DEPARTMENT OF BOTANY
Guru Ghasidas Vishwavidyalaya, Bilaspur
M. Sc. III Semester
LBC 902/LBT 302: Genetics and Breeding

Section A

Time : 3 hours **Maximum marks – 60**

Multiple choice questions **2 × 10 = 20**

1. (a) Most other scientists of the time were also using peas, so a lot was known about them
2. (b) test cross
3. (c) TtHH
4. (c) Ignored all characteristics except a few markedly contrasting ones in which he studied.
5. (c) Acrocentric — chromosome arms are identical in size
6. (a) Linkage
7. (d) all of these
8. (b) genetic modification through genetic engineering
9. (b) Blood relatives
10. (c) A founder effect

Section B

Descriptive answer type questions (attempts any four) **10 × 4 = 40**

1. **Give a brief account of the classical genetics of Mendel.**
   Mendelian Genetics
   ➢ Mendelian factors are now called genes
   ➢ Alleles are different versions of the same gene
   ➢ An individual with two identical alleles is termed homozygous
   ➢ An individual with two different alleles, is termed heterozygous
   ➢ Genotype refers to the specific allelic composition of an individual
   ➢ Phenotype refers to the outward appearance of an individual
   Genotype and Phenotype
   ➢ Hereditary traits are under the control of genes (Mendel called them factors).
   ➢ Genotype is the genetic makeup of an organism, a description of the genes it contains.
   ➢ Phenotype is the characteristics that can be observed in an organism.
   ➢ Phenotype is determined by interaction of genes and environment.
   ➢ Each trait carries two copies of a unit of inheritance, one inherited from the mother and the other from the father and alternative forms of traits are called alleles.
   MENDEL'S LAWS OF INHERITANCE
   ➢ His work, entitled "Experiments on Plant Hybrids" was published in 1866
   ➢ Like many great scientific discoveries, it was ignored for 34 years
   ➢ In 1900, Mendel's work was rediscovered by three botanists working independently : Hugo de Vriies off Holland, Carll Correns off Germany, –Erich von Tschermak off Austria
   **Keys to Mendel's Experiments**
   ➢ The garden pea was an ideal organism for study because: – Vigorous growth, – Self fertilization, – Easy to cross fertilize, – Produces large number of offspring each generation
   ➢ Mendel analyzed traits with discrete alternative forms (one of two options): – purple vs. white flowers, – yellow vs. green peas, – round vs. wrinkled seeds, – long vs. short stem length
   ➢ Mendel established pure/true breeding lines to conduct his experiments. – (traits remain constant from generation to generation)
   Monohybrid Crosses and Mendel's Principle of Segregation

Terminology used in breeding experiments:

➢ Parental generation is the P generation. – True breeding unless stated otherwise
➢ Progeny of P generation is the first filial generation, – designated F1.
➢ When F1 interbreed or are "selfed" – the second filial generation, F2, is produced, – F2 is always F1 x F1
➢ Subsequent interbreeding produces F3, F4, and F5 generations.
➢ A monohybrid cross involves true-breeding strains that differ in a single trait.
➢ To determine whether both parents contribute equally to the phenotype of a particular trait in offspring, – a set of reciprocal crosses is performed.
➢ In Mendelian genetics, offspring of a monohybrid cross will exactly resemble only one of the parents, – This is the principle of uniformity in F1 (Figure 1). – Complete dominance
➢ Mendel noted that traits that disappear in the F1 reappear in the F2.
➢ The F2 has a ratio of about three individuals with the phenotype of the F1 to one individual with the "reappearing" phenotype, – 3:1 ratio, – 3/4 to 1/4
➢ Mendel reasoned that information to create the trait was present in the F1 in the form of "factors", – now called genes (Figure 1).

