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T.Y. B.Sc. (Sem. IV) (CBCS)

MICROBIOLOGY

[503]: Molecular biology and genetic engineering

Unit 1

FUNDAMENTAL OF GENETICS

Prepared By

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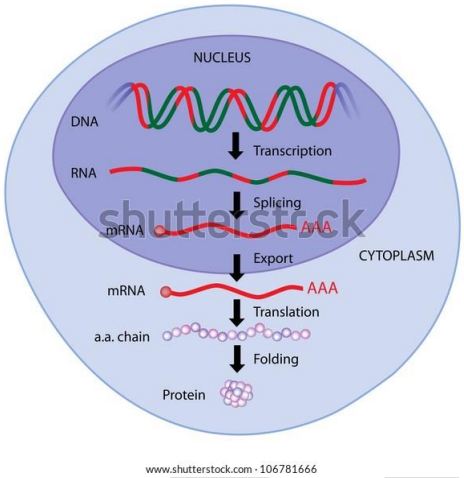
CONTENT

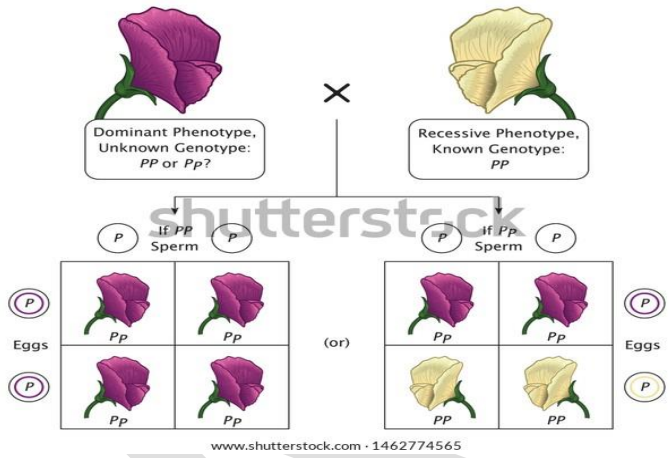
- History of genetics and central dogma of life
- Mendelian laws of inheriitance
- DNA is the universal genetic material & experimental evidance
- Gene structure and architechture in prokaryotes and eukaryotes
- Prokaryotic DNA replication: experiment, machineries, mechanisms & models

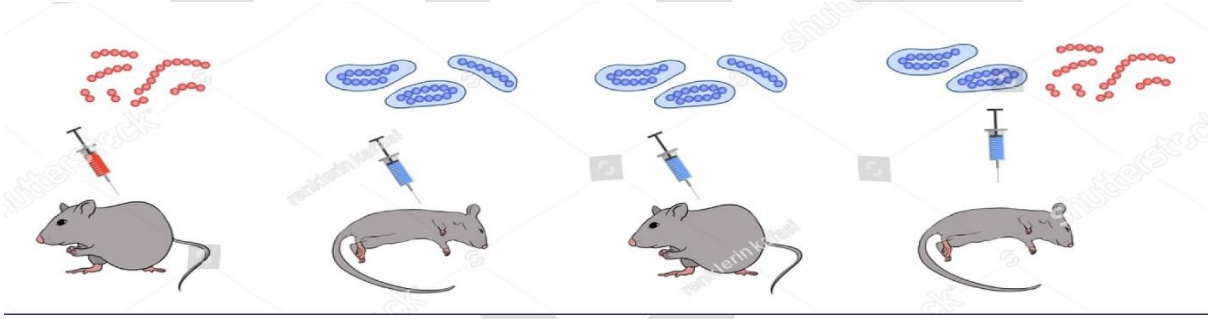
TITLE EXPLANATION

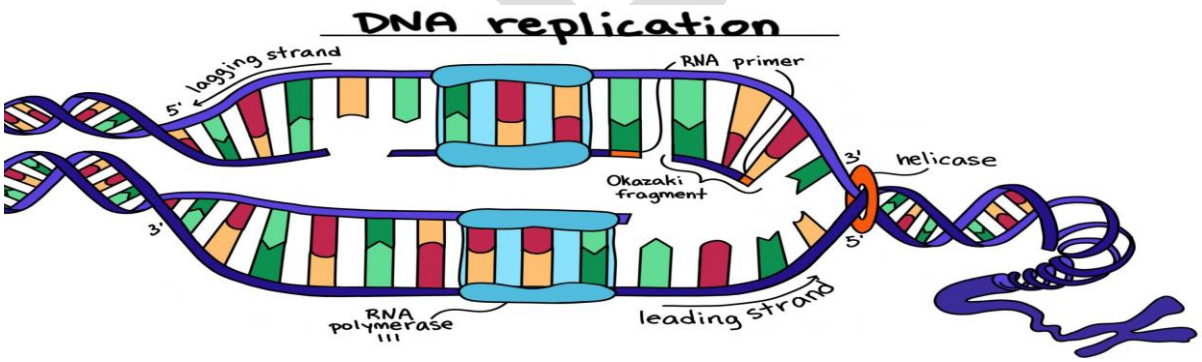
- In this unit we are going to learn and discuss about history of gentics and central dogma of life how DNA converts into RNA and how RNA converts into protein molecules in living organisms.
- Mendelian laws of independent assortment, law of segregation and law of dominance.
- Study of experiments which can prove that DNA is universal genetic material.
- Structure and architechture of gene.
- Study of How DNA prepare copy of its own by replication process.

LEARNING OUTCOME









1.1 History of genetics and central dogma of life

Late 19th Century: Evolution, Natural Selection, Particulate Inheritance and Nuclein

- Understanding origins is a constant pursuit of man. In the 1858, our understanding of the origin of species and how species variability arose was revolutionized by the research of Darwin and Wallace. They described how new species arose via evolution and how natural selection uses natural variation to evolve new forms.
- The importance of this discovery was reflected in the now famous quote of Dobzhansky: "Nothing in biology makes sense except in the light of evolution." - Theodore Dobzhansky, The American Biology Teacher, March 1973 A few years later, Gregor Mendel, an Austrian monk, summarized his years of research on peas in his famous publication. In that paper, he described the unit of heredity as a particle that does not change.
- This was in contrast to the prevailing "blending theory of inheritance." Equally important, Mendel formalized the importance of developing pure (genotypically homozygous) lines, keeping careful notes, and statistically analyzing the data.
- His approach of crossing individuals with variable phenotypes and following them in successive generations is still the only approach utilized to understand the genetic inheritance of a trait. Others in this century were concluding that statistical approaches to biology would help solve problems in biology and inheritance. Research in the 19th century was often performed in isolation.
- While Mendel was concluding that inheritance was particulate in nature, others were trying to figure out the physical nature of the particle. Haeckel correctly predicted that the heredity material was located in the nucleus.

- Miescher showed the material in the nucleus was a nucleic acid. Others observed the behavior of chromosomes and suggested they had a role in heredity. One wonders how concepts might have evolved if information was mobile at that time as it is today.

Early 20th Century: Mendelian Principles are extended and the Chromosomal Theory of Inheritance solidifies

- Except for his early adult years, Mendel did not have an active research program. Therefore, his groundbreaking research went largely unnoticed. It was not until 1900 that others, who had performed similar experiments to his, arrived at the same conclusions. Their publications cited his work, leading to a rediscovery of the Mendelian principles.
- Quickly following the rediscovery, other genetic principles such as linkage, lethal genes, and a bit later, maternal inheritance were described. In each case, the principles provided to be simple extensions of the Mendelian laws, providing further evidence of their importance. At the beginning of the century, the work on chromosomes coalesced into the chromosomal theory of inheritance.
- This theory focused research on the chromosome as the location of genes. The field of cytogenetics was based on this discovery. The first observations of chromosomal abnormalities (duplications, deletions, translocations, inversions) are reported. Observations such as position effect demonstrate that there is a direct link between chromosome structure and phenotype.
- All of these discoveries justified research to discover the physical basis of heredity.

Mid 20th Century: DNA is the stuff of life; the preeminence of the Darwinian theory of evolution via natural selection is confirmed

- As early as the 1870s, the material in the nucleus was determined to be a nucleic acid. From the 1920s through the mid-1950s, a series of experiments demonstrated that DNA was indeed the genetic material.

- The transformation experiments of Griffith demonstrated that a factor found in a lethal strain of bacteria could convert a non-lethal strain of the bacteria into a lethal strain. It was the careful experiments of Avery, MacLeod and McCarty that determined DNA, not protein or RNA was the factor responsible for the conversion.
- This was further confirmed by Hershey and Chase, although their experiments had flaws which prevented them from being definitive. Watson and Crick determined the structure of DNA, and others suggested that DNA contained a genetic code.
- By the mid 1960s that code was deciphered. Experiments involving the process of transcription and translation led to the development of the "central dogma of molecular biology" concept by Crick. The experiments of the early 19th century that confirmed that Mendelian principles could be extended to many gene systems became a major component of what was to be called the Modern synthesis (on neo-Darwinism).
- The experimental demonstration that mutations could be induced was also an important component of the solidification of the concept that natural selection was a major factor in evolution. Finally, the theories embodied in population genetics were also critical.
- The synthesis states that mutations create variation; recombination develops new forms, the variation extends through the population, and based on environmental constraints the variation is finally acted upon by the forces of natural selection to produce more fit individuals.

Mid-late 20th Century and the Early Days of the 21st Century: The Age of Molecular Genetics; Phylogenetics Studies Intensive; The Information Age; The Emergence of Genomics Science

- The discoveries of the mid to late 20th century defined processes that would provide the tools for molecular biology, recombinant DNA technology, and finally the biotechnology industry. The elucidation of the process of DNA

replication described the necessary components needed for the widely-used chain termination DNA sequencing procedure.

- Understanding replication helped determine those tools necessary for the radiolabelling of DNA. The development was necessary to support Southern hybridizations and the early molecular mapping experiments. Understanding replication also defined the role of the ligase enzyme that is so critical for DNA cloning.
- Restriction enzymes were discovered and used to construct recombinant DNA molecules that contained foreign DNA that could be grown in abundance in bacterial cells. The discovery of reverse transcriptase also enabled cDNA cloning which is essential for the modern EST(expressed Sequence tag) projects.
- Cloning is essential for the discovery of gene structure and function. It is also an essential step for all of the genome sequencing projects. The importance of the PCR procedure cannot be emphasized enough.
- The advent of protein and DNA sequencing launched a new era of phylogenetics. Species could now be compared at the molecular level. New procedures for the development of phylogenies are developed. The neutral theory of molecular evolution is proposed.
- This is a direct attack on preeminence of selection as the driving force of evolution. The theory suggests that most mutations are neutral and are fixed by genetic drift and not selection. It is debated whether the evolution of species is driven more by neutral effects or selection. Some feel the two theories are compatible and exert their effects on different genes. The information age is essential to genomics.
- The electronic analysis, distribution and storage of genomic data is a hallmark of the science. Critical to this was the development of computers, both large and small, which put computing power in the hands of all scientists.
- The free distribution of analytical software provided scientists with the tools to study the details of their experiments. The internet spawned the distribution of information from central databases. E-mail connected

scientists and fostered the rapid exchange of ideas. The advent of the WWW provided a new medium for the presentation of information.

