Chapter 1
Sexual Reproduction in Flowering Plants

Introduction:

Reproduction is a vital process without which species cannot survive for long. An individual increases its number by asexual or sexual means. Sexual mode of reproduction enables creation of new variants so that survival advantage is enhanced. All flowering plants show sexual reproduction, flowers and floral parts, shows an amazing range of adaptations to ensure formation of the end products of sexual reproduction, the fruits and seeds.

This chapter deals with morphology, structure and processes of sexual reproduction in flowering plants (angiosperms).

FLOWER – A FASCINATING ORGAN OF ANGIOSPERMS

Flower are objects of aesthetic, ornamental, social, religious and cultural value. Flowers are morphological and embryological marvels and the sites of sexual reproduction in angiosperms

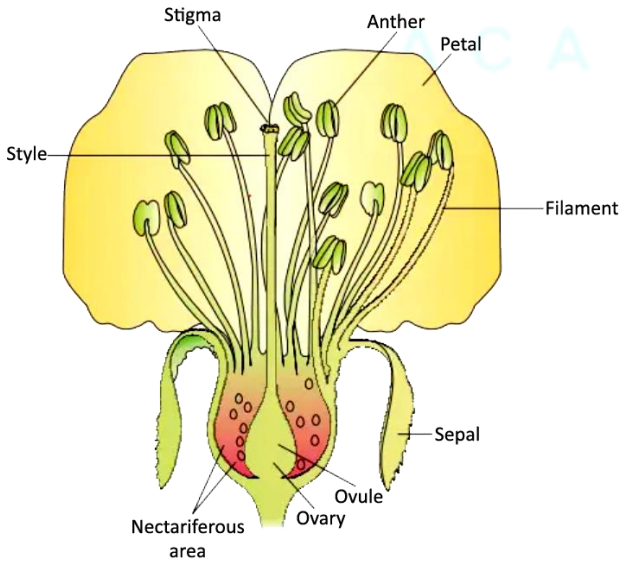


Fig. A diagrammatic representation of L.S. of a Flower

A typical angiospermic flower consists of four whorls of floral appendages attached on the receptacle.

1. **Calyx** (consists of sepals) :- Non – essential whorl (sterile)
2. **Corolla (consists of petals) :** Non - essential whorl (sterile)
3. **Androecium (consists of stamens)** – Male unit : Essential whorl (fertile)
4. **Gynoecium** (consists of carpels) – Female unit : Essential whorl (fertile)

The essential whorls androecium and gynoecium are the two most important units of sexual reproduction.

Much before the actual flower is seen on a plant, the decision that the plant is going to flower has taken place. A number of hormonal and structural transformations occur prior to initiation of flowering. Shoot apical meristem is transformed into reproductive meristem. Reproductive meristem grows to form inflorescence axis over which floral primordial develop. The primordial grow into floral buds and then flowers. In the flower, the androecium and gynoecium differentiate and develop.

Stamen, Microsporangium, Pollen Grain

A typical stamen consists of two parts :

1. **Anther :** It is terminal bilobed structure.
2. **Filament:** It is long slender stalk. Its proximal end remains attached to thalamus or the petal of the flower.

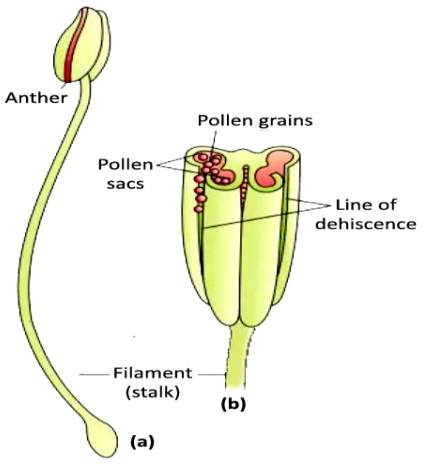


Fig : (a) A typical stamen, (b) Three – dimensional cut section of an anther

Structure of Typical Anther

i. A typical anther is bilobed. The two anther lobes are separated by a deep groove in front and are attached to each other by a band of vesiculated sterile tissue called **connective.**

ii. Each anther is a four-sided tetragonal structure consisting of four microsporangia located at corners, two in each lobe. Hence, a mature anther is **tetrasporangiate.**

iii. Microsporangia forms **pollen sacs** which on maturity become filled with pollen grains.

Structure of Microsporangium : In a transverse section, a typical microsporangium appears near circular in outline. It consists of homogenous mass of meristematic cells called primary sporogenous cells surrounded by anther wall. Primary sporogenous cells forms microspore mother cells (2n).

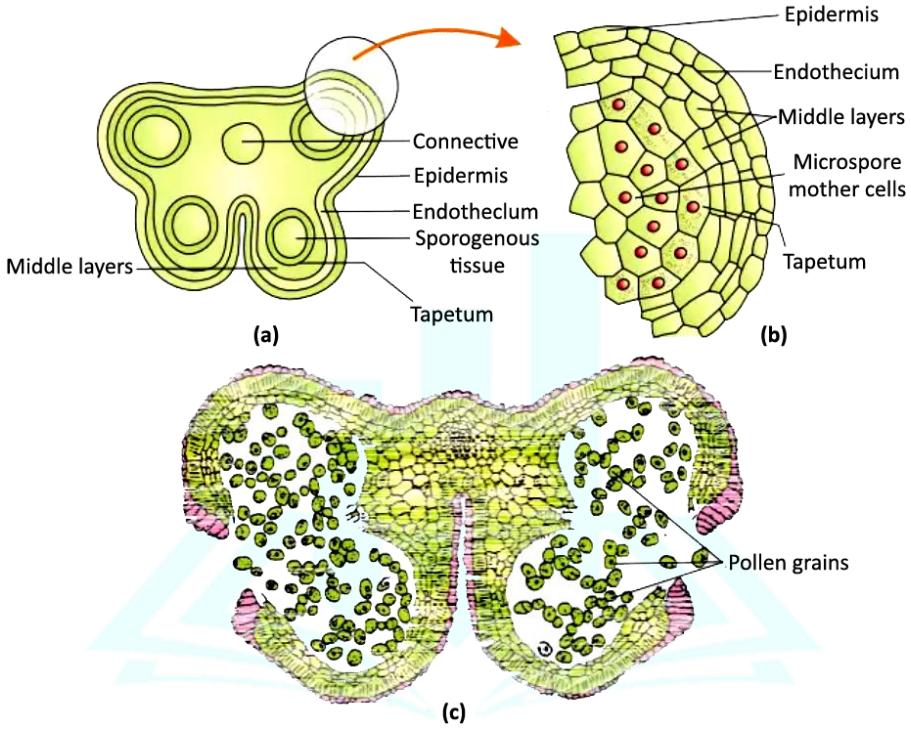


Fig : (a) T.S. of a young anther, (b) Enlarged view of one microsporangium (c) A Mature dehisced anther

Anther Wall Layers : Anther wall consists of following layers :

1. **Epidermis :** Outermost, single layered and protective in function.
2. **Endothecium :** Cells of this layer α – cellulosic fibrous bands arising from inner tangential wall which **help in dehiscence** of anther due to their hygroscopic nature. **Fibrous bands are absent in hydrophytes**.
3. **Middle layer :** Cells of this layer are ephemeral and are 1 – 3 layered. It degenerates at maturity .
4. **Tapetum :** This is the innermost layer of anther wall which surrounds the sporogenous tissue. Tapetal cells nourishes the developing pollen grains. Cells of the tapetum possess dense cytoplasm and generally have more than one nucleus. They are polyploid. The tapetal cells show increase in their DNA content.

Sprogenous Tissue : When the anther is young, a group of compactly arranged homogenous cells called the sporogeneous tissue occupies the centre of each microsporangium.

Microsporogenesis : The formation of haploid microspores from diploid microspore mother cell inside pollen sac by meiotic division is called as **microsporogenesis.** The haploid microspores formed from a single microspore mother cell (pollen mother cell) are arranged in the form of four cells called microspore tetrad (haploid).

As the anthers mature and dehydrate, the microspores dissociate from each other and develop into **pollen grains.** Inside each microsporangium, several thousands of microspores or pollen grains are formed that are released with the dehiscence of anther.

Pollen Grain

The pollen represents the male gametophytes.

1. These are generally spherical structures measuring about 25-50 micrometer in diameter.
2. The cell wall of pollen grain is called **sporoderm** which consists of two layers., i.e., **exine** and **intine.**

(a) **Exine :** It is hard outer layer made up of **sporopollenin (one of the most resistant organic**

materials known). It can withstand high temperature, strong acids and alkali. No enzyme that degrades sporopollenin is so far known.

(i) This also helps in **fossilization.** Pollen grains are well – preserved as fossils because of the presence of sporopollenin.

(ii) It is hard so that the pollen grains are well protected from hazardous environment when they are pollinated by biotic/abiotic agents.

(iii) It exhibits a fascinating array of patterns and designs which is of **taxonomic significance.**

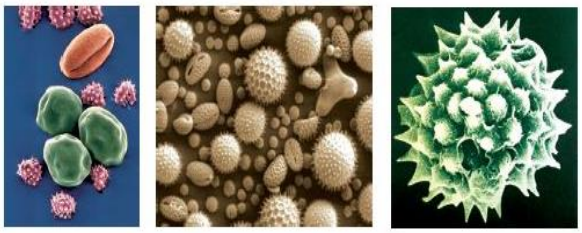


Fig : Scanning electron micrographs of a few pollens' grains

It has prominent apertures called **germ pores** where sporopollenin is absent.

(b) Intine : It is the inner wall which is thin and continuous and made up of **cellulose** and **pectin.**

(c) Cytoplasm of pollen grain is surrounded by plasma membrane.

Pollen Allergy : Pollen grains of many species (**especially anemophilous plants)** cause severe allergies and **bronchial affliction** in some people often leading to chronic respiratory disorders like asthma and bronchitis.

(i) Parthenium/ carrot grass (came into India as a contaminant with imported wheat).

(ii) Amaranthus.

(iii) Chenopodium

Pollen Products : Pollen grains are rich in nutrients. Pollen consumption has been claimed to increase the performance of athletes and race horses. It has become a fashion in recent years to use pollen tablets as food supplements. In western countries, a large number of pollen products in the form of tablets and syrups are available in market.

❖ **Pollen Viability :**

(a) The period for which the pollen grains retain the ability to germinate on landing on the stigma is called as pollen viability.

(b) It is highly variable and depends on prevailing temperature and humidity.

Pollen viability periods in plants :

1. 30 minutes ($^1/_2$ hour) – Rice, wheat (cereals)

2. Several months – Leguminosae, Rosaceae and Solanaceae

Pollen Banks : Storage of pollen grain for years in liquid N_2 (– 196°C) for later use in plant breeding programmes is called as cryopreservation. These centers for storage of pollens are called pollen banks.

Structure and Development of Male Gametophyte

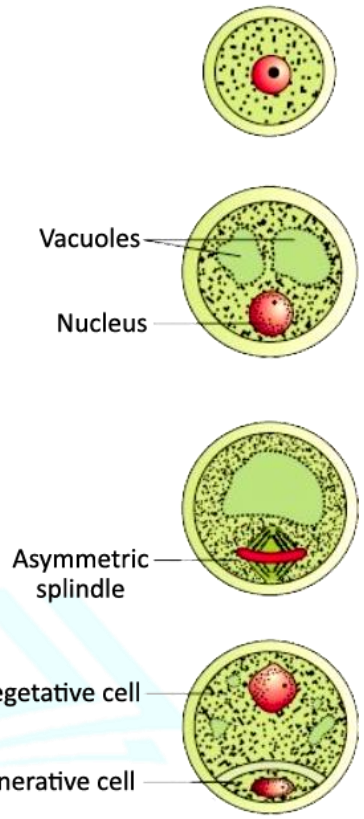


Fig : Development of Male Gametophytes

Pollen grain or microspore divides mitotically into **two cells :**

1. Vegetative cell : Bigger in size having abundant food reserve and a large irregularly shaped nucleus.

2. Generative cell : Small and floats in the cytoplasm of vegetative cell. It is spindle shaped with dense cytoplasm and a nucleus.

Pollination or shedding of pollen grains takes place at two – celled stage in 60 percent of angiosperms. In rest, the generative cell divides to form two male gametes and pollen is shed at three – celled stage. The male gametes are non – motile and amoeboid. They are slightly unequal in size; such a pollen will be called three celled pollen or mature male gametophyte.

Ovary has an ovarian cavity with one or more chambers (locules). The placenta is located inside the ovarian cavity. Arise from the placenta are the megasporangia, commonly called ovules. As ovary may have a single ovule as in **wheat , rice, mango** or many ovules as in **papaya, watermelon** and **orchids.**

II. Ovule (integumental indehiscent megasporangium)

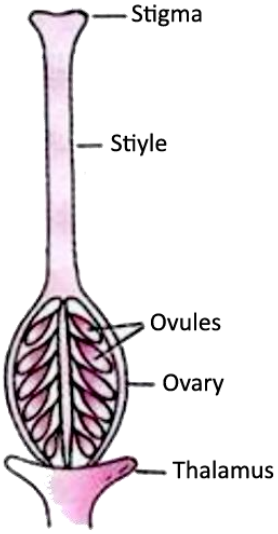


Fig. : Typical Pistil

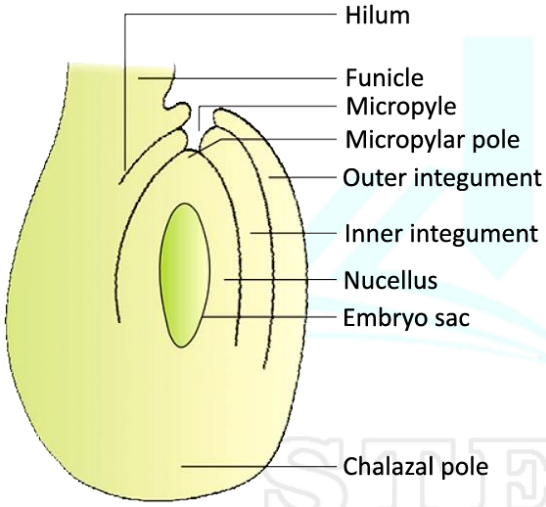


Fig : Typical Anatropous Ovule

The structure of ovule can be studies under the following headings :

(a) **Funicle :** The stalk of the ovule by which it remains attached to placenta.

(b) **Hilum :**It is the junction between ovule and funicle or the point of attachment of funiculus to the bod of ovule.

(c) **Integument :** The one or more protective envelops surrounding the body of ovule.

(d) **Micropyle :**The pore or passage present at the tip of ovule where the integument is absent.

(e) **Chalaza :** Opposite to micropylar end representing the basal part of ovule.

(f) **Nucellus :** The parenchymatous mass of tissue enclosed within the integuments and forms the body of ovule.

(g) **Embryo sac :** It is also called the **female gametophyte** and is located in the nucellus. An ovule generally has a **single embryo sac** formed from a megaspore.

III. Megasporogenesis

Definition : The process of formation of megaspores from megaspore mother cell (MMC) is called Megasporogenesis.

(i) Ovules generally differentiate a single megaspores megaspore mother cell (MMC) in the micropylar region of the nucellus.

(ii) MMC is a large cell containing **dense cytoplasm** and a **prominent nucleus.**

(iii) The MMC undergoes meiosis and forms a linear tetrad of four haploid megaspores. Out of which one remains functional (chalazal end) and three degenerate (micropylar end)

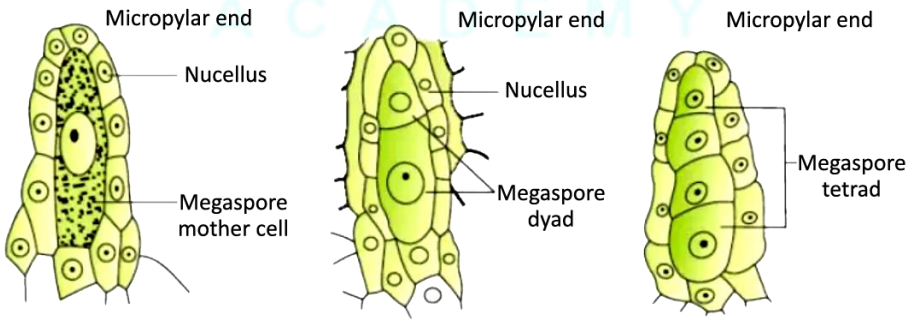


Fig: (A) Large megaspore mother cell, (B) A dyad of megaspore, (C) A tetrad of megaspore

Importance of meiosis in megaspore mother cell : To ensure the formation of a haploid female gamete before fertilization.

Polygons type of embryo sac is found in 80% flowering plants. This development has been studies in **Polygons** by **Strasburger.** The nucleus of **chalazal functional megaspore** (4th from micropyle) divides mitotically to form two nuclei which move to opposite poles, forming the 2 – nucleate embryo sac. Two more sequential mitotic nuclear divisions result in the formation of the 4 – nucleate

and later the 8 – nucleate stages of the embryo sac. One nucleus from each pole moves to the middle and they followed immediately by cell wall formation. At this stage, following changes occur :

(a) Three of the nuclei (n) organized as cells at micropylar end forming **egg apparatus.** One is the **egg call** (n) and two are **synergids (n)**

(b) Three nuclei get organized as antipodal cell (n) at chalazal end.

(c) Two nuclei in the centre are called **polar nuclei (n)**

This constitutes a **7 – celled and 8 – nucleated** embryo sac.

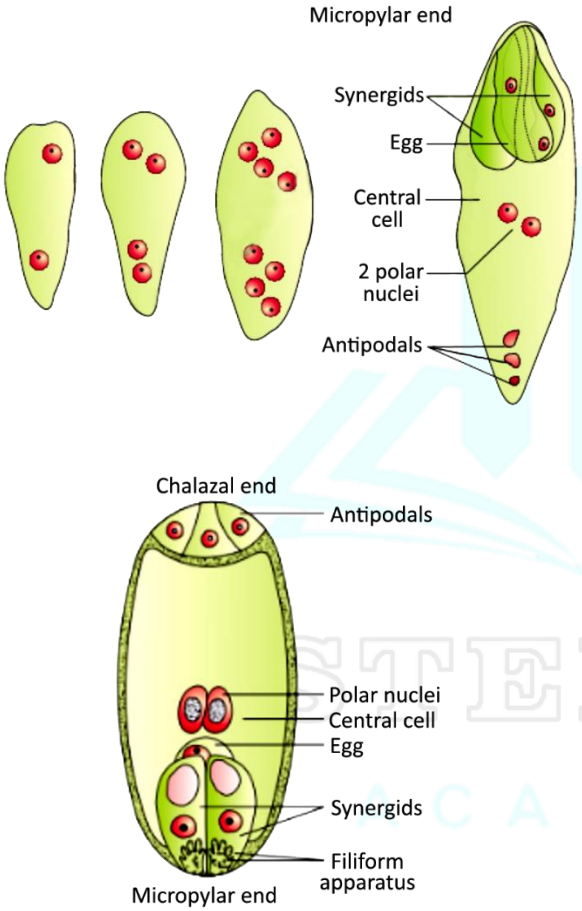


Fig : Development of female gametophyte (Polygonum type)

Organization of Embryo Sac

(a) Synergids or helper cells or co – operative cells: These cells generally possess a micropylar nucleus and a chalazal vacuole. The electron microscopic studies have revealed that the synergids lack a cell wall on their chalazal side at maturity. They are characterized by the presence of a **filiform apparatus** at the micropylar tip. It is in the form of finger like projections, each projection comprising a core of microfibrils enclosed in a sheath. Usually, one synergid starts to degenerate just with pollination. The synergids perhaps secrete some chemotropic substance and thus, direct the pollen tube growth inside embryo sac.

(b) Egg : The egg shows cytoplasmic polarity opposite to synergid and its wall is thicker at the micropylar end. Usually, the egg has a micropylar vacuole and a chalazal nucleus. Plasmodesmata connection is present in between egg and synergids.

(c) Antipodals or vegetative cells : These are vegetative cells of embryo sac. In most of the plants there are three antipodal cells.

(d) Central Cell : It is the largest cell of the embryo sac. It initially contains **two polar nuclei** which fuse just before fertilization to form a **secondary nucleus or definitive nucleus (2n).**

Pollination

In flowering plants, male and female gametes are produced in the pollen grain and embryo sac respectively. These gametes are non – motile, therefore, they have to be brought together for fertilization. Pollination is the mechanism to achieve this objective. The transfer of pollen to the stigma is called pollination.

Types of pollination : Depending on the source of pollen, pollination is of three types :

1. Autogamy : The transfer of pollen grain from the anther to the stigma of the same flower.

Adaptations seen in plant to ensure self – pollination :

(i) Bisexuality : Presence of both the essential whorls in the same flower.

(ii) Homogamy : Maturation of both androecium and gynoecium at the same time, i.e., there should be synchrony in release of pollen and stigma maturation.

(iii) Cleistogamy : A condition in which flower does not open. In such flowers, the anthers and stigma lie close to each other. When anther dehisced in the flower buds, pollen grains come in contact with stigma to effect pollination

→ Some plants like Viola (common pansy), Oxalis and Commelina produce both types of flowers, i.e., open flowers (chasmogamous) and closed flowers (cleistogamous).

Advantage of Cleistogamy :

(a) It ensures seed formation even in the absence of any pollinating agent.

(b) It is cheaper for the plant as there is no costly nectar or fragrance which the plant has to produce for pollination.

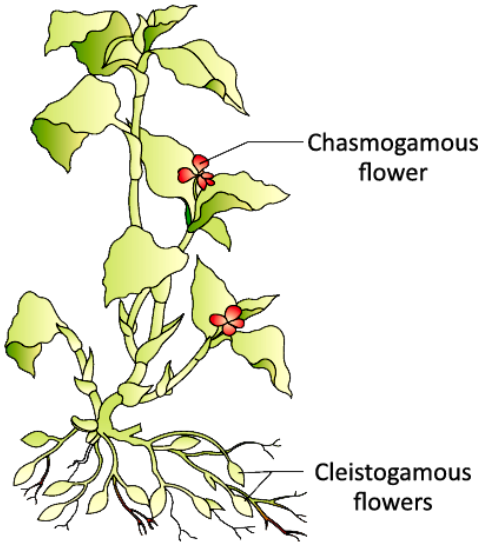


Fig : Cleistogamous Flowers

Disadvantage of Cleistogamy : The offsprings produced have limited genetic diversity

2. **Geitonogamy :** The transfer of pollen grains from anther to the stigma of another of the same plant. This transfer involves an agent of pollination, hence functionally it is cross – pollination. Genetically, it is similar to **autogamy** since the pollen grains come from the same plant.
3. **Xenogamy :** The transfer of pollen grains from anther to the stigma of another flower of different plant of the same species.

   This is the only type of pollination which brings genetically different types of pollen grains to stigma.

Agents of pollination

- Abiotic agents (Wind, Water) (Used only by a small proportion of plants)
- Biotic agents (Animals) (Used by majority of plants)

Abiotic Agents :

(1) Wind (Anemophily) : This is the more **common amongst abiotic pollinations.**

Characteristics of wind pollinated flowers:

(i) The pollen grains are light and non – sticky, so that they can easily by carried by air currents.

(ii) The flowers have well – exposed stamens, so that the pollens are easily dispersed into wind currents.

(iii) Flowers have large **feathery stigma** to easily trap the air borne pollen grains.

(iv) Nectarines absent.

(v) Presence of single ovule in each ovary.

(vi) Flowers packed into inflorescence.

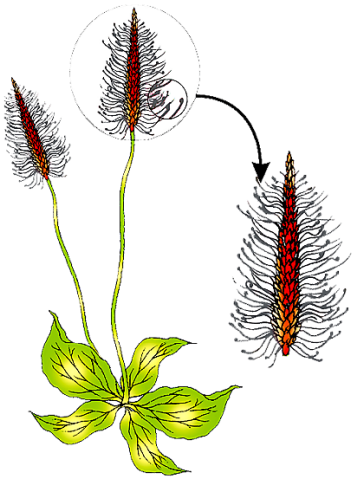


Fig. : A wind-pollinated plant showing compact inflorescence and well-exposed stamens

Examples of wind pollinated plants : It is quite common in grasses.

(2) **Water (Hydrophily) :** Pollination by water is quite rare in flowering plants and is limited to about 30 genera, mostly monocots, e.g., Fresh water plants like Vallisneria, Hydrilla, marine water plant like Zostera.

Characteristic Features :

(i) Light, unwettable pollen grains, generally surrounded by mucilaginous covering, hence protected from wetting.

(ii) Long, sticky unwettable stigma.

Pollination by water may occur at two places :

(i) On the surface of water (Epihydrophily) : Example: Vallisneria

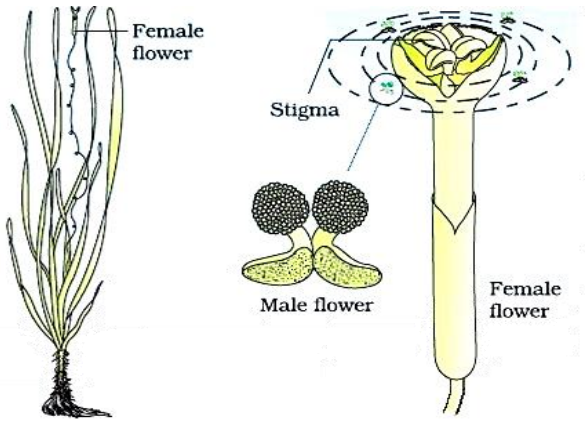


Fig : Pollination by water in Vallisneria

- The female flower have a very long pedicel, therefore it reaches the surface of water.

- Male flowers after breakage floats on the surface of water.
- Pollen grains are released on to the surface of water. They are carried passively by water currents, some of them eventually reach the female flowers and the stigma.

(ii) Beneath the surface of water (Hypo hydrophily): Example : Zostera (sea grass) Zostera is a marine water plant. Female flowers remain submerged in water. Pollen grains are long ribbon like and they are carried passively inside the water, some of them reach the stigma and achieve pollination.

In a majority of aquatic plants. The flower emerges above the level of water and are pollinated by insects or wind. e.g., Water hyacinth and water lily are pollinated by insects.

Biotic Agents :

Majority of flowering plants use a range of animals as pollinating agents. Bees, butterflies, flies, beetles, wasps, ants, moths, birds (sun birds and humming birds) and bats are the common pollinating agents.

Larger animals like some primates (Lemur), arboreal (tree – dwelling) rodents, or even reptiles (gecko lizard and garden lizard) have also been reported as pollinators in some species. E.g., Lemur in Ravenala plant and lizard in flax.

Insects (Entomophily) : These are the most common biotic agents of pollination.

Bees are the most common insect which acts as a pollinating agent. Other insect pollinators are butterflies, flies, beetles, wasps, ants, moths.

Characteristic features of flowers pollinated by insects

(1) Majority of insect – pollinated flowers are large sized.

(2) Small sized flowers are clustered into an inflorescence.

(3) Colorful, fragrance.

(4) Nectarines present.

(5) Sticky pollen grain.

(6) Foul – obdured flowers if pollinated by flies and beetles.

Floral rewards for insects :

(1) Nectar

(2) Pollen

(3) Safe place to lay eggs. For example, Amorphophallus (flower is 6 feet in height).

(4) **Pronubia moth lays its eggs** in the ovary of Yucca plant and its flowers get pollinated by the moth. Both the organisms cannot complete their life cycle without each other. The moth deposits its eggs in the locule of the ovary and the flower, in turn, gets pollinated by the moth. The larvae of the moth come out of the eggs as seeds start developing.

