UNIT - VII
CHAPTER – 5 : PRINCIPLES OF INHERITANCE AND VARIATION

Heredity is the transfer of character from parents to their offsprings. These hereditary characters are present on the chromosomes in the form of genes. These gene combinations express characters which may be more similar to one of its two parents.

The differences in characters of offspring mainly depend upon unique process of crossing over that occurs during meiosis. This is one of the main reasons of producing recombination.

Gregor Johann Mendel was born in 1822 in Heinzendorf, which was a part of Czechoslovakia. He began his genetic experiments on garden pea in 1856 in the garden at the monastery.

Selection of pea plant: The main reasons for adopting garden pea (*Pisum sativum*) for experiments by Mendel were –

- Pea has many distinct contrasting characters.
- Life span of pea plant is short.
- Flowers show self pollination, reproductive whorls being enclosed by corolla.
- It is easy to artificially cross pollinate the pea flowers. The hybrids thus produced were fertile.

Working method: Mendel's success was also due to his meticulous planning and method of work –

- He studied only one character at a time.
- He used all available techniques to avoid cross pollination by undesirable pollen grains.
- He applied mathematics and statistics to analyse the results obtained by him.

Mendel's work and results:

The results obtained by Mendel were studied and on their basis he proposed certain laws known as "Laws of heredity". These laws are discussed below:

1) Law of dominance:

This law states that when two contrasting genes for a character come together in an organism, only one is expressed externally and shows visible effect. It is called dominant and the other gene of the pair which does not express and remains hidden is called recessive.

2) Law of segregation or Purity of gametes:

This law states that both parental alleles (recessive and dominant) separate and are expressed phenotypically in F2 generation. When F2 generation was produced by allowing F1 hybrid to self pollinate, to find out segregation or separation it was observed that both dominant and recessive plants appeared in 3:1 ratio.

3) Law of Independent assortment:

The law of independent assortment states that inheritance of two or more genes when occur at one time, their distribution in the gametes and in the progeny of subsequent generations is independent of each other. To prove this, he did a dihybrid cross. He crossed homozygous dominant smooth and yellow seeded (YYRR) with homozygous recessive wrinkled and green seeded (yyrr) plants. The F1 hybrid was self pollinated and F2 generation was obtained with the phenotypic ratio of 9:3:3:1 and genotypic ratio of 1:2:1:2:4:2:1:2:1.

Test Cross:

A cross between F1 hybrid (Aa) and its homozygous recessive parent (aa) is called Test Cross. This cross is called test cross because it helps to find out whether the given dominant phenotype is homozygous or heterozygous.

Incomplete dominance:

When neither of the alleles of a character is completely dominant over the other and the F1 hybrid is intermediate between the two parents, the phenomenon is called incomplete dominance.

The most common example of incomplete dominance is that of flower colour in 4'O clock plant. Homozygous red (RR) flowered variety was crossed with white (rr) flowered variety. F1 offspring had pink flowers (Rr). This is called incomplete dominance. Incomplete dominance is also known to occur in snapdragon. The phenotypic ratio and genotypic ratio in F2 generation in case of incomplete dominance is 1:2:1.

Multiple Allelism / Codominance:

When a gene exists in more than two allelic forms, it shows the phenomenon of multiple allelism. A well known example is the inheritance of A, B and O blood groups in human being. The gene for blood group occurs in three allelic forms I^A, I^B and i. Any person carries two of these alleles. The gene I^A produces glycoprotein (sugar) A and the blood group is A. The gene I^B produces glycoprotein B and the blood group is B. The gene 'i' is unable to produce any glycoprotein and so the person homozygous for it, has O group blood. The genes I^A and I^B are dominant over 'i'. When I^A and I^B are present together, both are equally dominant and produce glycoproteins A and B and the blood group is AB. They are called codominant alleles.

Phenotypic (Blood group)	Genotype
A	I^AI^A / $I^A I^O$
B	I^BI^B / $I^B I^O$
AB	I^AI^B
O	I^OI^O

Chromosome theory of Inheritance:

Chromosome theory of inheritance was proposed by Sutton and Boveri independently in 1902. The two workers found a close similarity between the transmission of hereditary characters and behaviour of chromosomes while passing from the one generation to the next through agency of gametes.

Salient features of chromosome theory:

- Both chromosomes as well as genes occur in pairs in the somatic or diploid cells.
- A gamete contains only one chromosome of a type and only one of the two alleles of a character.
- The paired condition of both chromosomes as well as Mendelian factor is restored during fertilization.

Parallelism of behaviour between chromosomes and Mendelian factors:

- Both the chromosomes as well as Mendelian factors (whether dominant or recessive) are transmitted from generation to generation in an unaltered form.
- A trait is represented by only one Mendelian factor inside a gamete. A gamete similarly contains a single chromosome out of a pair of homologous chromosomes due to meiosis that occurs before the formation of gametes.
- An offspring contains two chromosomes of each type, which are derived from the two parents through their gametes that are involved in fusion and formation of zygote. It also contains two Mendelian factors for each character. The factors come from two different parents through their gametes.