- Whole genome are sequenced for the first time. For other species, the gene content is described using ESTs. Microarray analyses provided the first glimpse of global expression patterns. Proteomics begins to describe the protein component of the genome. Metabolomics is established. Massively parallel sequencing technology is introduced.
- This new technology greatly increases the amount of DNA sequence that can be collected in a short period. It will also dramatically decrease the cost of sequencing.
- Importantly it launches the age of individual genome sequencing which will support an era of individualized medicine. 4 Table 1. An annotated history of genetics and genomics.

Year	Discoverer	Discovery
1859	Charles Darwin	The origin of species
1866	Gregor Mendel	Inheritance of factors in pea plants
1888	Henrich Wilhelm Gottfried Waldeyer	The term chromosome is applied to the condensed version of material found in the nucleus
1900	Hugo de Vries	The term mutation is used to describe the apparent spontaneous appearance of new traits in evening primrose (*Oenothera*)
1902	Walter Sutton Theodor Boveri	Within a specific species, each chromosome is described as having unique physical characteristics. Chromosomal theory of inheritance
1905	William Bateson	The terms genetics, homozygote, heterozygote, epistasis, and allelomorph (shortened later to allele) were first used
1905	Nettie Stevens Edmund Wilson	Determine the behavior of sex chromosomes XX determines female and XY determines male
1908	G.H. Hardy W. Weinberg	The Hardy-Weinberg principle of genetic equilibrium was formulated
1909	Wilhelm Johannsen	Terms phenotype and genotype are coined. The term gene is also used for the 1st time
1910	Thomas Hunt Morgan	Genes reside on chromosomes
1913	Alfred Sturtevant	The first genetic map is developed in *Drosophila*
1927	H.J. Muller	X-ray induce mutations in *Drosophila*
1928	F. Griffith	Transformation of Pneumoccoci was obtained. This was the critical experiments that lead to the eventual discovery that DNA as the genetic material
1944	Oswald T Avery, Colin M MacLeod, Maclyn McCarthy	Extending the experiments of Griffith (1929), it is first shown that DNA is the genetic material
1950	Erwin Chargaff	Adenine, Thymine, Guanine, Cytocine and Uracil. It is demonstrated that within all DNA molecules, the number of adenines equals the number of thymine, and the number of guanine equals the number of cytosine
1953	James Watson Francis Crick	A structural model of DNA is presented that states it consists of two antiparallel chains held together by hydrogen bonds. The model suggests a model of DNA replication
1954	George Gamow	Suggested that DNA contains a code that is responsible for the production of proteins
1956	Joe Hin Tjio Albert Levan	Established the correct chromosome number in humans to be 46
1966	Marshall Nirenberg, H Gobind Khorana, Sydney Brenner, Francis Crick	Cracked the genetic code that triplet mRNA codons specify each of the twenty amino acids
1972	Paul Berg	The first recombinant DNA molecule was created by splicing together bacterial and viral DNA
1980	Sanger Group	The first complete genome sequence was published
1981	Krontiris and Cooper	Discovery of human oncogenes
1983	Kary B Mullis	Discovers the PCR enabling the easy amplification of DNA
1990	US Government	The 15-year Human Genome Program is launched. The goal is to "find all the genes on every chromosome in the body and to determine their biochemical nature."
1997	Ian Wilmut Roslin Institute Scotland	First cloning of a mammal (Dolly the Sheep)
2001	International Human Genome Sequencing Consortium Celera Corp	The human genome sequence was published
2003	British Columbia Cancer Agency	The SARS-associated coronavirus genome sequence released. The sequence is released <5 months after the disease began spreading worldwide
2004	Solexa (now illumina)	The sequencing-by-synthesis technology is developed. This is the first of the genome resequencing technologies developed. The age of personalized genome sequences is launched
2010	Pacific biosciences	Single molecule DNA sequencer released
2011	Sequencing in progress to date	In viruses, microbes, fungi, animals and plants

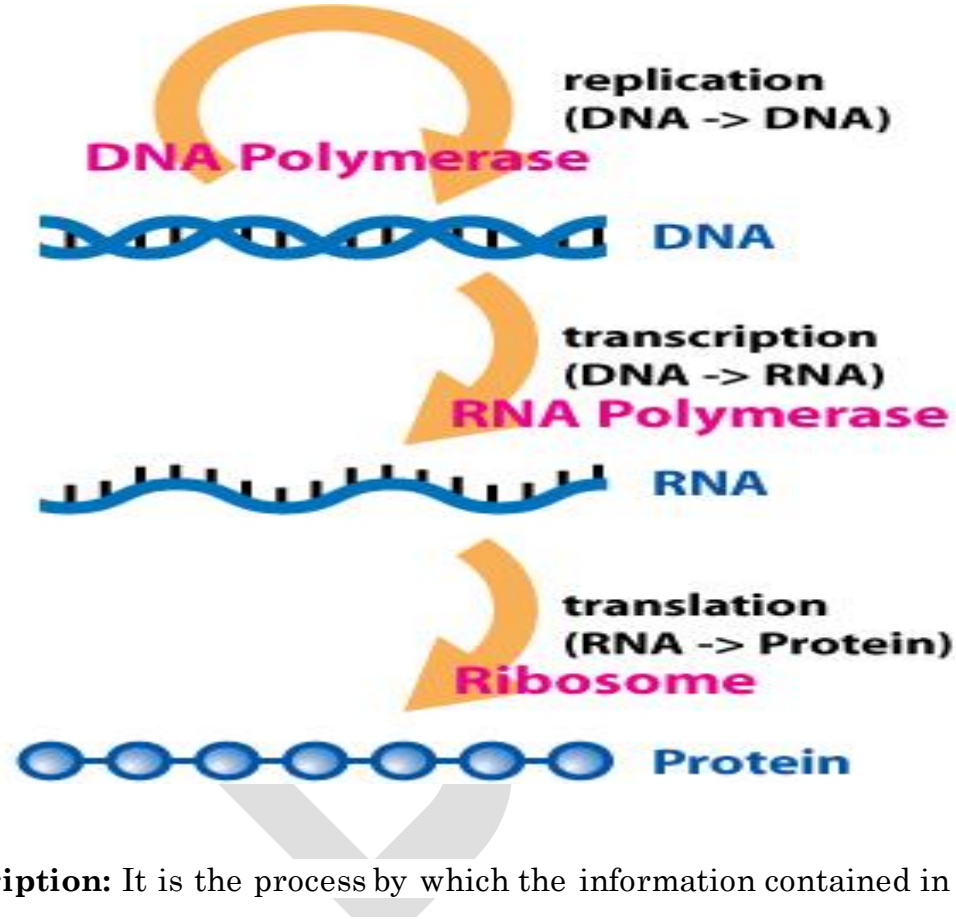
PCR=Polymerase chain reaction

SARS= severe acute respiratory syndrome

Sr. no.	Question	Answer
1	**Who gives theory of origin of species?**	**Charles darvin**
2	Full form of PCR	Polymerase chain reaction
3	who is father of genetics?	George mendle
4	Who invented PCR technique?	Kary B Mulis
5	Give chargaff rule	A=T, G=C
6	Who proposed DNA structure?	Watson and crick

CENTRAL DOGMA OF LIFE

The **central dogma of molecular biology** is an explanation of the flow of genetic information within a biological system. It is often stated as "DNA makes RNA, and RNA makes protein", although this is not its original meaning. It was first stated by Francis Crick in 1957, then published in 1958:



Transcription: It is the process by which the information contained in a section of DNA is replicated in the form of a newly assembled piece of messenger RNA (mRNA). Enzymes facilitating the process include RNA polymerase and transcription factors.

DNA → RNA

Translation: The mature mRNA finds its way to a ribosome, where it gets translated. In prokaryotic cells, which have no nuclear compartment, the processes of transcription and translation may be linked together without clear separation. In eukaryotic cells, the site of transcription (the cell nucleus) is usually separated from the site of translation

(the cytoplasm), so the mRNA must be transported out of the nucleus into the cytoplasm, where it can be bound by ribosomes.

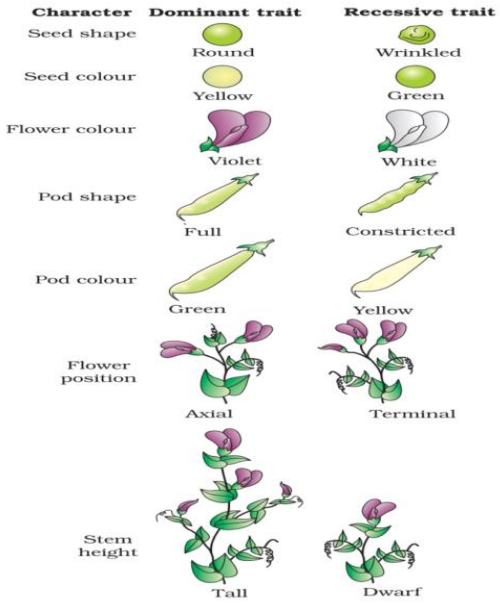
RNA → PROTEIN

Sr. no.	Question	Answer
1	**Who proposed central dogma of life?**	Francis Crick
2	What is central dogma of life?	DNA→RNA→Protein
3	Name of process which convert DNA to RNA.	Transcription
4	Name of process which convert RNA to Protein.	Translation
5	Which enzyme used in DNA replication?.	DNA polymerase
6	Which enzyme used in RNA synthesis?	RNA polymerase

1.2 MENDELIAN LAWS OF INHERITANCE

- It was during the mid-nineteenth century that headway was made in the understanding of inheritance. Gregor Mendel, conducted hybridisation experiments on garden peas for seven years (1856-1863) and proposed the laws of inheritance in living organisms. During Mendel's investigations into inheritance patterns it was for the first time that statistical analysis and mathematical logic were applied to problems in biology.
- His experiments had a large sampling size, which gave greater credibility to the data that he collected. Also, the confirmation of his inferences from experiments on successive generations of his test plants, proved that his res ults pointed to general rules of inheritance rather than being unsubstantiated ideas.
- Mendel investigated characters in the garden pea plant that were manifested as two opposing traits, e.g., tall or dwarf plants, yellow or green seeds. This allowed him to set up a basic framework of rules governing inheritance, which was expanded on by later scientists to account for all the diverse natural observations and the complexity inherent in them.
- Mendel conducted such artificial pollination/cross pollination experiments using several true-breeding pea lines. A truebreeding line is one that, having undergone continuous self-pollination, shows the stable trait inheritance and expression for several generations.
- Mendel selected 14 true-breeding pea plant varieties, as pairs which were similar except for one character with contrasting traits. Some of the contrasting traits selected were smooth or wrinkled seeds, yellow or green seeds, inflated (full) or constricted green or yellow pods and tall or dwarf plants**Figure 1, Table 1). Characters of pea plant**