Other biotic agents of pollination

Name of the agent	Term used
1. insect	Entomophily
2. Bats	Chiropterophily
3. Snails	Malacophily
4. Snakes	Ophiophily
5. Bird	Ornithophily

(2) If the female parents bear bisexual flowers in such crossing experiments, it is important to make sure that only the desired pollen grains are used for pollination.

(a) **Emasculation :** Removal of anthers from female parent flower buds before the anther dehisces.

(b) **Bagging :** Covering of emasculated flowers with a bag of suitable size generally made of butter paper to prevent contamination of stigma with unwanted pollen.

(3) Dusting of pollen grains from anthers of male parent on the stigma of female parent when stigma attains receptivity and then it is retagged.

(4) Fruits are then allowed to develop.

(5) If the female parent produces unisexual flowers, there is no need for emasculation.

DOUBLE FERTILIZATION

→ After entering one of the synergids, the pollen tube releases the two male gametes into the cytoplasm of the synergid. The following two events take place in the embryo sac :

(1) Syngamy :

♂ + ♀ ⟶ **Zygote** (2n) 2N

Male gamete (n) + Egg (n)

(2) Triple fusion :

♂ Male gamete (n) + Two fused polar nuclei (2n) ⟶ **PEN** primary endosperm nucleus 3N (Endosperm)

The other male gamete moves towards the two polar nuclei located in the central cell and fuses with them to produce a triploid primary endosperm nucleus (PEN).

The two types of fusion, i.e., **syngamy** and **triple fusion** occurs in an embryo sac, it is termed as **double fertilization.**

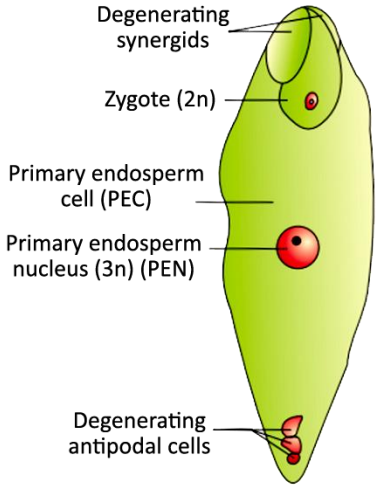


Fig : A Fertilized embryo sac showing zygote and PEN

POST – FERTILIZATION : STRUCTURES AND EVENTS

The post fertilization events include :

I. Endosperm development.
II. Embryo development
III. Ovules maturing into seed
IV. Ovary maturing into fruit

I. Endosperm

This is a product of triple fusion and develops from central cell of embryo sac. It is generally a **triploid tissue.** The cells of this tissue are filled with reserve food materials and are used for the nutrition of the developing embryo. It is absent in families such as Orchidaceae, Podostemaceae and Trapaceae.

Fate of Endosperm : Endosperm is meant for nourishing the embryo. There are two possibilities :

(i) Endosperm is completely consumed during development of embryo before seed maturation. Such seeds are called as **Ex albuminous** or **non-endospermic seeds. Examples :** Pea, Beans, Groundnut.

(ii) **Endosperm persists in mature seeds :** Such seeds are called as albuminous or endospermic seeds

Examples : Castor, coconut, rice, wheat, maize barley.

II. Embryo Development :

The development of embryo from a zygote is called as embryogeny.

(1) **Embryogeny in Dicot Plants**

(a) Zygote (oospore) divides into two unequal cells, larger suspensor cells micropyle and a smaller embryonal cell (terminal cell) towards antipodal region.

(b) The suspensor cell undergoes transverse divisions forming 6-10 celled long suspensor.

(c) The first cell of the suspensor (towards micropyle) is large and called haustorium or vesicular cell.

(d) The last cell of the suspensor towards embryo cell is hypophysis. It forms radical tip.

(e) Embryonal cell divides twice vertically and once transversely to produce two tiered eight – celled embryo.

(f) The epibasal tier forms two cotyledons and a plumule while hypo basal tier produces only hypocotyls and most of radical.

(g) For this the octant embryo undergoes periclinal divisions producing protoderm, procambium and ground meristem. It is initially globular but with the growth of cotyledons it becomes heart – shaped and then assumes the typical shape.

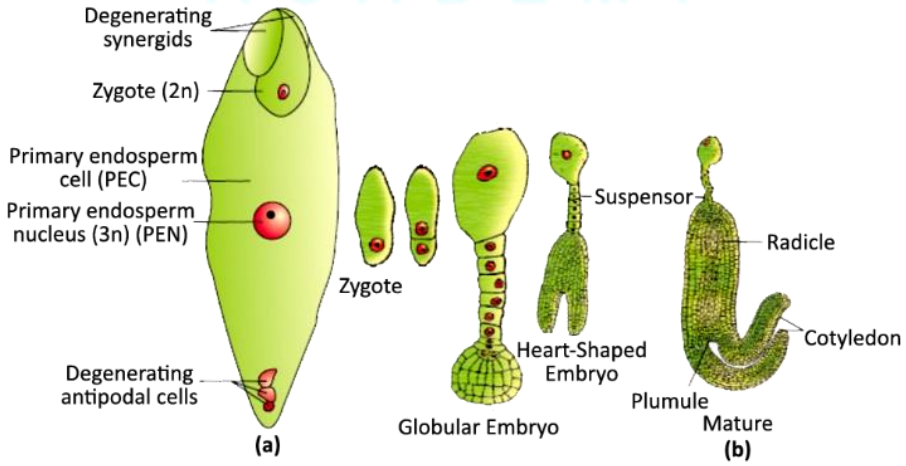
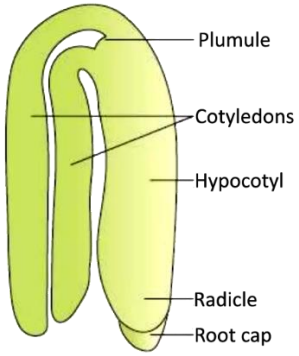


Fig. : (a) Fertilised embryo sac showing zygote and Primary Endosperm Nucleus (PEN); (b) Stages in embryo development in a dicot [shown in reduced size as compared to (a)]

Structure of a typical dicot embryo :



A typical dicotyledonous embryo consists of

(i)An embryonal axis(ii)Two cotyledons

Pasts of Embryonal axis :

(i) **Epicotyl :** The portion of embryonal axis above the level of cotyledons which terminates into **stem tip** or **plumule.**

(ii) **Hypocotyl :** The embryo cell divides transversely again into a top and a middle cell.

(iii) The terminal cell divides vertically and transversely into a globular embryo.

Structure of a typical monocot embryo :

(i) A single cotyledon called as **scutellum** that is situated towards one side (lateral) of embryonal axis.

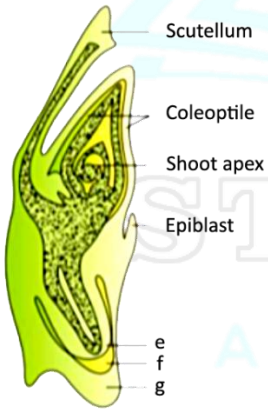


Fig : L.S. of an embryo of grass

(ii) At its lower end, the **embryonal axis** has radical and root cap enclosed in an undifferentiated sheath called **coleorhizae.**

(iii) **The portion of embryonal axis above the level of attachment of scutellum is the epicotyls. It has a shoot apex and few leaf primordial enclosed in a hollow foliar structure called** coleoptiles.

(iv) Remains of second cotyledon occur in some grasses. It is called as **epiblast.**

III. Seed

Definition : A fertilized ovule is called a seed.

Seeds may be :

Endospermic/Albuminous seeds : e.g., Wheat maize, barley, sunflower, coconut, castor.

(1) **Non – endospermic/Ex albuminous seeds :** e.g., Pea, bean, groundnut.

(2) **Peri spermic seeds :** Seeds in which remains of nucellus is seen. The residual, persistent nucleus is called perisperm, e.g., Black pepper, beet.

❖ **Structure of Seed :**

A typical seed consists of :

(1) **Seed coat :** Formed from integuments of ovule. Its function is to give protection to the embryo. **The outer layer of seed coat is called as Testa** and the **inner one is called tegmen.** The micropyle remains as a small pore is the seed coat. Facilitates entry of O_2 and water into the seed during germination.

(2) **Endosperm :** Present or absent.

(3) **Embryo :** It gives rise to the mature plant and maintains continuity of generation.

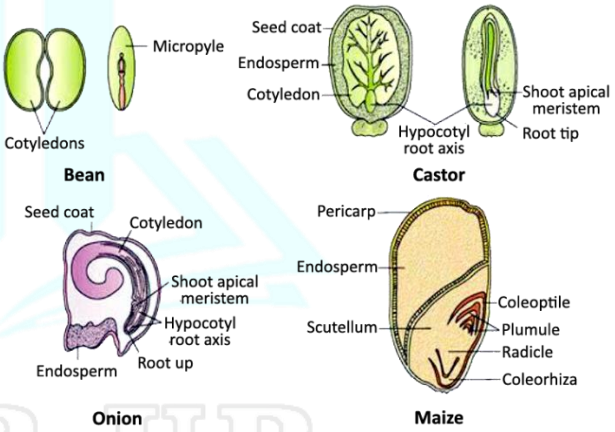


Fig : Structure of some seeds

⇨ Dormancy and Seed Germination

(1) Dormancy : It is a state of inactivity of embryo when the seed is not able to germinate. The moisture content of seed decreases and reaches 10 – 15%.

(2) Germination : The ability of a seed to produce a seeding in presence of favorable environmental condition like adequate moisture, oxygen and suitable temperature.

Advantages of Seed to Angiosperms

(1) Seeds have better adaptive strategies for dispersal to new habitual.

(2) It has sufficient food reserves for nourishment of young seedlings.

(3) Protection is provided to young embryo by the hard seed coat.

(4) Generate new genetic recombination as it is a product of sexual reproduction.

Seeds – Basis of our agriculture

Seeds form the basis of our agriculture as they show :

(1) Dehydration

(2) Dormancy

These two features help in storage of seeds which can be used as food throughout the also to raise crops in the next season.

Seed Viability :

The period for which the seeds retain their power of germination is called **seed viability.**

There are examples where seed lose viability within few months, e.g., Oxalis. Seeds of a large number of species live for several years. Some seeds can remain alive for hundreds of years. There are several records of very old yet viable seeds :

(1) The oldest is that of a **lupine.** Lupinus arcticus excavated from Arctic Tundra.

The oldest germinated and flowered after an estimated record of 10,000 years of dormancy.

(2) During an archaeological excavation at King Herod's Palace near the Dead Sea, a 2000 years old viable seed of date palm, Phoenix dactylifera was found.

The number of seeds in a fruit is generally equal to or less than the number of ovules in an ovary. It never exceeds the number of ovules :

(1) Orchid's fruit contains thousands of tiny seeds.

(2) Parasitic species like Orobranchial and Striga also contain many tiny seeds.

IV. Fruit

(1) A ripened ovary is called a fruit.

(2) The wall of the ovary forms the wall of the fruit which is called as **pericarp.**

(3) Fruits may be :

(i) **True fruit :** Fruit which develops from the ovary, e.g., Mango.

(ii) **False fruit :** The which develops from other floral parts and thalamus along with the development of ovary wall, e.g., Apple, strawberry, cashewnut.

(iii) **Parthenocarpic fruit :** When fruits develop without the process of fertilization. These fruits are seedless and can be produced through application of growth hormones like auxins. E.g., Banana.

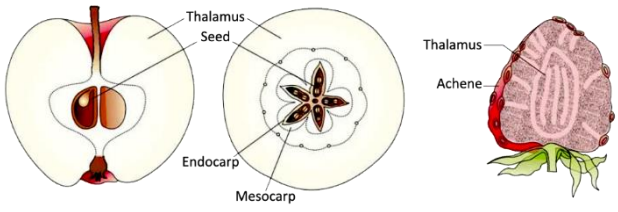


Fig : False fruits of apple and strawberry

The first stimulus for fruit development comes from pollination while second stimulus is received from developing seeds and the third stimulus is provided by the availability of nutrients.

APOMIXIS AND POLYMBRYONY

Apomixis

Although seeds, in general, are the products of fertilization a few flowering plants such as some species of Asteraceae and grasses, have evolved a special mechanism, to produce seeds without fertilization, called apomixes. It is a form of asexual reproduction that mimics sexual reproduction.

Types of Apomixis :

1. **Adventive Embryony (Sporophyte budding) :** Embryo arise from diploid saprophytic cells such as nucellus or integuments (other than egg), e.g., Citrus, Opuntia, Mango.
2. **Recurrent Agamospermy :** In this method, a diploid embryo sac is formed from megaspore mother cell of nucellar cell which has a diploid egg or ecosphere. The diploid egg develops into a diploid embryo, e.g., Apple, Pear.

Polyembryony

Occurrence of more than one embryo in a seed is referred as polyembryony.

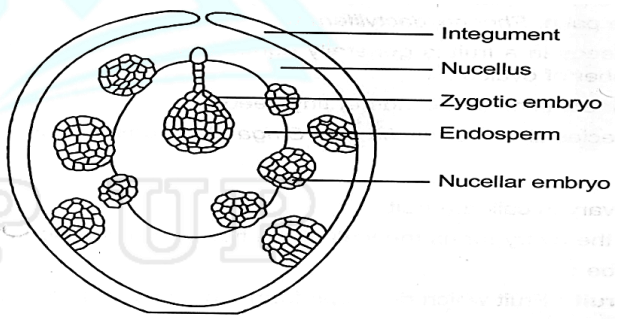


Fig. : Polyembryony

The diagram above shows several embryo formations,

The embryos shown are :

1. Zygotic
2. Nucellar
3. Integumentary

The nucellar and integumentary embryos are apomictic. The genetic nature of these embryo is diploid (2n) and they would be genetically identical to each other and can be called as a clone.

Potential of Apomictic Polyembryony :

Hybrid varieties are preferred by agriculturists because of their higher yield, vigor and resistance to stresses. They have increased crop productivity, e.g., Maize, Tomato, Cauliflower etc.

Major problem of using hybrid varieties: Hybrid characters are not maintained because they segregate in the progeny in the second generation. So, in order to obtain higher yield hybrid seeds have to be produced ever year. It is not economical for the farmers.

Therefore, agricultural scientists are for the search of various methods to maintain hybrid traits indefinitely. One of the possible methods is development of seeds though apomixes. Since embryo in apomictic seeds often develops from diploid cells, segregation of traits will not occur and the new seeds will contain all the traits of the hybrid variety.

Apomixis is genetically controlled. Genes controlling apomixes are being searched. As soon as they are located, efforts will be made to transfer them into hybrid varieties.

Chapter 2 Human Reproduction

Introduction:

Human are sexually reproducing and viviparous organisms. Their reproductive events include formation of gametes **(gametogenesis),** i.e., sperms in male and ovum in females, transfer of sperms into the female genital tract (**insemination)** and fusion of male and female gametes **(fertilisation)** leading to formation of zygote. This is followed by formation and development of blastocyst and its attachment to the uterine wall **(implantation),** embryonic development **(gestation)** and delivery of the baby **(parturition).**

THE MALE REPRODUCTIVE SYSTEM

The male reproductive system is located in the pelvic region. It includes a pair of **testes**, with **accessory ducts, glands** and the **external genitalia.**

The Testes

The testes are situated outside the abdominal cavity within a pouch called **scrotum.** The scrotum helps maintaining the low temperature of the testes (2 – 2.5°C) lower than the normal internal body temperature which is necessary for spermatogenesis. The slightly cooler temperature of the scrotum is necessary for the development of normal sperm. The testes start their development in the abdominal cavity. But during the 7th month of the fetal life, they descend into the scrotal sacs in presence of testosterone hormone. Hence, the testes of human males are extra abdominal. If they fail to descend, this condition is called **cryptorchidism** that leads to sterility. Scrotum remains connected with the abdomen or pelvic cavity by the **inguinal canal.** Blood vessels, nerves and conducting tubes pass through it. **Cremaster muscles** and connective tissues form spermatic cord and surround all structures passing through inguinal canal. Cremaster muscles and **dartos muscles** of the scrotal sac help in the positioning of testes. Whenever the outside temperature is low, these contract to move the testes close to the abdominal or pelvic cavity. When outside temperature is high, these relax moving the testes away.

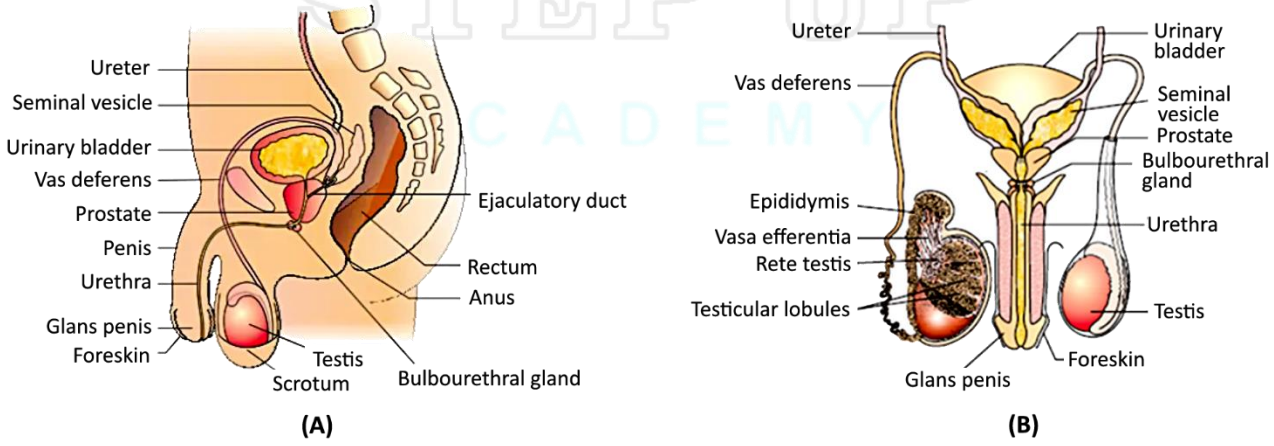


Fig. : (A) Diagrammatic sectional view of male pelvis showing reproductive system, (B) Diagrammatic view of male reproductive system (part of testis is open to show inner details)

In adults, each is oval in shape, with a **length** of about **4 to 5 cm** and **width** of about **2 to 3 cm.** The testis is covered by a dense covering. They are enclosed in an outer tough capsule of collagenous connective tissue, the **tunica albuginea.** Each testis has about 250 compartments called **testicular lobules,** these compartments contain highly coiled tubules called **seminiferous tubules.**

Each lobule contains one to three **seminiferous tubules** in

which the sperms are produced. Each seminiferous tubule is lined on its inside by two types of cells called **male germ cells** (spermatogonia) and **Sertoli cells.** Spermatogonia lining these tubules give rise to spermatozoa which are released into the lumen of the tubule. In between spermatogenic cells, Sertoli or **sustentacular** or nurse cells are present which provide nourishment to developing spermatozoa and regulate spermatogenesis by regulate spermatogenesis by releasing inhibin to check FSH over-activity. The other functions of sertoli cells are

(i) To absorb the parts being shed by developing spermatozoa.
(ii) To release anti mullerian factor (AMF) to prevent development of mullerian duct/oviduct in male.
(iii) To release Androgen Binding Protein (ABP).
(iv) To form blood-testis barrier.

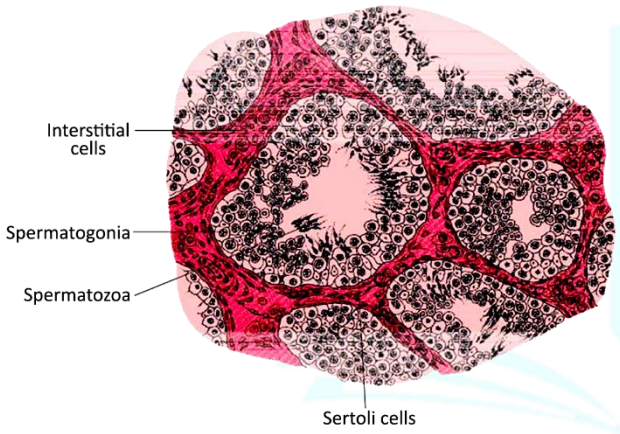


Fig. : Diagrammatic sectional view of seminiferous tubule

Groups of polyhedral cells **Interstitial cells of Leydig,** are located in the connective tissue around the seminiferous tubules. They constitute the endocrine tissue of the testis. Leydig cells synthesise and secrete testicular hormones called **Androgens** into the blood. Seminiferous tubules unite to form several straight tubules called **tubuli recti** which open into irregular cavities in the posterior part of the testis which is a highly anastomosing labyrinth of cuboidal epithelium lined channels called **rete testis.** Several tubes called vasa efferentia arise from it and conduct spermatozoa out of the testis.

The **extratesticular duct system** consists of tubes which conduct sperms from the testes to the outside. It starts with ducts known as vasa efferentia. From each testis. 10-12 vasa efferentia confluent to form a folded and coiled tube called **epididymis** behind each testis. The epididymis consists of three parts : (i) **Caput (ii) Corpus (iii) Cauda.**

The epididymis stores the sperms temporarily. From cauda epididymis, a partially coiled tube called **vas deferens** ascends into the abdomen through inguinal canal, passes over the urinary bladder, the ductus deferens/vas deferens dilates to form ampulla, which receives duct from the seminal vesicle behind the urinary bladder and forms an **ejaculatory duct.** The final portion of ampulla passes through the prostate to open into the urethra shortly after is origin from the urinary bladder.

Urethra

Male urethera provides a common pathway for the flow of urine and semen. It is much longer in male than in female, measuring about 20 cm.

(i) First part is surrounded by prostate gland and is called prostatic/glandular part of urethera.
(ii) Membranous urethera is the second part which is situated behind the lower part of public symphysis and is smallest.
(iii) Penile urethra is situated in the penis and is the longest part.

The urethra receives the ducts of the prostate and Cowper's glands, passes through the penis and opens to the outside.

Penis

This is the **copulatory organ** of man. It is a cylindrical, erectile, pendulous organ suspended from pubic region in front of scrotum. It remains small and limp (flaccid) but on sexual arousal, it becomes long, hard and erect, ready for copulation (coitus or intercourse). Erect human penis is, on an average, about 15 cm long.

The penile mass is itself encased in a fibrous sheath, called **tunica albuginea.** The interior of the penis is formed of three cylindrical cords of spongy, erectile (cavernous) tissues. Two of these cords are thicker and situated parallel on right and left sides, forming the thick part of penis that remains in front when penis is limp, but become superior-posterior when penis is erect. These two cords are called **corpora cavernosa.** The fibres of tunica albuginea surround both the cords jointly and also form a separate sheath around each cord. Some fibres form a partition called **septum penis** between these cords. The third, smaller cord forms that part of penis which remains inferio-anterior in erect penis. Urethra runs through this cord. Hence, this cord is called **corpus urethrae** of **spongiosum.**

The extended part of corpus spongiosum is enlarged, forming a bulging, conical structure called **glans penis.** The surface of glans is formed of thin, smooth and shiny, hairless skin. The base line of glans is referred to as the **neck** of the penis. The loose skin of penis becomes folder here to form a loose, retractile skin covering upon the glans, called **foreskin or prepuce.** At the tip of glans penis is the slit like **external urethral orifice** or **meatus** by which urethra open out and discharges urine of semen.

Tyson's gland or Preputial glands, present in the skin of penis neck, secrete a white sebaceous substance called **smegma.** Microbial infection in smegama can cause irritation due to inflammation.

Accessory Glands of male

1. **Seminal vesicles- (60%)** These are paired, tubular, coiled glands situated behind the blander. They secrete viscous fluid which constitutes the main part of the ejaculate. Seminal fluid contains fructose, citric acid, inositol and prostaglandins.
2. **Prostate gland :-(25%)** The prostate gland is chestnut shaped gland and is a collection of 30-40 tubuloalveolar glands which lie at the base of the bladder and surrounds the first part of the urethra. It contributes an alkaline component to the semen. (Although, the alkalinization of semen is primarily accompalished through secretion from the seminal vesicles.)

The alkaline secretions of prostate glands help the sperms to become active and counteract any adverse effects that the urine may have on the sperms. The prostatic fluid provides a characteristic odour to the seminal fluid. Prostate gland secretes citrate ions, calcium, phosphate ions and profibrinolysin.

Prostatitis : Inflammation of prostate gland.

3. **Bulbourethral glands or Cowper's glands :** The two bubourethral glands are pea sized structures lying adjacent to the urethra at the base of penis. They secrete a viscous mucus which acts as a lubricant.

The duct system, accessory glands and penis are secondary male sex organs. Their growth, maintenance and functions are promoted by testosterone, secreted by **Leydig cells.** On the other hand, the growth, maintenance and functions of **seminiferous tubules** and **Leydig cells** are regulated respectively by **FSH** and **CSH** of anterior pituitary.

Semen

Semen is a misture of sperms and seminal fluid which is the liquid portion of semen that consists of secretions of the seminiferous tubules, seminal vesicles, prostate gland and bulbourethral glands. The average volume of semen in an ejaculation is 2.5 – 5 ml, with a sperm count (concentration) of **200 to 300 million sperms**. Out of these sperms, for normal fertility, atleast 60 percent sperms must have normal shape and size and atleast 40 percent of them must show vigorous motility.

Semen has a slightly alkaline pH of **7.2 – 7.7** due to the higher pH and larger volume of fluid from the seminal vesicles. The prostatic secretion gives semen a milky appearance whereas the fluids from the seminal vesicles and bulbourethral glands give it a sticky consistency. Semen provides sperms with transportation medium and nutrients. It neutralizes the hostile acidic environment of the male urethra (due to presence of urine) and the female vagina.

Path Sperm through the Male Body

Seminiferous tubules → Rete testis → Vasa efferentia → Epididymis → Vas deferens → Ejaculatory duct → Urethra

Delivery of Sperm : The urethra passes through the penis, an erectile copulatory organ that deposits the semen in the female reproductive tract. The penis is the male external genitalia, made up of three cylinders of special spongy tissue. Filling of blood in these tissue helps in erection of the penis that facilitate insemination. The enlarged end of the penis is called the **glans penis,** covered with a loose fold of skin called **foreskin** or **prepuce.** Semen is forcefully expelled from the penis by the contractions of smooth muscles that line the urethra. This process is ejaculation.

THE FEMALE REPRODUCTIVE SYSTEM

The female reproductive system consists of a pair of **ovaries**, a **duct system** consisting of a pair of fallopian tubes (oviducts), a uterus, cervix and vagina. A pair of **mammary glands** are accessory **genital glands**.

Ovaries

The ovary is the primary female sex organ. It produces ova and secretes the female sex hormones, estrogens and progesterone which are responsible for the development of secondary female sex characters and cause marked cyclic changes in the uterine endometrium. The human ovaries are small, almond-like flattened bodies, about 2 to 4 cm in length and is connected to the pelvic wall and uterus by ligaments.