P generation

Smooth seeds ✕ Wrinkled seeds

F₁ generation   All smooth seeds

F₁ ✕ F₁ cross

✕

Self-cross of F₁ progeny plants

F₂ generation

5474 smooth seeds   and   1850 wrinkled seeds

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➢ By convention, letters may be used to designate alleles, – with the dominant a capital letter (T), – and the recessive in lowercase (t).
➢ Individuals with identical alleles are said to be homozygous for that gene, – (e.g., genotypes TT and tt), – because all their gametes will have the same allele for this trait.
➢ Individuals with different alleles are heterozygous, – (e.g., Tt), – because 1/2 of their gametes will contain one allele, and 1/2 the other.
➢ When Mendel had conducted experiments for the seven different traits in garden peas, he made these conclusions:
   1. Results of reciprocal crosses are always the same.
   2. The F1 resembled only one of the parents.
   3. The trait missing in the F1 reappeared in about ¼ of the F2 individuals.
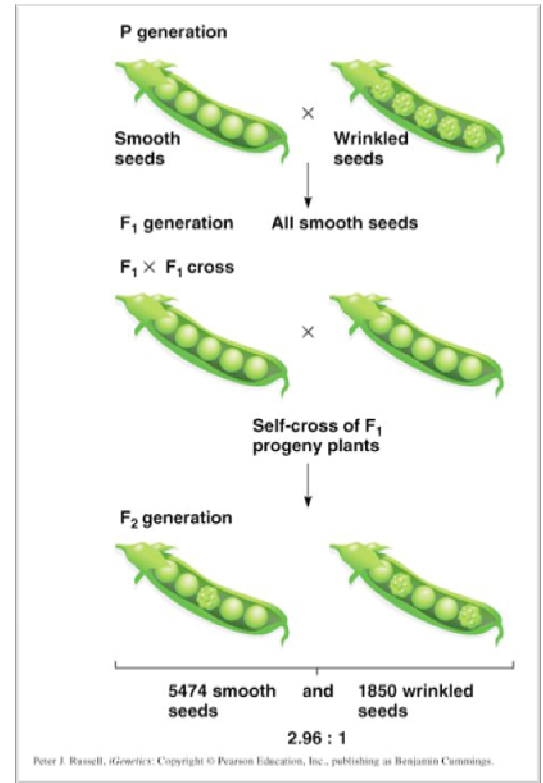
The Principle of Segregation
➢ The first Mendelian law, the principle of segregation, states: – "Recessive characters, which are masked in the F1 from a cross between two true-breeding strains, reappear in a specific proportion in the F2."
➢ This is because alleles segregate during anaphase I of meiosis – and progeny are then produced by random combination of the gametes.
➢ Mendel observed that plants with the recessive phenotype are all true breeding.
➢ Not true for the dominant phenotype –When plants with the dominant phenotype are self crossed
   • 1/3 are true-breeding,
   • and 2/3 produce progeny with both phenotypes

F2 x F2 self fertilization	TT x TT	Tt x Tt
F3 progeny	All TT (Smooth)	¾ Smooth (Tt)
		¼ Wrinkled (tt)

➢ A more common way to determine whether an individual with the dominant phenotype is homozygous or heterozygous is to perform a testcross; – By definition you must cross the individual with one that is homozygous recessive

2. **Write an essay on chromosomal theory of inheritance and sex chromosomes and its determinants.**

Chromosome theory of heredity

➢ William Sutton and Theodor Boveri proposed the chromosome theory of heredity: the idea that genes are parts of chromosomes.

➢ Some evidence that supported the theory:

- Nuclei of egg and sperm are equal in size and contain coloured bodies that are easily visible during cell division
- The number of chromosomes is consistent from cell to cell within an organism but varies between organisms
- The behaviour of the chromosomes at meiosis correlates with the behaviour of Mendel's hypothetical particles

Evidence from chromosome segregation

➢ Calvin Bridges' hypothesis

- If genes are located on chromosomes then unusual, unexpected genetic results should correspond to unusual, abnormal chromosome arrangements
- Working with *Drosophila melanogaster* Bridges noticed that among the progeny of the cross w/w;+;+;+ X +/Y;+;+;+ about 1 of 2000 was a white-eyed ♀ or a red-eyed ♂!
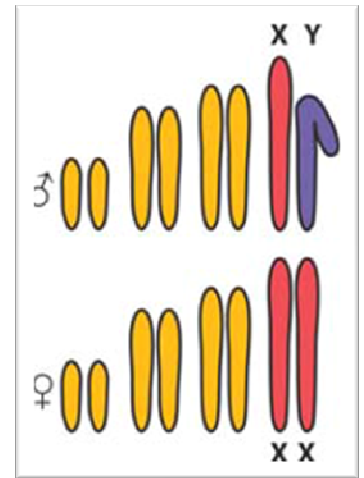
Drosophila