Linkage and Recombination:

Linkage is the phenomenon, where two or more linked genes are always inherited together and their recombination frequency in a test cross progeny is less than 50%.

A pair of genes may be identified as linked, if their recombination frequency in a test cross progeny is lower than 50 percent. All the genes present on one chromosome form a linkage group and an organism possesses as many linkage groups as its haploid number of chromosomes. If the two genes are fully linked, their recombination frequency will be 0%.

Sex Determination by chromosomes:

Those chromosomes which are involved in the determination of sex of an individual are called sex chromosomes while the other chromosomes are called autosomes.

1) XX – XY type: In most insects including fruit fly Drosophila and mammals including human beings the females possess two homomorphic sex chromosomes, named XX. The males contain two heteromorphic sex chromosomes, i.e., XY. Hence the males produce two types of gametes / sperms, either with X-chromosome or with Y-chromosome, so they are called Heterogamety.

2) ZZ – ZW type: In birds and some reptiles, the males are represented as ZZ (homogamety) and females are ZW (heterogamety).

3) XX – XO type: In round worms and some insects, the females have two sex chromosomes, XX, while the males have only one sex chromosomes X. There is no second sex chromosome. Therefore, the males are designated as XO. The females are homogametic because they produce only one type of eggs. The males are heterogametic with half the male gametes carrying X-chromosome while the other half being devoid of it.

Sex determination in Humans:

Human beings have 22 pairs of autosomes and one pair of sex chromosomes. All the ova formed by female are similar in their chromosome type (22+X). Therefore, females are homogametic. The male gametes or sperms produced by human males are of two types, (22+X) and (22+Y). Human males are therefore, heterogametic. The two sexes produced in the progeny is 50:50 ratio.

Mutation:

It is a phenomenon which results in alteration of DNA sequences and consequently results in changes in the genotype and phenotype of an organism.

Gene / Point mutation: Due to change in a single base pair of DNA. Ex. Sickle cell anemia (GAG→GUG).

Chromosomal mutation: Due to change in structure or number of chromosomes. Ex. Down's syndrome.

Mutagens: The chemical and physical factors that induce mutations are known as Mutagens. Ex. UV rays.

Genetic Disorders:

Pedigree analysis: It is a system to analyse the distribution and movement of characters in the family tree.

Mendelian Disorders: These are mainly determined by alteration or mutation in the single gene. These disorders are transmitted to the offspring on the same line as the principle of inheritance.

Examples : Haemophilia, Cystic fibrosis, Sickle cell anemia, Colour blindness, Phenylketonuria, Thalesemia, etc.

Haemophilia: It is a sex linked recessive disease, which shows its transmission from unaffected carrier mother to some of the male progeny. Haemophilia is a disorder in which a vital factor for clotting of blood is lacking. So clotting of blood is abnormally delayed and it can be fatal. Bleeding can be checked by transfusion of the entire volume of blood or the clotting factor in concentrated form.

Sickle cell anemia: It is an autosome linked recessive trait. It is due to a mutant allele on chromosome 11 (autosome), that causes change of glutamine (GAG) to valine (GUG) at the sixth position of β-chain of haemoglobin. The disease is controlled by a single pair of allele, HbA HbA (normal) ; HbA HbS (carrier) and HbS HbS (diseased). The patient has sickle shaped RBCs with defective haemoglobin. They are destroyed more rapidly than normal RBCs.

Phenylketonuria: It is due to a recessive mutant allele on chromosome 12 (autosome). The affected individual lacks an enzyme (phenylalanine hydroxylase) that converts the amino acid phenylalanine into tyrosine. As a result, this phenylalanine and its derivatives accumulate in the cerebrospinal fluid leading to mental degeneration (retardation) and are excreted in the urine due to its poor absorption by kidney.

Chromosomal Disorders: Due to absence or excess or abnormal arrangement of one or more chromosomes.

A change in the number of chromosomes in an organism arises due to non-disjunction of chromosomes, during gamete formation.

Aneuploidy: This arises due to loss or gain of one or more chromosomes during gamete formation. Ex. Down's syndrome (47) and Turner's syndrome (45).

Polyploidy: In this, the number of chromosomes is the multiple of the number of chromosomes in a single set (haploid). Accordingly, these may be haploid, diploid and polyploid.

Down's Syndrome: It was first described by Langdon Down (1866). It is due to trisomy of 21st chromosome, arising from non-disjunction. As the maternal age increases, the instances of non-disjunction increase. When such an ovum containing two 21st chromosomes (24) is fertilized by a normal sperm (23), the zygote (47) comes to possess three copies of 21st chromosome.
Symptoms: Short statured with small round mouth, palm is broad with characteristic palm crease, physical, psychomotor and mental development is retarded.