1. **Location :** Ovaries are located near kidneys and remain attached to the lower abdominal cavity through **mesovarium.**

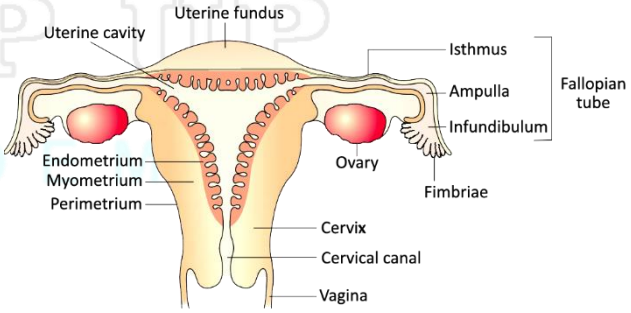


Fig. : Diagrammatic sectional view of the female reproductive system

2. **Structure :** The free surface of the ovaries is covered by a **germinal epithelium** composed of a single layer of cubical cells. This epithelium is continuous with the mesothelium lining called **peritoneum**. The epithelium encloses the ovarian stroma. The stroma is divided into two zones – a peripheral cortex and an inner medulla. Immediately below the germinal epithelium, the cortex is covered by a connective tissue called **tunica albuginea.**

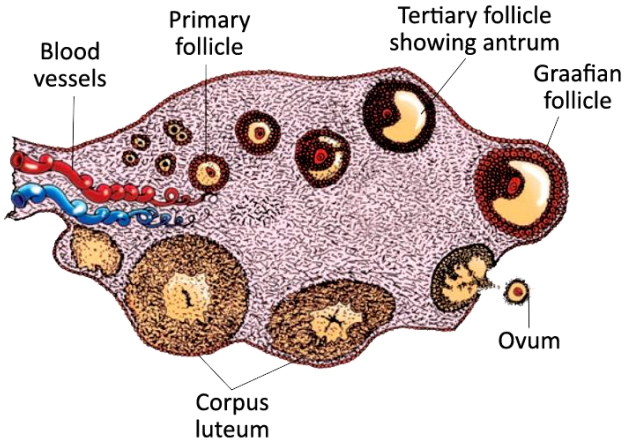


Fig. : Diagrammatic Section view of ovary

The **cortex** contains numerous spherical or oval, sac-like masses of cells known as **ovarian follicles**. The **medulla** consists of loose connective tissue, elastic fibres, numerous blood vessels and some smooth muscle fibres.

Internal Structure :

(a) Ovarian follicle : The ovarian follicle contains a large, centrally placed ovum, surrounded by several layers of granular cells **(follicular granulose** or discus, proligerus or cumulus oophorus). It is suspended in a small cavity called the antrum. Antrum is filled with a fluid known as **liquor foliculi**. The secondary oocyte in the tertiary follicle also forms a new membrane called **zona pelucida**. The follicle bulges onto the surface of the ovary. Such a follicle is called the mature **Graafian folicle** (**after de Graf,** who reported them in 1672 and considered them to be eggs).

(b) Corpus – luteum : The ovum is shed from the ovary by rupture of the follicle. The shedding of the ovum is called **ovulation** and occurs nearly 14 days before the onset of the next menstrual cycle.

After the extrusion of the ovum, what remains in the Graafian follicle is called **corpus, luteum(yellow body).** The cytoplasm of the corpus luteum is filled with a yellow pigment called **lutein**. The corpus luteum grows for a few days and if the ovum is fertilized and pregnancy results, it continues to grow. But if the ovum is not fertilized, the corpus luteum persists only for about 14 days and during this period, it secretes progesterone and small amount of estrogen. At the end of its functional life, the corpus luteum degenerates and is converted into a mass of fibrous tissue called **corpus albicans (white body).**

Fallopian Tubes (Oviducts)

These are one pair of long (10 to 12 cm), ciliated, muscular and tubular structures which extend from theperiphery of each ovary to the uterus. Each oviduct is suspended by mesosalpinx and is differentiated into three parts :

(i) Infundibulum : The part of oviduct closer to the ovary is the funnel shaped infundibulum. The edgesof infundibulum possess finger-like projections called fimbriae. Fimbriae help in the collection of the ovum after ovulation. Infundibulum opens-into the abdominal cavity by an aperture called ostium.

(ii) Ampulla : The infundibulum leads to a wider part of the oviduct called ampulla.

(iii) Isthmus : It is the last and narrow part having narrow lumen that links to the uterus.

The tube is involved in conduction of the ovum or zygote towards the uterus by peristalsis and ciliary action. It is also the site of fertilization. (Fertilization occurs **at the junction of ampulla and isthmus).**

Uterus (Hystera/Womb)

It is a large hollow, muscular, highly vascular and inverted pear shaped structure present in the pelvis between the bladder and rectum. It is suspended by a mesentery, the **mesometrium.** It has the following three parts.

(i) Fundus : It is upper, dome-shaped part above the opening of fallopian tubes.

(ii) Corpus/Body : It is the middle and main part of uterus.

(iii) Cervix : It is lower, narrow part which opens in body of uterus by **Internal os** and in vagina below by **external os. It is mainly formed of the most powerful sphincter muscles in the body.** The cavity of the cervix is called **Cervical canal** which along with along with vagina forms the **birth canal.**

Wall of uterus : The wall of uterus is formed of outer peritoneal layer, **perimetrium**; middle muscular **myometrium** of smooth muscle fibres, and inner highly vascular and glandular **endometrium.** The endometrium undergoes cyclical changes during menstrual cycle while myometrium exhibits strong contractions during delivery of the baby. Implantation of embryo in uterine fundus.

It is the site of growth pregnancy. It also takes part in placenta formation and expulsion of the baby during parturition.

Vagina

It is a long (8.5 cm) fibro-muscular tube. It extends backward in front of rectum and anal canal from cervix to the vestibule. It is a highly vascular tube lined internally by mucus membrane which is raised into transverse folds called vaginal-rugae. It is lined with stratified, squamous epithelium. (Non Keratinised). Vagina is devoid of glands.Vaginal orifice is covered partially by a membranous diaphragm called **hymen**. The hymen is often ruptured during the first coitus (intercourse). However, it can also be broken by a sudden fall or jolt, insertion of a vaginal tampon, active participation in some sports like

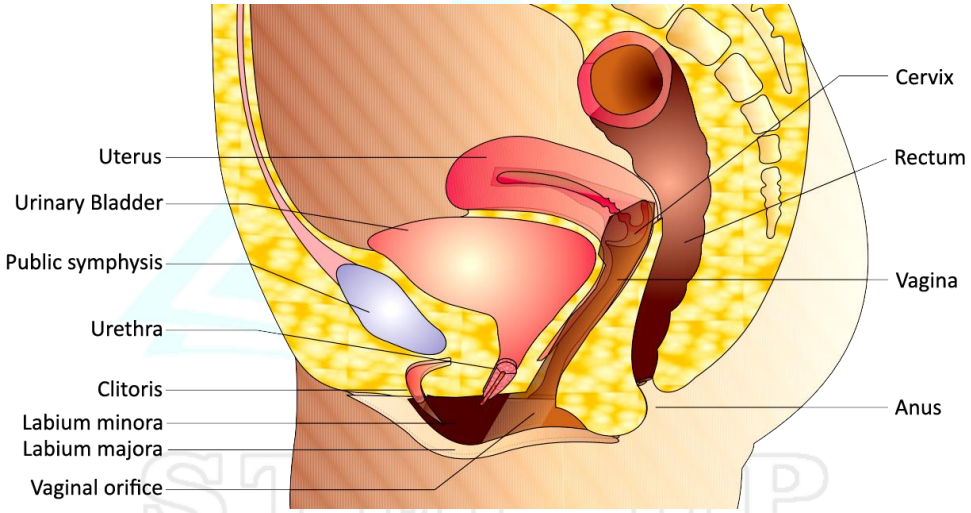
horseback riding, cycling etc. In some women the hymen persists even after coitus, In fact, the presence or absence of hymen is not reliable indicator of virginity or sexual experience.

Vagina acts both as **copulation canal** (as it receives the sperms from penis during copulation) and as **birth canal** along with cervix (during parturition).

External Genitalia

The external genital structures of the female reproductive system are collectively called the **vulva**. The female external genitalia or vulva includes mons pubis, labia majora, labia minora, hymen and clitoris. **Mons pubis**is a cushion of fatty tissue covered by skin and pubic hair. The **labia majora** are fleshy folds of skin, which extend down from mons pubis and surround the vaginal opening. The **labia minora** are paired folds of tissue in the form of lips under the **labia majora**. The opening of vagina is often covered partially by a membrane called **hymen**. The clitoris is a tiny finger-like structure which lies at the upper junction of the two labia minora above the urethra! opening. It is formed of two erectile bodies and is covered by skin fold called **prepuce**. It has a depression, the vestibule, in front of anus. Vestibule has **two apertures-upper external urethra lorifice** and lower **vaginal orifice.**

Vestibule is bounded by two pairs of moist skin folds called **labia minora** and **labia majora. Is homoglous to scrotum.** Labia minora fuse anteriorly to form a skin fold called **prepuce** in front of a small erectile organ, the **clitoris** which is homologous to penis as both are supported by corpora cavernosa. Labia the minora also fuse posteriorly to form a membranous fold called **fourchette**. The area between the fourchette and the anus is called perineum. There is fleshy elevation above the labia majora and is known as **mons pubis** which has pubic hair.



Accessory Glands

Vestibular Glands : These are of two types-greater and lesser. Greater vestibular or **Bartholin's** glands are a pair of small reddish glands on each side of vaginal orifice and secrete alkaline secretion for lubrication and neutralizing urinary acidity. **Lesser vestibular glands** or paraurethral or **skene's** glands are small mucus glands present between urethral and vaginal orifices.

Mammary Glands/ Breasts

There are a pair of rounded prominences present over the pectoralis major muscles on the front wall of the thorax. These remain in rudimentary form in male. in females, these remain undeveloped till puberty. At puberty, these start developing under the influence of oestrogen and progesterone hormones. On the external side, each breast has a projection, the 'nipple' surrounded by rounded hyper pigmented area called areola and appear deep pink or light brown. On the surface of the areola, numerous sebaeceus glands, called areolar glands are present.

Internally, the breast consists of the glandular tissue forming mammary glands, the fibrous tissue (connective tissue) and the fatty or adipose tissue. Mammary glands are modified sweat glands.

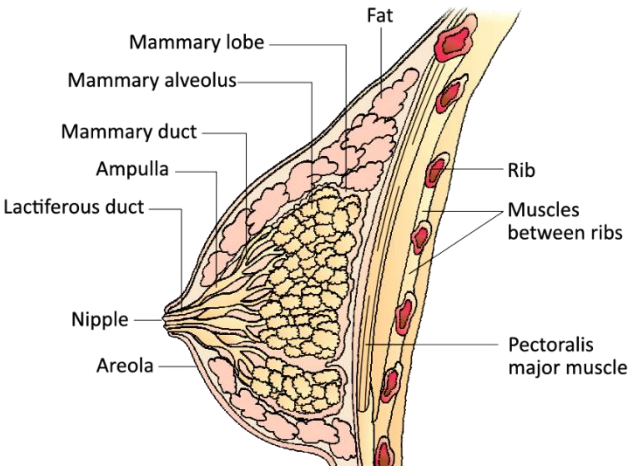


Fig.: Female's Breast in Sagittal Section

(a) The **glandular tissue** comprises about 15-20 lobes in each breast. Each lobe is made up of a number of **lobules**. Each lobule is composed of grape like clusters of milk secreting glands termed **alveoli**. When milk is produced it passes from the alveoli into the **mammary tubules** and then into the **mammary ducts**. Near the nipple, mammary ducts expand to form mammary ampullae (lactiferous sinuses) where some milk may be stored before going to **lactiferous ducts**. Each lactiferous duct typically carries milk from one of the lobes to exterior.

Mammary alveoli →Mammary tubule → Mammary duct → Mammary ampulla → Lactiferous duct.

(b) The **fibrous tissue** (connective tissue) supports the alveoli and the ducts.

(c) The **fatty** or **adipose tissue** is found between the lobes and covers the surface of the gland. The amount of the adipose tissue determines the size of the breasts.

Main functions of the mammary glands are secretion and ejection (release) of milk. These functions are called **lactation**. Lactation is associated with pregnancy and child birth. Milk production is stimulated largely by the hormone prolactin secreted by anterior lobe of the pituitary gland. The ejection of milk is stimulated by the hormone **oxytocin,** released from the posterior lobe of the pituitary gland.

Human milk consists of water and organic and inorganic substances. Its main constituents are **fat** (fat droplets). c**asein** (milk protein), **lactose** (milk sugar), mineral salts (sodium, calcium, potassium, phosphorus, etc) and vitamins. Milk is poor in iron content. Vitamin C is present invery small quantity in milk. The process of milk secretion is regulated by the nervous system. It is also influenced by the psychic state of the mother. The process of milk production is also influenced by hormones of the pituitary gland (already mentioned), the ovaries and other endocrine glands. A nursing woman secretes 1 to 2 liters or milk per day.

GAMETOGENESIS

The primary sex organs - the testis in the males and the ovaries in the females, produce gametes i.e. sperms and ovum respectively, by the process called **gametogenesis.**

Spermatogenesis

In testis, the immature male germ cells, spermatogonia produce sperms by a process Spermatogenesis that begins at puberty. Spermatogenesis occurs in four stages: (i) **Spermatocytogenesis**, (ii) **Meiosis-I**, (iii) **Meiosis-II** and (iv) **Spermiogenesis**.

(i) Spermatocytogenesis : In Spermatocytogenesis, the **spermatogonia** present on the inside wall of the seminiferous tubules multiply by mitotic division, and increase in number. Each **spermatogonia** is diploid containing **46 chromosomes**. Some spermatogonia undergo changes they grow, increase in size by accumulating nourishing materials and are called **primary spermatocytes** which periodically undergo meiosis and others remain as **spermatogonia**.

(ii) Meiosis-I : A primary spermatocyte is diploid, (2n) with 44 + XY (total 46) chromosomes. It completes the first meiotic division (reduction division) leading to the formation of two equal, haploid cells called **secondary spermatocytes**, which have only 23 chromosomes each

i.e. 22 + X or 22 + Y.

(iii) Meiosis-II : The secondary spermatocytes undergo the second meiotic division to produce four equal, haploid spermatids. The number of chromosomes in each spermatid is 23.

(iv) Spermiogenesis : Transformation of spermatid into sperm is **termed Spermiogenesis**. A spermatid is non-motile and heavy. It has organelles like mitochondria, Golgi bodies, centrioles, nucleus etc. During Spermiogenesis, the weight of gamete is reduced along with the development of locomotory structures. Nucleus becomes compact forming the major part of head of spermatozoa. **Golgi complex of spermatid gives rise to acrosome. The two centrioles of the spermatids become arranged one after the other behind the nucleus. Mitochondria** from different parts of spermatid get arranged in **the middle piece around axial filament**. Much of the cytoplasm of a spermatid is lost. It forms a thin layer around middle piece. A typical mammalian sperm is flagellated, consisting of four parts namely **head, neck,middle piece** and **tail**. The human sperm was first seen by **Hamm** and **Leeuwenhoek**. After Spermiogenesis the sperm heads become embedded in the **Sertoli cellls**, and are finally released from the seminiferous tubules by the process called **spermiation.**

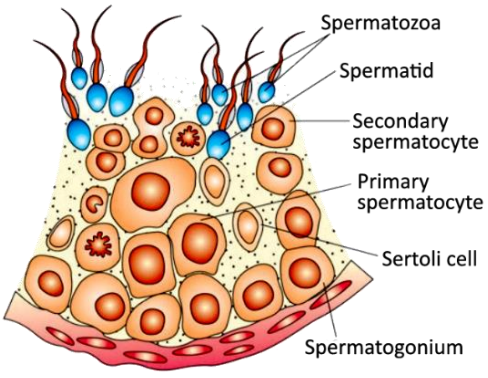


Fig. : Diagrammatic sectional view of a seminiferous tubule

In Spermatogenesis from one primary spermatocyte four haploid sperms are formed.

Hormonal Control of Male Reproductive System : Spermatogenesis starts at the age of **puberty** due to significant increase in the secretion of gonadotropin releasing hormone (GnRH) from hypothalamus. The increased levels of GnRH then acts at the **anterior pituitary** gland and stimulates secretion of two gonadotropins – **luteinizing hormone** (LH) and follicle **stimulating hormone** (FSH). LH acts at the Leydig cells and stimulated.

synthesis and secretion of androgens. Androgens, in turn, stimulate the process of spermatogenesis. FSH acts on the Sertoli cells and stimulates secretion of some factors which help in the process of **Spermiogenesis.**Sertoli cells also secrete another protein, hormones called **inhibin**, which suppresses FSH synthesis. So, FSH along with testosterone stimulate the sperm production in the seminiferous tubules

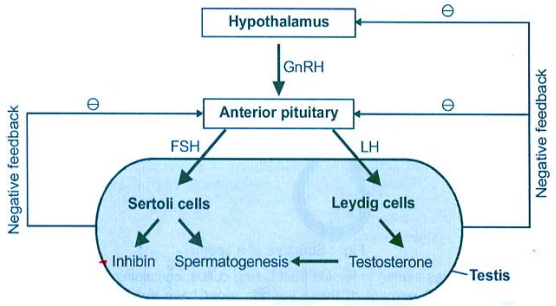


Fig. Hormonal control of the testes

Structure of Mature Sperm: Mature sperm cell consists of a **head**, a **neck**, a **middle piece** and a **tail**. A plasma membrane envelop the whole body of sperm. The sperm **head** contains a very little cytoplasm, an elongated haploid nucleus, the anterior portion of which is covered by a cap-like structure, **acrosome**. The acrosome is filled with enzymes that help in fertilisation of ovum. These enzymes called **sperm lysins** that dissolve the membranes enveloping the ovum and help the sperm cell to enter the ovum. **Acrosome**is derived from **golgi apparatus**. Its membrane extends down the outer surface of nucleus. The **short neck,** contains two distinct granules - the p**roximal and distal centrioles**. The **proximal centriole** plays a crucial role during the first cleavage of the fertilized ovum. The **distal centriole** gives rise to the axial filament of the long tail of the sperm. The **middle piece** possesses numerous mitochondria (25 to 30 arranged spirally) which produce energy for the movement of tail that facilitates sperm motility essential for fertilisation, that is whyit is called as the **power house** of the sperm. The **tail** is made up of a central axial filament surrounded by a small amount of cytoplasm and cell membrane as external sheath. The sperms move by **swimming** at the rate of 1.5 to 3 mm per minute to reach the site of fertilisation within 30 minutes.

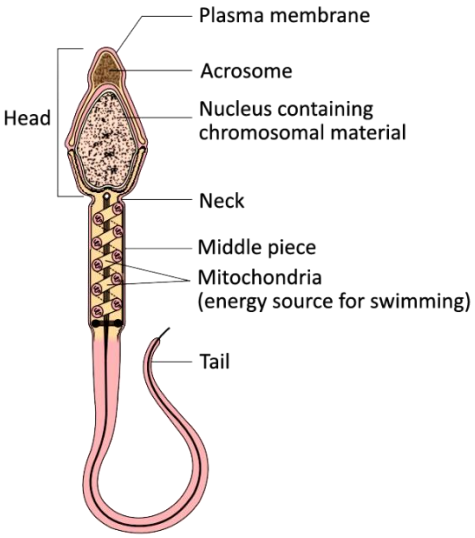


Fig. : Structure of a sperm

The human male ejaculates semen in female tract during coitus, containing 200 to 300 million sperms. **For normal fertility, at least 60 percent of these sperms must have normal shape and size and at least 40 percent of them must show vigorous motility.**

Sperms released from seminiferous tubules, are transported by the accessory ducts, secretions of epididymis, vas deferens, seminal vesicle and prostate are essential for maturation and motility of sperms. **The functions of the male accessory ducts and glands** are maintained by the testicular hormones (androgens).

Oogenesis

The process of formation of a mature female gamete is called **oogenesis** which is markly different from spermatogenesis. **Oogenesis** is initiated during the **embryonic** development stage when a couple of million gamete mother cells (**oogonia)** are formed within each fetal ovary ; no more oogonia are **formed** or **added** after birth. Scattered ovarian follicles are embedded in the stroma of cortex.

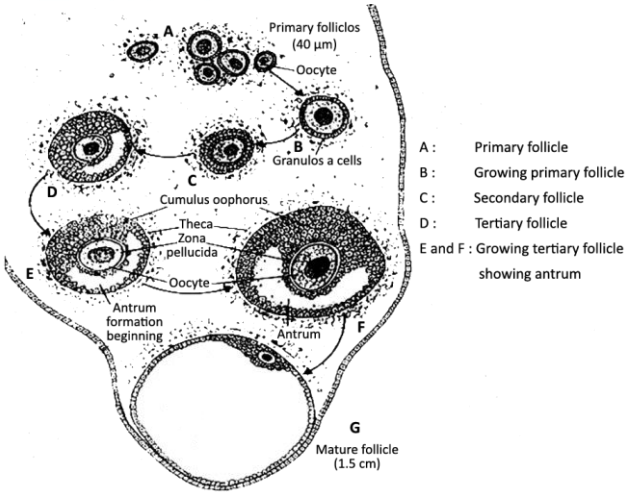


Fig. : Development of a human oocyte and ovarian follicle

An ovarian follicle consists of an oocyte, surrounded by one or more layers of follicular (flat epithelial) cells, the granulosa cells, which are derived from the germinal epithelium lining the ovary. The oogonial cells start division and enter into prophase-1 of the meiotic division, and get temporarily arrested at this stage called **primary oocytes**. Each primary oocyte gets surrounded by a layer of granulosa cells and then called **primary follicle.**

A large number of these follicles degenerate from birth to puberty. Degeneration of ovarian follicles is called **follicular atresia** and their disposal is done by phagocytes. Therefore, **at puberty only 60,000 to 80,000 primary follicles are left in each ovary.**

With the onset of puberty, a primary follicle begins to mature with each ovarian cycle. The follicular cells become cuboidal, divide by mitosis to form a stratified epithelium, the granulose layer. So the primary follicles get surrounded by more layers of granulosa cells and a new theca, called **secondary follicles. Granulosa** cells rest on a basement membrane and the-surrounding stromal cells form the theca folliculi. The **secondary follicle** soon transforms into a **tertiary follicle** which is characterized by a fluid filled cavity **antrum,** which appears between the granulosa cells. Initially, the antrum is cresent shaped, but with time it greatly enlarges.The fluid of antrum is liguor folliculi. As the follicles grow, the theca folliculi become organised into inner layer of secretory cells, the **theca interna** and an outer layer of connective tissue cells containing fibroblast-like cells, the **theca externa**. The maturing oocytes adhere to the wall of the follicle through a pedicel/stalk, **cumulus oophorus**, formed by, granulosa celjs, and remains suspended in liquor folliculi. Theca interna is composed of cells having characteristics of steroid secretion, rich in blood vessels and theca externa gradually merges with ovarian stroma.

The primary oocyte within the tertiary follicle grows in size and **completes** its **first meiotic division** at puberty. It is an unequal division resulting in the formation of a large **haploid secondary oocyte** and a tiny first polar body.The **secondary** oocyte retains the bulk of nutrient rich cytoplasm of the **primary oocyte.** The tertiary follicle changes into the mature follicle or **Graafian follicle.**

The secondary oocyte forms a new membrane called Zona pellucida surrounding it. This thick coat of zona pellucida is composed of **glycoproteins** and synthesised by oocyte. Later, the granulosa cells lying in close vicinity of the ovum and zona pellucida, become elongated to form the **corona radiata**. In the presence of LH hormone, the **Graafian follicle** now ruptures to release the secondary oocyte developing (ovum) from the ovary by the process called ovulatjon. After ovulation the ruptured follicle left in the ovary is converted to a structure called **corpusluteum**, which secretes mainly **progesterone**.

- **What accounts for the large difference in the size of sperm and eggs?**

  During spermatogenesis excess cytoplasm of sperm is also absorbed by the sertoli cell during spermiogenesis. The difference in the sizes of sperm and eggs is due to the difference between the process of sperm formation (spermatogenesis) and egg formation (oogenesis). During spermatogenesis, equal divisions of the cytoplasm follow meiosis-1 and meiosis-11, resulting in four equal-sized sperm cells having little cytoplasm. During oogenesis, unequal divisions of the cytoplasm follow meiosis-1 and meiosis-11, resulting in formation of one large egg and two or three small polar bodies. Polar bodies later on degenerate.

- **Does the first polar body born out of first meiotic division divide further or degenerate?**

  In human beings and most vertebrates, the 1st polar body does not undergo meiosis-II, and usually dies. However, in some species 1st polar body undergoes meiosis-II.

- **Why is unequal division of cytoplasm seen in meiotic division?**

  Due to the unequal division of cytoplasm, the mature egg or ovum, retains most of the cytoplasm, which provides nutrients for the ovum during the early stages of development.

- In human beings from one primary oocyte, a single ovum and two polar bodies are formed. The ovum is released from the ovary in secondary oocyte stage after the release of 1st polar body.

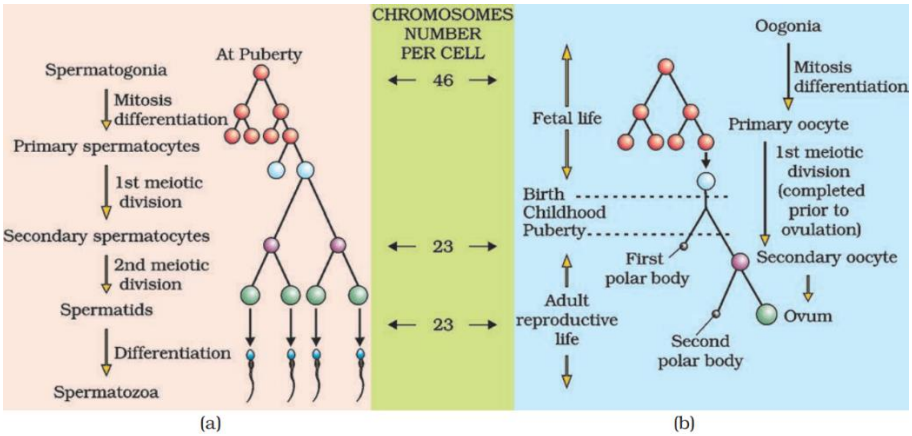


Fig. : Schematic representation of (a) Spermatogenesis; (b) Oogenesis

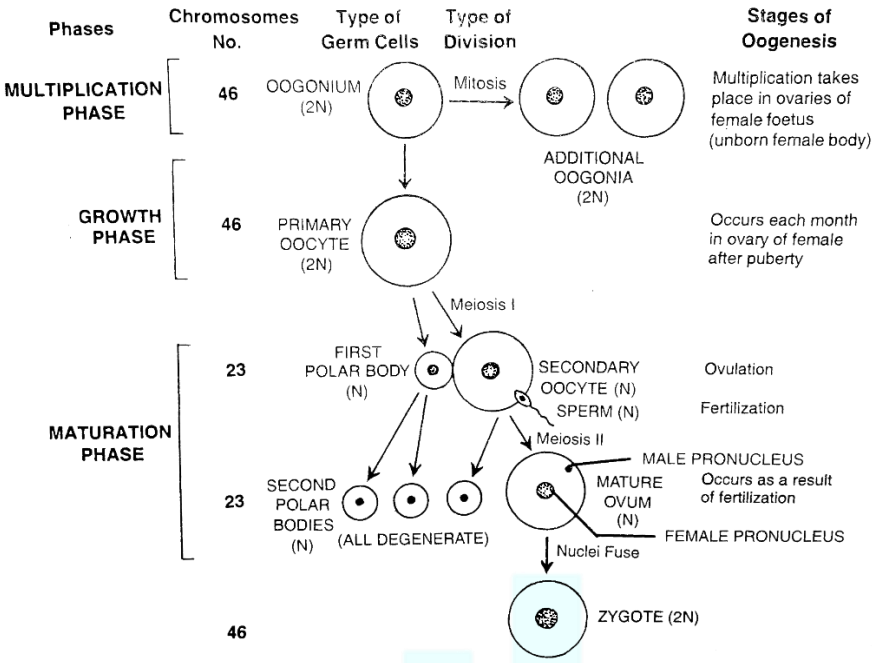


Fig. : Stages in oogenesis (diagrammatic).