- *Drosophila melanogaster* has 4 chromosomes: 1 pair of sex chromosomes and 3 pairs of autosomes
- semi-colons are used to indicate when genes are on different chromosomes – Eg: w;+;+;+ 1;2;3;4

Chromosomal Sex Determination in *Drosophila*

➢ Although both mammals and fruit flies produce XX females and XY males, their chromosomes achieve these ends using very different means.

➢ The sex-determining mechanisms in mammals and in insects such as *Drosophila* are very different.

➢ In mammals, the Y chromosome plays a pivotal role in determining the male sex. Thus, XO mammals are females, with ovaries, a uterus, and oviducts (but usually very few, if any, ova).

➢ In *Drosophila*, sex determination is achieved by a balance of female determinants on the X chromosome and male determinants on the autosomes.

➢ Normally, flies have either one or two X chromosomes and two sets of autosomes. If there is but one X chromosome in a diploid cell (1X:2A), the fly is male.

➢ If there are two X chromosomes in a diploid cell (2X:2A), the fly is female

➢ Thus, XO Drosophila are sterile males. In flies, the Y chromosome is not involved in determining sex. Rather, it contains genes active in forming sperm in adults.

➢ Table 1 shows the different X-to-autosome ratios and the resulting sex.

Ratios of X chromosomes to autosomes in different sexual phenotypes *in Drosophila melanogaster*

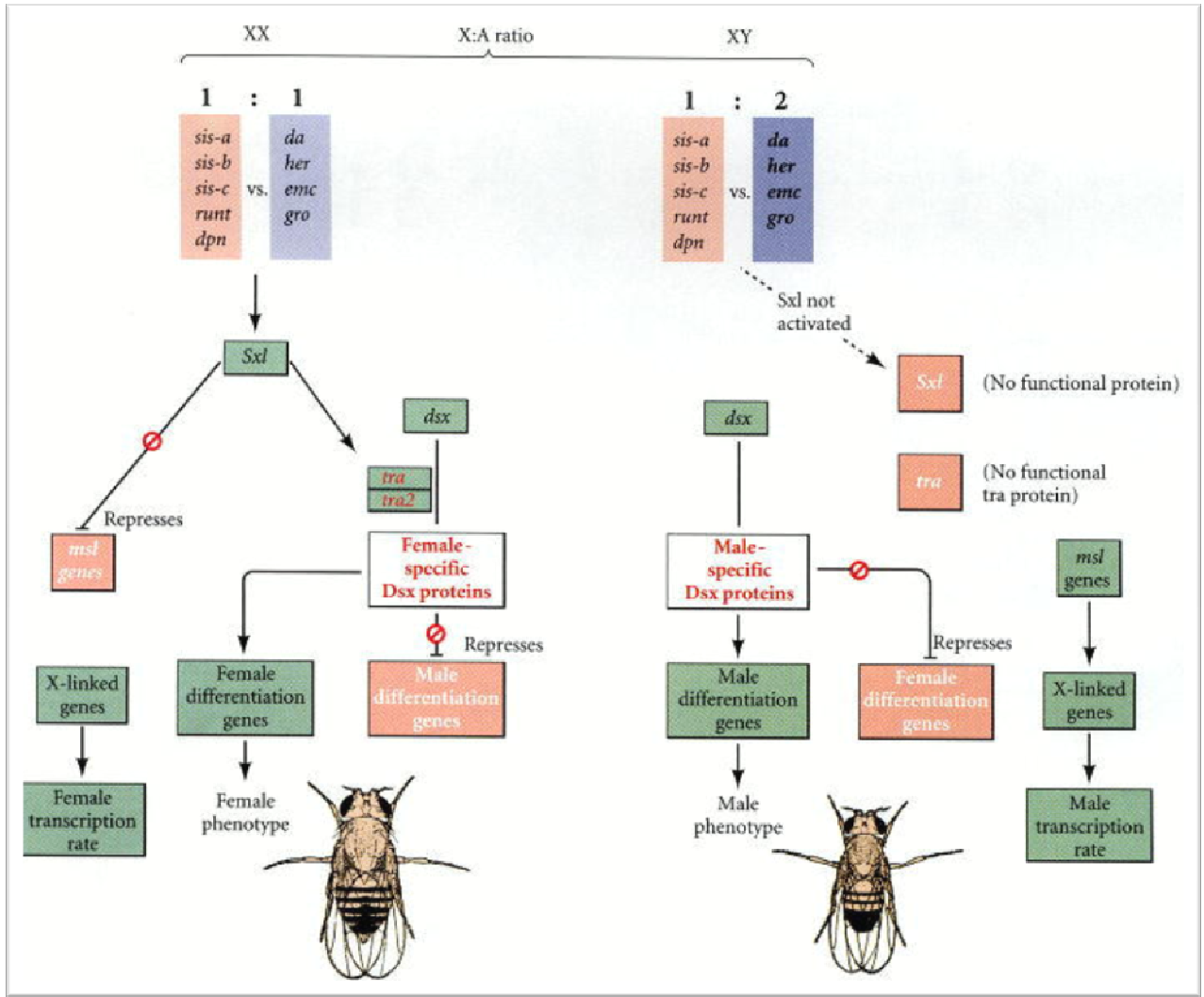| X Chromosome	Autosome set	(A)X:A ratio	SEX
3	2	1.50	Metafemale
4	3	1.33	Metafemale
4	4	1.00	Normal female
3	3	1.00	Normal female
2	2	1.00	Normal female
2	3	0.66	Intersex
1	2	0.50	Normal male
1	3	0.33	Metamale