Klinefelter's syndrome: It arises due to non-disjunction of X-chromosomes during ova formation. When an ovum containing two X-chromosomes is fertilized by a Y-carrying sperm, XXY individual (47) appears.
Symptoms: A male with underdeveloped breasts (gynaecomastia), sparse body hair, mentally retarded and sterile.

Turner's Syndrome: It arises due to non-disjunction of X-chromosomes during ova formation. When an ovum carrying no X-chromosome is fertilized by a sperm carrying X- chromosome, a zygote with XO appears.
Symptoms: A female with rudimentary ovaries, short stature, lack of secondary sexual characters, they are sterile.

IMPORTANT TERMS:

1. Heredity: - It can be defined as the transmission of characters from one generation to successive generations of living organisms.
2. Alleles: - The various forms of a gene are called alleles.
3. Phenotype: - The external / observable characteristics of an organism constitute its phenotype.
4. Genotype: - The genetic constitution of an organism is its genotype.
5. Homozygote: - It is an individual organism in which the members of a pair of alleles for a character are similar.
6. Heterozygote: - It is an individual organism in which the members of a pair of alleles of a character are different.
7. Dominant character: - The form of the character which is expressed in the F1 hybrid is called dominant character.
8. Recessive character: - The form of the character which is suppressed in the presence of the dominant character in a hybrid is called recessive character.
9. Monohybrid cross: - It is a cross between individuals of the same species, in which the inheritance of contrasting pairs of a single trait is considered.
10. Dihybrid cross: - It is a cross between two individuals of the same species, in which the inheritance of contrasting pairs of two traits is considered.

CHAPTER – 6 : MOLECULAR BASIS OF INHERITANCE

Structure of DNA:

Watson and Crick proposed a double helical model for DNA, based on X-ray crystallography of the molecule. Each strand (helix) is a polymer of nucleotides, each nucleotide consisting of a deoxyribose sugar, a nitrogen base and a phosphate. The sugar – phosphate chain is on the outside and act as back bone and the bases are on the inside (like in ladder). The two strands are held together by weak hydrogen bonds between the nitrogen bases. A purine base, always pairs with a pyrimidine base, i.e., adenine (A) pairs with thymine (T) and guanine (G) pairs with cytosine (C). So the two strands are complementary to each other and run in antiparallel direction with one chain having 5' – 3' orientation and the other having a 3' – 5' orientation. The purine and pyrimidine bases are stacked 0.34 nm apart in the chain and the helix makes a turn after ten base pairs, i.e., 3.4 nm.

Central dogma of molecular biology:

Crick proposed the Central dogma in molecular biology, which states that the genetic information flows from DNA --> RNA --> Protein. In some viruses like retroviruses, the flow of information is in reverse direction that is from RNA --> DNA --> mRNA --> Protein.

Packaging of DNA helix:

In prokaryotes, negatively charged DNA is held with some positively charged proteins and form as nucleoid.

In eukaryotes, negatively charged DNA is held with positively charged proteins called Histones (octomer) and form a structure called Nucleosome.

The search for Genetic Material:

1. Bacterial Transformation (Transforming Principle) :

Fredrick Griffith conducted his experiment on *Streptococcus pneumoniae*, the pneumonia causing bacterium. He observed that there are two strains of this bacterium, one forming smooth colonies (S-type) with capsule (virulent) and the other forming rough colonies (R – type) without capsule (avirulent).

Experiment:

a) Smooth type bacteria were injected into mice. These mice died as a result of pneumonia caused by bacteria.
b) Rough type bacteria were injected into mice. These mice lived and pneumonia was not produced.
c) Smooth type bacteria which normally cause disease were heat killed and then injected into the mice. The mice lived and pneumonia was not caused.
d) Rough type bacteria (living) and heat killed S-type were injected together into mice. The mice died due to pneumonia and virulent smooth type living bacteria could also be recovered from their bodies.

This indicates that some factor from the dead S-cells converted the live R-cells into S-cells (transformation).

Later Avery, MacLeod and McCarty (1944) found out that when DNA isolated from the heat killed S-cells was added to R-cells in a culture, the R-cells changed into S-cells and pathogenic.

Evidence from experiments with bacteriophage:

This experiment was devised by Hershey and Chase with two different preparations of T_2 phage. In one preparation, the protein part was made radioactive and in the other, nucleic acid (DNA) was made radioactive. These two phage preparations were allowed to infect the culture of E.coli. Soon after infection, before lysis of cells, the *E.coli* cells were gently agitated in a blender, to loosen the adhering phage particles and the culture was centrifuged. The heavier infected bacterial cells pelleted to the bottom and the lighter viral particles were present in the supernatant. It was found that when T_2 phage containing radioactive DNA was used to infect *E.coli*, the pellet contained radioactivity. If T2 phage containing radioactive protein coat was used to infect *E.coli*, the supernatant contained most of the radioactivity. This suggests that during infection by the virus, the viral DNA enters the bacterial cell and that has the information for the production of more viral particles. It proves that DNA and not proteins, is the genetic material in bacteriophage.