MENSTRUAL CYCLE

The reproductive cycle in the female primates (e.g., monkeys, apes and human beings) is called **menstrual cycle.** The first menstruation begins at puberty and is called **menarche.** In human females, menstruation is repeated at an average interval of about 28/29 days, and the cycle of events from one menstruation till next one is called the **menstrual cycle.** One ovum is released during the middle of each menstrual cycle of 28 days. The major events of the menstrual cycle are shown in the figure.

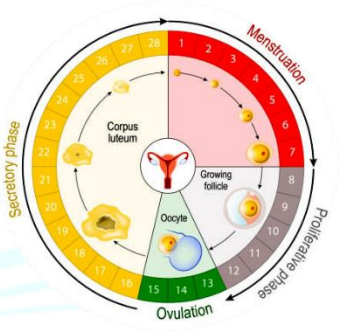


Fig. : Schematic representation of menstrual cycle.

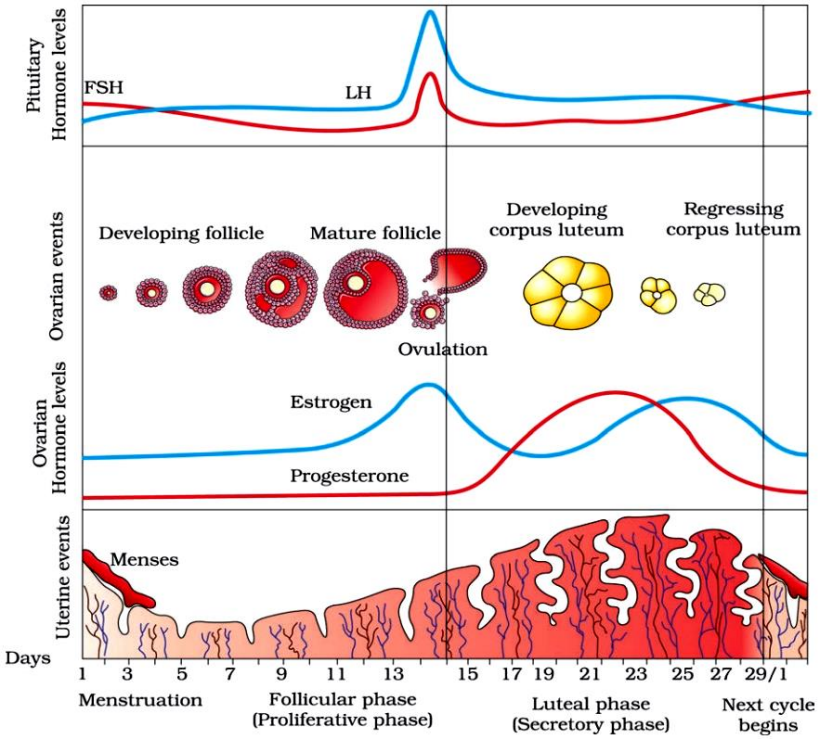


Fig. : Diagrammatic presentation of various events during a menstrual cycle

Phases of menstrual cycle

(i) Menstrual phase
(ii) Follicular phase
(iii) Ovulation
(iv) Luteal/Secretory phase

(i) Menstrual phase : Menstrual flow in this phase and it lasts for 3 to 4 days. This flow results due to the breakdown of endomentrial lining of the uterus and its blood vessels which forms a liquid and flows out through the vegina. Menstruation usually occurs about 14 days after ovulation, if the released ovum is not fertilized. Lack of menstruation may be indicative of pregnancy.

The total amount blood discharged in one cycle is 30 to 50 ml. This blood forms clot in the uterus, later fibrinolytic enzyme from the uterus dissolves the clot so the blood in the menses remains in liquid state.

(ii) Follicular phase : The menstrual phase is followed by the follicular phase. During this phase, primary follicles in ovary grow to become a fully mature **Graafian follicle** and simultaneously the endometrium of uterus regenerates through proliferation. These changes in the ovary and the uterus are induced by changes in the levels of pituitary **gonadotropins** and **ovarian hormones**. Secretion of gonadotropins (LH and FSH) increases gradually during the follicular phase, and stimulates follicular development and secretion of estrogens by the growing follicles. FSH hormone stimulates follicular growth. The follicular cells secrete estrogen, a sex hormone that also aids in the growth of the follicle. Estrogen hormone stimulates mitotic divisions of the cells in the lining of uterus, and helps to repair the broken tissue and blood vessels. It also causes the thickening of the endometrium. Both FSH and LH attain a peak level in the middle of each cycle, on 14th day of 28th day cycle. During this phase, the estrogen level in the blood continues to rise until it reaches the peak and the Graafian follicle moves to the surface of ovary. The elevated estrogen levels acts as positive feedback mechanism by stimulating the anterior lobe of pituitary to secrete luteinising (LH) hormone, which initiates the next stage of menstrual cycle. Rapid secretion of LH leading to its maximum level during the mid-cycle called as **LH surge** induces the rupture of Graafian follicle and thereby the release of ovum (ovulation).

(iii) Ovulation : LH induces ovulation which usually occurs on 14th day in the 28 days cycle. The Graafian follicle ruptures and secondary oocyte (ovum) is released.

Day of ovulation = Number of days in M cycle – 14

(iv) (Luteal phase/Secretory phase: Following ovulation, an egg is swept into the fallopian tube, where it awaits fertilisation as it travels through the tube towards uterus. The egg has stored nutrients to survive about 24 hours. The ovulatory phase is followed by luteal phase during which the remaining parts of Graafian follicle transform as **Corpus luteum** in the ovary.

Corpus iuteum secretes large amounts of progesterone which is essential for **maintenance** of endometrium which is thickened by estrogen. In luteal phase, the endometrium further thickens due to estrogen hormone also secreted by corpus luteum. LH hormone causes the cells of the ruptured follicle to form corpus luteum. A corpus luteum is a yellowish mass of follicular cells that functions like an endocrine structure. LH hormone also stimulates the corpus luteum to secrete estrogen and progesterone. Estrogen and progesterone inhibit the release of FSH and LH. This prevents the development of new follicles during the luteal phase. Luteal phase lasts for 14 days. During this phase, the levels of estrogen and progesterone will rise, while FSH and LH levels drop. Low level of LH causes, degeneration of corpus luteum leading to sudden decline in progesterone level that causes menstruation.

Maintenance of endometrium by progesterone is necessary for implantation of the fertilised ovum and maintenance of pregnancy. During pregnancy all the events of the menstrual cycle stop and there is no menstruation due to high level of progesterone.

In the absence of fertilisation, the corpus luteum degenerates; the level of progesterone hormone will fall. This causes **disintegration of** the endometrium leading to menstruation, marking a new cycle. In human beings, menstrual cycles ceases around 50 years of age, termed as **menopause**. Cyclic menstruation is an indicator of normal reproductive phase and extends between menarche and menopause.

FERTILISATION AND IMPLANTATION

During copulation (coitus) semen is released by the penis into the vagina of female, called **insemination**. A human sperm can live for many weeks in male genital duct. Once ejaculated in the semen, it lives only for 48 to 72 hours outside the body. Sperms move in the liquid medium secreted by female genital tract at a speed of 1.5 - 3.0 mm/minute. Prostaglandings of semen help in the movement of spermatozoa.

Once the sperms are released **Capacitation** of sperm occurs in the female genital system and involves :

(1) Removal of membrane cholesterol present over acrosome, weakening the membrane cover.

(2) Dilution of decapacitation factors.

(3) Entry of Ca^{2+} into sperms causing rapid whiplash movements of the tail part. They swim through the vagina, cervix, uterus and finally reach the junction of the isthmus and ampulla called ampullary-isthmic junction of the **fallopian tubes**. The ovum released by the ovary is also transported to the **ampullaryisthmic junction where fertilisation occurs**. Ovum is released in the secondary oocyte stage (arrested in metaphase-II). Due to ciliary current produced by fimbriae portion of oviduct, ovum is drawn in through ostium. It reaches ampulla, the site of fertilization, by the ciliary action of ciliated columnar epithelial lining of oviduct. Fertilisation can only occur if the ovum and the sperms are transported simultaneously to the ampullary isthmic-junction. This is the reason why not all copulations lead to fertilisation and pregnancy.

The process of fusion of a sperm with the ovum is called **fertilisation.**

Fusion of gametes/Syngamy : The various steps involved are :

Acrosomal reaction : A number of sperms adhere to the surface of egg (**Agglutination**). The acrosome starts releasing its hydrolytic enzymes or sperm lysins which include

(a) Hyaluronidase : Dissolves the hyaluronic acid responsible for cementing of follicle cells or granulosa cells.

(b) Corona penetrating enzyme (CPE) : Dissolves corona radiata.

(c) Zona lysin/Acrosin : Digests the zona pellucida.

Contact of acrosome stimulates development of an outgrowth by the oocyte called **fertilisation cone or cone of reception.**

Cortical and zona reactions : As the sperm head comes in contact with the fertilization cone, it causes opening of Na^{+} channels to cause depolarization of ovum membrane (**fast block to check polyspermy)** and Ca^{2+} move into the egg. Sperm and egg membranes dissolve. Complete sperm enters cytoplasm of egg and the envelope is left out. Ca^{2+} influx causes extrusion of cortical granules **(cortical reaction)** and **zona reactions** which make the zona pellucida impervious to second sperm by destroying sperm receptors.

Cortical reaction and zona reaction constitute**s (low block to check polyspermy)**

The entry of sperm into the ovum induces completion of the meiotic division of the secondaryoocyte. Entry of sperm causes breakdown of metaphase promoting factor (MPF) and turns on anaphasepromoting complex (APC). This results in completion of meiosis-II. The second meiotic division is also unequaland results in the formation of a **second polar body** and a **haploid ovum** (ootid). Soon the haploid nucleusof the sperm and that of ovum fuse together to form a **diploid zygote**.

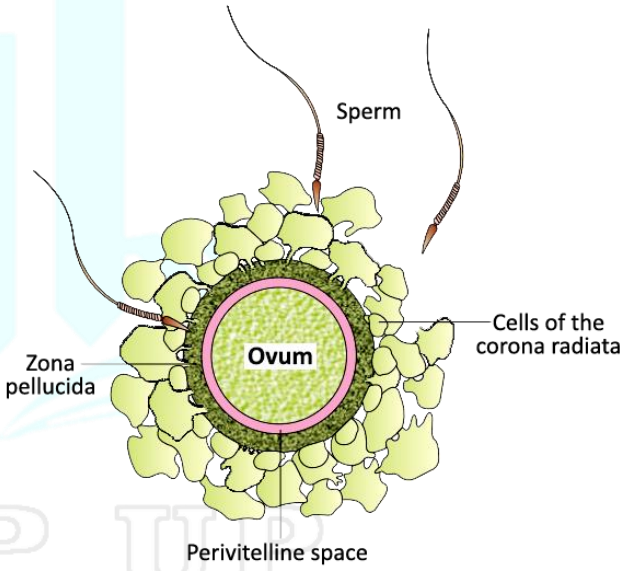


Fig. : Ovum surrounded by few sperms Embryonic Development

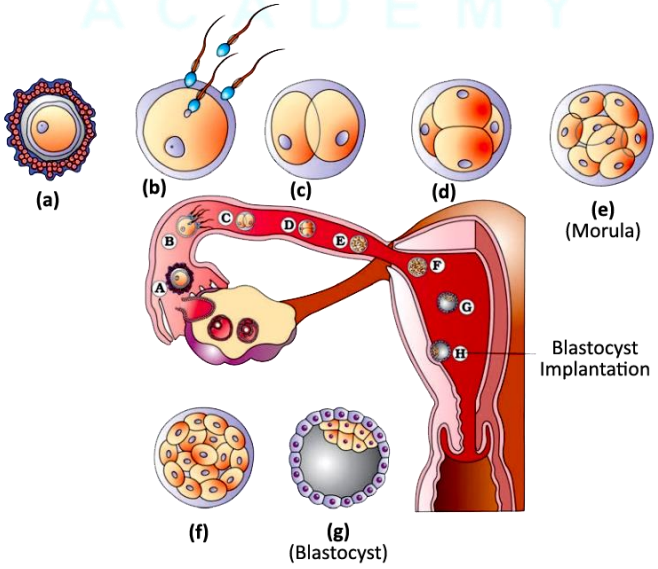


Fig. : Transport of ovum, fertilization and passage of growing embryo through fallopian tube.

It includes cleavage, blastulation, implantation, gastrulation and organogenesis.

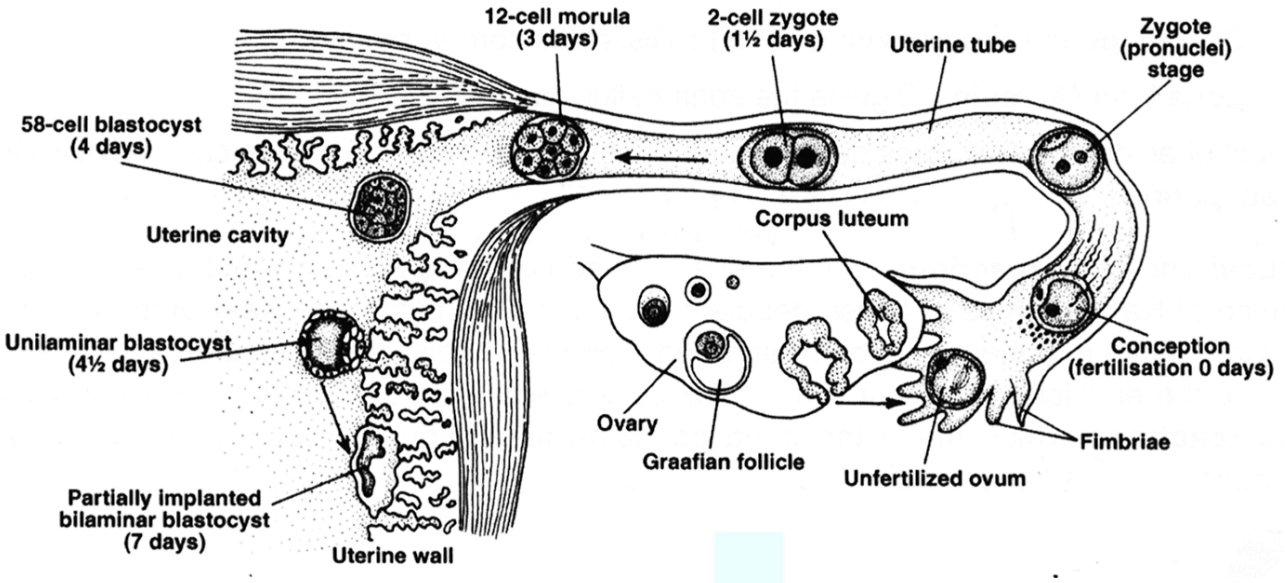


Fig. : Transport of ovum, fertilization and passage of growing embryo through fallopian tube.

Cleavage : First cleavage is completed after 30 hours of fertilization. Cleavage furrow passes from animal vegetal axis as well as centre of zygote (Meridional plane). It divides the zygote completely into the blastomeres (Holoblastic cleavage). Second cleavage is completed after 60 hours of fertilization. It is and meridional but at right angle to the first one. It is completed earlier in one of the two blastomeles result in a **transient 3-celled stage**. Third cleavage is horizontal forming 8 blastomeres. It is slightly unique. There after the rate and pattern of cleavage is non specific. In mammals, including humans, cleavage divisons are among the slowest in animal kingdom. Also, the cleavage divisons 'asynchronous. The number of results blastomeres increased following arithmetic progression.

Morula : Cleavage results in a solid ball of cells, **Morula** having 8-16 cells. Zona pellucida still forms the outer cover. Morula undergoes compaction. The outer/peripheral cells are smaller/flat with tight junctions while the inner cell mass consists of slightly large, rounded cells with gap junctions. Morula descends slowly towards uterus in 4-6 days and corona radiata detaches during this period.

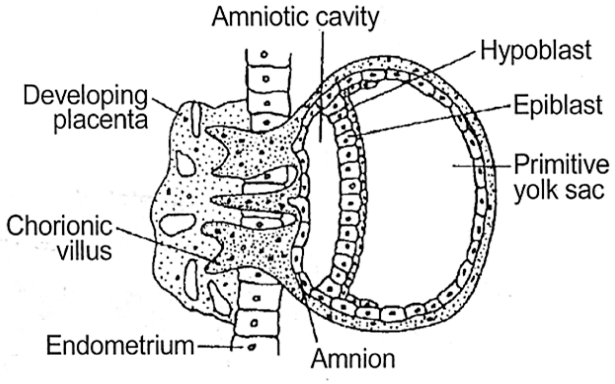


Fig. : Implanted blastocyst

Blastulation of Blastocyst Formation : Endometrium secretes a nutrient fluid and its mucosal cells become enlarged with stored nutrients. As the morula enters uterus, it gets a rich supply of nutrients. Outer peripheral cells enlarge and flatten further. They form **trophoblast or trophoectoderm.** Trophoblast cells secrete a fluid into the interior creating a cavity called **blastocoels.** The **inner cell mass** now comes to lie on one side as **embryonal knob.** With the formation of blastocoels, morula is converted into **blastula** which is called **blastocyst** in mammals because of different nature of surface layer and eccentric inner cell mass.

Due to pressure of growing blastocyst, a slit is produced in zona pellucida through which it squeezes out. The growing blastocyst comes out of this slit. At times, it gets broken into two parts which gives rise to identical wins or **monozygotic twins.**

Trophoblast cells in contract with embryonal knob are called cells of Rauber. Area of embryonal knob represents **animal pole.** The opposite side is **abembryonal pole.** Soon embryonal knob shows rearrangement to form **embryonal disc.** Cells of trophoblast layer divide periclinally. This gives rise to two layers, outer **syncytiotrophoblast** and inner **cytotrophoblast.** The two layers later form chorion, amnion and foetal part of placenta.

Implantation : It is embedding of the blastocyst comes in contact with the endometrium in the rigion of embryonal knob or embryonic disc and adheres to it. The surface cells of trophoblast secrete lytic enzymes which cause corrosion of endometrial lining. They also give rise to finger-like outgrowths called villi. Villi not only help in fixation but also in absorption of nutrients. Implantation causes nutrient

enrichment, enlargement of cells and formation of uterine part of placenta called deciduas (L. deciduos-falling off).

Decidua has three regions : (i) **Decidua Basalis –** Part of decidua underlying the chorionic villi and overly the myometrium. (ii) **Decidua Capsularis –** Lying between embryo and lumen of uterus and (iii) **Decidua Parietalis** the part of decidua that lines the uterus at places other than the site of attachment of embryo.

Throphoblast covering secretes a hormone called **human chorionic gonadotropin** (hCG). Detection of HCG in the urine is the basis of pregnancy/Gravidex test. hCG maintains the corpus luteum beyond its normal life times when it is called corpus luteum of pregnancy. It continues to secrete progesterone which prevents menstruation and maintains the uterine lining in nutrient rich state. Progesterone induces the cervical glands to secrete viscous mucus for filling the cervical canal to form a protective plug. Progesterone is also called pregnancy hormone as it is essential for maintenance of pregnancy. The hormone is secreted by placenta as well.

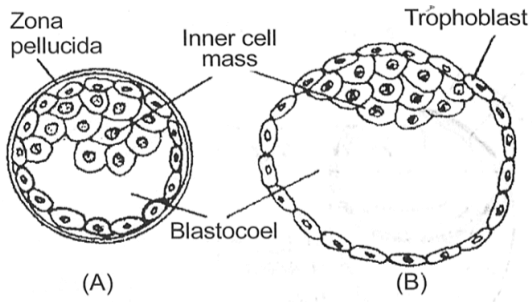


Fig. : Development of blastocyst

Gastrulation

It is characterized by movements of cells in small masses or sheets so as to form primary germinal layers. There are three primary germinal layers – endoderm, ectoderm and mesoderm. The cell movements that occur during gastrulation are called **morphogenetic movements** since they lead to initiation of morphogenesis. The product of gastruction is called gastrula.

A space appears between the ectoderm (below) and the trophoblast. This is the amniotic cavity, filled by amniotic fluid.

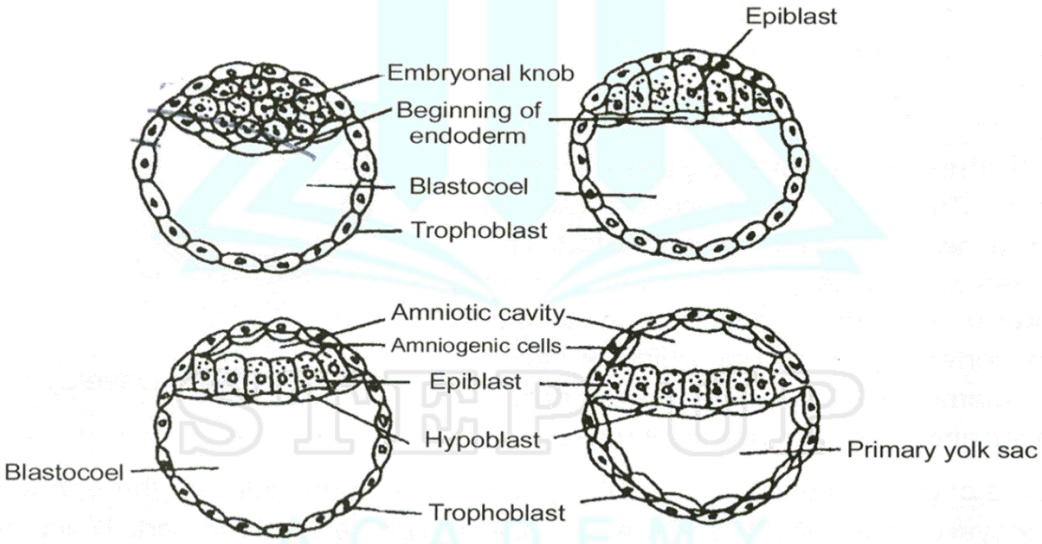
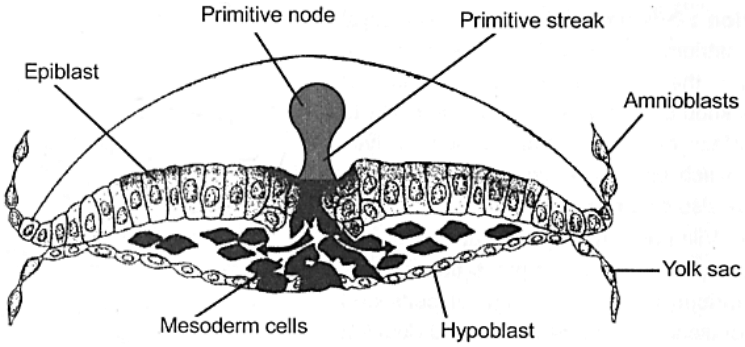


Fig. : Formation of endoderm and amniotic cavity

Formation of Primary Germinal Layers : Cells of the inner cell mass or embryonal get rearranges to form a flat **embryonic** or **germinal disc.** The latter differentiates into two layers, an outer epibast of larger columnar cells and inner hypoblast of smaller cuboidal cells. Gastrulation begins with the formation of primitive streak on the surface of the epiblast.



1. The diagram shows a cross section the cranial region of the streak at 15 days showing movement of epiblast cells. The first to move inward displace the hypoblast to create the definitive endoderm.
2. Once definitive endoderm is established, inwardly moving epiblast forms mesoderm.
3. Cells remaining in the epiblast then form ectoderm. Thus **the epibast is the source of all the germ layers in the embryo.**

⇨ **Fate of Germ Layers :**

Zygote → **Blastula** → **Gastrula**

Ectoderm	Mesoderm	Endoderm
Epidermis	Dermis	Gut
Cutaneous glands	Muscular tissue	Visceral organs
Nervous system	Connective tissue	Glands of stomach and intestine
(Brain and spinal cord)	Endoskeleton	Tongue
Eye (Retina, lens and cornea)	Vascular system	Lungs, trachea and bronchi
Nasal epithelium	Blood (heart and blood vessels)	Urinary bladder
Internal ear and external ear	Kidneys	Gills
Lateral line sense organ	Gonads	Liver and pancreas
Stomodaeum	Urinary and genital ducts	Thyroid gland
Proctodaeum	Coelom and coelomic epithelium	Parathyroids
Pituitary	Coelom and coelomic epithelium	Thymus
Pineal gland	Choroid and sclerotic coats of eye	Eustachian tube
Adrenal medulla	Adrenal cortex	
	Spleen	

Important developmental Changes in the Human Embryo

1. After one month of pregnancy, the embryo's heart is formed. The first sign of growing foetus may be noticed by listening to the heart sound carefully through the stethoscope.
2. By the end of the second month of pregnancy, the foetus develops limbs and digits.
3. By the end of 12 weeks (first trimester), most of the major organ systems are formed, for example, the limbs and external genital organs are well developed.
4. The first movements of the foetus and appearance of hair on the head are usually observed during the fifth month.
5. By the end of about 24 weeks (end of second trimester), the body is covered with fine hair, eye-lids separate, and eyelashes are formed.
6. By the end of nine months of pregnancy, the foetus is fully developed and is ready for delivery.

Parturition and Lactation

The average duration of human pregnancy is about 9 months which is called the gestation period. Vigorous contraction of the uterus at the end of pregnancy causes expulsion/delivery of the foetus. This process of delivery of the foetus (childbirth) is called **parturition.** Parturition is induced by a complex neuroendocrine mechanism. The signals for parturition originate from the fully developed foetus and the placenta which induce mild uterine contractions called **foetal ejection reflex.** This triggers release of oxytocin from the maternal pituitary. Oxytocin acts on the uterine muscle and causes stronger uterine contractions, which in turn stimulates further secretion of oxytocin. The stimulatory reflex between the uterine contraction and oxytocin secretion continues resulting in stronger and stronger contractions. This leads to expulsion of the baby out of the uterus through the birth canal – parturition. Soon after the infant is delivered, the placenta is also expelled out of the uterus. What do you think the doctors inject to induce delivery?

The mammary glands of the female undergo differentiation during pregnancy and starts producing milk towards the end of pregnancy by the process called **lactation.** This helps the mother in feeding the new born. The milk produced during the initial few days of lactation is called **colostrums** which contains several antibodies absolutely essential to develop resistance for the new-born babies. Breast-feeding during the initial period of infant growth is recommended by doctors for bringing up a healthy baby.

Chapter 3 Reproductive Health

REPRODUCTIVE HEATH (PROBLEMS & STRATEGIES)

Population explosion and Birth control

Growth of population

Increased health facilities & better living conditions.

World population

	1900	2 billion
	2000	6 billion

India 1947 – 35 crore (350 million)

May 2000 – 1 billion (Every sixth person is an Indian)

Probable reason :

(1) Rapid decline in death rate.
(2) MMR (Maternal mortality rate)
(3) IMR (Infant mortality rate)
(4) Increase in number of people in reproducible age.

RCH programme (Reproductive & child health care) :

Bring down the population growth rate it was only marginal.

Census - Population growth rate

2001 - 1.7% (17/1000/year)

(Rate at which over population could double in 33 years)

This alarming growth rate dead to scarcity of food, shelter, Clothing.

Types of Contraceptive methods :

- Natural/Traditional
- Barrier
- Oral contraceptives
- Implants
- Surgical methods.

Natural method :

Periodic abstinence – Avoiding coitus during unsafe period

Withdrawal coitus interruptus – Withdrawal of penis from vagina just before ejaculation.

Lactational amenorrhea – This method is effective only up to a maximum period of six-month following parturition.