Figure: Proposed regulation cascade for *Drosophila* somatic sex determination. Arrows represent activation, while a block at the end of a line indicates suppression. The *msl*loci, under the control of the *Sxl* gene, regulate the dosage compensatory transcription of the male X chromosome.

3. What is coupling and repulsion hypothesis? Describe it with suitable example.

➢ Bateson, in 1905, described a cross in sweet pea, where a deviation from independent assortment was exhibited. Plants of a sweet pea variety having blue flowers (*B*) and long pollen (*L*) were crossed with those of another variety having red flowers (*b*) and round pollen (l). F1 individuals (*BbLl*) had blue flowers and long pollen. These were crossed with plants having red flowers and round pollen (*bbll*). (In this case character for blue colour of flowers is dominam over red colour and long pollen character is dominant over round pollen).

➢ Normally if independent assortment takes place, we should expect 1:1:1:1 ratio in a testcross. Instead, 7:1:1:7 ratio was actually obtained, indicating that there was a tendency in dominant alleles to remain together. Similar was the case with recessive alleles. This deviation was, therefore, explained as gametic coupling by Bateson. Similarly, it was observed that when two such dominant alleles or two recessive alleles come from different parents, they tend to remain separate. This was called gametic repulsion.

➢ In Bateson's experiment in repulsion phase, one parent would have blue flowers and round pollen (*BBll*) and the other would have red flowers and long pollen (*bbLL*). The results of a testcross in such a repulsion phase were similar to those obtained in coupling phase giving 1:7:7:1 ratio instead of expected 1:1:1:1. Bateson explained the lack of independent assortment in the above experiments by means of a hypothesis known as coupling and repulsion hypothesis. Although coupling and repulsion as explained above were later

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discovered to be the two aspects of the same phenomenon called linkage, the terms coupling phase and repulsion phase are still considered to be useful terms in scientific literature.