Properties of Genetic Material:

a) It should be able to generate its replica (replication)
b) It should chemically and structurally be stable.
c) It should provide the scope for slow changes (mutation) that are required for evolution.
d) It should be able to express itself in the form of 'Mendelian Characters'.

Replication:

The Watson – Crick model of DNA immediately suggested that the two strands of DNA should separate. Each separated or parent strand now serves as a template (model) for the formation of a new but complementary strand. Thus, the new or daughter DNA molecules formed would be made of one old or parental strand and another newly formed complementary strand. This method of formation of new daughter DNA molecules is called semi-conservative method of replication.

The Experimental Proof:

Meselson and Stahl conducted an experiment to prove that DNA replication is semi conservative. They grew bacterium E. coli in a medium containing nitrogen salts ($^{15}NH_4Cl$) labeled with radioactive ^{15}N. ^{15}N was incorporated into both the strands of DNA and such a DNA was heavier than the DNA obtained from *E.coli* grown on a medium containing ^{14}N. Then they transferred the *E.coli* cells on to a medium containing ^{14}N. After one generation, when one bacterial cell has multiplied into two, they isolated the DNA and evaluated its density. Its density was intermediate between that of the heavier ^{15}N-DNA and the lighter ^{14}N-DNA. This is because during replication, new DNA molecule with one ^{15}N-old strand and a complementary ^{14}N-new strand was formed (semi-conservative replication) and so its density is intermediate between the two.

Mechanism of DNA replication:

The intertwined DNA strands start separating from a particular point called origin of replication (single in prokaryotes and many in eukaryotes). This unwinding is catalysed by enzymes called Helicases. Enzymes called Topoisomerases break and reseal one of the strands of DNA, so that the unwound strands will not wind back. When the double stranded DNA is unwound upto a point, it shows a Y-shaped structure called Replication Fork. Enzyme DNA dependent DNA polymerase catalyses the joining of Deoxyribonucleotides (A, G, C and T) in the 5' – 3' direction. The enzyme forms one new strand in a continuous stretch (leading strand) in the 5' – 3' direction, on one of the template strands. On the other template strand, the enzyme forms short stretches (discontinuous) strand of DNA also in the 5' – 3'. The discontinuous fragments are later joined by DNA-ligase to form a leading strand. The two strands are held together by hydrogen bonds between nucleotides.

Transcription:

Transcription is the process by which DNA gives rise to RNA. It can also be defined as, the process of copying genetic information from one strand of the DNA into RNA is termed as Transcription.

Transcription Unit:

A transcription unit in DNA is defined primarily by the three regions in the DNA;

- A Promoter
- The Structural gene
- A Terminator

Mechanism of Transcription:

Transcription involves the binding of RNA-polymerase at the promoter site on DNA. As it moves along (through structural gene), the DNA unwinds and one of the two strands acts as template to synthesize a meaningful RNA and other strand act as non-coding. A complementary RNA strand is synthesized with A, U, C and G as bases. RNA synthesis is terminated when the RNA-polymerase falls off a Terminator sequence on the DNA.

Transcription Unit and the Gene:

A gene is defined as the functional unit of inheritance. In eukaryotes, DNA consists of both coding and non-coding sequences of nucleotides. The coding sequences / expressed sequences are defined as Exons. Exons are said to be those sequence that appear in mature / processed RNA. These exons are interrupted by non-coding sequences called Introns. These introns do not appear in mature RNA.

Types of RNA:

In prokaryotes, a single RNA polymerase enzyme (composed of different subunits) catalyses the synthesis of all types of RNA(mRNA, tRNA and rRNA) in bacteria.

Where as in eukaryotes, there are three different RNA polymerase enzymes I, II and III, they catalyse the synthesis of all types of RNA.

RNA polymerase I – rRNAs
RNA polymerase II - mRNA
RNA polymerase III – tRNA

Process of transcription in Prokaryotes:

RNA polymerase binds to promoter and initiates transcription. RNA polymerase associates with initiation factor and termination factor to initiate and terminate the transcription respectively. In prokaryotes, since the mRNA does not require any processing, the transcription and translation take place in the same compartment and can be coupled.

Process of transcription in Eukaryotes:

In eukaryotes, the primary RNA contains both the exons and introns and is non-functional. Hence, these non-coding introns will be removed by the process called Splicing. Then this mature RNA undergoes ***Capping*** (addition of unusual nucleotide methyl guanosine triphosphate at 5' –end) and ***Tailing*** (addition of adenylate residues at 3' –end). Now, this fully matured RNA will be transported out of the nucleus for translation.

Genetic Code:

Genetic code refers to the relationship between the sequence of nucleotides (nitrogen bases) on mRNA and the sequence of amino acids in proteins. Each code is known as Codon with three nucleotides (triplet). It has been deciphered by Nirenberg, Khorana, Severo Ochoa and Crick.