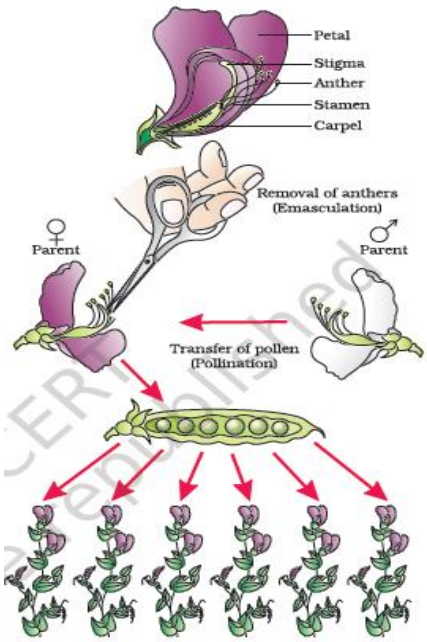
S.No.	Characters	Contrasting Traits
1.	Stem height	Tall/dwarf
2.	Flower colour	Violet/white
3.	Flower position	Axial/terminal
4.	Pod shape	Inflated/constricted
5.	Pod colour	Green/yellow
6.	Seed shape	Round/wrinkled
7.	Seed colour	Yellow/green

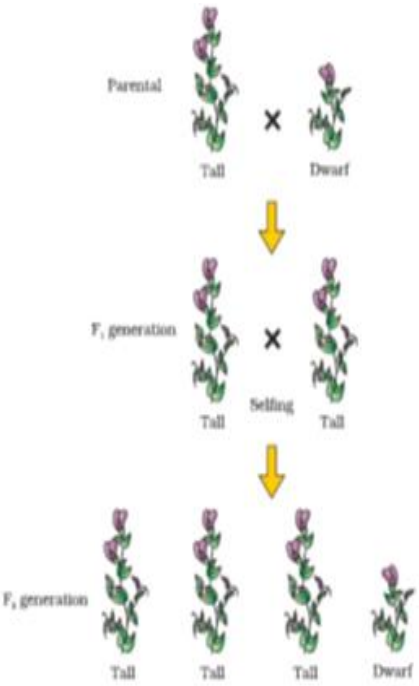


INHERITANCE OF ONE GENE

- Let us take the example of one such hybridisation experiment carried out by Mendel where he crossed tall and dwarf pea plants to study the inheritance of one gene (Figure 2).
- He collected the seeds produced as a result of this cross and grew them to generate plants of the first hybrid generation. This generation is also called the Filial progeny or the F1. Mendel observed that all the F1 progeny plants were tall, like one of its parents; none were dwarf (Figure 3).
- He made similar observations for the other pairs of traits – he found that the F1 always resembled either one of the parents, and that the trait of the other parent was not seen in them.
- Mendel then self-pollinated the tall F1 plants and to his surprise found that in the Filial2 generation some of the offspring were 'dwarf'; the character that was not seen in the F1 generation was now expressed.

Figure 2: Steps in making a cross in pea **figure 3:** Diagrammatic representation of monohybrid cross





- ❖
- ❖
- ❖ The proportion of plants that were dwarf were 1/4th of the F2 plants while 3/4th of the F2 plants were tall. The tall and dwarf traits were identical to their parental type and did not show any blending, that is all the offspring were either tall or dwarf, none were of in between height (Figure.3).
- ❖ Similar results were obtained with the other traits that he studied: only one of the parental traits was expressed in the F1 generation while at the F2 stage both the traits were expressed in the proportion 3:1. The contrasting traits did not show any blending at either F1 or F2 stage.
- ❖ Based on these observations, Mendel proposed that something was being stably passed down, unchanged, from parent to offspring through the gametes, over successive generations. He called these things as 'factors'. Now we call them as genes. Genes, therefore, are the units of inheritance.
- ❖ They contain the information that is required to express a particular trait in an organism.
- ❖ Genes which code for a pair of contrasting traits are known as alleles, i.e., they are slightly different forms of the same gene. If we use alphabetical symbols for each gene, then the capital letter is used for the trait expressed at the F1 stage and the small alphabet for the other trait.

- For example, in case of the character of height, T is used for the Tall trait and t for the 'dwarf', and T and t are alleles of each other. Hence, in plants the pair of alleles for height would be TT, Tt or tt. Mendel also proposed that in a true breeding, tall or dwarf pea variety the allelic pair of genes for height are identical or homozygous, TT and tt, respectively.
- TT and tt are called the genotype of the plant while the descriptive terms tall and dwarf are the phenotype. What then would be the phenotype of a plant that had a genotype Tt? As Mendel found the phenotype of the F1 heterozygote Tt to be exactly like the TT parent in appearance, he proposed that in a pair of dissimilar factors, one dominates the other (as in the F1) and hence is called the dominant factor while the other factor is recessive . In this case T (for tallness) is dominant over t (for dwarfness), that is recessive.
- He observed identical behaviour for all the other characters/trait-pairs that he studied. It is convenient (and logical) to use the capital and lower case of an alphabetical symbol to remember this concept of dominance and recessiveness. (Do not use T for tall and d for dwarf because you will find it difficult to remember whether T and d are alleles of the same gene/character or not).
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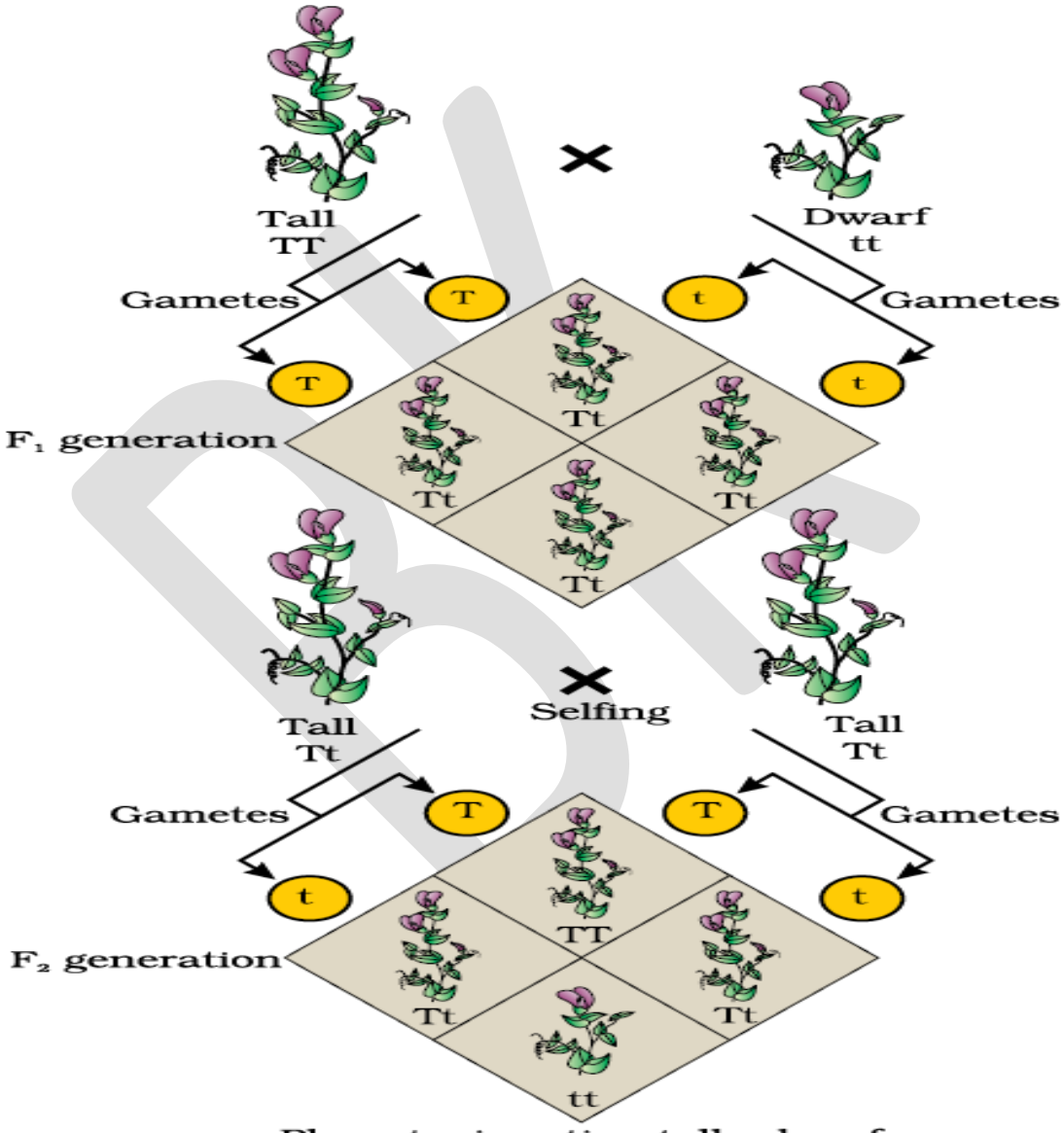
- Alleles can be similar as in the case of homozygotes TT and tt or can be dissimilar as in the case of the heterozygote T t. Since the Tt plant is heterozygous for genes controlling one character (height), it is a monohybrid and the cross between TT and tt is a monohybrid cross.
- From the observation that the recessive parental trait is expressed without any blending in the F2 generation, we can infer that, when the tall and dwarf plant produce gametes, by the process of meiosis, the alleles of the parental pair separate or segregate from each other and only one allele is transmitted to a gamete.
- This segregation of alleles is a random process and so there is a 50 per cent chance of a gamete containing either allele, as has been verified by the results of the crossings. In this way the gametes of the tall TT plants have the allele T and the gametes of the dwarf tt plants have the allele t.

- During fertilisation the two alleles, T from one parent say, through the pollen, and t from the other parent, then through the egg, are united to produce zygotes that have one T allele and one t allele. In other words the hybrids have Tt.

figure 4: A Punnett square used to understand a typical monohybrid cross conducted by Mendel between true-breeding tall plants and true-breeding dwarf plants



Phenotypic ratio : tall : dwarf
3 : 1
Genotypic ratio : TT : Tt : tt
1 : 2 : 1

- Since these hybrids contain alleles which express contrasting traits, the plants are heterozygous. The production of gametes by the parents, the formation of the zygotes, the F1 and F2 plants can be understood from a

diagram called Punnett Square as shown in Figure 4. It was developed by a British geneticist, Reginald C. Punnett.

- It is a graphical representation to calculate the probability of all possible genotypes of offspring in a genetic cross.
- The possible gametes are written on two sides, usually the top row and left columns. All possible combinations are represented in boxes below in the squares, which generates a square output form.
- The Punnett Square shows the parental tall TT (male) the Tt plant is heterozygous for genes controlling one character (height), it is a monohybrid and the cross between TT and tt is a monohybrid cross.
- From the observation that the recessive parental trait is expressed without any blending in the F2 generation, we can infer that, when the tall and dwarf plant produce gametes, by the process of meiosis, the alleles of the parental pair separate or segregate from each other and only one allele is transmitted to a gamete.
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- In other words the hybrids have Tt. Since these hybrids contain alleles which express contrasting traits, the plants are heterozygous. The production of gametes by the parents, the formation of the zygotes, the F1 and F2 plants can be understood from a diagram called Punnett Square as shown in Figure .4.
- It was developed by a British geneticist, Reginald C. Punnett. It is a graphical representation to calculate the probability of all possible genotypes of offspring in a genetic cross. The possible gametes are written on two sides, usually the top row and left columns.