➤ T.H. Morgan, while performing an experiment with *Drosophila*, in 1910, found that coupling or repulsion was not complete. He proposed that two genes are found in coupling phase or in repulsion phase, because these were present on same chromosome (coupling) or on two different, but homologous chromosomes (repulsion). Such genes are then called linked and the phenomenon is called linkage. He further suggested that the strength of linkage will, be determined by distance between two genes in question. The greater this distance, lower will be the linkage strength. The linkage is broken down due to the phenomenon of crossing over occurring during meiosis. Crossing over will be relatively more frequent, if distance between two genes is more than in a case where the distance between two genes is less. The phenomenon of crossing over involves exchange of chromosome segments.

Figure: Exchange of chromosome segments during crossing over. The first figure represents a pair of normal chromosomes, the second figure represents exchange due to double crossing over

➤ In order to make the phenomenon of linkage and crossing over easily understandable, let us take another hypothetical example, where genes *A* and *B* are involved, *a* and *b* being their recessive alleles respectively.

➤ A cross *AB/AB* x *ab/ab* would give rise to a F1 dihybrid *AB/ab*. This dihybrid will then be crossed with double recessive parent *ab/ab* to get the testcross progeny. The formation of gametes from dihybrid *(AB/ab)* in presence and in absence of crossing over is shown in Figure. The derivation of test cross progeny is also represented.

➤ Depending upon distance between any two genes which is inversely proportional to strength of linkage, non-crossovers will vary from 50%-100% (100% non-crossovers is a state where no crossing over takes place as in male *Drosophila*. The crossovers will similarly vary from 0-50% and will never exceed 50%. The easiest way of finding out the proportion of non-crossovers and crossovers is to make a test cross *(AB/ab* x *ab/ab)* as shown in Figure. In such a situation, since phenotypic ratio will depend upon ratio of different kinds of gametes coming from F1 *(AB/ab),* the relative proportion of non-crossovers and, crossovers can be easily determined from phenotypic ratio. This is illustrated in the next section using an example from maize.
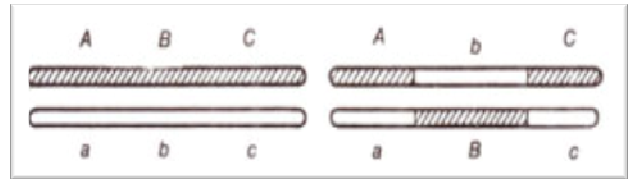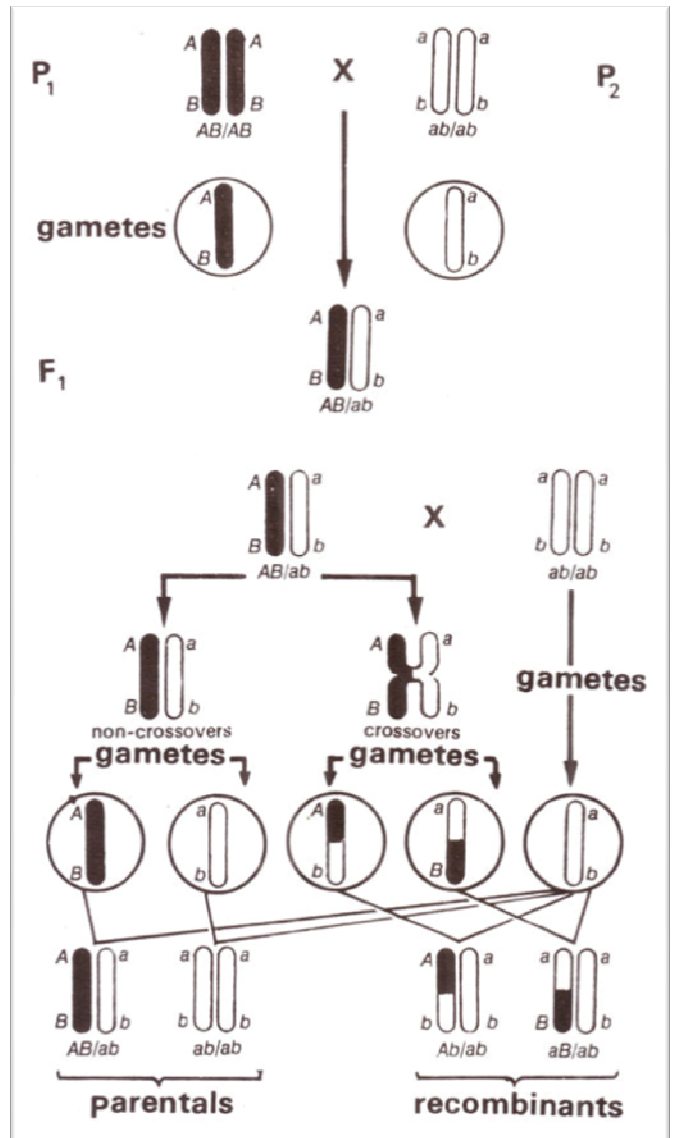