Salient features of Genetic code:

- The codon is triplet. 61 codons code for 20 different amino acids and 3 codons do not code for any amino acids, hence they function as Stop codons (UAG, UGA and UAA).
- One codon codes for only one amino acid, hence, it is unambiguous and specific.
- Some amino acids are coded by more than one codon, hence the code is degenerate.
- The codon is read in mRNA in a contiguous fashion. There are no punctuations.
- The code is nearly universal. For example, from bacteria to human, UUU would code for Phenylalanine (phe) amino acid.
- AUG has dual function. It codes for Methionine (met), and it also act as Initiator codon.

Mutations and Genetic Code:

Mutation caused due to insertion / deletion of single base pair is known as Point mutation. Effect of point mutations that inserts or deletes a base in structural gene can be better understood by following simple example;

Consider a statement that is made up of the following words each having three letters like genetic code;

RAM HAS RED CAP

If we insert a letter B in between HAS and RED and rearrange the statement, it would read as follows;

RAM HAS BRE DCA P

Similarly, if we now insert two letters at the same place, say BI'. Now it would read,

RAM HAS BIR EDC AP

Now we insert three letters together, say BIG, the statement would read,

RAM HAS BIG RED CAP

The conclusion is, insertion or deletion of one or two bases changes the reading frame from the point of insertion or deletion. Insertion or deletion of three or its multiple bases insert or delete one or multiple codon hence one or multiple amino acids, and reading frame remains unaltered from that point onwards. Such mutations are referred to as ***Frame-shift insertion or deletion mutations.***

Structure of t-RNA : The Adapter Molecule:

tRNA molecule appears like a clover leaf , but in actual structure, the tRNA is a compact molecule which looks like inverted L.

tRNA has three loops,

a) an anticodon loop that has bases complementary to the codon.
b) An amino acid accepter end to which it binds to amino acids.
c) Ribosomal binding loop.

tRNAs are specific for each amino acid. There are no tRNAs for stop codons.

Translation:

It refers to the process of polymerization of amino acids to form a polypeptide. The order and sequence of amino acids are defined by the sequence of bases in the mRNA. The amino acids are joined by a bond which is known as a peptide bond.

It involves four steps namely

- Activation of amino acids (charging of tRNA / aminoacylation of tRNA)
- Initiation of polypeptide synthesis
- Elongation of polypeptide synthesis
- Termination of polypeptide synthesis

a) *Activation of amino acids*: In this process, a particular amino acid becomes to a specific tRNA molecule.

b) *Initiation of polypeptide chain*: The initiator methionyl-tRNA charged with amino acid methionine and anticodon UAC interacts with the initiation codon by codon-anticodon interaction. With the initiator methionyl-tRNA at P site, the larger subunit binds to the smaller subunit, thus forming an initiation complex.

c) *Elongation of polypeptide chain*: A second tRNA charged with an appropriate amino acid enters the ribosome at the A site, close to the P site. A peptide bond is formed between the first amino acid and the second amino acid. Then the first tRNA is removed from the P-site and the second tRNA at the A site, now carrying a dipeptide, is pulled along with mRNA to the P-site (translocation). Now the A-site is occupied by a third codon and an appropriate aminoacyl tRNA will bind to it. This process of peptide bond formation and translocation will be repeated and the polypeptide chain grows in length.

d) *Termination of polypeptide chain*: When untranslated regions / termination codons come at the A-site, no amino acid would be added, as it is not recognized by any tRNA. So protein synthesis will stop. At the end, a release factor binds to the stop codon, terminating translation and releasing the complete polypeptide from the ribosome.

Regulation of Gene Expression:

All the genes are not needed constantly. The genes needed only sometimes are called regulatory genes and are made to function only when required and remain non-functional at other times. Such regulated genes, therefore required to be switched 'on' or 'off' when a particular function is to begin or stop.

The Lac operon:

Jacob and Monod (1961) proposed a model of gene regulation, known as operon model. Operon is a co-ordinated group of genes such as structural genes, operator genes, promoter genes, regulater genes and repressor which function or transcribed together and regulate a metabolic pathway as a unit.

There are three structural genes, lac Z, lac Y and lac A, coding for galactosidase, permease and transacetylase respectively. These three genes are controlled by a single switch called operator. The operator switch is controlled by the repressor protein which coded by the regulator gene.

When the repressor binds to the operator, the genes are not expressed (switched off). When the operator switch is on, the three structural genes transcribe a long polycistronic mRNA catalysed by RNA – polymerase.

A few molecules of lactose (inducer) enter the cell by the action o enzyme permease. They are converted into an active form of lactose which binds to the repressor and changes its configuration and prevents it from binding to the operator. Beta-galactosidase breaks lactose into glucose and galactose. (Fig. Text book p.117).

Human Genome Project:

Goals of HGP:

- Identify all the approximately 20,000-25,000 genes in human DNA;
- Determine the sequences of the 3 billion chemical base pairs that make up human DNA
- Store this information in databases;
- Improve tools for data analysis;
- Transfer related technologies to other sectors, such as industries.