- All possible combinations are represented in boxes below in the squares, which generates a square output form. The Punnett Square shows the parental tall TT (male) and dwarf tt (female) plants, the gametes produced by them and, the F1 Tt progeny.
- The F1 plants of genotype Tt are self-pollinated. The symbols & and % are used to denote the female (eggs) and male (pollen) of the F1 generation, respectively. The F1 plant of the genotype Tt when self-pollinated, produces gametes of the genotype T and t in equal proportion.
- When fertilisation takes place, the pollen grains of genotype T have a 50 per cent chance to pollinate eggs of the genotype T, as well as of genotype t. Also pollen grains of genotype t have a 50 per cent chance of pollinating eggs of genotype T, as well as of genotype t.
- As a result of random fertilisation, the resultant zygotes can be of the genotypes TT, Tt or tt. From the Punnett square it is easily seen that 1/4th of the random fertilisations lead to TT, 1/2 lead to Tt and 1/4th to tt.
- Though the F1 have a genotype of Tt, but the phenotypic character seen is 'tall'. At F2, 3/4th of the plants are tall, where some of them are TT while others are Tt. Externally it is not possible to distinguish between the plants with the genotypes TT and Tt.
- Hence, within the genopytic pair Tt only one character 'T' tall is expressed. Hence the character T or 'tall' is said to dominate over the other allele t or 'dwarf' character.
- It is thus due to this dominance of one character over the other that all the F1 are tall (though the genotype is Tt) and in the F2 3/4th of the plants are tall (though genotypically 1/2 are Tt and only 1/4th are TT). This leads to a phenotypic ratio of 3/4th tall : (1/4 TT + 1/2 Tt) and 1/4th tt, i.e., a 3:1 ratio, but a genotypic ratio of 1:2:1.
- The 1/4 : 1/2 : 1/4 ratio of TT: Tt: tt is mathematically condensable to the form of the binomial expression (ax +by)2, that has the gametes bearing genes T or t in equal frequency of ½. The expression is expanded as given below :
- (1/2T + 1/2 t)2 = (1/2T + 1/2t) X (1/2T + 1/2t) = 1/4 TT + 1/2Tt + 1/4 tt

- Mendel self-pollinated the F2 plants and found that dwarf F2 plants continued to generate dwarf plants in F3 and F4 generations. He concluded that the genotype of the dwarfs was homozygous – tt.
- What do you think he would have got had he self-pollinated a tall F2 plant? From the preceeding paragraphs it is clear that though the genotypic ratios can be calculated using mathematical probability, by simply looking at the phenotype of a dominant trait, it is not possible to know the genotypic composition.
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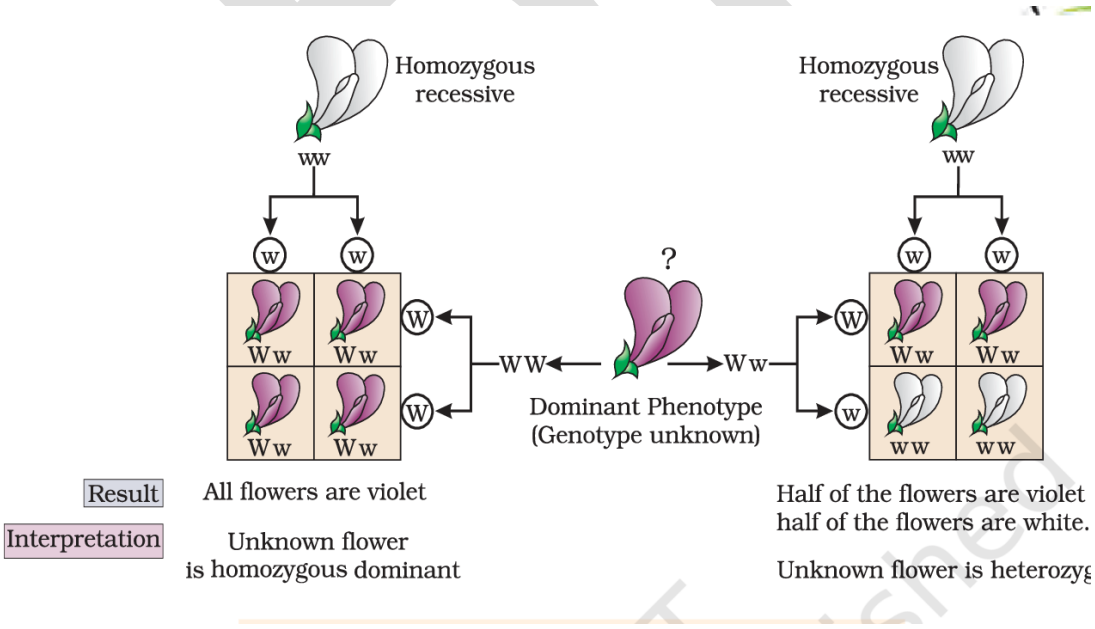
- That is, for example, whether a tall plant from F1 or F2 has TT or Tt composition, cannot be predicted. Therefore, to determine the genotype of a tall plant at F2, Mendel crossed the tall plant from F2 with a dwarf plant. This he called a test cross.
- In a typical test cross an organism (pea plants here) showing a dominant phenotype (and whose genotype is to be determined) is crossed with the recessive parent instead of self-crossing.
- **Figure 5: Diagrammatic representation of a test cross**



- The progenies of such a cross can easily be analysed to predict the genotype of the test organism. Figure 5 shows the results of typical test cross where violet colour flower (W) is dominant over white colour flower (w).
- *Using Punnett square, try to find out the nature of offspring of a test cross.*

What ratio did you get? Using the genotypes of this cross, can you give Based on his observations on monohybrid crosses Mendel proposed two general rules to consolidate his understanding of inheritance in monohybrid crosses.

- Today these rules are called the Principles or Laws of Inheritance: the First Law or Law of Dominance and the
- Second Law or Law of Segregation.

Law of Dominance

- Characters are controlled by discrete units called factors.
- Factors occur in pairs.
- In a dissimilar pair of factors one member of the pair dominates
- (dominant) the other (recessive).
- The law of dominance is used to explain the expression of only one of the parental characters in a monohybrid cross in the F1 and the expression of both in the F2. It also explains the proportion of 3:1 obtained at the F2.

Law of Segregation

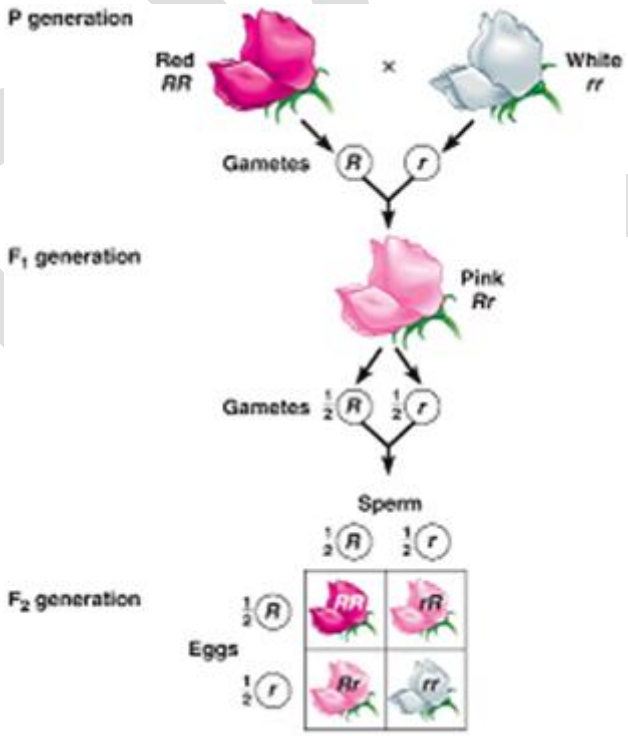
- This law is based on the fact that the alleles do not show any blending and that both the characters are recovered as such in the F2 generation though one of these is not seen at the F1 stage.
- Though the parents contain two alleles during gamete formation, the factors or alleles of a pair segregate from each other such that a gamete receives only one of the two factors. Of course, a homozygous parent produces all gametes that are similar while a heterozygous one produces two kinds of gametes each having one allele with equal proportion.

Sr. no.	**Question**	**Answer**
1	**Which plant is used by mendel?**	**Pea**
2	Which character is used by mendel?	Height of plant
3	Which symbol is used for showing height?	Alphabet
4	Which alphabet is used for dwarft plant?	t
5	Example of monohybrid cross.	TT and tt

6	What is proportion of law of dominance?	3:1

Incomplete Dominance

- When experiments on peas were repeated using other traits in other plants, it was found that sometimes the F1 had a phenotype that did not resemble either of the two parents and was in between the two.
- The inheritance of flower colour in the dog flower (snapdragon or *Antirrhinum sp*.) is a good example to understand incomplete dominance.
- **Figure 6 incomplete dominance**



-
- In a cross between true-breeding red-flowered (RR) and truebreeding white-flowered plants (rr), the F1 (Rr) was pink (Figure 5.6). When the F1 was self-pollinated the F2 resulted in the following ratio 1 (RR) Red: 2 (Rr) Pink: 1 (rr) White. Here the genotype ratios were exactly as we would expect in any mendelian monohybrid cross, but the phenotype ratios had changed from the 3:1 dominant : recessive ratio.
- What happened was that R was not completely dominant over r and this made it possible to distinguish Rr as pink from RR (red) and rr (white).

Explanation of the concept of dominance: What exactly is dominance? Why are some alleles dominant and some recessive? To tackle these questions, we must understand what a gene does. Every gene, as you know by now, contains the information to express a particular trait.

- In a diploid organism, there are two copies of each gene, i.e., as a pair of alleles. Now, these two alleles need not always be identical, as in a heterozygote.
- One of them may be different due to some changes that it has undergone (about which you will read further on, and in the next chapter) which modifies the information that particular allele contains.
- Let's take an example of a gene that contains the information for producing an enzyme. Now there are two copies of this gene, the two allelic forms. Let us assume (as is more common) that the normal allele produces the normal enzyme that is needed for the transformation of a substrate S.
- Theoretically, the modified allele could be responsible for production of the normal/less efficient enzyme, or a non-functional enzyme, or no enzyme at all Figure 5.6 Results of monohybrid cross in the plant Snapdragon, where one allele is incompletely dominant over the other allele.
- In the first case, the modified allele is equivalent to the unmodified allele, i.e., it will produce the same phenotype/trait, i.e., result in the transformation of substrate S. Such equivalent allele pairs are very common.
- But, if the allele produces a non-functional enzyme or no enzyme, the phenotype may be effected.
- The phenotype/trait will only be dependent on the functioning of the unmodified allele. The unmodified (functioning) allele, which represents the original phenotype is the dominant allele and the modified allele is generally the recessive allele.
- Hence, in the example above the recessive trait is seen due to non-functional enzyme or because no enzyme is produced.