Coupling Cross Chi-Square Test

F_1 Gamete	Observed	Expected	$(O-E)^2/E$
$pr^+ vg^+$	1339	709.75	557.9
$pr^+ vg$	151	709.75	439.9
$pr\ vg^+$	154	709.75	435.2
$pr\ vg$	1195	709.75	331.8
Total	**2839**	**2839**	$X^2=1764.8$

Repulsion Cross Chi-Square Test

F_1 Gamete	Observed	Expected	$(O-E)^2/E$
$pr^+ vg^+$	157	583.75	312.0
$pr^+ vg$	965	583.75	249.0
$pr vg^+$	1067	583.75	483.3
$pr vg$	146	583.75	328.3
Total	**2335**	**2335**	**X^2=1372.6**

It is quite clear that both of these large chi-square values indicate that neither of these ratios fit the 1:1:1:1

4. **How can you differentiate between the terms haploidy and monoploidy? How can monoploids be produced and utilized in plant breeding?**

➢ The term "haploid" refers to individuals with the genetic number, but they may have arisen from a diploid or a polyploid. The term "monoploid" is used only for those that arise from a diploid and hence possess a single genome.

➢ Gametes (sperm and ova) are haploid cells. The haploid gametes produced by most organisms combine to form a zygote with n pairs of

> **Figaure:** A testcross *(AB/ab* x *ab/ab)* showing crossover and non-crossover gametes (from *AB/ab)* and progenies

chromosomes, one from each gamete, i.e. 2n chromosomes in total. The chromosomes in each pair, one of which comes from the sperm and one from the egg, are said to be homologous.

➢ Cells and organisms with pairs of homologous chromosomes are called diploid. For example, most animals are diploid and produce haploid gametes. During meiosis, sex cell precursors have their number of chromosomes halved by randomly "choosing" one member of each pair of chromosomes, resulting in haploid gametes. Because homologous chromosomes usually differ genetically, gametes usually differ genetically from one another.

➢ All plants and many fungi and algae switch between a haploid and a diploid state, with one of the stages emphasized over the other. This is called alternation of generations. Most fungi and algae are haploid during the principal stage of their lifecycle, as are plants like mosses. Most animals are diploid, but male bees, wasps, and ants are haploid organisms because they develop from unfertilized, haploid eggs.

➢ In some cases there is evidence that the n chromosomes in a haploid set have resulted from duplications of an originally smaller set of chromosomes. This "base" number – the number of apparently originally unique chromosomes in a haploid set – is called the monoploid number. As an example, the chromosomes of common wheat are believed to be derived from three different ancestral species, each of which had 7 chromosomes in its haploid gametes.

➢ The monoploid number is thus 7 and the haploid number is $3 \times 7 = 21$. In general n is a multiple of x. The somatic cells in a wheat plant have six sets of 7 chromosomes: three sets from the egg and three sets from the sperm which fused to form the plant, giving a total of 42 chromosomes. As a formula, for wheat $2n = 6x = 42$, so that the haploid number n is 21 and the monoploid number x is 7. The gametes of common wheat are considered to be haploid, since they contain half the genetic information of somatic cells, but they are not monoploid, as they still contain three complete sets of chromosomes (n = 3x). [11]

➢ In the case of wheat, the origin of its haploid number of 21 chromosomes from three sets of 7 chromosomes can be demonstrated.