Methodologies:

The methods involved two major approaches. One approach focused on identifying all the genes that expressed as RNA referred as ***Expressed Sequence Tags*** (ESTs). The other approach is blind approach of simply sequencing the whole set of genome that contained all the coding and non-coding sequence, and later assigning different regions in the regions in the sequence with functions, referred as ***Sequence Annotation.***

Steps involved in sequencing:

a) Isolation of total DNA from a cell and converted into random fragments.
b) Cloning of DNA fragments can be performed by using cloning vectors like BAC (Bacterial Artificial chromosomes) and YAC (yeast artificial chromosomes).
c) The fragments were sequenced using automated DNA sequencers that worked on the principle of a method developed by Frederick Sanger.
d) These sequences were then arranged based o some overlapping regions present in them.

Salient features of Human Genome:

a) The human genome contains 3164.7 million nucleotide bases.
b) The average gene consists of 3000 bases, but sizes vary greatly, with the largest known human gene being dystrophin at 2.4 million bases.
c) Less than 2 per cent of the genome codes for proteins.
d) Repeated sequences make up very large portion of the human genome.
e) Repetitive sequences are stretches of DNA sequences that are repeated many times, sometimes hundred to thousand times.
f) Chromosome 1 has most genes (2968), and the Y has the fewest (231).
g) Scientists have identified about 1.4 million locations where single base DNA differences (SNPs – single nucleotide polymorphism) occur in humans.

DNA Fingerprinting:

DNA fingerprinting involves identifying differences in some specific regions in DNA sequence called as repetitive DNA, because in these sequences, a small stretch of DNA is repeated many times. These repetitive DNA are separated from bulk genomic DNA as different peaks during density gradient centrifugation. The bulk DNA forms a major peak and the other small peaks are referred to as satellite DNA. These sequence

show high degree of polymorphism (variation at genetic level) and form the basis of DNA fingerprinting.

Polymorphism can be defined as, an inheritable mutation is observed in a population at high frequency, it is referred to as DNA polymorphism.

The technique of DNA fingerprinting was initially developed by Alec Jeffreys. He used a satellite DNA as probe that shows very high degree of polymorphism. It was called Variable Number of Tandem Repeats (VNTRs).

Mechanism of DNA fingerprinting :

Extraction: DNA is extracted from the small amounts of blood, semen or hair bulbs available.
Amplification: Many copies of this DNA are made by a technique called Polymerase Chain Reaction (PCR).
Restriction Digestion: DNA is cut into desired reproducible segments using restriction enzymes.
Separation: These DNA sequences (restriction fragments) are separated by Gel Electrophoresis.
Southern Blotting: The separated DNA sequences are transferred from Gel onto a nitrocellulose membrane.
Hybridisation with probe, the DNA sequence complementary to VNTR sequences.
Exposure of the membrane to X-ray film, whose specific bands are developed.

Applications:
It is used effectively in forensic science for identifying;

a) the biological father (in case of paternity disparity)
b) the criminals such as murderers and rapists.

CHAPTER – 7 : EVOLUTION

Theory of Special Creation:

According to this theory, life originated on this earth from super natural powers like god. He created all plants and animals, which appeared on earth in the form they exist today.

Theory of Spontaneous generation or Abiogenesis:

According to this theory life originated on earth from non-living objects spontaneously by a process called *Abiogenesis* (origin of life from non-living matter). It was believed that fishes and frogs originated from mud, maggots arouse from decaying meat, Insects from plant juices and microorganisms from air & water. But later *Louis Pasteur* disproved this theory and stated that life originate from pre-existing life.

Conditions of Primitive earth/ Origin of life:

It is believed that earth has originated about 4,600 million years ago. It is formed by the condensation and cooling from a cloud of gases and dust. At first the earth was very hot and had various gases and vapour of several elements. With the passage of time, the earth gradually cooled down and gases condensed. Thus a solid crust of earth was formed. There were torrential rains for thousands of years resulting in the formation of large water bodies like oceans.

The earth's atmosphere at the time was a reducing atmosphere and not an oxidizing one as today. There were large quantities of hydrogen, nitrogen, water vapour, carbon monoxide, methane and ammonia in the primitive atmosphere. However, free oxygen was not present, so the atmosphere is known as reducing atmosphere and this led to the continuous series of chemical reactions among the gases to form amino acids. Hence life originates from reducing atmosphere.

The present atmosphere is oxidizing one and no life is originating today because oxygen will not allow any continuous series of chemical reaction and if any product is formed among the gases that will be oxidized.

A.I. Oparin and J.B.S. Haldane believed that methane, ammonia and water vapours contain the kinds of atoms needed to form various substances such as alcohol and amino acids. Accumulation of such organic compounds within the oceans, lakes, ponds, pools, etc. over million of years must have produced a kind of 'hot soup'. In this 'hot soup' or 'Darwin's warm little pond' smaller organic compounds must have combined together to form larger organic compounds and various macromolecules like polypeptides, proteins, nucleic acids, carbohydrates etc. These compounds then interacted to produce the first living cell. So according to them, the first living cell arose from simple inorganic and organic non-living elements – a process called Abiogenesis.