Co-dominance

- Till now we were discussing crosses where the F1 resembled either of the two parents (dominance) or was in-between (incomplete dominance).
- But, in the case of co-dominance the F1 generation resembles both parents. A good example is different types of red blood cells that determine ABO blood grouping in human beings.
- ABO blood groups are controlled by the gene *I*. The plasma membrane of the red blood cells has sugar polymers that protrude from its surface and the kind of sugar is controlled by the gene.
- The gene (*I*) has three alleles *IA*, *IB* and *i*. The alleles *IA* and *IB* produce a slightly different form of the sugar while allele *i* does not produce any sugar. Because humans are diploid organisms, each person possesses any two of the three *I* gene alleles.
-
- *IA* and *IB* are completely dominant over *i*, in other words when *IA* and *i* are present only *IA* expresses (because *I* does not produce any sugar), and when *IB* and *i* are present *IB* expresses.
- But when *IA* and *IB* are present together they both express their own types of sugars: this is because of co-dominance. Hence red blood cells have both A and B types of sugars.
- Since there are three different alleles, there are six different combinations of these three alleles that are possible, and therefore, a total of six different genotypes of the human ABO blood types (Table 5.2). *How many phenotypes are possible?*
- Table .2: Table Showing the Genetic Basis of Blood Groups
- in Human Population Allele from Allele from Genotype of Blood Parent 1 Parent 2 offspring types of offspring.
- **Table 2 blood group and genes**

in Human Population

Allele from Parent 1	Allele from Parent 2	Genotype of offspring	Blood types of offspring
I^A	I^A	$I^A I^A$	A
I^A	I^B	$I^A I^B$	AB
I^A	i	$I^A i$	A
I^B	I^A	$I^A I^B$	AB
I^B	I^B	$I^B I^B$	B
I^B	i	$I^B i$	B
i	i	$i\ i$	O

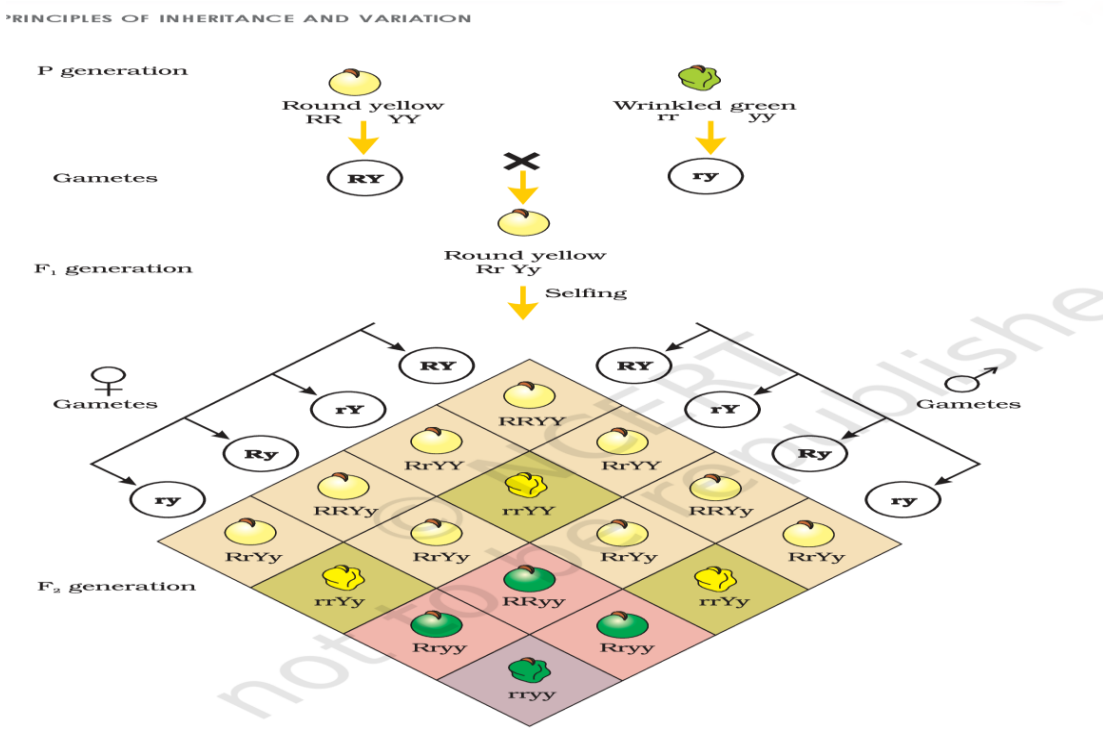
- Do you realise that the example of ABO blood grouping also provides a good example of multiple alleles? Here you can see that there are more than two, i.e., three alleles, governing the same character.
- Since in an individual only two alleles can be present, multiple alleles can be found only when population studies are made. Occasionally, a single gene product may produce more than one effect.
- For example, starch synthesis in pea seeds is controlled by one gene. It has two alleles (B and b). Starch is synthesised effectively by BB homozygotes and therefore, large starch grains are produced.
- In contrast, bb homozygotes have lesser efficiency in starch synthesis and produce smaller starch grains. After maturation of the seeds, BB seeds are round and the bb seeds are wrinkled.
- Heterozygotes produce round seeds, and so B seems to be the dominant allele. But, the starch grains produced are of intermediate size in Bb seeds. So if starch grain size is considered as the phenotype, then from this angle, the alleles show incomplete dominance.
- Therefore, dominance is not an autonomous feature of a gene or the product that it has information for.
- It depends as much on the gene product and the production of a particular phenotype from this product as it does on the particular phenotype that we

choose to examine, in case more than one phenotype is influenced by the same gene.

Sr. no.	Question	Answer
1	**How many types of dominance?**	**Two**
2	Which character is used for incomplete dominance?	Color
3	Which character is used for co-dominance?	Blood group
4	Which type of blood group system is used?	ABO
5	Which alphabet is used for gene?	I and i
6	Which alphabet is used for homozygote seed in pea plant?	BB and bb

❖ INHERITANCE OF TWO GENES

- ❖ Mendel also worked with and crossed pea plants that differed in two characters, as is seen in the cross between a pea plant that has seeds with yellow colour and round shape and one that had seeds of green colour and wrinkled shape (Figure.7). Mendel found that the seeds resulting from the crossing of the parents, had yellow coloured and round shaped seeds.
- ❖ *Here can you tell which of the characters in the pairs yellow/green colour and round/wrinkled shape was dominant?* Thus, yellow colour was dominant over green and round shape dominant over wrinkled.
- ❖ These results were identical to those that he got when he made separate monohybrid crosses between yellow and green seeded plants and between round and wrinkled seeded plants.
- ❖ Let us use the genotypic symbols Y for dominant yellow seed colour and y for recessive green seed colour, R for round shaped seeds and r for wrinkled seed shape.
- ❖ **Figure 7 inheritance of two genes**



❖

❖ The genotype of the parents can then be written as RRYY and rryy. The cross between the two plants can be written down as in Figure 5.7 showing the genotypes of the parent plants.

❖ The gametes RY and ry unite on fertilisation to produce the F1 hybrid RrYy.

❖ When Mendel self hybridised the F1 plants he found that 3/4th of F2 plants had yellow seeds and 1/4th had green.

❖ The yellow and green colour segregated in a 3:1 ratio. Round and wrinkled seed shape also segregated in a 3:1 ratio; just like in a monohybrid cross.

Sr. no.	**Question**	**Answer**
1	**How many character used by mendel?**	**Two**
2	Which character is used by mendel ?	Shape and color
3	What is segregated ratio of color character?	3:1
4	What is segregated ratio of shape character?	3:1

5	Which alphabet is used for wrinkled shape?	r

Law of Independent Assortment

- In the dihybrid cross (Figure 7), the phenotypes round, yellow; wrinkled, yellow; round, green and wrinkled, green appeared in the ratio 9:3:3:1. Such a ratio was observed for several pairs of characters that Mendel studied. The ratio of 9:3:3:1 can be derived as a combination series of 3 yellow: 1 green, with 3 round : 1 wrinkled.
- This derivation can be written as follows: (3 Round : 1 Wrinkled) (3 Yellow : 1 Green) = 9 Round, Yellow : 3 Wrinkled, Yellow: 3 Round, Green : 1 Wrinkled, Green Based upon such observations on dihybrid crosses (crosses between plants differing in two traits) Mendel proposed a second set of generalisations that we call Mendel's Law of Independent Assortment.
- The law states that 'when two pairs of traits are combined in a hybrid, segregation of one pair of characters is independent of the other pair of characters'.
- The Punnett square can be effectively used to understand the independent segregation of the two pairs of genes during meiosis and the production of eggs and pollen in the F1 RrYy plant.
- Consider the segregation of one pair of genes R and r. Fifty per cent of the gametes have the gene R and the other 50 per cent have r. Now besides each gamete having either R or r, it should also have the allele Y or y.
- The important thing to remember here is that segregation of 50 per cent R and 50 per cent *r* is *independent* from the segregation of 50 per cent Y and 50 per cent y. Therefore, 50 per cent of the r bearing gametes has Y and the other 50 per cent has y.
- Similarly, 50 per cent of the R bearing gametes has Y and the other 50 per cent has y. Thus there are four genotypes of gametes (four types of pollen and four types of eggs).

- The four types are RY, Ry, r*Y* and ry each with a frequency of 25 percent or 1/4th of the total gametes produced.
- When you write down the four types of eggs and pollen on the two sides of a Punnett square it is very easy to derive the composition of the zygotes that give rise to the F2 plants (Figure .7).

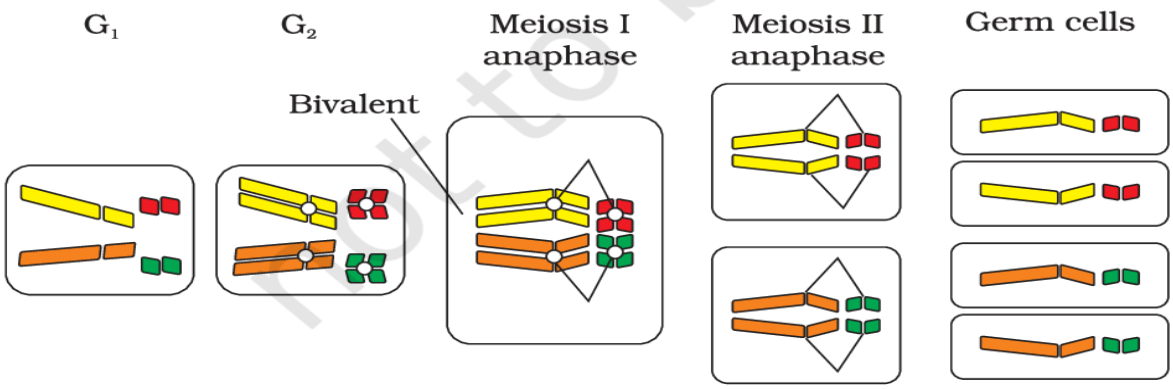
Sr. no.	Question	Answer
1	crosses between plants differing in two traits is known as……..	**Dihybrid cross**
2	Ratio of phenotype is…..	9:3:3:1
3	Which are four types of gametes are produced?	RY, Ry, rY and ry
4	What is the frequency of gametes?	¼

Chromosomal Theory of Inheritance

- Mendel published his work on inheritance of characters in 1865 but for several reasons, it remained unrecognised till 1900. Firstly, communication was not easy (as it is now) in those days and his work could not be widely publicised.
- Secondly, his concept of genes (or factors, in Mendel's words) as stable and discrete units that controlled the expression of traits and, of the pair of alleles which did not 'blend' with each other, was not accepted by his contemporaries as an explanation for the apparently continuous variation seen in nature.
- Thirdly, Mendel's approach of using mathematics to explain biological phenomena was totally new and unacceptable to many of the biologists of his time.
- Finally, though Mendel's work suggested that factors (genes) were discrete units, he could not provide any physical proof for the existence of factors or say what they were made of. In 1900, three Scientists (de Vries, Correns and von Tschermak) independently rediscovered Mendel's results on the inheritance of characters.