Barrier method :

Condom : Made up of latex sheath (Both male and female condom)

It is used to cover penis & ejaculate semen remain in condom & not enter in female reproductive tract.

Diaphragm, cervical cap and vaults : are made up rubber, used to cover the cervix during coitus.

Intra uterine device (IUD) :

- Non medicated IUDs (Lippes's loop) – Increase phagocytosis of sperms within the uterus.
- Copper releasing IUDs (**Cu T, Cu7, Multiload 375**) – Suppress sperm motility
- Hormone releasing IUDs (**Progestasert, LNG – 20**) – Female uterus unsuitable for implantation & cervix hostile to sperm.

Oral contraceptive pills (OCP) :

Contain small doses of either progesterone or progesterone – estrogen combination inhibit ovulation. Daily pills – Male D, Mala N (Taken daily from 5^{th} day to 21 days after gap of 7 days again repeated) Weekly pill – Saheli/Centa chroman (non-steroidal, very few side effect) and high contraceptive value.

⇨ **Injection or Implants :** Mode of action is similar to pills effective is much longer.

⇨ **Surgical method/Terminal method :**

Male – Vasectomy

Females – Tubectomy

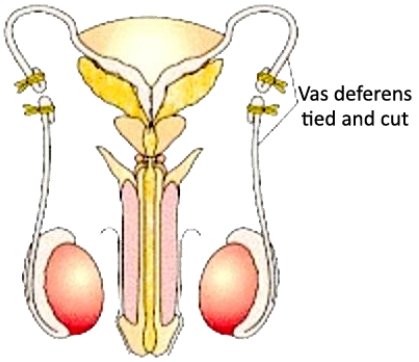


Fig : Vasectomy

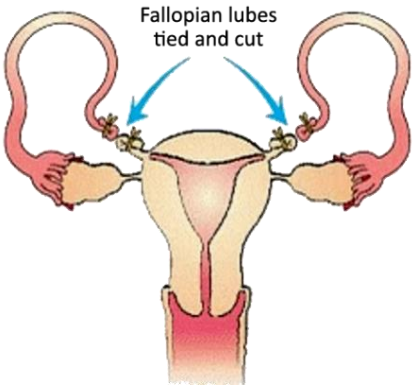


Fig : Tubectomy

Infertility : Couple unable to produce children inspite of unprotected sexual co – habitation.

Assisted reproductive technologies (ART)

(1) In vitro fertilisation (IVF) : fertilisation is done outside the body.

ZIFT (Zygote intra follopian transfer) – Zygote or early embryo (Upto 8 blastomers) is transferred into the fallopian tube.

IUT (Intra uterine transfer) :

Embryo with more than 8 blastomere (commonly 32 blastomeres) is transferred into the uterus.

ICSI (Intra cylopasmic sperm injection) : Sperm injected into the ovum & then transferred into the fallopian tube.

(2) **In vivo fertilisation :** Fusion of gametes within the female.

GIFT (Gamete intra fallopian transfer) : Ovum collected from a donar is tranfered into the fallopian tube of another female.

Artificial insemination :

Semen collected either from the husband or a healthy donar is artifiacilly introduced either into the vagina or uterus of the female.

It is parformed when male partner is unable to inseminate the female or sperm count is very low.

Amniocentesis –

Meaning and Use : Amniocentesis is a foetal sex and disorder determination test based on the chromosomal pattern of the embryo's cells in the amniotic fluid surrounding the developing embryo.

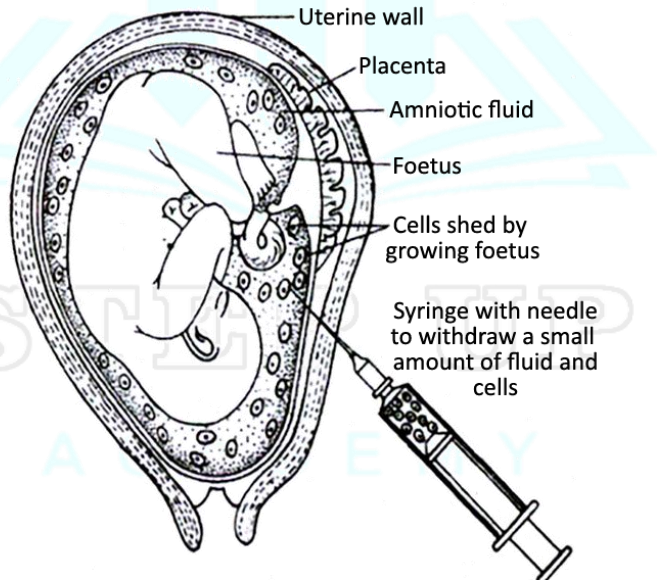


Fig : Amniocentesis

Procedure : Amniotic fluid contains cells from the skin of the foetus and other sources. These cells can be used to determine the sex of the infant, to identify some abnormalities in the number of chromosomes and to detect certain biochemicals and enzymatic abnormalities. If it is established that the child is likely to suffer from a serious incurable congenital defect, the mother should get the foetus aborted.

Misuse of Amniocentesis : It is being misused to know the sex of unborn baby followed by medical termination of foetus, in case its female.

Medical Termination of Pregnancy (MTP)

It is voluntary or interntional abortion, induced and performed to end pregnancy before the completion of full term. Worldwide, nearly 20 % of the total pregnancies get aborted. The number of MTPs is 40-50 million/yr. Therefore, MTPs have a significant role in

containment of population though they are not perfomed for this purpose. They are mainly meant for removing unsustainable pregnancies. Many countries do not have a

law about MTPs because the latter involve emotional, ethical, religious and social issues. However, in India there is a proper act, **Medical Termination of Pregnancy Act, 1971.** It is mainly meant for preventing unnatural maternal deaths due to unsafe abortions (8.9% of the total maternal deaths). The act was amended in 2002. Underthis act, **termination of pregnancy can be done upto 20 weeks,** if the pregnancy is likely to produce a congenitally malformed child is a result of rape or contraceptive failure or is likely to harm the mother.

MTP is safe if it is performed upto **12 weeks (first trimester) of pregnancy.** Misoprostol (prostaglandin) alongwith mifepristone (antiprogesterone) is an effective combination. Vacuum aspiration and surgical procedures are adopted there after. Second trimester abortions are risky. They are generally performed after testing the sex of the baby through amniocentesis or sonography. It has resulted in large scale female foeticide and complications due to unsafe abortions in the hands of untrained persons. To prevent such mis – happenings, the government has enacted a law, **Pre – natal diagnostic techniques (Regulation and Prevention of Misuse) Act, 1994** with amendments in 2003. It prohibits preconception and prenatal sex determination.

SEXUALLY TRANSMITTED DESEASES

The general term **sexually transmitted disease (STD)** is applied to any of the large group of diseases that can be spread by sexual contact. The group includes conditions traditionally specfied as **venereal diseases (VD),** such as **chlamydia, gonorrhoea, syphilis, and genital herpes. AIDS and hepatitis are** sexually transmitted diseases that may be contracted by other ways also. STDs are also called RTI (Reproductive tract infections).

Except for hepatitis – B, genital herpes and HIV infections, other STDs are completely curable if detected early and treated properly

Early symptoms include itching, fluid discharge, slight pain, swallings etc, in the genital region. Later complications include pelvic inflammatory disease (PID), abortions, still births, ectopic pregnancies, infertility or even cancer of the reproductive tract.

Chapter 4 Principles of Inheritance and Variation

Principles of Inheritance & Variation, Molecular Basis of inheritance

- Genetics deals with the inheritance, as well as the variation of characters from parents to offsprings.
- Inheritance is the process by which characters are passed on from parent to progeny.
- Variation is the degree by which progeny differs from their parents.

GENETICAL TERMS

- **Genes (Factors) :** They are the units of inheritance, which contain the information that is required to express a particular character, in an organism.
- **Alleles :** Genes which code for a pair of contrasting traits and present on a same locus on the homologous chromosome, are known as alleles. i.e. They are the slightly different forms of the same gene.
- **Homozygous (Pure) :** Identical pair of alleles (TT or tt)
- **Heterozygous (Hybrid) :** Dissimilar pair of alleles (Tt)
- **Phenotype :** External & morphological appearance of character.
- **Genotype :** Genetic makeup of an organism.
- **Punnett square :** It is a graphical representation to calculate the probability of all possible genotypes of offspring in a genetic cross.

MENDELISM

- Gregor Johann Mendel, conducted hybridization experiments on garden pea (**Pisum sativum).**
- He studied seven pairs of contrasting characters.

S.No.	Characters	Dominant/Recessive	Chromosome No.
1	Seed/Cotyledon colour	Yellow/Green	1st
2	Flower colour	Violet/White	1st
3	Pod Shape	Inflated/Constricted	4th
4	Flower position	Axial/Terminal	4th
5	Stem length/Height	Tall/Dwarf	4th
6	Pod colour	Green/Yellow	5th
7	Seed shape	Round/Wrinkled	7th

- Mendel uses Emasculation, Bagging, & Tagging technique for hybridization.

Inheritance of one gene (Monohybrid Cross)

- Study of inheritance of one character at a time in an organism is called as monohybrid cross.
- Phenotypic / Mendelian Ratio = **3 : 1**
- Genotypic / Real Ratio = **1 : 2 : 1**

Conclusions of monohybrid cross :

(i) Postulate of Dominance :

- **Characters** are controlled by discrete units celled factors.
- Factors occur in pairs.

In a dissimilar pair of factors one member of the pair dominates the other.

A dominant allele is wild type or unmodified allele, which produces normal enzyme / protein, that forms a character.

A modified allele is a mutant allele, which could be responsible for the production of less – efficient enzyme or non – functional enzyme or no enzyme at all. Thus it will be a recessive allele.

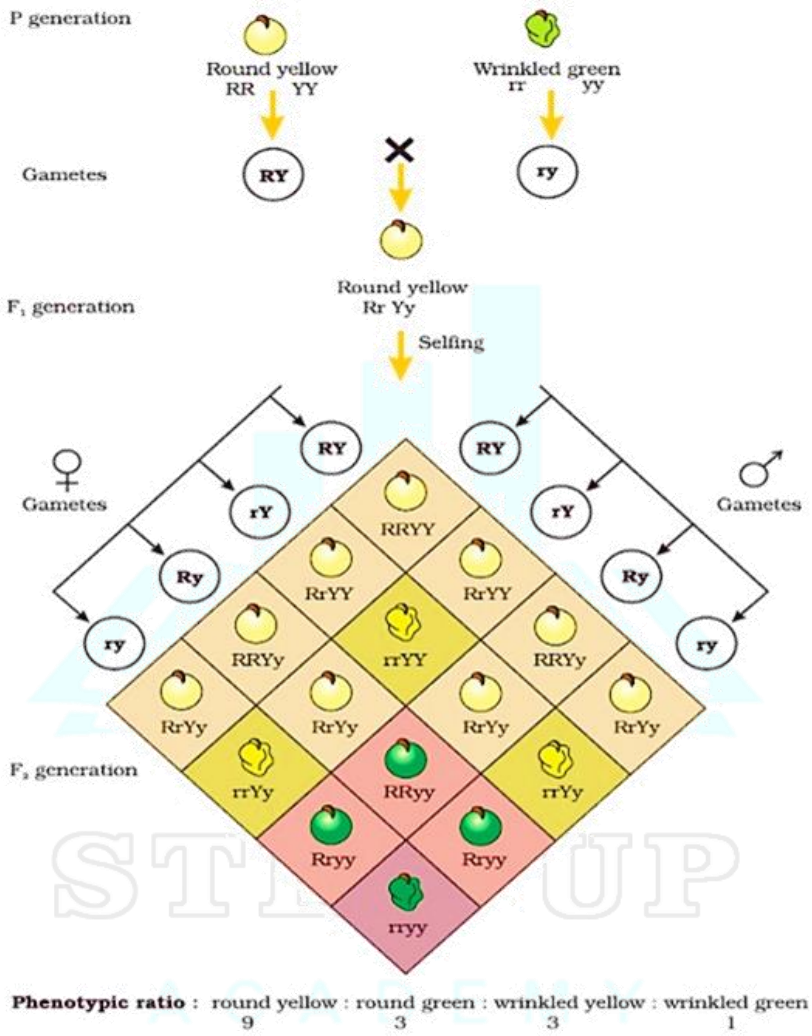
(ii) Law of segregation (Law of purity of gametes) :

- 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 F_2 generation.

- Through 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. **Thus gametes are always pure.**
- The 3 : 1 ratio obtained in F_2 is mainly due to segregation, although there is the role of dominance also.

Inheritance of two genes (Dihybrid cross)

- Study of inheritance of two characters at a time, in an organism in called as dihybrid cross.
- Phenotypic ratio = **9 : 3 : 3 : 1**
- Genotypic ratio = **1 : 2 : 1 : 2: 4 : 2 : 1 : 2 : 1**



Results of a dihybrid cross where the two parents differed in two pairs of contrasting traits: seed colour and seed shape

Conclusion of Dihybrid cross :

(i) Law of independent assortment :

This 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.

- Each character is inherited in monohybrid pattern (3 : 1)
- It is applicable only when both the genes are present on separate homologous pair of chromosomes.

Important formulae (Applicable in case of selling only)

- **2^n = Type of gametes / Type of phenotypes**
- **3^n = Type of genotypes**
- **4^n = Total possible number of zygote**
- **n = Number of heterozygous condition.**

Back cross :

- It is cross of F_1 individuals with any of their parents.
- It is of two types :

(i) Out cross : F_1individual x Homozygous dominant parent.

Phenotypic ratio = All dominant

Genotypic ratio = 1 : 1 (For monohybrid cross)

(ii) Test cross : F_1individual x Homozygous recessive parent.

It is used to **find out the genotype of F_1 individual.**

Phenotypic ratio = 1 : 1 (For monohybrid cross)

Genotypic ratio = 1 : 1 (For monohybrid cross)

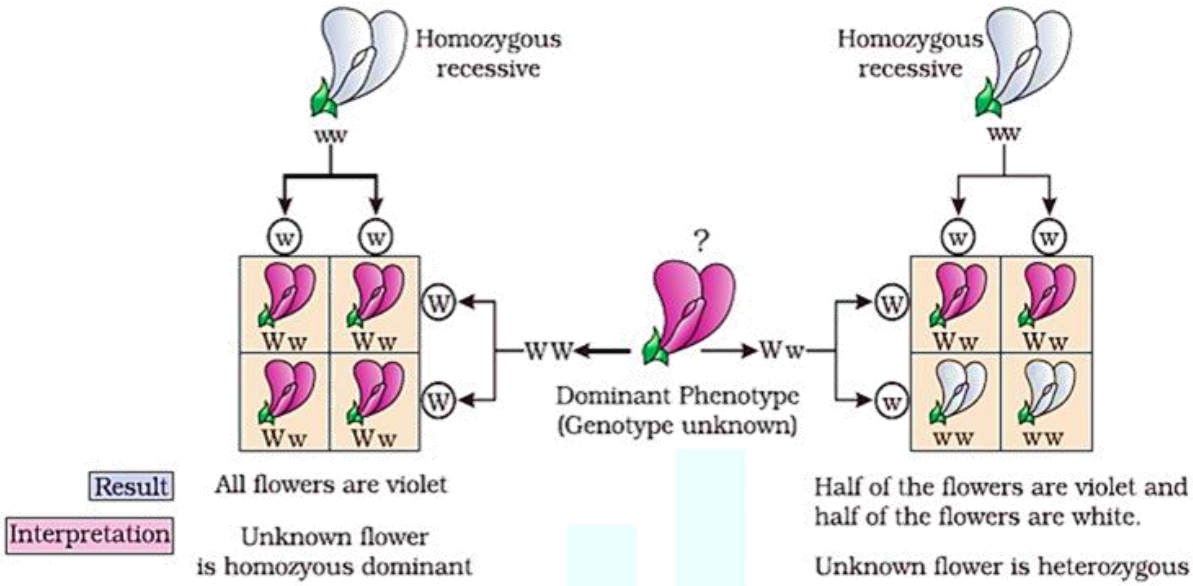


Fig. : Diagrammatic representation of a test cross.

Reciprocal cross :

- A pair of crosses in which male & female sexes are reversed.
- In Mendelian cross, results of reciprocal crosses were same.

GENE INTERACTION :

It is of two types :

1. Allelic interactions / Intragenic interactions

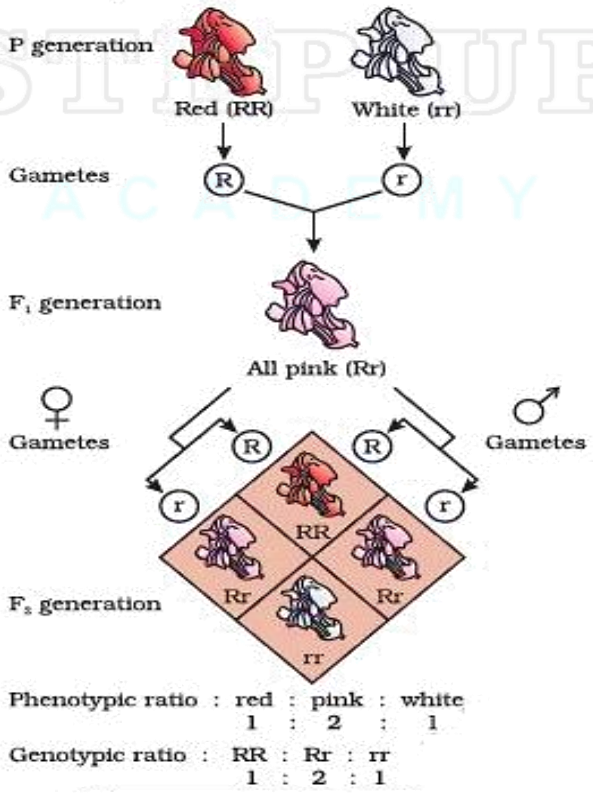
(i) Incomplete dominance :

F_1 phenotype did not resemble either of the two parents & was in between the two.

Phenotypic & Genotypic ratio = 1 : 2 : 1

e.g. Flower colour in **Mirabilis Jalapa Antirrhinum** (Snapdragon).

Starch grain size in **Pisum sativum.**



(ii) Co – dominance :

F_1 phenotype resembles both parents.

Phenotypic & Genotype ratio = 1 : 2 : 1

e.g. Coat colour in cattle.

AB blood group ($I^A I^B$)

Carrier of sickle cell anemia ($Hb^A Hb^S$)

Table showing the Genetic Basis of Blood Groups 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

(iii) Multiple Alleles :

Existence of more than two alleles of a gene.

Since in an individual only two alleles can be present, multiple alleles can be found only when population studies are made.

Number of possible genotypes = $\frac{n(n+1)}{2}$ [n = number of alleles]

e.g. Blood group inheritance in human = 3 alleles.

(iv) Lethal genes :

They affect viability of an organism & causes death in homozygous condition.

Phenotypic ratio becomes 2 : 1 instead of 3 : 1

e.g. Coat colour in mice

Leaf colour in Snapdragon

Sickle cell anemia in human.

(v) Pleiotropic genes :

Genes which controls more than one character.

e.g. Most of genetical disorders / syndrome in human.

In pea plant, single gene influence seed coat colour. Flower colour & red spot in leaf axil.

In pea plant, single gene influence seed shape & size of starch grains.

2. Non – Allelic interactions / Intergenic interactions:

(i) Epistasis :

When a gene prevents the expression of another non – allelic gene.

(a) **Dominant epistasis :** e.g. Hair colour in Dogs.

Phenotypic ratio = 12 : 3 : 1

(b) **Recessive epistasis :** e.g. Coat colour in mice

Phenotypic ratio = 9 : 3 : 4

(ii) Complementary gene :

e.g. Flower colour in **Lathyrus** Phenotypic ratio = 9 : 7

(iii) Supplementary genes :

e.g. Coat colour in mice Phenotypic ratio = 9 : 3 : 4

POLYGENIC INHERITANCE

- Inheritance of characters in which one character is controlled by many genes.
- Intensity of character depends on number of dominant alleles i.e. dominant alleles are contributory in nature.

  e.g. **Kernal colour of wheat** = 2 genes (Ratio = 1 : 4 : 6 : 4 : 1)

  Skin colour of human = 3 genes (Ratio = 1 : 6 : 15 : 20 : 6 : 1)

CYTOPLASMIC INHERITANCE (By C.Correns)

- It is the inheritance of those characters which are controlled by cytogenesis.
- This inheritance occurs only through female (also called maternal inheritance). Thus reciprocal cross results will be affected.
- It is mainly due to some cell organelles like plastids & mitochondria (Organellar inheritance),

  e.g. Plastid inheritance in Mirabilis Jalapa.

  Male sterility in Maize (Mitochondrial)

CHROMOSOMAL THEORY OF INHERITANCE

By Walter Sutton & Theodore Bovary

- They noted that the behaviour of chromosomes was parallel to the behavior of genes & used chromosome movement to explain Mendel' laws.
- A comparison between bahaviour of chromosome & genes :

A (Gene)	B(Chromosome)
Occur in pairs	Occur in pairs
Segregate at the time of gamete formation such that only one of each pair is transmitted to a gamete	Segregate at gamete formation and only one of each pair is transmitted to a gamete
Independent pairs segregate independently of each other	One pair segregates independently of another pair

- The experimental verification of chromosomal theory inheritance was done by T.H. Morgan & his colleagues.

LINKAGE & RECOMBINATION

- Inheritance of genes in group is called linkage.
- Linked genes are located on some chromosome & all the genes located on one of homologous chromosome form one linkage group.
- In linkage, the proportion of parental gene combinations are much higher than the non – parental type.
- Tightly linked genes shows very low recombination while loosely linked genes shows higher recombination.
- Linked genes may either cis arrangement [AB & ab] or trans arrangement [Ab & aB].
- Linkage may be complete (C.O. absent) or incomplete (C.O. present).
- % Recombination / Recombination frequency / Distance between genes.
- % Recombination $= \frac{\text{Numberofrecombinantorganisms}}{\text{Totalorganisms}} x\ 100$
- [1% CO = 1 centiMorgan(cM)]

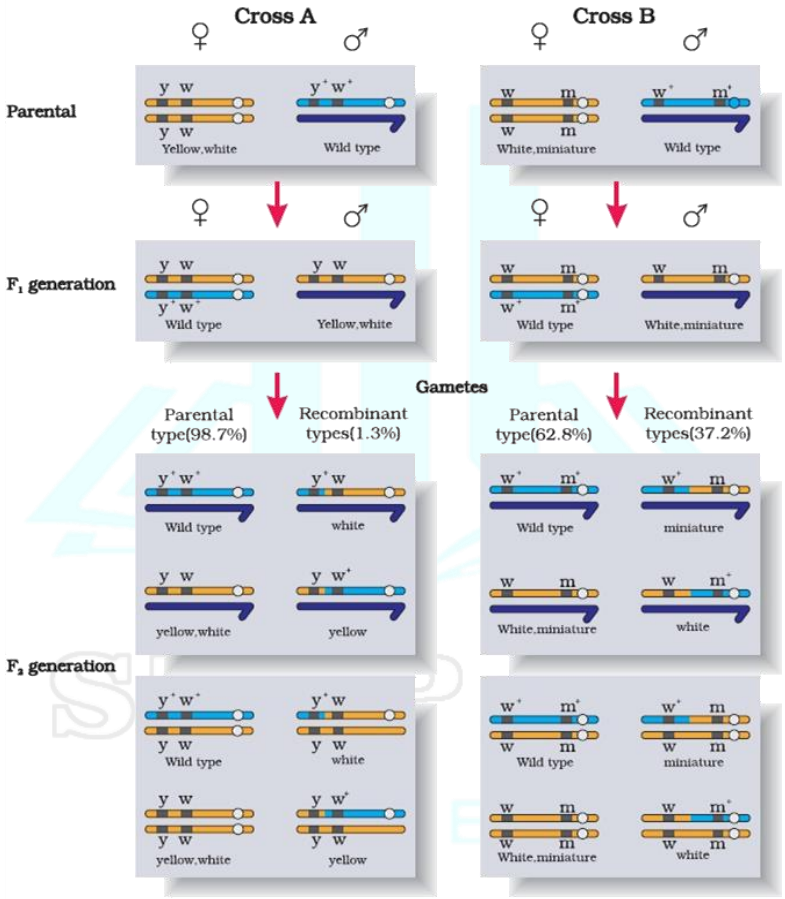


Fig. : Linkage; Results of two dihybrid crosses conducted by Morgan. Cross a show crossing between gene y and w; Cross B shows crossing between genes w and m. Here dominant wild type alleles are represented with (+) sign in superscript. Note: The strengths of linkage between y and w are higher than w and m.

SEX LINKAGE (By Morgan)

- Gene present on sex chromosomes shows sex linkage.
- Sex linked characters shows two types of inheritance.

(i) Criss – cross inheritance
- Digenic — Father → Daughter → Grandson ⟹
- Dendric — Mother → Son → Granddaughter ⟹

(ii) Non-Criss – cross inheritance
- Hologynic — Mother→ Daughter → Granddaughter ⟹
- Holandric — Father → Son → Grand ⟹

SEX DETERMINATION :

- Establishment of sex through differential development in an individual at an early stage of life.

(1) Allosomic determination of sex :

Sex chromosomes are responsible for sex determination.

(a) XX (♀) - XY (♂) type (Leagues type) : e.g. **Human, Drosophila**, Coccinea & Melandrium plants.

(b) XY (♀) - XX(♂) type (or ZW –ZZ type) : e.g. **Birds**, Fishes, reptiles & Fragaria plant.

(c) XX (♀) - XO (♂) type (Protenor type) : e.g. Grasshopper, Cockroach, Ascaris.

(2) Genic Balance theory (By C.B.Bridges)

- It is on the basis of Drosophila.
- In this sex determination takes place by sex index ratio.

Sex index ratio (SIR) = $\frac{Number\ of\ X-chromosome}{Number\ of\ sets\ of\ Autosome} = \frac{X}{A}$

If SIR = 1 → Female

= 0.5 → Male

= > 1 → Super female

= <0.5 → Super male

= 0.5 → Intersex

(3) Environmental determination of sex :

e.g. Bonellia, Crepidula, Crocodile, Turtles etc.