➢ In many other organisms, although the number of chromosomes may have originated in this way, this is no longer clear, and the monoploid number is regarded as the same as the haploid number. Thus in humans, x = n = 23.

> Monoploidy has been applied in plant biotechnology to rapidly develop plants from anthers that have a fixed genotype. F1 plants derived from a cross of two parents are grown and anther tissue is used to regenerate new plants using tissue culture techniques.
> The plants that are derived from this tissue will be monoploid, and the genetics of these individuals can be studied or they can be treated with a chemical to double the chromosome number.

5. **Define gene frequency. Briefly describe the procedure for estimation of gene frequency in a given population.**

> **Gene frequency or allele frequency** is the proportion of a particular allele (variant of a gene) among all allele copies being considered.
> **Formal definition:** Allele or gene frequency is the percentage of all alleles at a given locus in a population gene pool represented by a particular allele.
> In other words, it is the number of copies of a particular allele divided by the number of copies of all alleles at the genetic place (locus) in a population. It is usually expressed as a percentage. Inpopulation genetics, allele frequencies are used to depict the amount of genetic diversity at the individual, population, and species level. It is also the relative proportion of all alleles of a gene that are of a designated type.

Given the following:
1. a particular locus on a chromosome and the gene occupying that locus
2. a population of N individuals carrying n loci in each of their somatic cells (e.g. two loci in the cells of diploid species, which contain two sets of chromosomes)
3. different alleles of the gene exist
4. one allele exists in a copies

Estimation of gene frequency
> **Gene frequency** can be calculated in two equivalent ways: from the observed numbers of different genotypes at a particular locus or from the genotype frequencies. First, we can calculate the allele frequencies directly from the numbers of genotypes. In this method, we count the number of alleles of on type at a particular locus and divide it by the total number of alleles at that locus in the population. This method is called gene counting and works for a wide variety of cases, including X-linked genes and mitochondrial genes. Expressing the gene counting method as a formula for a nuclear gene with two alleles, we get

Gene frequency = number of copies of a given allele/sum of counts of all allele in the population
> For example, imagine a population of 1000 diploid individual with 300 AA, 400 Aa, and 150 aa individuals. Each individual has two A alleles, whereas each Aa heterozygote possesses only a single A allele. Therefore, the number of A allele in the population is (2x the number of AA homozygotes)+ (the number of Aa heterozygotes), or (2x300)+400 = 1000. Since every diploid individual has two alleles, the total number of alleles in the population is twice the number of individuals, or 2x1000 for autosomal genes. Using the equation just given, the allele frequency is 1000/2000= 0.50.
> When two alleles are present at a locus, we can use the following formula to calculate allele frequencies:

p=f(A)= (2xcount of AA)+(count of Aa)/2xtotal number of individuals
> The second method of calculating allele frequencies goes through the step of first calculating genotype frequencies as demonstrated previously.

p= f(A)= (frequency of the AA homozygote)+(1/2 x frequency of the Aa heterozygote)

q= f(a)= (frequency of the AA homozygote)+(1/2 x frequency of the Aa heterozygote)

Gene frequency with multiple alleles

➢ Suppose we have three allele – A^1, A^2, and A^3- at a locus.

The formula:

p= f(A^1)= (2x count of A^1A^1) + (A^1A^2) +A^1A^3)/ (2x total number of individuals)

q= f (A^2) = (2x count of A^2A^2) + (A^1A^2) + (A^2A^3)/ (2x total number of individuals)

r = f (A^3) = (2x count of A^3A^3) + (A^1A^3) + (A^2A^3)/ (2x total number of individuals)

Gene frequency at an X- linked locus

p = f (X^A) = (2x X^AX^A female) + ($X^A X^a$ females) + (X^AY males)/ (2x number of female) + (number of males)

6. **What is allele frequency? Calculate the allele frequency from the following population data.**

   Genotype AA=68, Aa=42, aa=24 and Total number is 134.