- Also, by this time due to advancements in microscopy that were taking place, scientists were able to carefully observe cell division. This led to the discovery of structures in the nucleus that appeared to double and divide just before each cell division.
- These were called chromosomes (*colored bodies*, as they were visualised by staining). By 1902, the chromosome movement during meiosis had been worked out.
- Walter Sutton and Theodore Boveri noted that the behaviour of chromosomes was parallel to the behaviour of genes and used chromosome movement (Figure .8) to explain Mendel's laws (Table 3).
- Recall that you have studied the behaviour of chromosomes during mitosis (equational division) and during meiosis (reduction division). The important things to remember are that chromosomes as well as genes occur in pairs. The two alleles of a gene pair are located on homologous sites on homologous chromosomes.
- **Figure .8 Meiosis and germ cell formation in a cell with four chromosomes**

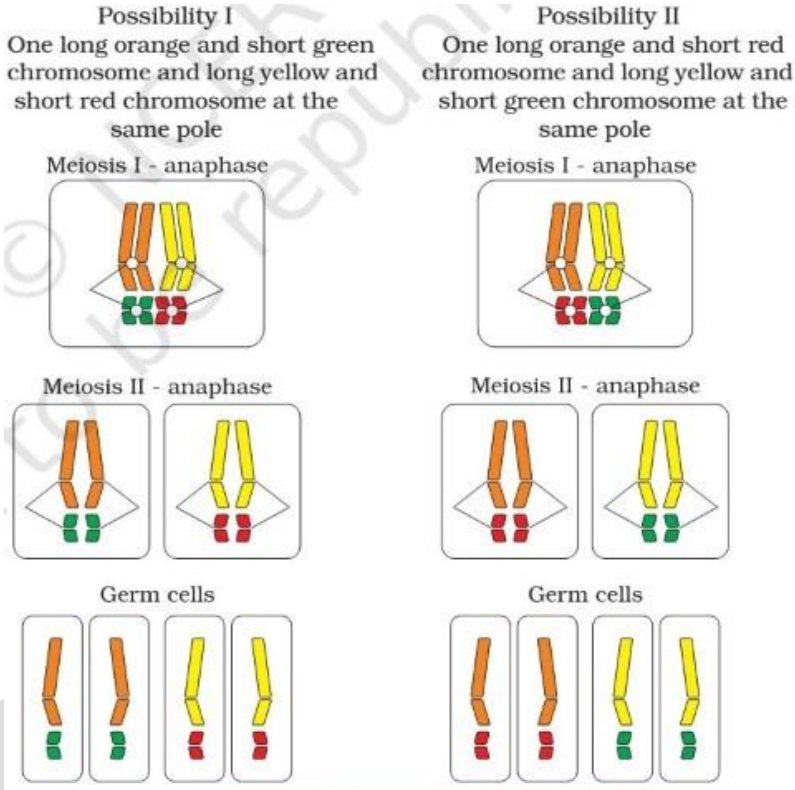
sites on homologous chromosomes.



-
- Can you see how chromosomes segregate when germ cells are formed?

- Possibility I Possibility II
- One long orange and short green One long orange and short red chromosome and long yellow and chromosome and long yellow and short red chromosome at the short green chromosome at the same pole same pole

❖ **Figure 9 Independent assortment of chromosomes**



Independent assortment of chromosomes

❖

❖

❖ *Can you tell which of these columns A or B represent the chromosome*

❖ *and which represents the gene? How did you decide?*

❖

❖ During Anaphase of meiosis I, the two chromosome pairs can align at the metaphase plate independently of each other (Figure 5.9).

❖ To understand this, compare the chromosomes of four different colour in the left and right columns. In the left column (Possibility I) orange and green is segregating together.

❖ But in the right hand column (PossibilityII) the orange chromosome is segregating with the red chromosomes.

❖ Table: A Comparison between the Behaviour of Chromosomes

❖ and Genes

❖ A

❖ *Occur in pairs*

❖ *Segregate at the time of gamete*

❖ *formation such that only one of each*

- *pair is transmitted to a gamete*
- *Independent pairs segregate*
- *independently of each other*
- B
- *Occur in pairs*
- *Segregate at gamete formation and only*
- *one of each pair is transmitted to a*
- *gamete*
- *One pair segregates independently of*
- *another pair*

PRINCIPLES OF INHERITANCE AND VARIATION

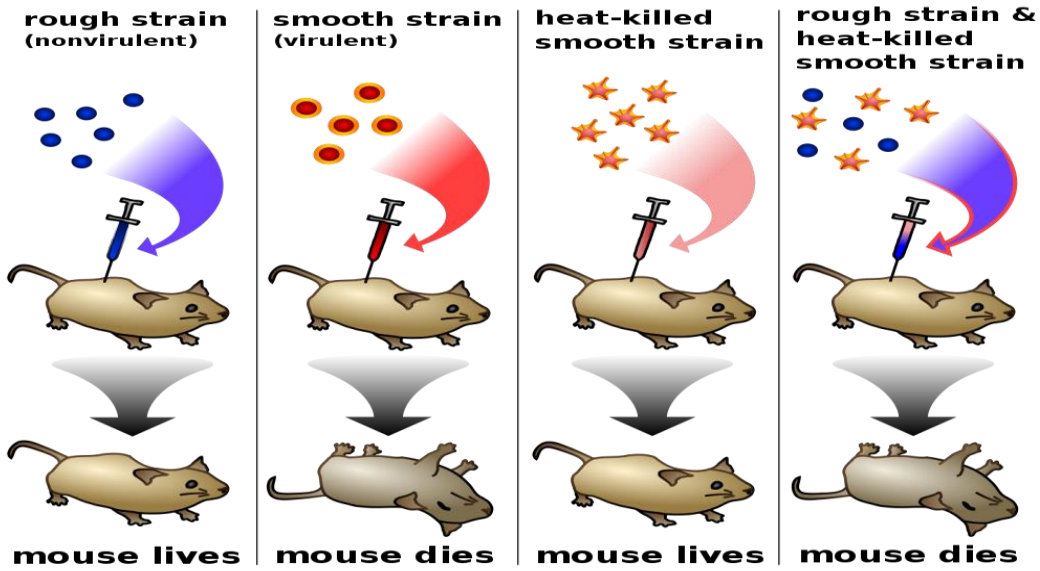
- *melanogaster* (a) Male
    - Female
- Sutton and Boveri argued that the pairing and separation of a pair of chromosomes would lead to the segregation of a pair of factors they carried. Sutton united the knowledge of chromosomal segregation with Mendelian principles and called it the chromosomal theory of inheritance.
- Following this synthesis of ideas, experimental verification of the chromosomal theory of inheritance by Thomas Hunt Morgan and his colleagues, led to discovering the basis for the variation that sexual reproduction produced.
- Morgan worked with the tiny fruit flies, *Drosophila melanogaster* (Figure 5.10), which were found very suitable for such studies. They could be grown on simple synthetic medium in the laboratory.
- They complete their life cycle in about two weeks, and a single mating could produce a large number of progeny flies Also, there was a clear differentiation of the sexes – the male and female flies are easily distinguishable.
- Also, it has many types of hereditary variations that can be seen with low power microscopes.

Sr. no.	Question	Answer
1	**Which scientist rediscover mendel's work?**	de Vries, Correns and von Tschermak
2	Miosis is also known as…….	reduction division
3	Who discover the basis for the variation that sexual reproduction produced.	Thomas Hunt Morgan and his colleagues
4	Mitosis is also known as…….	equational division
5	Morgan did his work using which organisms?	Fruit fly
6	What is the scientific name of fruit fly?	*Drosophila melanogaster*

1.3 DNA IS THE UNIVERSAL GENETIC MATERIAL & EXPERIMENTAL EVIDENCES

Our modern understanding of DNA's role in heredity has led to a variety of practical applications, including forensic analysis, paternity testing, and genetic screening. Thanks to these wide ranging uses, today many people have at least basic awareness of DNA. It may be surprising, then to realize that less than a century ago, even the best educated members of the scientific community did not known that DNA was the hereditary material.

Frederic Griffith's Experiment: bacterial transformation

- **Griffith's experiment**, reported in 1928 by Frederick Griffith, was the first experiment suggesting that bacteria are capable of transferring genetic information through a process known as transformation.
- Griffith's findings were followed by research in the late 1930s and early 40s that isolated DNA as the material that communicated this genetic information.
- **Figure 10 Griffiths experiment**
- 

- Pneumonia was a serious cause of death in the wake of the post-WWI Spanish influenza pandemic, and Griffith was studying the possibility of creating a vaccine.
- Griffith used two strains of pneumococcus (*Streptococcus pneumoniae*) bacteria which infect mice – a type III-S (smooth) which was virulent, and a type II-R (rough) strain which was nonvirulent.
- The III-S strain synthesized a polysaccharide capsule that protected itself from the host's immune system, resulting in the death of the host, while the II-R strain did not have that protective capsule and was defeated by the host's immune system.
- A German bacteriologist, Fred Neufeld, had discovered the three pneumococcal types (Types I, II, and III) and discovered the quellung reaction to identify them *in vitro*. Until Griffith's experiment, bacteriologists believed that the types were fixed and unchangeable, from one generation to another.
- In this experiment, bacteria from the III-S strain were killed by heat, and their remains were added to II-R strain bacteria. While neither alone harmed the mice, the combination was able to kill its host.
- Griffith was also able to isolate both live II-R and live III-S strains of pneumococcus from the blood of these dead mice.
- Griffith concluded that the type II-R had been "transformed" into the lethal III-S strain by a "transforming principle" that was somehow part of the dead III-S strain bacteria.
- Today, we know that the "transforming principle" Griffith observed was the DNA of the III-s strain bacteria. While the bacteria had been killed, the DNA had survived the heating process and was taken up by the II-R strain bacteria.
- The III-S strain DNA contains the genes that form the smooth protective polysaccharide capsule. Equipped with this gene, the former II-R strain

bacteria were now protected from the host's immune system and could kill the host.

- The exact nature of the transforming principle (DNA) was verified in the experiments done by Avery, McLeod and McCarty and by Hershey and Chase.