The energy for such chemical reactions must have come from the heat of the atmosphere and from the electrical energy of lightening.

The most important compound that initially formed is a nucleoprotein (nucleic acid and protein) since it is the chemical characteristic of genes. They might have aggregated in various combinations and must have formed the colloidal masses at the base of oceans. They formed the small globules. They are then covered by fatty acids to form their surface membranes. This membrane also became selectively permeable so a specific organization inside was maintained. Experimental evidences have also shown that such types of cells formed are called as coacervates (pre-cell) and then they gradually transformed into a living cell.

Then enzymes and other important compounds inside were formed. In the present day cells, all these macromolecules are formed by the actions of enzymes. But the enzymes are protein in nature. So initially all macromolecules were formed by non-enzymatic actions.

Urey and Miller experiment:

Stanley Miller and Harold C. Urey in 1953 tested the Oparin-Haldane theory. They made an apparatus to circulate methane, ammonia, water vapour and hydrogen gases. All these gases were put in a flask fitted with electrodes. In another flask, water was being boiled continuously. The electrical charges were used to provide energy similar to lightening, they were passed for one week or more. After that they collected and analysed the contents of the apparatus. He was able to get a number of amino acids, some of which are known to be present in the proteins e.g., glycine, alanine, aspartic acid and glutamic acid. Miller also got several of the simple acids that are

known to occur in the living organisms such formic acid, acetic acid, propionic acid, lactic acid and succinic acid.

Hence they proved the Oparin and Haldane theory and now it is clear that reducing atmosphere was essential for such abiotic synthesis.

Organic evolution:

It is defined as the process of gradual and orderly changes in organisms from one form to another over a period of millions of years. It is a slow and continuous process.

Morphological Evidences:

Homologous organs (Divergent Evolution):

Organs having similar embryonic origin and basic plan, but differing in their functions are known as homologous organs. E.g., The arm of man, the leg of a horse, the wing of a bat, the wing of a bird and the flippers of a seal have the same basic plan of development but they are used for different works. All of them possess humerus in upper arm, radius and ulna in the forearm, carpals in the wrist, metacarpals in the palm and phalanges in digits. They also show similarities in the arrangement of the muscles and nerves and also show same pattern of embryonic development.

Homology in plants:

In plants, the homologous organs are a thorn of *Bougainvillea* and a tendril in *Cucurbita* both arising in the axillary position, but perform different functions, Thorn for protection and tendril for support.

Analogous organs (Convergent Evolution):

Organs having similar functions but different in their basic plan of development are known as analogous organs. For example, the wing of insects and that of birds or bats are analogous structure. Their basic plan of development is different but has a similar function of flying. In insects wing is an extension of the integument whereas a bird's wing is formed of bones covered with flesh, skin and feathers.

In plants:

- In Opuntia / Cactus, a stem is modified to look like a leaf and may perform the function of a leaf (photosynthesis).
- In potato and sweet potato, potato is a stem tuber and sweet potato is a root tuber. Storage of food is the same function.

Geological time scale:

It shows the ages of the various eras and periods together with the major groups of plants and animals that are believed to have existed during that period. It helps in the study of palaeontology.

It has been divided into 6 eras which are further divided into periods or epochs. Each being characterised by some specific living forms and climatic changes geological time scale is the calender of earth past history indicating the evolution of life through time recorded in sequence of rocks.

Biological Evolution:

The essence of Darwinian theory about evolution is natural selection. Branching descent and natural selection are the two key concepts of Darwinian Theory of Evolution.

Lamarck's concept of evolution/Inheritance of acquired characters:

This theory states that characters are acquired by animals in two ways,

1. The effects of environment
2. Use and disuse of body parts.

For example, the long neck of giraffe is explained by Lamarck on the same principle. Giraffe, which lived in the dry and arid deserts of Africa, tried to reach the foliage high up on the trees to eat them as there was no vegetation on the ground. In the process its neck and forelegs got stretched a bit and this was inherited to the next generation. Then in the next generation same efforts were continued. Gradually through many successive generations, we got giraffe having such a long neck and forelegs.

Lamarck's idea of the use and disuse of body parts and the inheritance of acquired characters was not accepted by the scientists. It was disproved by *August Wiesmann.* He showed that even after cutting the tail of rats for several generations, no rat was born without a tail.

Darwin's Theory:

Charles Darwin and Alfred Russel Wallace independently gave the theory of evolution. This theory is known as 'Darwin's theory of natural selection' and is published in a book, "Origin of Species by Natural Selection". The main features of this theory are as follows,

Reproduction: All organisms reproduce and multiply enormously. Eg. A pair of mice produces dozens of young ones, insects lay thousands of eggs and plants also produce thousands of seeds.