Allele frequency

➢ Allele frequency is the concept used to quantify genetic variation. It is defined as a measure of the commonness of a given allele in a population, that is, the proportion of all alleles of that gene in the population that are specifically this type

➢ For calculating the allele frequency. Twice the number of homozygous genotypes with that allele (because homozygotes carry two copies each of the same allele), plus the number of heterozygous genotypes with that allele (because heterozygotes carry only one copy of a particular allele), divided by two times the total number of individuals in the sample (because each individual carries two alleles per locus).

For the given population the frequency is as followes.

p=f(A)= (2xcount of AA)+(count of Aa)/2xtotal number of individuals

p= f (A) = (2 x 68) + (42)/ 2 x 134 = 136 + 42 / 268 = 178/268 = **0.664**

q = f (a) = (2 x 24) + (42)/ 2 x 134 = 48 + 42/ 268 = **0.335**

7. **Comment on followings :**

   (i) Inbreeding depression

➢ Inbreeding depression is the reduced fitness in a given population as a result of breeding of related individuals. It is often the result of a population bottleneck.

➢ In general, the higher the genetic variation within a breeding population, the less likely it is to suffer from inbreeding depression.

➢ Inbreeding depression seems to be present in most groups of organisms, but varies across mating systems. Hermaphroditic species often exhibit lower degrees of inbreeding depression than outcrossing species, as repeated generations of selfing is thought to purge deleterious alleles from populations.

➢ For example, the outcrossing nematode *Caenorhabditis remanei* has been demonstrated to suffer severely from inbreeding depression, unlike its hermaphroditic relative *C. elegans*, which experiences outbreeding depression.

- With some exceptions, inbreeding reduces offspring fitness in essentially all naturally outcrossing plants and to a lesser extent in selfing species. The negative effects of mating between relatives have been noticed for many centuries.
- The careful breeding studies of Darwin (1876) first empirically demonstrated inbreeding depression in a wide variety of taxa. The negative effects of inbreeding have since been observed in both outcrossing and selfing species for a variety of traits with consequences for offspring fitness.
- Examples of traits shown to be subjects to inbreeding depression include pollen quantity, number of ovules, amount of seed, germination rate, growth rate and competitive ability.
- Genetic models have been developed to functionally explain reduction in fitness-related traits caused by inbreeding.
- The primary genetic consequence of inbreeding is increased homozygosity. Two hypotheses for the genetic basis of inbreeding depression are put forth, both of which depend on the idea that homozygosity will increase with inbreeding.
- Distinguishing between the two genetic mechanisms is complicated by linked sets of deleterious recessives that imitate over-dominance effects.

(ii) Polyploid breeding

- Polyploids are organisms with multiple sets of chromosomes in excess of the diploid number. Polyploidy is common in nature and provides a major mechanism for adaptation and speciation.
- Approximately 50-70% of angiosperms, which include many crop plants, have undergone polyploidy during their evolutionary process.
- Flowering plants form polyploids at a significantly high frequency of 1 in every 100,000 plants.

Implications of ploidy in plant breeding

- Heterosis or hybrid vigor is the difference between the hybrid and the mean of the two parents and is characterized by increased vigor and superior qualitative or quantitative traits.
- Over the last several decades, breeders have increased the world food production by utilizing the concept of heterosis in hybrid cultivars.
- The process of hybrid formation for polyploids is not without setbacks. Many interspecific hybrids have low fertility and viability due to hybrid incompatibilities. Hybrid incompatibility results from genes that are functionally diverged in the respective hybrid forming species.
- This may lead to silencing of protein encoding genes and has been reported in interspecific hybrids of *Arabidopsis*.
- To increase the heterosis, fertility and viability of interspecific hybrids, several factors should be considered. The parents used should be of diverse genetic background and preferably heterozygous.
- Self pollination is an important method for attaining homozygosity in breeding. Through this process, it is possible to fix desired alleles in the background of a crop.
- Fixing a trait controlled by a single gene in an autotetraploid, would require four identical alleles to achieve homozygosity. In a segregating, tetraploid F2 population the proportion of the homozygous loci would be 1:18.
- An immediate consequence of polyploidy is the change in gametic and filial frequencies.
- This is because polyploids have multiple alleles associated with a single locus. For example, a hexaploid has six alleles per locus while a tetraploid has four.