Sr. no.	Question	Answer
1	**Who proposed transformation theory?**	**Griffith**
2	Which organism used by Griffith?	Mice
3	Which microorganisms used by Griffith?	Bacteria
4	Which bacteria used by Griffith?	*Streptococcus pneumoniae*
5	Which character used by Griffith?	Smooth and rough strain

-

Avery, McCarty, and Macleod: identifying the transforming principle

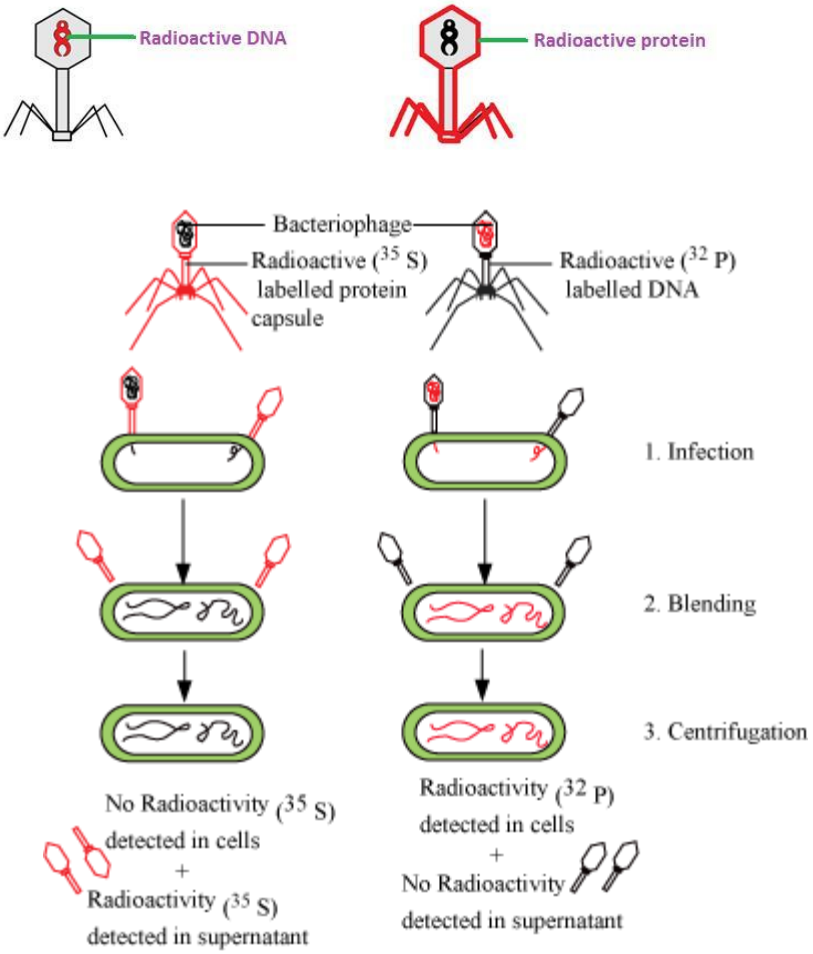
- **Principle:** in 1944, three Canadian and American researchers Oswald avery, maclyn mccart and colin macleod, set out to identify griffith's "transformation principle".
- to do so, they began with large cultures of heat killed S cells and through a long series of biochemical steps progressively purified the transforming principle by washing away, separating out, or enzymatically destroying the other cellular components.
- By this method they were able to obtain small amounts of highly purified transforming principle, which they could then analyze through other tests to determine its identity.
- Several times of evidence suggested to avery and his collegues that the transforming principle might be DNA.

- The purified substance gave a negative result in chemical test known to detect DNA.
- The elemental composition of the purified transforming principle closely resembled DNA in the ratio of nitrogen and phosphorus.
- Protein and RNA-degrading enzymes had little effect on the transforming principle, but enzyme able to degrade DNA eliminated the transforming activity.
- These results all pointed to DNA as the likely transforming principle. However, Avery was caution in interpreting his results. He realized that it was still possible that some contaminating substance present in small amounts. Not DNA was the transforming principle.
- Because of this possibility, debate over DNA's role continued until 1952. When Alfred Hershey and chase used a different approach to conclusively identify DNA as the genetic material.
- **R cells + purified S cell polysaccharide → R colonies**
- **R cells + purified S cell protein → R colonies**
- **R cells + purified S cell RNA → R colonies**
- **R cells + purified S cell DNA → S colonies**
- **S cell extract + protease+ R cells → S colonies**
- **S cell extract + Rnase + R cells → S colonies**

Sr. no.	Question	Answer
1	**Who prove that DNA is universal genetic material?**	**Avery,McCarty and Macleod**
2	Which result obtained if we use R cells with purified S cell DNA?	S colonies
3	Which result obtained if we use S cells with purified R cell DNA?	R colonies
4	Which result obtained if we use S cells with purified R cell polysaccharide?	S colonies
5	What is universal genetic material?	DNA

The Harshey-chase experiments

- Hershey-Chase experiment was performed in 1952 to further confirm that DNA was the genetic material. They experimented with Bacteriophages. Bacteriophages are the viruses that infect & replicate within bacteria. Bacteriophages were grown in two different mediums.
  - Some bacteriophages were grown in **radioactive phosphorus medium**. It was found that these Bacteriophages came up with **radioactive DNA**
  - Some bacteriophages were grown in **radioactive sulfur medium**. It was found that these Bacteriophages with **radioactive protein**.
  - **Figure 11 harshey chase experiment**



- Bacteriophages with Radioactive DNA were brought in contact with bacteria

- Bacteria got infected
- Agitated in a blender to separate phage particles from bacterial cells
- Centrifugation leaves Phage particles as supernatant
- Bacterial cells were found to be radioactive
- No radioactivity was detected in the phage particles

- Bacteriophages with Radioactive protein were brought in contact with bacteria
- Bacteria got infected
- Agitated in a blender to separate phage particles from bacterial cells
- Centrifugation leaves Phage particles as supernatant
- Phage particles were found to be radioactive
- No radioactivity was detected in the bacterial cells
- It was therefore concluded that it was not the proteins, rather DNA which entered into the bacteria. Therefore, DNA causes the replication of viruses inside the bacteria.
- DNA was thus proved to be the genetic material.

Sr. no.	**Question**	**Answer**
1	**Harshey chase experiment also known as ……..**	**Blender experiment**
2	Which microorganism used by harshey and chase?	Bcteriophage
3	Which technique used by harshey and chase?	Radio labelling
4	What is result obtained by harshey and chase?	DNA is genetic material not Protein

1.4 Gene structure and architechture in prokaryotes and eukaryotes

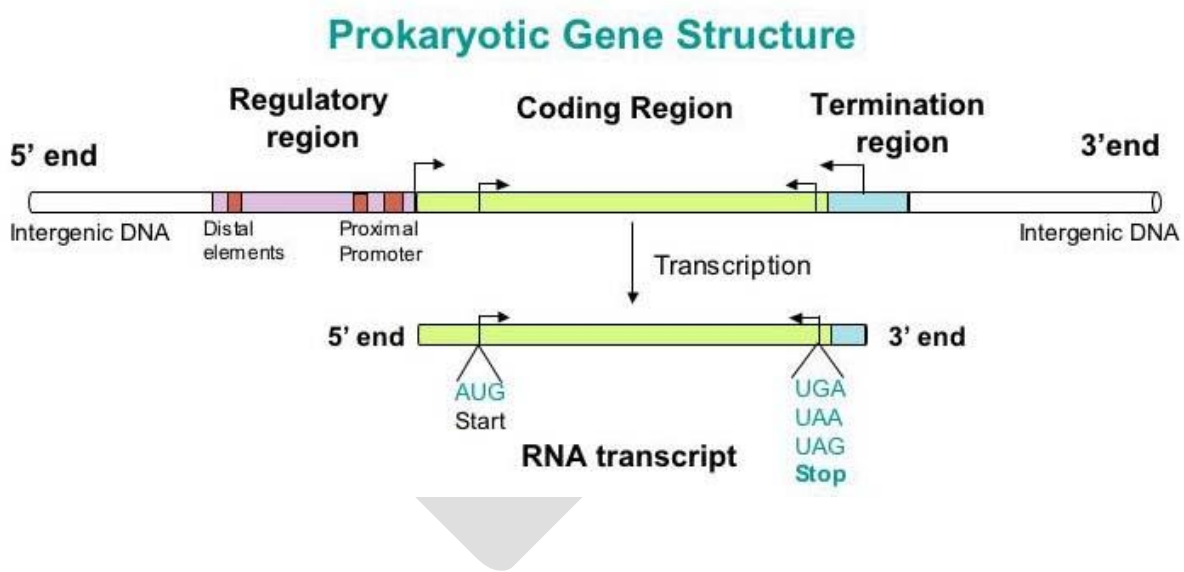
- **A gene is the entire nucleic acid sequence that is necessary for the controlled production of its final product (RNA or protein)**

  Cistron: a section of a DNA or RNA molecule that codes for a specific polypeptide in protein synthesis.

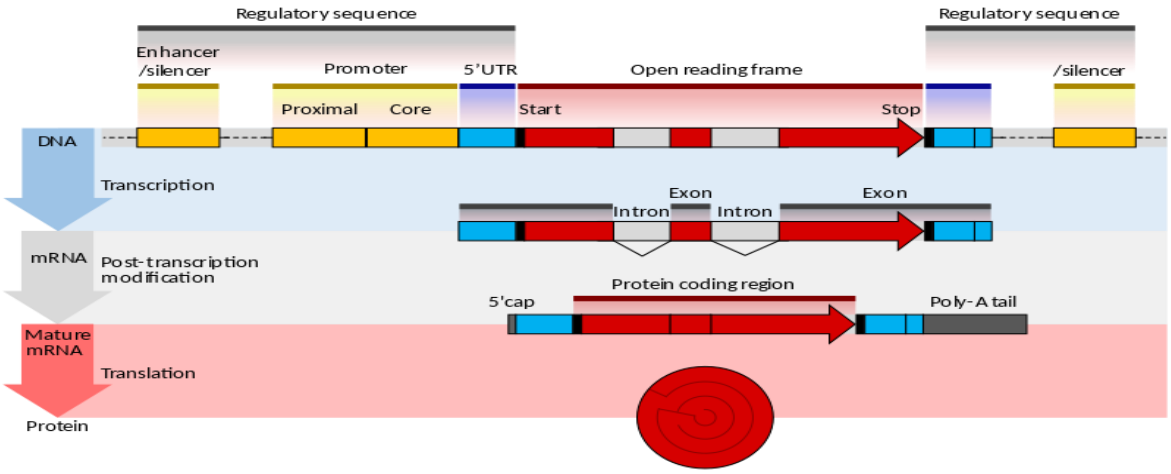
  Intron: a segment of a DNA or RNA molecule which does not code for proteins and interrupts the sequence of genes.