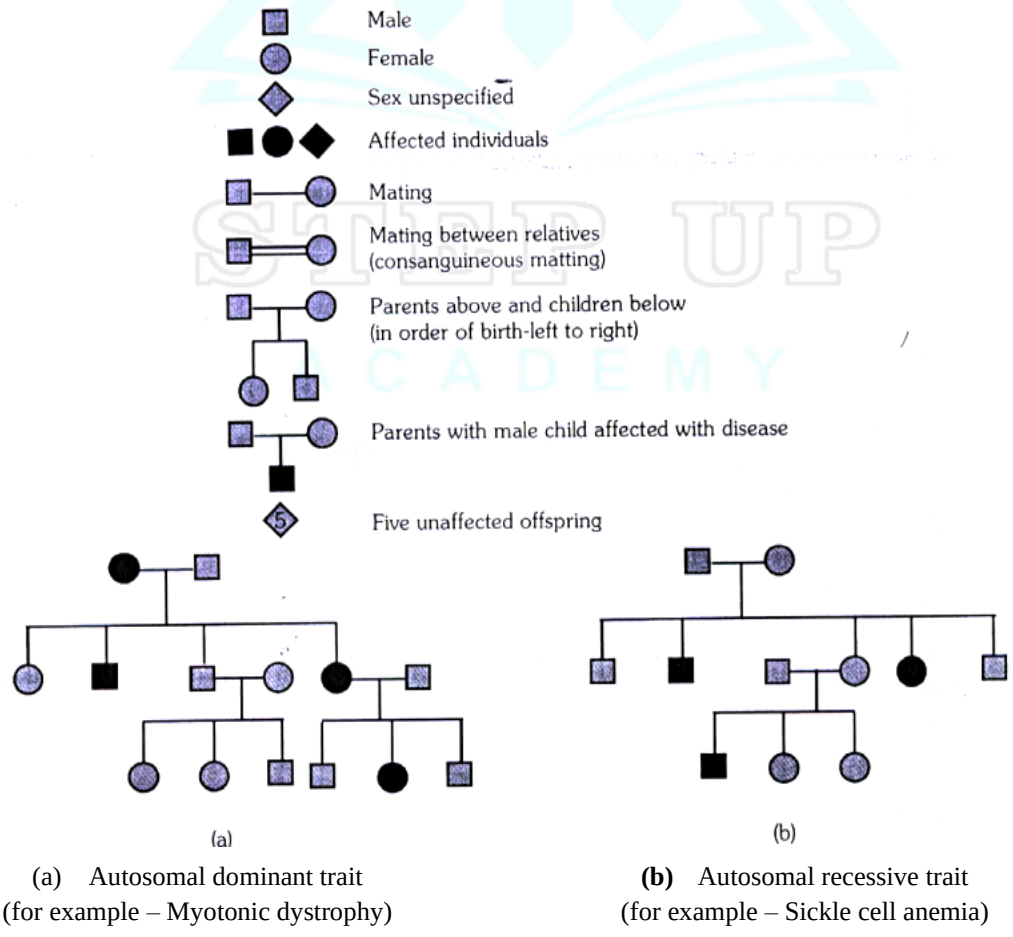
(4) Sex determination by hormone :

Sex hormone are required for sex differentiation. It can be observed in **Free martin** development in cattle.

- In honey bee, ants etc. Haploid (♂) - Diploid (♀) method found.
- Gynandromorph (Half(♂) & Half (♀) body) in Drosophila is due to loss of one X-chromosome during first zygotic division.
- Cytological basis of sex determination – Formation of Barr body in Mammalian(♀).

HUMAN GENETICS

- In human direct genetical studies are possible. For this different indirect methods are used. **Pedigree analysis** in one such important method.
- Study of the family history for the inheritance of particular trait in several generation of a family is called the pedigree analysis.
- In pedigree analysis, symbols are used for study. Some of the important standard symbols used in the pedigree analysis are as follows :



(a) Autosomal dominant trait (for example – Myotonic dystrophy)

(b) Autosomal recessive trait (for example – Sickle cell anemia)

Fig : Representation of Pedigree analysis of gene expression

- **One gene – one enzyme hypothesis was given by Beadle & Tatum.**
- According to this, each gene produces a particular type of enzyme.
- They worked on **Neurospora crass.**
- **Prototroph :** It is the wild type **Neurospora** which can easily grow on minimal nutrient medium.
- **Auxotroph :** These are the nutritional mutants which are unable to grow on minimal nutrient medium.
- Later on one gene – one enzyme hypothesis has been modified into **one gene – one polypeptide** hypothesis.

Types of Human Genetic Disorders :

We know that each and every feature in any organism is controlled by one or the other gene located on the DNA present in the chromosome. DNA is the carrier of genetic information. It is transmitted from one generation to the other without any change or alteration. However, changes do take place occasionally. A number of disorder in human beings have been found to be associated with the inheritance or changed or altered genes or chromosomes.

2. Mendelian Disorders

These are mainly determined by mutation in the single gene, therefore also called gene related human disorders. They are transmitted to the offspring as per Mendelian principles. The pattern of inheritance of such disorders can be traced in a family by the pedigree analysis. Some common and prevalent Mendelian disorders are as follows:

S.No.	Disorder	Dominant/Recessive	Autosomal/ Sex linked
1.	Hemophilia	Recessive	X – linked
2.	Colour blindness	Recessive	X – linked
3.	Sickle cell anemia	Recessive	Autosomal
4.	Phenylketonuria	Recessive	Autosomal
5	Cystic fibrosis	Recessive	Autosomal
6	Thalassemia	Recessive	Autosomal
7	Myotonic dystrophy	Dominant	Autosomal

(a) Colour blindness : Colour blindness is a recessive sex – linked trait. The normal gene and its recessive allele are carried by X – chromosome. In female, Colour blindness, like any other sex – linked trait, shows **cress – cross inheritance** (i.e., a male transmits his trait to his grandson through daughter, while a female transmits the traits to her grand – daughter through her son or it is transfer of trait from one sex to the offspring of the opposite sex).

It should be very much clear, colour blindness does not mean not seeing any colour at all, it means that those who are colorblind have trouble in seeing the differences between certain colors.

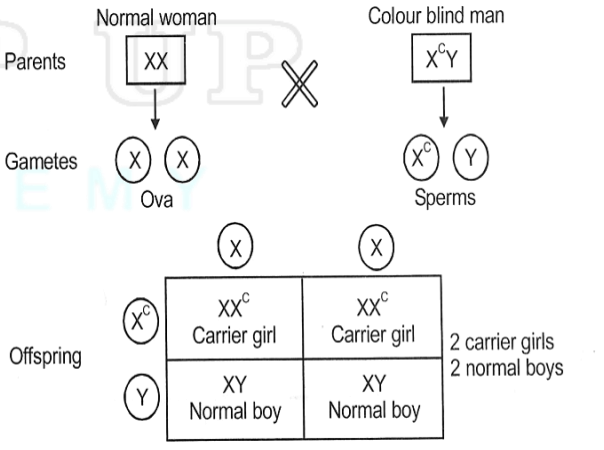
$X^C X^C$ → Colourblind female

$X^C X$ → Carrier female

$X^C Y$ → Colourblind male

Most colorblind people cannot tell the difference between red or green. That does not mean that they cannot do their normal work. In fact, they can also drive they learn to respond to the way the traffic single lights up the red light is generally on the top and green is on the bottom.

If a colorblind man (X^cY) marries a girl with normal vision (XX), the daughters would have normal vision but would be carrier, while sons would also be normal **(shown in cross a).**



Cross (a)

If the carrier girl heterozygous for colour blindness (X^cX) now marries a colour blind man X^cY, the offspring would show 50% females and 50% males. Of the females, 50 % would be carries for colour blindness and the rest 50% would be colour blind. Of the males, 50% would have normal vision and the 50% would be colour blind **(shown in cross (b)**

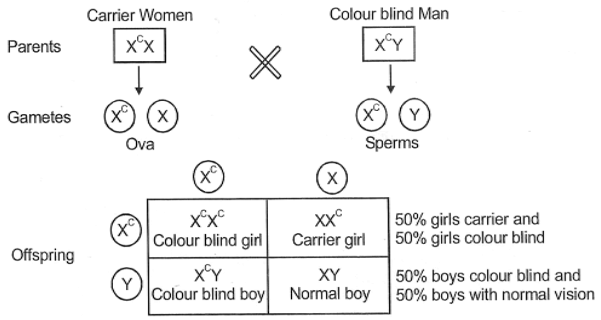


Fig : Sex – linked inheritance of colour blindness – cross (a) and cross (b)

(b) Hemophilia : It is X – linked recessive trait therefore shows its transmission from normal carrier female (heterozygous) to male progeny. Due to presence of defective form of blood clotting factor (protein), exposed blood of affected individuals fails to coagulate.

The possibility of a female becoming a hemophilic is extremely rare because mother of such a female has to be at least carrier and the father should be hemophilic (unviable in the later stage of life). Hemophilic female dies before birth. The family pedigree of Queen Victoria shows a number of hemophilic descendants as she a carrier of the disease.

The person suffering from this disease synthesize a normal blood protein called antihemophilic globulin (AHG) required for normal blood clotting (Hemophilia A – more severe). Therefore, even a very small cut may lead to continuous bleeding for a long time. This gene is located on X chromosome and is recessive. It remains latent in carrier females.

$X^h X^h$ → Haemophilic female

$X^h X$ → Carrier female

$X^h Y$ → Haemophilic male

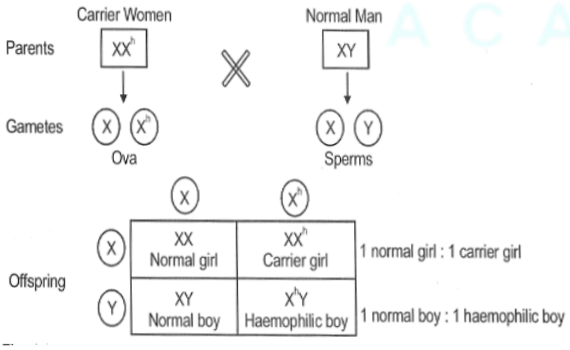


Fig : Inheritance of hemophilia when the mother is carrier and the father is normal

If a normal man marries a girl who is carrier for hemophilia, the progeny would consist of 50% females and 50 % males. Of the females, 50% would be normal and the rest 50% would be hemophilia carrier. Of the males 50% would be normal and the rest would be hemophilic.

Hemophilia – B (Christmas disease) – plasma thromboplastin in absent inheritance is just like Hemophilia A.

(c) Sickle–cell anemia : As it is autosomal recessive disease therefore it can be transmitted from parents to the offspring when both male and female individuals are carrier (heterozygous) for the gene. The disease is controlled by a single pair or allele, Hb^A and Hb^S. Thus three genotypes are possible in population.

(i) Hb^AHb^A (Normal, homozygous)

(ii) Hb^AHb^S (Normal, carrier)

(iii) Hb^sHb^S (Diseased, die before attaining maturity)

Heterozygous (Hb^AHb^S) individuals appear apparently unaffected but they are carrier of the disease as there is 50% probability of transmission of the mutant gene to the progeny. Thus exhibiting sickle – cell trait.

The disease / defect is caused by mutation (transversion) of the gene controlling β – chain. It replaces glutamic acid (Glu) present at 6th position of the β – chain by amino acid valine (Val). The mutant hemoglobin molecule undergoes polymerization under low O_2 tension causing the change in the shape of the RBC from biconcave disc to elongated sickle – like structure.

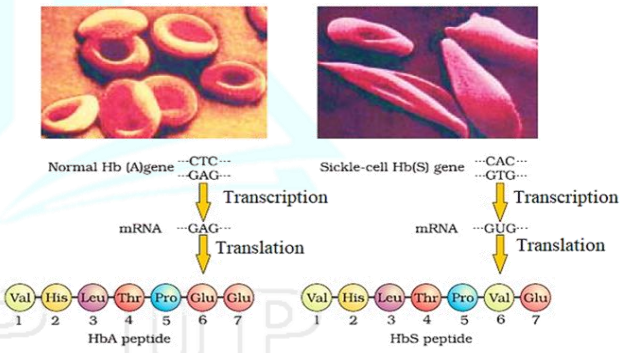


Fig : Micrograph of the red blood cells and the amino acid composition of the relevant portion of β – chain of hemoglobin : (a) From a normal individual : (b) From an individual with sickle – cells anemia

Phenylketonuria : This inborn error of metabolism is also inherited as the autosomal recessive trait. The affected individual lacks a liver enzyme called **phenylalanine hydroxylase** that convert the amino acid phenylalanine into tyrosine. As a result of this phenylalanine is accumulated and converted into phenyl pyruvic acid and other derivatives. Accumulation of these in brain results in mental retardation. These are also excreted through urine because of its poor absorption by kidney.

(d) Thalassemia : Thalassemia is a recessive autosomal genetic defect, originated in Mediterranean region – by their mutation or deletion recessive autosomal. Thalassemia's are a group of disorders caused by defects in the synthesis of globin polypeptide in RBC. Absence or reduced synthesis of one of the globin

chains results in an excess of the other. In this situation free globin chains, which are insoluble, accumulate inside the red cells and form precipitates which damage the cell, causing cell lysis and resulting in anemia. There are two main types of thalassemia's in which synthesis of α or β globin is defective. It is common in Mediterranean, Middle East, Indian subcontinent and in south – east Africa.

(e) Alkaptonuria : This was one of the first inborn metabolic diseases described by Garrod in 1908. In is an inherited autosomal, recessive, metabolic disorder produced due to *deficiency of an oxidase enzyme required for breakdown of tyrosine. Its toxic by product homogentisic acid* (also called **alkapton)** accumulates. The disease is called alkaptonuria (also written as alkaptonuria). Lack of the enzyme is due to the absence of the normal form of gene on chromosome 3 that controls the synthesis of the enzyme. Hence, homogentisic acid accumulates in the tissues and is also excreted in the urine. The most commonly affected tissues are heart valves, cartilages (ochronotic), capsules of joints, ligaments and tendons. The urine of these patients if allowed to stand for some hours in air, turns black due to oxidation of homogentisic acid. AA and Aa are normal but aa is alkaptonuria. The major defects are heart problems, arthritis, kidney and prostate stones. Nanterinone gives relief.

3. Chromosomal Disorders :

Mendelian disorders like hemophilia, sickle – cell anemia and phenylketonuria are due to the mutant allele and their defective products. However, disorders can also be created by imbalance in chromosome number and chromosomal rearrangements. These are called as chromosomal disorders. Down's syndrome, Klinefelter's syndrome and Turner's syndrome are common examples of chromosomal disorders.

(a) Down's syndrome : It was first described in 1866 by Langdon Down. The disorder develop due to trisomy of chromosome number 21. Trismic condition arises due to the formation of n+1 male or female gamete by non – disjunction and the subsequent fertilization by a normal (n) gamete. It is characterized by :

- **(i)** Short stature
- **(ii)** Small round head
- **(iii)** Furrowed tongue
- **(iv)** Partially open mouth
- **(v)** Broad palm with characteristic palm crease
- **(vi)** Many 'loops' on finger tips
- **(vii)** Big and wrinkled tongue

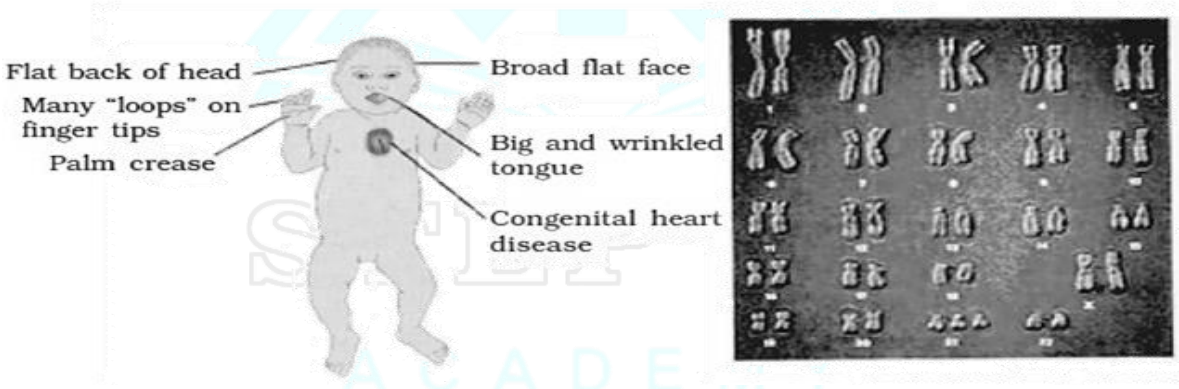


Fig : A representative figure showing an individual inflicted with Down's syndrome

(b) Klinefelter's syndrome : It is caused due to the presence of an additional copy of X – chromosome resulting into 44 + XXY type chromosome complement. The defect appears due to union of an abnormal egg (22+XX)) and a normal sperm (22 + Y) or normal egg (22 + X) and abnormal sperm (22 + XY). Such persons are sterile males with overall masculine development and some female characteristics (e.g., Feminine pitched voice, development of breast or gynecomastia).

(c) Turner's syndrome : The disorder is due to monosomy. It appears due to fusion of abnormal egg (22 + 0) and a normal sperm (22 + X) or a normal egg (22 + X) and abnormal sperm (22 + 0). Such females are sterile as ovaries are rudimentary besides other features including lack of other secondary sexual characters.

(d) Patau syndrome : trisomy of 13.

(e) Edward syndrome : Trisomy of 18.

Chapter 5
Molecular Basis of Inheritance

THE DNA

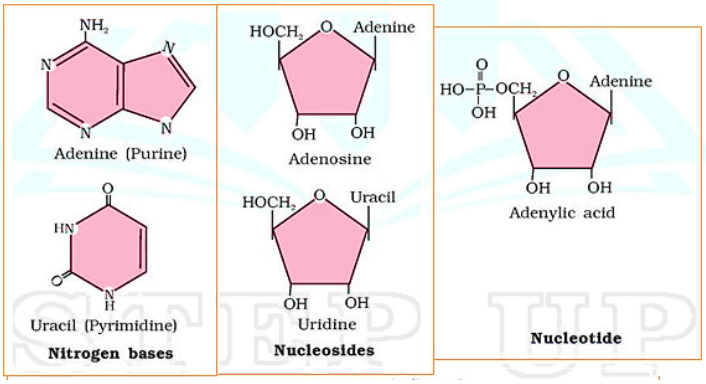
DNA is a long polymer of deoxyribonucleotides. It is an acidic substance present in nucleus, which was **first identified by Friedrich Miescher in 1869.** He named it as "**Nucleon". Altmann** found these substances to be acidic in nature, hence he named it nucleic acid. The **length of DNA** is usually defined as number of nucleotides or a pair or nucleotide referred to as base pairs (bp) present in it. **This also is the characteristic of an organism.**

Structure of Polynucleotide Chain

The basic unit of DNA is a nucleotide which has three components – a **nitrogenous base, a pentose sugar** (deoxyribose) and a phosphate group. There are two types or nitrogenous bases:

(i) **Purines :** Heterocyclic, 9 – membered double – ring structure with N at position 1, 3, 7 and 9, e.g., Adenine (A) and Guanine (G)

(ii) **Pyrimidines :** Heterocyclic, 6 – membered single – ring structure with N at 1 and 3 position e.g., Cytosine (C), Thymine and Uracil. Cytosine is common in both DNA and RNA; thymine is present in DNA and uracil is present in RNA at the place of thymine.



A polynucleotide chain shows following types of linkage or bond in its components :

(i) **N – glycosidic linkage :** A nitrogenous base in linkage to the pentose sugar through a N – glycosidic linkage to form a nucleoside. Purine nucleosides have 1 – 9 glycosidic linkage (carbon 1' of sugar and 9 position of A/G). Pyrimidine nucleosides have 1' – 1 linkage i.e., sugar carbon 1' and 1 position of T/C.)

(ii) **Phosphoester linkage :** When a phosphate group is linked to 5' – OH of a nucleoside through phosphoester linkage a corresponding nucleotide is formed. Two nucleotides are linked through 3' – 5' phosphodiester linage to form a dinucleotide.

A polymer thus formed has a free phosphate moiety at 5' – end of sugar, which is referred as 5' – end of polynucleotide chain. Similarly, at the other end of the polymer the sugar has a free 3' – OH group which is referred to as 3' – end of polynucleotide chain. The backbone in a polynucleotide chain is formed due to sugar and phosphates. The nitrogenous base linked to sugar moiety projects from the backbone.

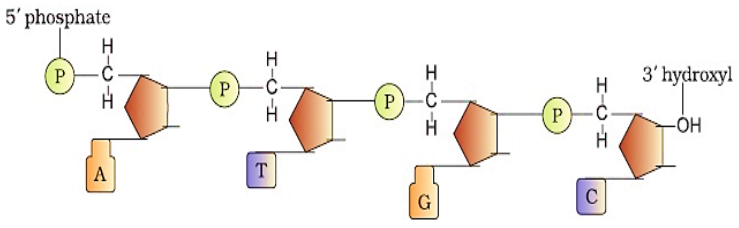


Fig : A polynucleotide chain

Types of Nucleosides in DNA	Types of Nucleotides in DNA
(i) Deoxyadenosine (A+S)____+ P = dAMP (deoxyadenosine monophosphate)	
(ii) Deoxyguanosine (G+S)____+ P = dGMP (deoxyadenosine monophosphate)	
(iii) Deoxycytidine (C+S)____ + P = dCMP (deoxycytidine monophosphate)	
(iv) Deoxythymidine (T+S)____+ P = dTMP (deoxythymidine monophosphate)	
Types of Nucleosides in DNA	**Types of Nucleotides in RNA**
(i) Adenosine (A + S) _____+ P = AMP (adenosine monophosphate)	
(ii) Guanosine (G + S) _____+ P = GMP (Guanosine monophosphate)	
(iii) Cytidine (C + S) _____+ P = CMP (Cytidine monophosphate)	
(iv) Uridine (U + S) _____+ P = UMP (Uridine monophosphate)	

DNA Structure

Two lines of investigations helped in derivation of DNA structure i.e.,

(a) **X – ray Crystallography and**

(b) **Chargaff's rule**

(a) **X –ray Crystallography : Maurice Wilkins and Rosalined Franklin** obtained very fine X – ray diffraction pictures of DNA. It was suggested that structure of DNA was sort of helix with 3.4 Å periodicity. But they had not proposed a definitive model for DNA.

(b) **Erwin Chargaff's Rules :** Chargaff's along with his colleagues, performed base composition studies and put forward certain generalisations for double – stranded DNA, called as Chargaff's rule (Not applicable for single standed DNA).

(i) Purines and pyrimidines occur in equal amounts.

(ii) Purines found in DNA are adenine and guanine. Pyrimidines of DNA are thymine and cytosine. A + G = T + C

(iii) $\frac{A+G}{T+C} = 1$**,this value is constant for all species**.

(iv) Base ratio $\frac{A+T}{C+G}$ is specific for a species. It is used to identify the species. It is less than one in prokaryotes, e.g., E. Coli = 0.92 and more than one in eukaryotes, e.g., Human = 1.52.

(v) Sugar deoxyribose and phosphate residues occur in equal number.

(vi) Purine adenine is equimolar with pyrimidine thymine.

(vii) Purine guanine is equimolar with pyrimidine cytosine.

James Watson and Francis Crick on the basis of previous information's proposed a very simple but famous double helix model for the structure of DNA. Salient features of the double helix structure of DNA are :

(i) DNA consists of two polynucleotide chains. The backbone is constituted by sugar – phosphate and the bases project inside.

(ii) The two chains of DNA run in anti – parallel fashion with 5' →3' polarity in one and 3' → 5' polarity in other chain.

(iii) The bases in two strands are paired through hydrogen bonds forming base pairs (bp). Adenine forms two H – bonds with thymine from opposite strand and vice-versa. Similarly, guanine is bonded with cytosine with three H – bonds. As a result, always a purine comes opposite to a pyrimidine. This generates approximately uniform distance between the two strands of helix.

DNA types	Base pairs per turn (n)	Rotation	Vertical rise per bp	Helical diameter bp (h)
A	11	Right-handed	2.56 Å	23 Å
B	10	Right-handed	3.4 Å	20 Å
C	9.33	Right-handed	3.3 Å	19 Å
Z	12	Left-handed	3.8 Å	18.4 Å

(i) Types of DNA and their comparison.

(ii) Linear double – stranded DNA in eukaryotes and PPLO (Monerans)

(iii) Repetitive DNA : It is the part of DNA which contain the same sequence of nitrogen bases repeated more than once in genome. The area with long sequence of short repetitive DNA is called satellite DNA because it separates out during density gradient ultracentrifugation as small dark bands.

5'_____3'
A A A A A A A
T T T T T T T
3'_____5'

(iv) Palindromic DNA : It has base sequence which reads the same on both strands either in 5' →3' or 3' → 5' direction. Different types of palindromic sequences are recognized by restriction endonucleases, e.g.,

5' – G A A T T C – 3'
3' – C T T A A G – 5'

(v) Denaturation and Renaturation : Separation of two strands of DNA from each other due to breakage of H – bonds when it is exposed to high temperature, acid, or alkali is called denaturation or melting. Reassociation of separated DNA by H – bonds formation is called renaturation or annealing. DNA with more A = T has low melting areas and denatured more easily. DNA with more G ≡ C than A = T has high melting areas.

(vi) C –value : Total amount of DNA per genome.

(vii) The amount of DNA is expressed in pictogram.

$1\ pg = 10^{-12}\ gm.$

(viii) DNA functions :

(i) Hereditary information

(ii) Variations : It occurs due to crossing over at the time of meiosis.

(iii) Mutations : Sudden inheritance variations due to change in genetic material.

(iv) Autocatalytic function or DNA replication i.e., DNA →DNA synthesis

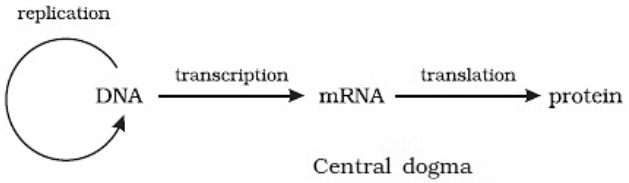
(v) Heterocatalytic function : DNA →DNA, proteinsm, hormones synthesis

(vi) Control of metabolism. Growth and differentiation

(vii) DNA fingerprinting

Central Dogma of Molecular Biology

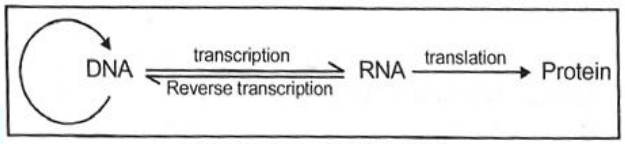
It explains one way or unidirectional flow of information from master copy DNA to working copy RNA and from RNA to building molecule or trait expressing molecule polypeptide Central dogma of molecular biology was proposed by Francis Crick.



Central dogma

Reverse Central Dogma or Teminism

An exception to this one-way flow of information was reported in 1970 by H. Temin and D. Baltimore. They independently discovered reverse transcription in some viruses. These viruses produce and enzyme reverse transcriptase which can synthesize DNA over RNA template. This discovery was important in understanding cancer and, hence, these two scientists were awarded Nobel prize. The modified flow of information now of information now can be shown as follows:



Reverse flow of information

Packaging of DNA Helix

The distance between two consecutive pairs is 0.34 nm (0.34×10^{-9} m) then length of DNA for a human diploid cell is 6.6×10^{9} bp x 0.34×10^{-9} m = 2.2 meters. This length is far greater than the dimension of a typical nucleus which is approximately 10^{-6} m.

Similarly, the number of base pairs in E. coli is 4.6×10^{6} so the total length comes out to be 1.36 mm which is placed in a cell having size 1 um. So, the long-sized DNA can be accommodated in small area only through packaging or compaction.

DNA Packaging in Prokaryotes

In prokaryotes, DNA is not scattered throughout the cell although they do not have a defined nucleus. DNA is found in cytoplasm in super coiled stage. The coils are maintained by non – histone basic protein polyamines which have positive charge. The packaged structure of DNA is called nucleoid or genophore.

DNA Packaging in Eukaryotes

In eukaryotes, this organization is much more complex and is carried out by a set of positively charged basic proteins called histones. Histones are rich in the basic amino acids residues lysine and arginine with charged side chains. There are five types of histone proteins i.e., H_1, H_2A, H_2B, H_3 and H_4 Four of them occur in pairs to produce histone octamer or nu – body (two copies of each H_2A, H_2B, H_3 and H_4) The negatively charged DNA is wrapped around the positively charged histone octamer to form a structure called nucleosome.