Variations: No two individuals are alike. They differ from each other in size, shape, behaviour, etc. even the offspring of the same parent are never exactly alike except identical twins.

Struggle for existence: All the offspring are not able to reach adulthood. When offspring
become adulthood, then they start to reproduce. This reproductive capacity varies from animal to animal; some reproduce more and some minimum. This differential capacity of reproduction is known as *differential reproduction.*

Since the number of individuals is far more than actually can survive, so they compete among themselves for food, shelter and space.

Survival of fittest/ Natural selection: Only those individuals which have favourable variations survive and reproduce while others not suited by the environment perish away. Thus nature exercises its selection and only those individuals that are 'fit' to survive and reproduce successfully.

Origin of Species/Speciation: This continuous process of variation and natural selection will ultimately result in elimination of certain individuals; while others will gradually establish. In this process new characters, which are good, will set in. Thus new species may be produced in due course of time.

Mechanism of Evolution:

Hugo deVries believed that it is mutation which causes evolution and not the minor variations (heritable) as Darwin said. Mutations are random and directionless while Darwinian variations are small and directional. Evolution for Darwin was gradual while deVries believed mutation caused speciation and hence called it Saltation (single step large mutation).

Hardy-Weinberg Principle:

- According to this law, if all the factors / conditions remain constant, the frequency of particular genes and their alleles will remain constant in a population of sexually reproducing organisms from generation to generation.
- The difference between the observed frequencies of alleles and those predicted by Hardy-Weinberg Principle indicates the degree of evolutionary change. Evolution occurs when the genetic equilibrium is disturbed.

Factors affecting Hardy-Weinberg Equilibrium:

- Gene migration / Gene flow
- Genetic drift
- Mutation
- Genetic recombination
- Natural Selection

Examples of Natural Selection:

1.Industrial melanism.

A case of natural selection was seen in Great Britain in a peppered moth (*Biston betularia)*. This moth had two forms: grey colour and black colour (Carbonaria). In the early part of the nineteenth century only the grey coloured forms of moths were present; the dark forms were rare. The grey coloured moths were seen on the tree trunks covered with lichens and so they were able to escape from their enemies. Later on, due to the development of industries the lichens were killed and the tree trunks looked dark due to the deposition of industrial soot. Birds now were able to spot these moths and feed upon them. So the grey coloured moths were eaten by the birds and the dark coloured moths escaped from the birds. Then now the coal is replaced by the

industries and oil and electricity is used. This has reduced the soot production and ultimately less deposition of soot on the tree trunks. These tree trunks have, now, again become grey in colour. Consequently, grey coloured moths have again increased in number. This example clearly brings out the action of natural selection.

2.Resistanceof mosquitoes to pesticides.

When DDT was introduced to control mosquitoes it was tremendously successful. Most of the mosquitoes were sensitive to DDT and were therefore killed. In that population of mosquitoes, few mosquitoes became resistant to DDT and survived. They multiplied and now almost total population of mosquitoes became resistant to DDT.

Hence the principle of natural selection shows that the chemical insecticides can remain effective only for a limited period.

Adaptive Radiation:

The Process of evolution of different species in a given geographical area starting from a point and literally radiating to other areas of geography (habitats) is called Adaptive radiation. Ex. Darwinian Finches, Australian Marsupials.

A Brief Account of Evolution:

- About 2000 million years ago the first cellular forms of life appeared on earth. Some of these cells had the ability to release O_2.
- Slowly single-celled organisms became multi-cellular forms and by the time 500 mya, invertebrates were formed and active.
- Jawless fish evolved around 350 mya.
- Organisms started to invade from water to land. Fish with stout and strong fins could move on land and go back to water. These fishes evolved into the first amphibians
- Later, these amphibians evolved into reptiles. They lay shelled eggs. Then reptiles of different shapes and sizes dominated on earth (dinosaurs).
- Some of the reptiles evolved into birds and later some of them to mammals. Mammals were viviparous and more intelligent in sensing and avoiding danger at least.

Origin and Evolution of Man:

- About 15 mya, primates called Dryopithecus and Ramapithecus were existing. They were hairy and walked like gorillas and chimpanzees. Ramapithecus was more man like and Dryopithecus was more ape-like.
- Two mya, Australopithecines probably lived in East aftrican grasslands. Evidence shows that they hunted with stone weapons but essentially ate fruits.
- Fossils of first human like being the hominid were found and their brain capacity were between 650-800cc, they were called as *Homo habilis*. They did not eat meat.
- Fossils discovered in Java 1891 revealed the next stage, i.e., *Homo erectus* about 1.5 mya and had a large brain around 900 cc and they ate meat.
- Neanderthal man with a brain size of 1400 cc. They used hides to protect their body and buried their dead.
- *Homo sapiens* arose in Africa and moved across continents and developed into distinct races.
- During ice age between 75,000-10,000 years ago modern *Homo sapiens* arose.

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