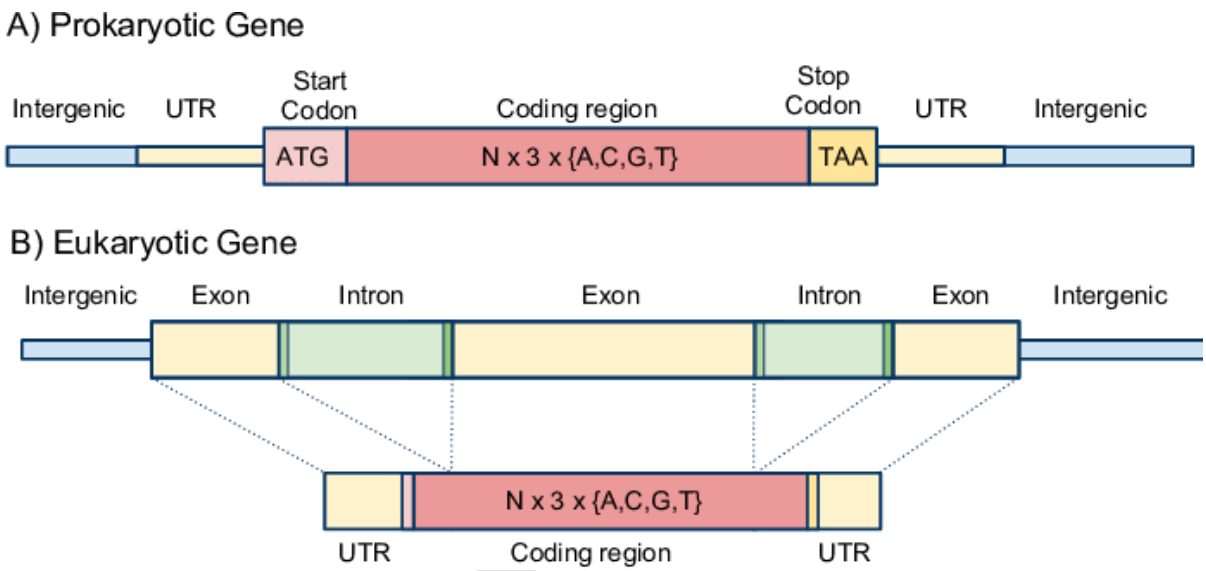
  Exon: a segment of a DNA or RNA molecule containing information coding for a protein or peptide sequence

- **figure 12 prokaryotic and eukaryotic gene structure**



Eukaryotic gene structure





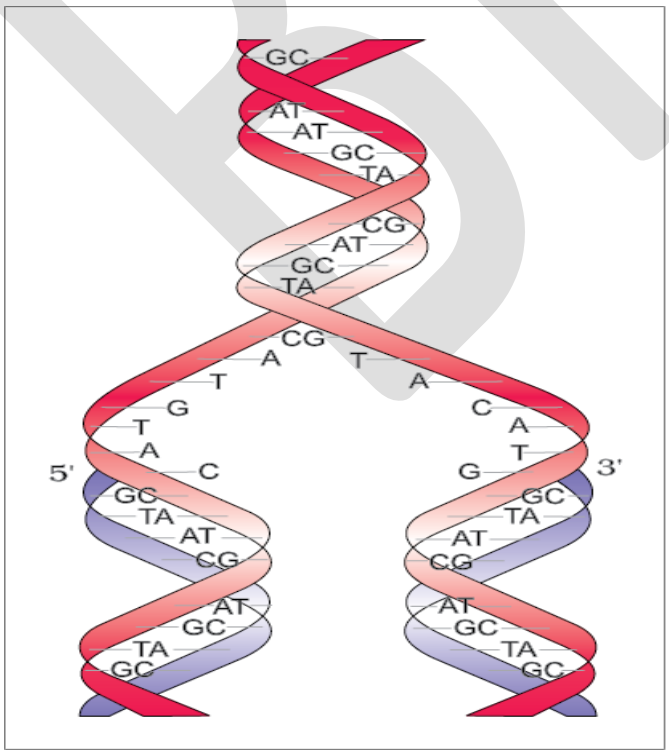
- **In eukaryotes, genes lie amidst a large expanse of noncoding DNA with unknown function and genes may also span regions of DNA unrelated to the gene.**
- **If a gene is incapable of producing a final gene product= pseudogene.**
- **Bacterial operons produce polycistron mRNA while most eukaryotic mRNAs are monocistronic and contain introns.**

Sr. no.	**Question**	**Answer**
1	**Non coding region is known as….**	**Intron**
2	Coding region is known as…..	Exon
3	polyocistronic mRNA produced by……	Bacteria
4	Pseudogene is present in ……gene.	Eukaryotic gene

1.5 Prokaryotic DNA replication: experiment, machineries, mechanisms & models

REPLICATION

- While proposing the double helical structure for DNA, Watson and Crick had immediately proposed a scheme for replication of DNA.
- To quote their original statement that is as follows: ‘‘It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material” (Watson and Crick, 1953).
- The scheme suggested that the two strands would separate and act as a template for the synthesis of new complementary strands. After the completion of replication, each DNA molecule would have one parental and one newly synthesised strand.
- This scheme was termed as **semiconservative DNA replication** (Figure 13).
- **Figure 13 semiconservative DNA replication**



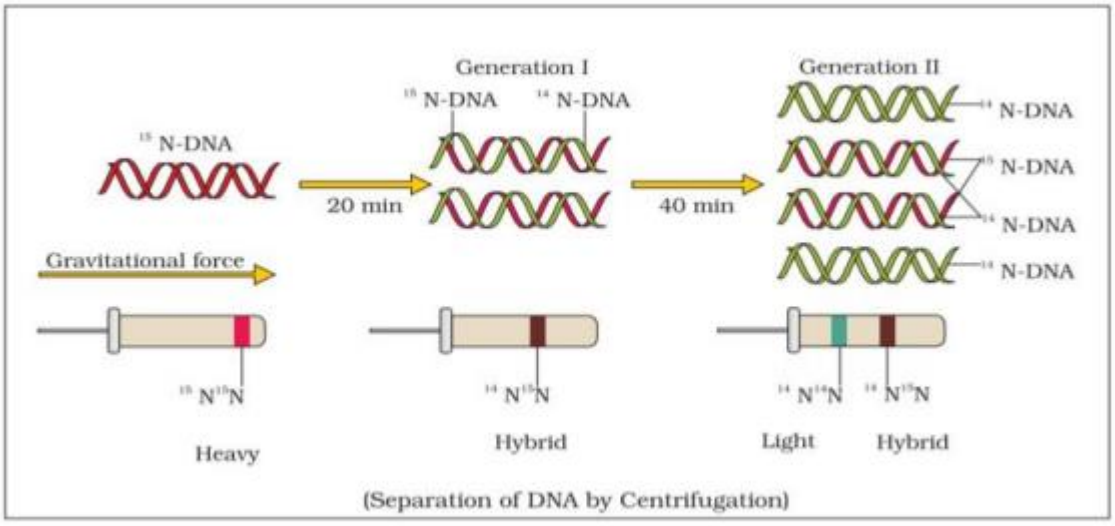
The Experimental Proof

- It is now proven that DNA replicates semiconservatively. It was shown first in *Escherichia coli* and subsequently in higher organisms, such as plants and human

cells. Matthew Meselson and Franklin Stahl performed the following experiment in 1958:

- They grew *E. coli* in a medium containing $^{15}NH_4Cl$ (^{15}N is the heavy isotope of nitrogen) as the only nitrogen source for many generations. The result was that ^{15}N was incorporated into newly synthesised DNA (as well as other nitrogen containing compounds). This heavy DNA molecule could be distinguished from the normal DNA by centrifugation in a cesium chloride (CsCl) density gradient (Please note that ^{15}N is not a radioactive isotope, and it can be separated from ^{14}N only based on densities).
- Then they transferred the cells into a medium with normal $^{14}NH_4Cl$ and took samples at various definite time intervals as the cells multiplied, and extracted the DNA that remained as double-stranded helices. The various samples were separated independently on CsCl gradients to measure the densities of DNA (Figure 2). *Can you recall what centrifugal force is, and think why a molecule with higher mass/density would sediment faster?*

- The results are shown in Figure 2.
- **Figure 13 result of experiment**



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- (iii) Thus, the DNA that was extracted from the culture one generation after the transfer from ^{15}N to ^{14}N medium [that is after 20 minutes; *E. coli* divides in 20 minutes] had a hybrid or intermediate density. DNA extracted from the culture after another generation [that is after 40 minutes, II generation] was composed of equal amounts of this hybrid DNA and of 'light' DNA.
- *If E. coli was allowed to grow for 80 minutes then what would be the proportions of light and hybrid densities DNA molecule?*

- Very similar experiments involving use of radioactive thymidine to detect distribution of newly synthesised DNA in the chromosomes was performed on *Vicia faba* (faba beans) by Taylor and colleagues in 1958. The experiments proved that the DNA in chromosomes also replicate semi conservatively.

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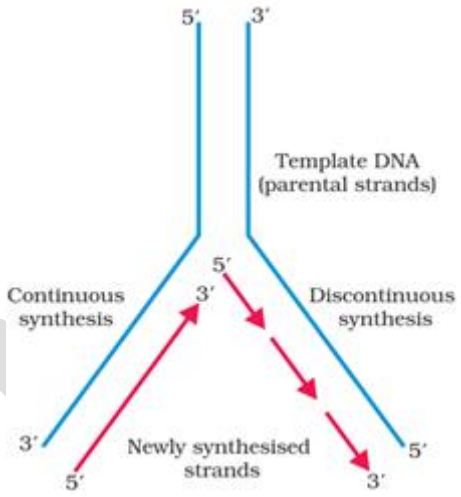
Sr. no.	Question	Answer
1	**Which mode of replication is approved?**	**Semi conservative**
2	Who proposed DNA replication?	Whatson and crick
3	Which medium used for centrifugation?	CsCl
4	Which centrifugation technique is used?	Density gradient
5	Which isotope is used in experiment?	N14

The Machinery and the Enzymes

- In living cells, such as *E. coli*, the process of replication requires a set of catalysts (enzymes). The main enzyme is referred to as DNA-dependent DNA polymerase, since it uses a DNA template to catalyse the polymerisation of deoxynucleotides.
- These enzymes are highly efficient enzymes as they have to catalyse polymerisation of a large number of nucleotides in a very short time. *E. coli* that has only 4.6×10^6 bp (compare it with human whose diploid content is 6.6×10^9 bp), completes the process of replication within 18 minutes; that means the average rate of polymerisation has to be approximately 2000 bp per second.
- Not only do these polymerases have to be fast, but they also have to catalyse the reaction with high degree of accuracy. Any mistake during replication would result into mutations. Furthermore, energetically replication is a very expensive process.
- Deoxyribonucleoside triphosphates serve dual purposes. In addition to acting as substrates, they provide energy for polymerisation reaction (the two terminal phosphates in a deoxynucleoside triphosphates are high-energy phosphates, same as in case of ATP).
- In addition to DNA-dependent DNA polymerases, many additional enzymes are required to complete the process of replication with high degree of accuracy. For long DNA molecules, since the two strands of DNA cannot be separated in its entire length (due to very high energy requirement), the replication occur within a small opening of the DNA helix, referred to as replication fork.

- The DNA-dependent DNA polymerases catalyse polymerisation only in one direction, that is 5′à3′ . This creates some additional complications at the replicating fork. Consequently, on one strand (the template with polarity 3′à5′), the replication is continuous, while on the other (the template with polarity 5′à3′), it is discontinuous.
- **Figure 14 replication**



- The discontinuously synthesised fragments are later joined by the enzyme DNA ligase (Figure 3). The DNA polymerases on their own cannot initiate the process of replication. Also the replication does not initiate randomly at any place in DNA.
- There is a definite region in *E. coli* DNA where the replication originates. Such regions are termed as origin of replication. It is because of the requirement of the origin of replication that a piece of DNA if needed to be propagated during recombinant DNA procedures, requires a vector.
- The vectors provide the origin of replication. Further, not every detail of replication is understood well.
- In eukaryotes, the replication of DNA takes place at S-phase of the cell-cycle. The replication of DNA and cell division cycle should be highly coordinated. A failure in cell division after DNA replication results into polyploidy(a chromosomal anomaly).

Sr. no.	Question	Answer
1	**E.coli has hoe much base pair?**	4.6×10^6 bp
2	Which enzyme catalyse 5’-3’ polymerization?	DNA dependent DNA polymerase
3	In eukaryotes replication of DNA take place at which stage?	S phase

4	Polyploidity is known as…….	Chromosomal abnormality
5	Discontinuous replication is in which direction?	5’- 3’
6	Continuous replication is in which direction?	3’-5’