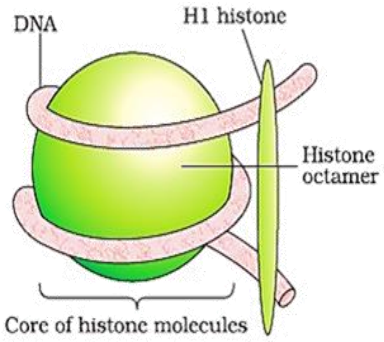


Fig : Nucleosome

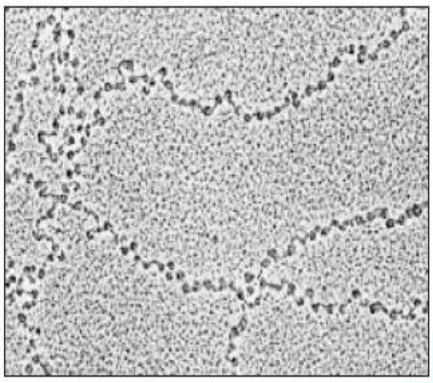


Fig : EM picture – "Beads – on – String

About 200 bp of DNA is wrapped over nu – body to complete about $1^3/_4$ turns. This forms nucleosome of size 110 x 60 A. DNA present Å. DNA present between two adjacent nucleosome is called linker DNA with about 80 bp. The nucleosome constitutes repeating unit of a structure in nucleus called chromatin. The nucleosomes in chromatin gives a 'beads on string' appearance under electron microscope. The nucleosomes further coils to form solenoid/ chromatin fibre. It has diameter of 30 mm. Chromatin fibers are further coils and condensed at metaphase stage of cell division to form chromosomes. The packaging of chromatin at higher level requires additional set of proteins that collectively are referred as Non – histone chromosomal (NHC) proteins.

Chromatin is differentiated into two regions, on the basis of staining behavior in a typical nucleus :

1. **Heterochromatin**
   - (i) It is darkly stained region
   - (ii) Chromatin is densely packed
   - (iii) Transcriptionally it is inactive
2. **Euchromatin**
   - (i) Lightly stained region
   - (ii) Loosely packed chromatin
   - (iii) Transcriptionally it is active

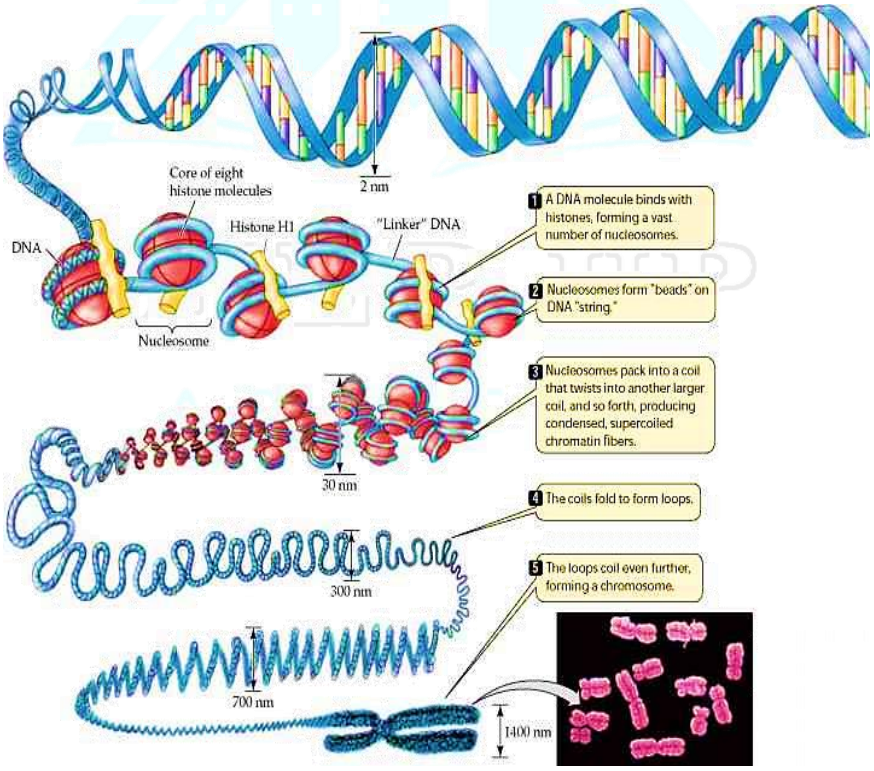


Fig : Various steps in the folding and super folding of basic chromatin components to generate a eukaryotic chromosome

THE SEARCH FOR GENETIC MATERIAL

Even though the discovery of nuclein by Miescher and the proposition for principle of inheritance by Mendel were almost at the same time, but that the DNA acts as a genetic material took long to be discovered and proven.

The experiments given below prove that DNA is the genetic material :

1. **Transforming Principle :**The transformation experiments, conducted by Frederick Griffith in 1928, are of great evidence in establishing the nature of genetic material. He performed series of experiments by selecting two strains of bacterium Streptococcus pneumonia (also Pneumococcus) namely, S – III and R – II

(i) S – III strain/smooth or capsulated type have a mucous (Polysaccharide) coat and produce smooth shiny colonies in culture plate. These are virulent and cause pneumonia.

(ii) R – II strain/rough or non – capsulated type has no mucous coat and produce rough colonies. These are non – virulent and do not cause pneumonia.

The experiment can be described in following four steps :

(a) S strain ⟶ injected into mice → Mice die

(b) R strain ⟶ injected into mice → Mice live

(c) S strain (heat – killed) ⟶ injected into mice → Mice live

(d) S – strain (heat – killed + R – strain (live) ⟶ injected into mice → Mice die

2. Evidence from Experiments with Bacteriophage : The unequivocal proof that DNA is the genetic material came from the experiments of Alfred Hershey and Martha Chase (1952). They worked with virus (T_2 bacteriophage) that infects bacterium Escherichia coli and multiples inside it. T_2 phage is made up of DNA and protein coat. Thus, it the most suitable material to determine whether DNA or protein contains information for the production of new virus particles. The functions of DNA and proteins could be found out by labeling them with radioactive tracers. DNA contains phosphorus but not sulphur. Therefore, phage DNA was labeled with P^{32} by growing bacteria infected with phages in culture medium containing $^{32}PO_4$. Similarly, protein of phage contains sulphur but not phosphorus. Thus, the phage protein coat was labeled with S^{35} by growing bacteria infected with phages in another culture medium containing $^{35}SO_4$. After labeling, three steps were followed i.e., **infection, blending** and **centrifugation.**

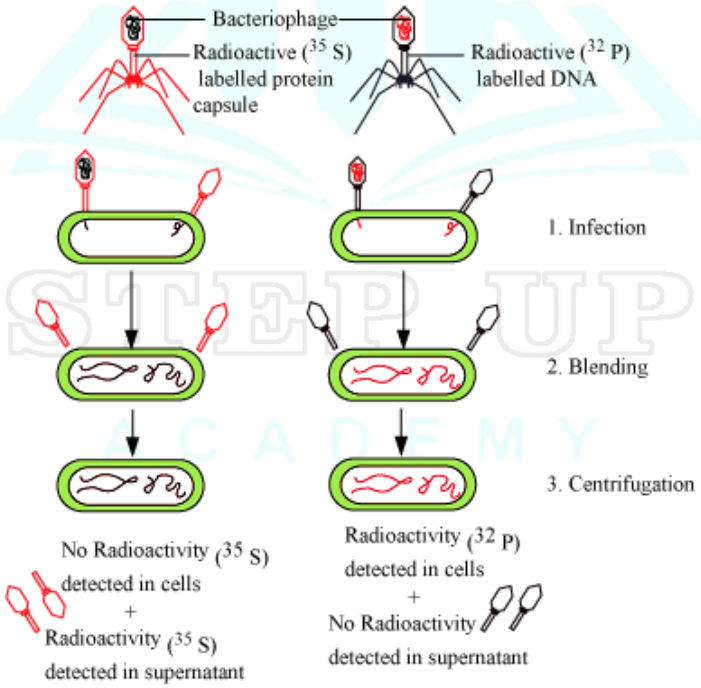


Fig : The Hershey – Chase Experiment

Properties of Genetic Material (DNA versus RNA)

Now it is clear that the debate between protein versus DNA as the genetic material was unequivocally resolved from Hershey – Chase experiment. However, it subsequently become clear that in some viruses RNA is the genetic material e.g., Tobacco Mosaic viruses, QB bacteriophage etc.

A molecule that can act as genetic material must fulfill the following criteria :

(i) It should chemically and structurally be stable.

(ii) It should be able to generate its replica (replication).

(iii) It should provide the scope for slow mutation that are required for evolution.

(iv) It should be able to express itself in the form of Mendelian characters.

The genetic material should enough not to change with different stages of life cycle, age or with change in physiology of the organism. DNA being more stable is preferred as genetic material, as

(a) Free 2'OH of RNA makes it more labile and easily degradable. Therefore, DNA in comparison is more stable.

(b) Presence of thymine (5 – Methyl uracil) at the place of uracil also confers additional stability to DNA.

(c) RNA being unstable, mutates at a faster rate. Consequently, viruses having RNA genome can directly code for the synthesis of proteins hence can easily express the characters.

RNA WORLD

RNA was the first genetic material. There are evidences to suggest that essential life processes, such as

Metabolism, translation, splicing etc. evolved around RNA. RNA used to act as a genetic material as well as a catalyst. There are some important biochemical reactions in living systems that are catalysed by RNA catalyst (ribozyme) and not by protein enzymes e.g., Ribonuclease P (Cleavage), Snurps (Splicing), Peptidyl transferase (peptide bond formation). But RNA being a catalyst was reactive and hence unstable. Therefore, DNA has evolved from RNA with chemical modifications that make it more stable. DNA being double stranded and having complementary strand further resists changes by evolving a process of repair. RNA is adapter, structural molecule and in some cases catalytic. From above discussion it is very much clear both RNA and DNA can function as genetic material, but DNA being stable is preferred for storage of/genetic material. For the transmission of genetic information RNA is better material.

REPLICATION

Watson and Crick had immediately proposed a scheme for DNA replication while proposing the double helical structure of DNA. The scheme suggested that the two strands would separate and act as template for the synthesis of new complementary strands. After the completion of replication, each DNA molecule would have one parental and one newly synthesized strand. This scheme was termed as semiconservative DNA replication.

The Experimental Proof

The following experiment suggests that DNA replication is semiconservative :

A. **Mathew Meselson and Franklin Stahl (1958)** performed following experiment using heavy nitrogen (^{15}N) in E. coli.

B. They grew E. coli in a medium containing $^{15}NH_4CI$ as the only nitrogen source for many generations. ^{15}N is the heavy isotope of nitrogen. ^{15}N was incorporated into newly synthesized 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 (CsCI) density gradient. A dense solution of CsCI, on centrifugation, forms density gradient bands of a solution of lower density at the top that increases gradually towards bottom with highest density.

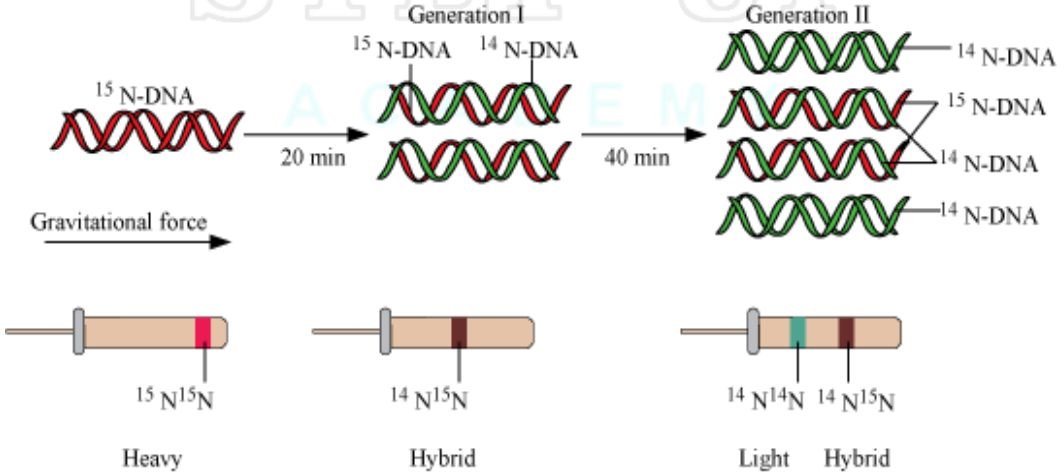


Fig : Meselson and Stahl's Experiment

C. **Taylor et. Al.** have proved semiconservative mode of chromosome replication in eukaryotes using tritiated thymidine (^3H – thymidine) in root of Vicia faba (Faba beans).

The Machinery and the Enzymes/DNA Replication Mechanism

The process of replication in living cells requires a set of enzymes. The main enzyme is referred to as DNA –

dependent DNA polymerase. It is highly efficient with the ability to polymerise some 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. The whole genome of Escherichia coli having 4.6 x 10^6 bp is replicated within 38 minutes. DNA replication completes in following steps.

(i) **Origin of Replication :** Replication begins at a particular region of DNA which is called 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, Prokaryotes have single origin of replication. It is called ORI – c in E. coli. On the other hand, eukaryotes have several thousand origins of replication.

(ii) Activation of deoxyribonucleotides : Four types of deoxyribonucleotides, namely, dAMP, 'dGMP, dTMP and dCMP' are activated by phosphate, energy, and enzyme phosphorylase into triphosphate state. Deoxyribonucleoside triphosphates serve dual purposes. In addition to acting as substrates, they provide energy for polmerisation reaction, because the two terminal phosphates in a deoxynucleoside triphosphates are high energy phosphates, same as in case of ATP.

$$\left.\begin{matrix}\text{dAMP}\\ \text{dGMP}\\ \text{dCMP}\\ \text{dTMP}\end{matrix}\right] + 2H_3PO_4 \xrightarrow[\text{Energy}]{\text{Phosphorylase}} \begin{matrix}\text{dATP}\\ \text{dGTP}\\ \text{dCTP}\\ \text{dTTP}\end{matrix}$$

(iii) Unbinding of helix : Unwinding of double helical parental, molecule is brought about by enzyme helicase, which is ATP dependent.

Unwinding of DNA molecule into two strands results in the formation of Y – shaped structure, called replication fork. These exposed single strands are stabilized with the help of single strand binding proteins (SSBP). Due to unwinding, a supercoiling gets developed on the end of DNA opposite to replicating fork. This tension is released by enzyme topoisomerase. In prokaryotes, DNA gyrase has topoisomerase activity.

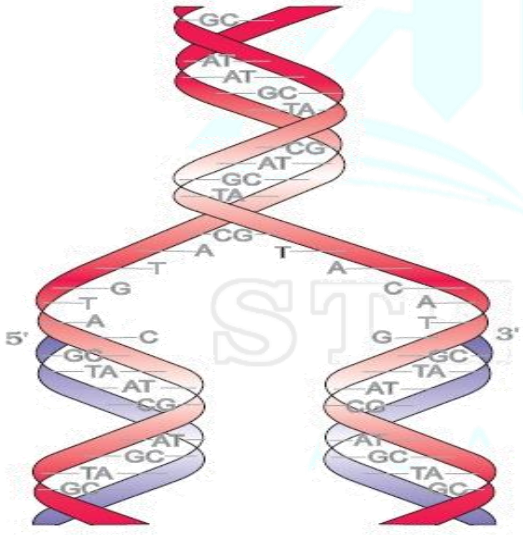


Fig. : Watson-Crick model for semiconservative DNA replication

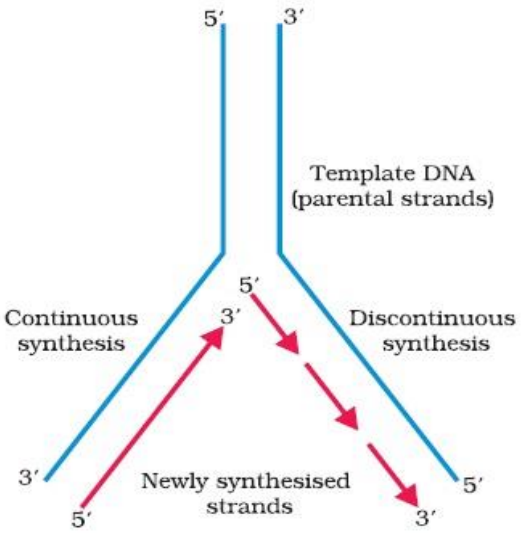


Fig. : Replication Fork

(iii) Formation of primer strand : A new strand is now to be synthesized opposite to the parental strands. DNA polymerase III is the true replicase in E. coli which is incapable of initiating DNA synthesis, i.e., it is unable to deposit the first nucleotide in a daughter strand. Another enzyme, known as primase, synthesized a short primer strand of RNA. The primer strand then serves as a stepping stone to start errorless replication. Once the initiation of DNA synthesis is completed, this primer RNA strand is then removed enzymatically.

(iv) Elongation of new strand : The DNA dependent DNA polymerases catalyse polymerization only in one direction that is 5' → 3'. This creates some additional complications at the replication fork. Consequently, the replication is continuous on one template strand with polarity 3' → 5'. It is now known as leading daughter strand. The replication is discontinuous in the form of short Okazaki fregments on other synthesized are latter joined by the enzyme DNA ligase.

In eukaryotes, the replication of DNA takes place at S – phase of 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.

TRANSCRIPTION

The process of copying genetic information from one strand of the DNA into RNA is known as transcription. Like DNA replication, the principle of complementarily governs the process of transcription, except the adenosine which forms base pair with uracil instead of thymine.

But, unlike DNA replication where total DNA of an organism gets duplicated, in transcription only a segment of DNA and only one of the strands is copied into RNA. Here only one strand is template strand while in replication both strands are template.

There are two explanations for both the strands of DNA not being copied during transcription.

(1) If both strands act as template, they would code for RNA molecule with different sequences. And in turn, if they code for proteins, the sequence of amino acids in the proteins would be different. Hence, one segment of the DNA would be coding for two different proteins. This would complicate the genetic information transfer machinery.

(2) The two RNA molecules if produced simultaneously would be complementary to each other, hence would form a double – stranded RNA. This would prevent the translation of RNA into protein.

Transcription Unit

The segment of DNA that takes parts in transcription is called transcription unit. Its components.

(i) A promoter

(ii) The structural gene

(iii) A terminator

Template Strand and Coding strand

There is a convention in defining the two strands of the DNA in the structural gene of a transcription unit. Since the two strands have opposite polarity and the DNA – polymerase also catalyse the polymerization in only one direction i.e., 5' → 3' polarity. The strand that has the polarity 3' → 5' acts as template, and is called template strand or non – coding strand. The other strand with polarity 5' → 3' and the sequence same as RNA, except thymine at the place of uracil, is displaced during transcription. And this strand is called coding strand or sense strand or non – template strand.

Structural genes are flanked on both sides by a promoter and a terminator in transcription unit.

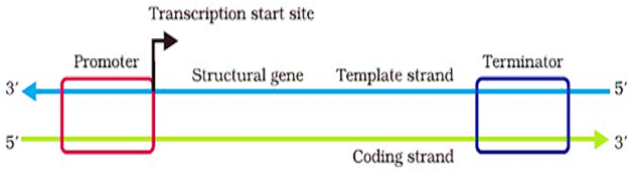


Fig Schematic structure of a transcription unit

Promoter sequences are present upstream towards 5' end of the structural gene of transcription unit (the reference is made with respect to the polarity of coding strand). It is a DNA sequence that provides binding site for DNA polymerase. It is the presence of a promoter in a transcription unit that also defines the template and coding strands. By switching its position with terminator, the definition of template and coding strand could be reversed. The binding sites for RNA polymerase lie within the promoter sequence.

Certain short sequence within the promoter sites is conserved, known as recognition sequence. (See knowledge could).

The terminator is present at 3' end (downstream) of coding strand and it usually defines the end of the process of transcription.

Transcription Unit and the Gene

A gene is defined as the functional unit of inheritance. Genes are located on the DNA and it is difficult to literally define a gene in terms of DNA sequence. The DNA sequence coding for tRNA or rRNA molecule also define a gene. Cistron is defined as a functional unit of gene, it is a segment of DNA coding for a polypeptide. The structural gene in a transcription unit is monocistronic (mostly in eukaryotes) and polycistronic (mostly in prokaryotes or bacteria). Monocistronic gene synthesizes one type of polypeptide or protein. Polycistronic gene synthesizes different proteins polypeptides.

The monocistronic structural genes have interrupted coding sequences i.e., the genes in eukaryotes are split. The coding sequences or expressed sequences are defined as exons which appear in mature or processed RNA. The exons are interrupted by introns. Introns are intervening sequences that do not appear in mature or processed RNA. The split – gene arrangement further complicates the definition of a gene in terms of a DNA segment.

Types of RNA and Process of Transcription

S.No.	mRNA	rRNA	tRNA
1.	5 % of total RNA in cell	80 %	15 %
2.	Longest	Smaller	Smallest
3.	It is called template/nuclear/ messenger or informational RNA as it carries genetic information provided by RNA	Has structural (forms ribosome) and catalytic role during translation)	Soluble or adapter RNA and carries amino acids

Thus, all three RNAs are needed to synthesise protein in a cell.

(A) **Transcription in Prokaryotes :** It occurs in cytoplasm with the help of transcripting enzyme.

The transcripting enzyme i.e., DNA – dependent RNA polymerase is only of one type and transcribe all types or RNAs i.e., mRNA, tRNA and rRNA. All three RNAs are needed to synthesize a protein in a cell.

RNA polymerase is a holoenzyme that is made of polypeptides σ. The enzyme without σ subunit is referred to as core enzyme. The process of transcription completes in 3 – steps:

(i) **Initiation :** It is catalysed by sigma (σ) factor or initiation factor. It binds to the promoter site of DNA and confers specificity. In the absence of σ – factor, transcription starts non – specifically by core enzyme at any base on DNA.

(ii) **Elongation :** The RNA polymerase (core enzyme) is only capable of catalyzing that process of elongation.

(iii) **Termination :** Rho factor (p) **is required for termination of transcription.**

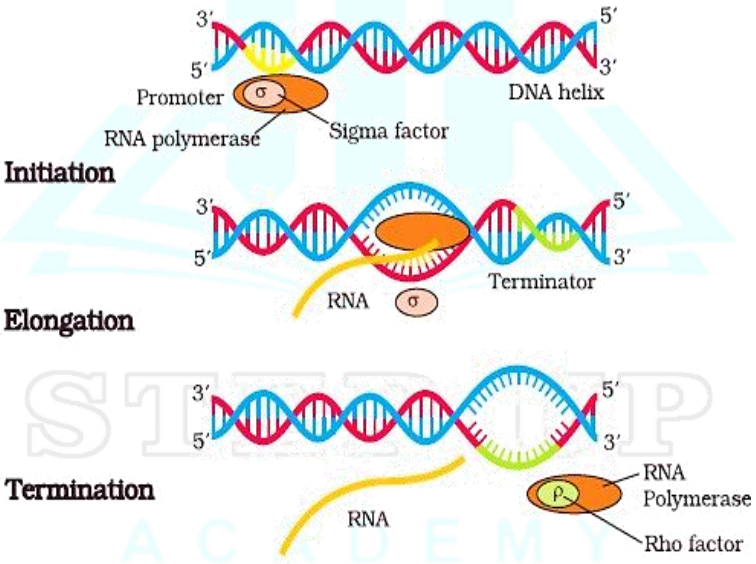


Fig Process of Transcription in Bacteria

This primary transcript is converted into functional mRNA after post – transcriptional processing which involves 3 steps :

(i) **Modification of 5' end by capping :** Capping at 5' end occurs rapidly after the start of transcription. An unusual nucleotide i.e. methyl guanosine triphosphate is added to the 5' – end of hnRNA. It is catalysed by guanyl transferase. Cap is essential for formation of mRNA – ribosome complex. Translation is not possible if cap is lacking because cap is identified by 18rRNA of

(ii) **Tailing and Splicing :** Tailing is the addition of adenylate residues about 200 – 300 at 3' end in a template – independent manner on newly formed hnRNA with the help of Poly a polymerase. Splicing is the process of removal of introns and joining of exons in a defined order'. Introns are removed by small nuclear RNA (SnRNA) and protein complex called small nuclear ribonucleoproteins or SnRNPs (Snurps).

The fully processed hnRNA is now called mRNA and it is transported out of the nucleus for translation.

The split – gene arrangements represent probably an ancient feature of genome. The presence of introns is reminiscent of antiquity, and the process of splicing represents the dominance of RNA – world.

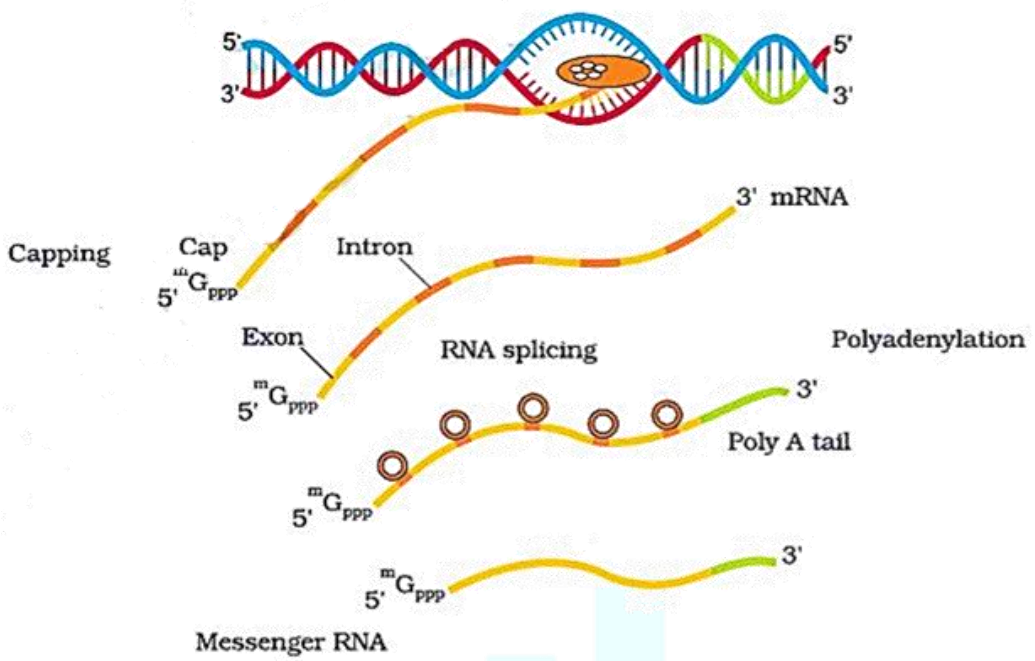


Fig. : Process of Transcription in Eukaryotes

GENETIC CODE

DNA (or RNA) carries all genetic information. It is expressed in the form of proteins which are made up of 20 different types of amino acids. The information about the number and sequence of these amino acids forming protein is present in DNA and is passed on to mRNA during transcription. Thus, genetic code is inter – relationship between nucleotides sequence of DNA or mRNA and amino acid and consists of 3 nucleotides.

The proposition and deciphering of genetic code were most challenging. In a very true sense, it required involvement of scientists from several disciplines – physicists, organic chemists, biochemists and geneticists. It was George Gamow, a physicist, who coined the term genetic code and argued since there are only 4 bases and if they have to code for 20 amino acids, the code should constitute a combination of bases. He suggested that in order to code for all the 20 amino acids, the code should be made up of 3 nucleotides. This was a very bold proposition, because a permutation of 4 x 4x 4 (4^3) would generate 64 codons, generating many more codons than require.

Salient Features of Genetic Code

(i) **Triplet code :** Each codon is made of three adjacent nitrogen bases. 61 codons code for amino acids and 3 codons do not code for any amino acids hence they function as stop codons.

(ii) **Non – ambiguous and specific codons :** One codon code for valine but at initiating position, codes for methionine)

(iii) **Comma less nature :** The codon is read in mRNA in a contiguous fashion without any punctuations.

