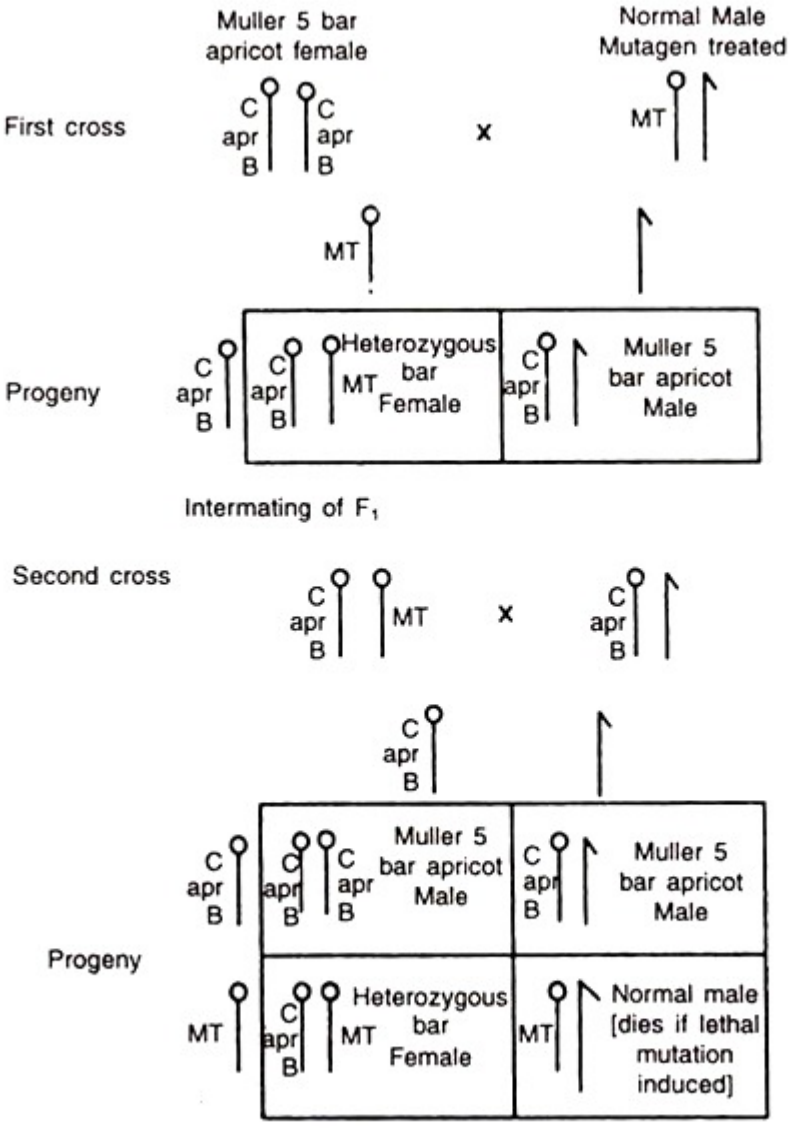


Fig. 14.3. Muller's 5 method of for detection of induced mutation in X-chromosome of **Drosophila.** Mt denotes mutagen treated X-chromosome.

a. A homozygous bar apricot female is crossed with mutagen treated male. In F_1 we get two types of progeny, viz., heterozygous bar females and bar apricot (Muller) males.

b. These F_1 are inter-mated. This produces four types of individuals. Half of the females are homozygous bar apricot, and half are bar heterozygous. Among the males, half are bar apricot (Muller 5) and half should be normal. If a lethal mutation is induced, the normal male will be absent in the progeny.