(i) **Degeneracy of code :** Some amino are coded by more than one codon, hence the code is degenerate e.g., serine, arginine and threonine by 4 codons etc.

(ii) **Universal code :** The code is nearly universal, e.g., UUU would code phenylalanine in all organisms. Some exceptions to this rule have been found in mitochondria and protozoa. (See knowledge could).

(iii) **Initiation codon/start signal :** AUG has dual functions, its codes for methionine, and it also acts as initiator codon.

(iv) **Stop signals :** Polypeptides chain termination is signaled by three termination codons **– UAA (ochre), UGA (amber) and UAG (opal).** They do not specify any amino acids, hence called as nonsense codons or stop codons.

(v) **Non – overlapping codon :** Each codon is independent and one codon does not overlap the next codon.

MUTATIONS AND GENETIC CODE

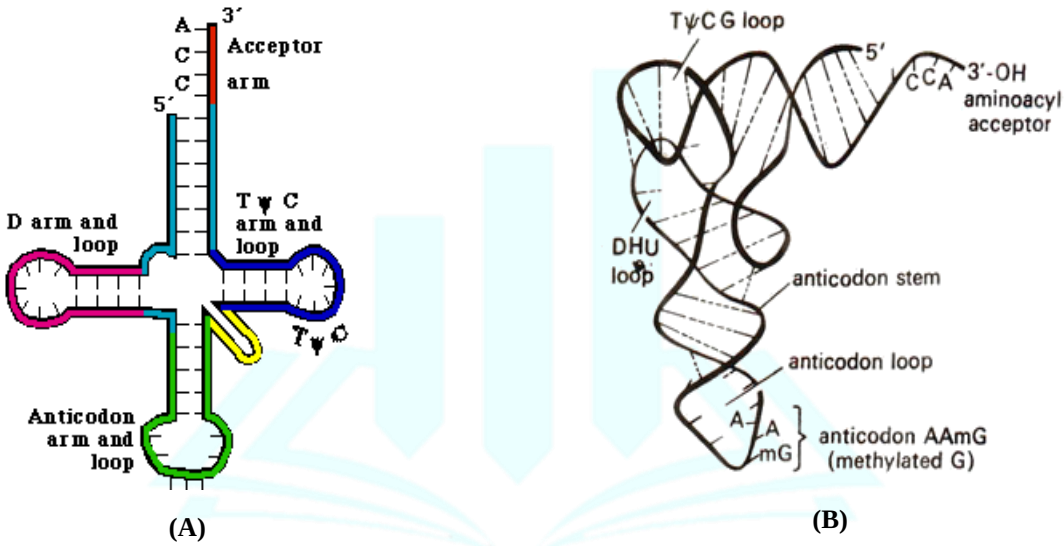
The relationship between genes and DNA are best understood by mutation studies. Effects of large deletions and rearrangements in a segment of DNA are easy to comprehend. It may result in loss or gain of a gene and so a function. A classical example of gene mutation or point mutation is a change of single base pair in the gene for beta globin chain that results in the change of amino acid residue glutamate to valine. It results into a diseased condition called as sickle cell anemia. Insertion or deletion of one or two bases changes the reading frame from the point of insertion of deletion. Insertion or deletion of three or its multiple bases the 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 mutations. This forms the genetic basis of proof that codon is a triplet and it is read in a contiguous manner.

tRNA – The Adapter Molecule

The existence of RNA was postulated by Francis Crick. It was also known as soluble RNA (sRNA) before the genetic code was postulated. These constitute about 15 % of the total cellular RNA.

Crick postulated the presence of an adapter molecule that would on one hand read the code and on other hand would bind to specific amino acid. It acts as intermediate molecule between triplet code of mRNA and amino acid sequence of polypeptide chain. All tRNA's have almost same basic structure. There are over 60 types of tRNA. The three – dimensional structure of the tRNA was proposed to be inverted : - shaped (by Kim and Klug). This is the actual structure of tRNA. The secondary structure of tRNA has been depicted that it looks like a clover – leaf. All tRNA molecules have a guanine residue at its 5' terminal end. At its 3' end, unpaired – CCA sequence is present. Amino acid gets attached at this end only tRNAs are specific for each amino acid. For initiation, there is another specific tRNA that is known as initiator tRNA. There are no tRNAs for stop codons.



(A) (B)

Fig. : Structure of tRNA

A. Clover leaf model to show basic plan of tRNA secondary structure or 2D structure

B. Three – Dimensional Structure showing inverted L – shaped configuration.

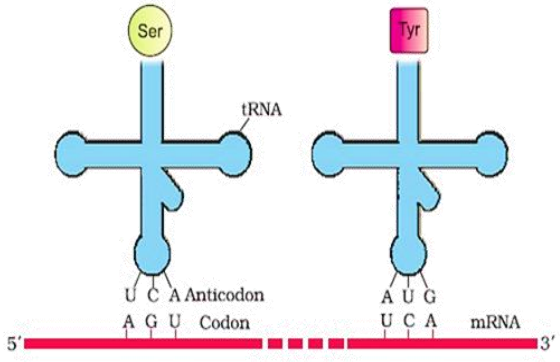


Fig. : tRNA – The adapter molecule

There are three loops in tRNA :

(i) Aminoacyl synthetase binding loop or DHU loop (dihydroxyuridine loop) – 1st loop 5' end.

(ii) Ribosomal binding loop with 7 unpaired bases – it is 1st loop from 3' end also called as tΨC loop.

(iii) Anticodon loop with 7 unpaired bases. Out of the 7 bases in anticodon loop, 3 bases act as anticodon for a particular triplet codon present on mRNA.

1. In prokaryotes recognition sequence is present in promoter region at upstream for RNA polymerase binding e.g.,

(i) **TATAAT or Pribnow box** – 10 sequence or 10 bp upstream from the start point)

(ii) **TAGACA : - 35** sequence as recognition sequence

2. In eukaryotes. TATA box or Hogness box (7 bp long – TATATAT or TATAAAT) located 20 bp upstream to the start point, another sequence is CAAT box present between - 70 and – 80 bp.

3. **Wobble hypothesis :** A change in nitrogen base at the 3rd position of a codon does not normally cause any change in the expression of the codon because the codon is mostly read by the first two nitrogen bases. The position of the third nitrogen base in a codon

which does not influence the reading of the codon is termed as Wobble position. It helps one tRNA to read more than one codon and thus, provides economy in number of tRNA molecules at the time of translation.

4. **Shine Dalgarno (SD) sequence :** It is 5' – AGGAGGU – 3' sequence at 5' – end near initiation codon in prokaryotes. It helps in binding of 30S subunit or ribosome on it.
5. Ribosomal RNA (rRNA) is most stable type of RNA and is constituent of ribosome.

- **In eukaryotes, rRNAs are of 4 types i.e., 5S, 5.8S 28S and 18S.**
- **In prokaryotes – 5S, 23S, 16S types of rRNA.**

6. **Genetic RNA:** RNA is genetic material in most plant viruses.

   Beadle and Tatum put forward a theory one – gene – one enzyme, they conducted experiments on the nutritional strains of pink mould. Neurospora crassa.

TRANSLATION

It refers to the 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 cellular factory responsible for synthesizing proteins is the ribosome. The ribosome consists of structural RNAs and about 80 different proteins. In its inactive state, it exists as two sub units, a large subunit and a small sub unit. Ribosomes have two sites for binding aminoacyl tRNA, P – site (peptidyl site) and A – site (aminoacyl). When the small subunit encounters an mRNA the process of translation of the mRNA to protein begins.

(a) **Activation of amino acids :** In the presence of enzyme aminoacyl – tRNA synthetase (E), specific amino acid (AA) binds with ATP.

$$AA_1 + ATP \xrightarrow{E_1\ mg2+} AA_1 - AMP - E \text{ complex} + PPi$$

(b) **Charging of tRNA :** The AA_1 – AMP – E complex reacts with specific tRNA. Thus, amino acid is transferred to tRNA. As a result, the enzyme and AMP are liberated. It is also called as aminoacylation of tRNA.

AA_1 – AMP – E_1 complex + tRNA → AA – tRNA + AMP + E_1 (change tRNA)

(c) **Formation of polypeptide chain :** It complete in three steps :

(1) Chain initiation : It requires 3 initiation factors in prokaryotes and 9 initiation factors in eukaryotes.

(i) Binding of mRNA with smaller subunit of ribosomes (30S/40S)

30S + mRNA → 30S – mRNA complex

In eukaryotes, there is formation of 40S – mRNA complex.

(ii) Binding of 30S – mRNA with $tRNA_{meth}$, non – formylated methionine is attached with tRNA in eukaryotes and formylated in prokaryotes.

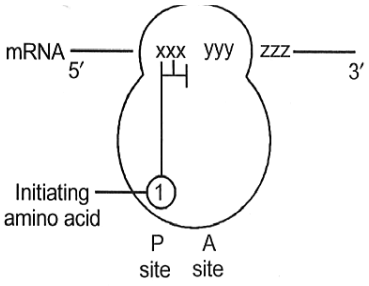
30S –mRNA + $tRNA_{fmet\ GTP}$ 30S- mRNA – $tRNA_{f\ met}$

(iii) Attachment of larger subunit of ribosome. It is 50S in prokaryotes & 60S in eukaryotes.

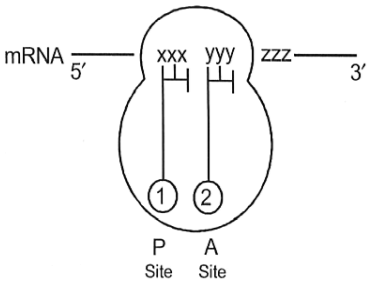
(2) Chain elongation : After the formation of complete ribosome – mRNA – tRNA complex, and aminoacyl acceptor site (A – site) is established next to the P – site. It exposes mRNA codon next to the initiation codon. A new aminoacyl tRNA complex reaches the A – site and a forms codon – anticodon bonding. This requires elongation factor and energy i.e., GTP. A peptide bond is formed between COOH group of first amino acid (methionine) and NH_2 group of second amino acid. If two charged tRNAs are close enough the formation of peptide bond between them would be favored energetically. The presence of a catalyst would enhance the rate of peptide bond formation. It is catalysed by enzyme peptidyl transferase (a type of ribozyme - catalytic RNA i.e. 23S rRNA in bacteria and 28 SrRNA in eukaryotes). The elongation factors are required in this process. Translocation is movement of ribosome on mRNA. The ribosome moves from codon to codon along the mRNA. Amino acids are added one by one, translated into polypeptide sequences dictated by DNA and represented by mRNA.

A translational unit in mRNA is the sequence of RNA that is flanked by the start codon (AUG) and the stop codon and codon for a polypeptide. An mRNA also has some additional sequences that are not translated and are referred as untranslated region (UTRs) . The UTRs are present at both 5' – end before start codon and 3' end after stop codon. They are required for efficient translation process.

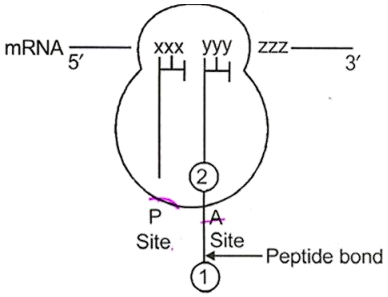
(3) Chain termination : The termination of polypeptide is signaled by one of the three termination codons (UAA, UAG,UGA). A GTP – dependent factor known as release factor is associated with translation and releasing the complete polypeptide from the ribosome.



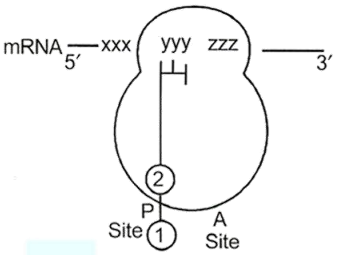
(A) Binding of first charged tRNA at P site



(B) Binding of next charged tRNA at A site



(C) Peptide bond formation by peptidyl transferase



(D) Translocation movement of ribosome on mRNA, so peptidyl tRNA is at P site. A site is open for the next charged t-RNA

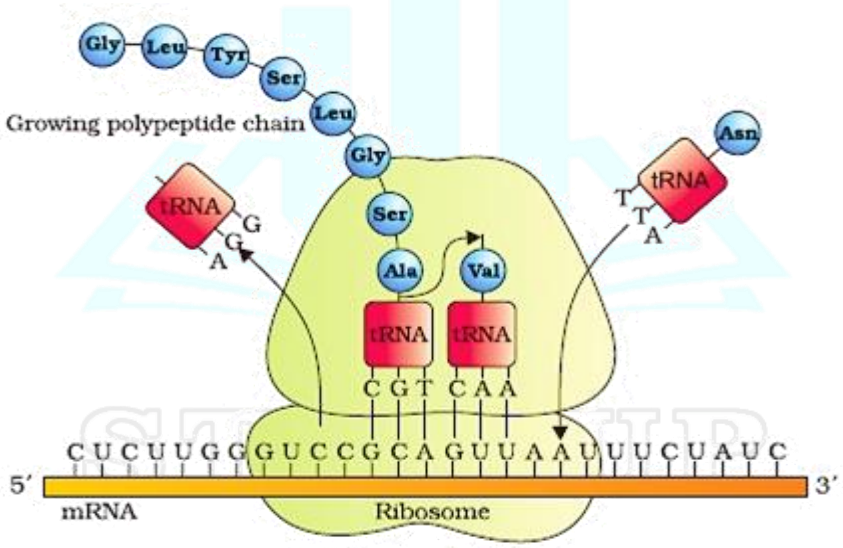


Fig. : Translation

REGULATION OF GENE EXPRESSION

Regulation of gene expression refers to a very broad term that may occur at various levels. Considering that gene expression results in the formation of a polypeptide, it can be regulated at several levels.

In eukaryotes, the regulation of gene expression could be exerted at four levels.

(i) Transcriptional level : Formation of primary transcript.

(ii) Processing level : Regulation of splicing.

(iii) Transport of mRNA from nucleus to the cytoplasm.

(iv) Translational level.

The genes in a cell are expressed to perform a particular function or a set of functions. In eukaryotes, functionally related genes do not represent do not represent an operon but are present on different sites, chromosomes. Here structural gene is called split gene which is a mosaic of exons and introns, i.e., the base triplet – amino acid matching is not continuous. The entire split gene is transcribed to form a continuous strip of mRNA. The removal of non – coding intronic part and fusion of exonic coding parts of RNA is called RNA splicing. About 50 – 90 % of primary transcribed RNA is discarded during processing. The development and differentiation of embryo into adult organisms are also a result of the coordinated regulation of expression of several sets of genes.

It is metabolic, physiological or environmental condition that regulates the expression of gene.

In prokaryotes, control of the rate of transcriptional

initiation is the predominant site for control of gene expression. In a transcription unit, the activity of RNA polymerase at a given promoter is regulated by interaction with accessory proteins, which affects its ability to recognize start sites. These regulatory proteins can act as both activators (positively) and repressor (negatively). The functioning of operator depends upon the protein products.

OPERON CONCEPT

Francis Jacob (a geneticist) and Jacques Monod (a biochemist) proposed a model of gene regulation, known as operon model in bacteria. Operon is a co – ordinate group of genes such as structural gene, operator gene, promoter gene, regulator gene which function together and regulate a metabolic pathway as a unit e.g., lac operon, try operon, ara operon, his operon, val operon etc.

(i) **Regulator gene :** It synthesizes a biochemical or regulator protein which can act positively as activator and negatively as repressor. It controls, the activity of operator gene.

(ii) **Operator gene :** It is a gene which receives the product of regulator gene. It allows the functioning of the operon when it is not covered by the biochemical produced by regulator gene.

(iii) **Promoter gene :** Provides attachment site for RNA polymerase.

(iii) **Structural gene :** Transcribes mRNA for polypeptide synthesis.

THE LAC OPERON

The lac operon (lac refers to lactose) consists of one regulatory gene or inhibitor gene (i), one promoter gene, one operator gene and three structural genes. A polycistronic structural gene is regulated by a common promoter and regulatory gene.

In E. coli, breakdown of lactose requires three enzymes. These are synthesized together in a co – ordinate manner by functional unit of DNA i.e., lac operon. Since the addition of lactose itself stimulates the production of required enzymes, thus it is called inducible system.

LAC OPERON GENES

1. **Structural genes :** Three structural genes are :

(i) **lac z :** The z gene codes for β – galactosidase which is primarily responsible for the hydrolysis of the disaccharide, lactose into its monomeric unit's galactose glucose.

(ii) **lac y :** The y gene codes for permease, with increases permeability of the cell to β – galactosidase.

(iii) **lac a :** The a gene codes for transacetylase which can transfer acetyl group to β – galactoside.

2. **Operator gene :** It interacts with a protein molecule or regulator molecule, which prevents the transcription of structural genes.
3. **Promoter gene :** The gene possess site for RNA polymerase attachment.
4. **Regulator gene (i) :** The gene codes for a protein known as repressor protein, it is synthesized all the time from the I – gene, that's why it is constitutive gene which is functional always.

   The operon is switched off when repressor protein produced by regulatory or inhibitor gene binds to operator gene. RNA polymerase gets blocked, so there would be no transcription.

Repressor protein + Operator gene → Switched off

Regulation of lac operon by repressor is referred to as negative control or regulation.

If lactose is provided in the growth medium of the bacteria, the lactose is transported into the cells through the action of permease. A very low level of expression of lac operon has to be present in the cell all the time, otherwise lactose cannot enter the cells. In the

presence of an inducer, such as lactose or allolactose, the repressor is inactivated by interaction with inducer. This allows RNA polymerase access to the promoter and transcription proceeds.

Inducer (Lactose) + Repressor → Switched on

Lac operon is under control of positive regulation as well.

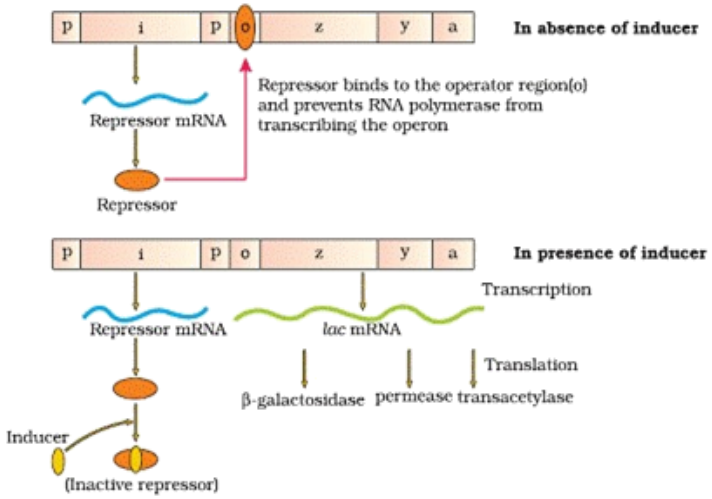


Fig. : The Lac Operon

HUMAN GENOME PROJECT (HGP)

Genetic make – up of an organism or an individual lies in the DNA sequences. The differences in two individuals will naturally be reflected in the differences of their nucleotide sequences. They can be known only if the entire human genome is mapped. With the establishment of genetic – engineering techniques where it was possible to isolate and clone any piece of DNA and availability of simple and fast techniques for determining DNA sequences, a very ambitious project of sequences, a very ambitious project of sequencing human genome was launched in the year 1990.

HGP was the international, collaborative research program whose goat was the complete mapping and understanding of all the genes of human beings. All genes together of haploid set of chromosomes are known as genome.

Human genome project as "Mega project" was a 13 – year – project, co – ordinate by the US Department of Energy and the National Institute of Health. Soon Welcome Trust (UK) joined the project as major partner, additional contributions from Japan, France, Germany, China and other. The project was complete in 2003. HGP has been called a megaproject due to

(i) Huge cost estimated to be 9 billion US dollars, the cost of sequencing 1 bp is US$3.

(ii) Very large number of base pairs (3 x 10^9 bp) to be identified and sequenced.

(iii) Requires a large number of scientists, technicians and supporting staff.

(iv) Storage of data generated which requires some 3300 books, each with 1000 pages and each page having 1000 typed letters. However, high – speed

The science of Bioinformatics also developed during this period and helped HGP.

Goals of HGP

Following are the important goals of HGP :

(i) Identification of all the approximately 20,000 –

(ii) 25,000 genes in human DNA.

(iii) To determine the sequences of the 3 billion chemical base pairs that make up human DNA.

(iv) To store this information in databases.

(v) To improve tools for data analysis.

(vi) Transfer – related technologies to other sectors, such as industries.

(vii) **ELSI** : To solve any ethical , legal and social issues.

(viii) **Bioinformatics** i.e., close association of HGP with the rapid development of a new area in biology.

METHODOLOGIES

The methods involved two major approaches:

(i) **ESTs/Expressed Sequence Tags :** Identifying all genes that are expressed as RNA.

(ii) **Sequence Annotation :** Sequencing the whole set of genomes that contained all the coding and non – coding sequences and later assigning different regions in the sequence with functions.

For sequencing, the total DNA from a cell is isolated and converted into random fragments of relatively smaller sizes (recall DNA is a very long polymer, and there are technical limitations in sequencing very long pieces of DNA) and cloned in suitable host using specialized vectors. The cloning resulted into amplification of each piece of DNA fragment so that it subsequently could be sequenced with ease. The commonly used hosts were bacteria and yeast, and the vectors were called as BAC (bacterial artificial chromosomes), and YAC (yeast artificial chromosomes).

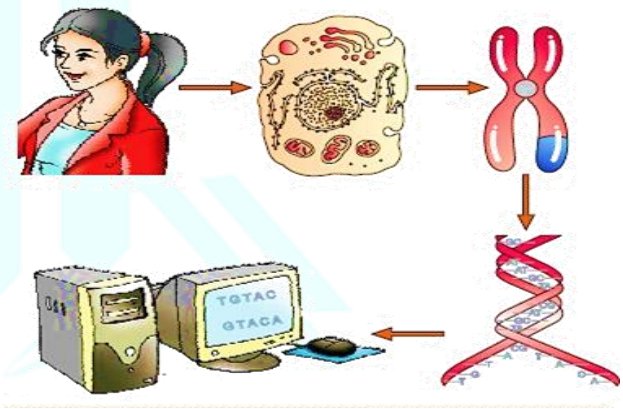


Fig. A representative diagram of human genome project

The fragments were sequenced using automated DNA sequencers that worked on the principle of a method developed by Frederick Sanger. Sanger is also credited for developing method for determination of amino acid sequence in proteins. These sequences were then arranged based on some overlapping regions present in them. This required generation of overlapping fragments for sequencing. Alignment of these sequences was humanly not possible. Therefore, specialized computer – based programs were developed. These sequences were subsequently only in May 2006 (this was the last of the 24 human chromosomes – 22 autosomes and X and Y – to be sequenced).

SALIENT FEATURE OF HUMAN GENOME

Some of the salient observations drawn from human genome project are as follows :

(i) The human genome contains 3164.7 million nucleotide bases.

(ii) The average gene consists of 3000 bases, but size varies greatly, with the largest known human gene being dystrophin as 2.4 million bases and TDF gene as smallest gene with 14 bases.

(iii) The total number of genes is estimated at 30,000 much lower than previous estimates of 80,000 to 1,40,000 genes. Almost all (99.9 percent) nucleotide bases are exactly the same in all people.

(iv) The functions are unknown for over 50 percent of discovered genes.

(v) Less than 2 percent of the genome code of proteins.

(vi) Repeated sequences make up very large portion of the human genome.

(vii) Repetitive sequences are stretches of DNA sequences that are repeated many times, sometimes hundred to thousand times. They are thought to have no direct coding functions, but they shed light on chromosome structure, dynamics and evolution.

(viii) Chromosome 1 has most genes (2968) and the Y has the fewest (231).

(ix) Scientists have identified about 1.4 million location where single DNA differences occur in humans. This is known as SNPs - single nucleotide polymorphisms. Pronounced as 'snips. This information promises to revolutions the process of finding chromosomal locations for disease – associated sequences and tracing human history.

DNA FINGERPRINTING

It is the technique used for determining nucleotide sequences of certain areas of DNA which are unique to each individual. DNA fingerprinting can distinguish one human being from another with the exception of monozygotic twins' 99.9 percent of base sequences among humans is the same. They 0.1% of genome or 3 x 10^6 differences in the base sequence in the base sequence. The differences occur not only in genes but also in repetitive DNA.

DNA fingerprinting involves identifying differences in some specific regions in DNA sequence called as repetitive DNA. 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.

Satellite DNA is classified into following categories on the basis of base composition (A : T rich or G : C rich) length of segment and number of repetitive units. These categories are :

1. **VNTRs (Variable Number of Tandem Repeats)** or mini-satellites surrounded by conserved restriction sites. A small DNA sequence is arranged tandemly in many copy numbers. The copy numbers varies from chromosome to chromosome in an individual. The number of repeats show very high degree of polymorphism. As a result, the size of VNTR varies from 0.1 to 20 kb.
2. **SSRs** (Single Sequence Repeats) or STRs (Short Tandem Repeats) or microsatellites with 1 – 6 bp.

These sequences normally do not code for any proteins, but they form a large portion of human genome. These the variation at genetic level. Since DNA from every tissue (such as blood, hair – follicle, skin, bone, saliva, sperm etc.), from an individual show the same degree of polymorphism, they become very useful identification tool in forensic application. Further, as the polymorphisms are inheritable from parents to children, DNA fingerprinting is the basis of paternity testing, in case of disputes.

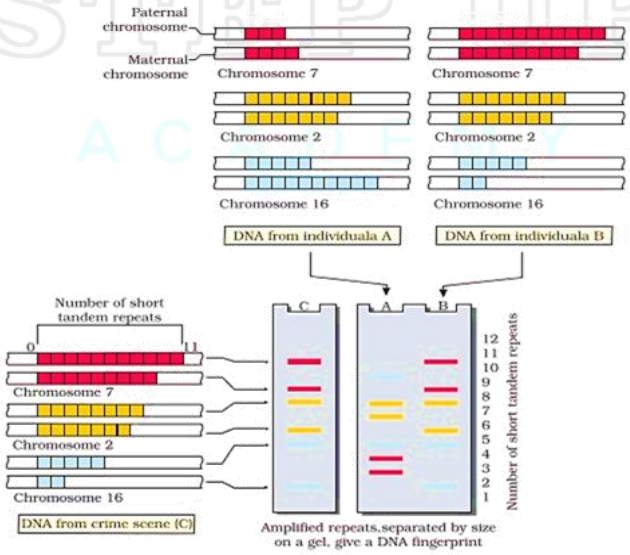


Fig.: Schematic representation of DNA fingerprinting: Few representative chromosomes have been shown to contain different copy number of VNTR. For the sake of understanding different schemes have been used to trace the origin of each bad in the gel. Two alleles (paternal and maternal) of a chromosome also contain different copy numbers of VNTR. It is clear that the banding pattern of DNA from crime scene matches with individual B, and not with A

As polymorphism in DNA sequence is the basis of genetic mapping of human genome as well as of DNA fingerprinting it is essential that we understand that what DNA polymorphism means in simple terms. Polymorphism (variation at genetic level) arises due to mutations. Allelic sequence variation has traditionally been described as a DNA polymorphism if more than one variant (allele) at a locus occurs in human population at high frequency it is referred to as DNA polymorphism. The probability of such variation to be observed in non – coding DNA sequence would be higher as mutations in these sequences may not have any immediate effect in an individual reproductive ability. These mutations keep on accumulating generation after generation and form one of the basis of variability/ polymorphism. There is a variety of different types of polymorphisms ranging from single nucleotide change to very large-scale changes. For evolution and speciation, such polymorphism play very important role.

C-value : Total amount of DNA per genome.

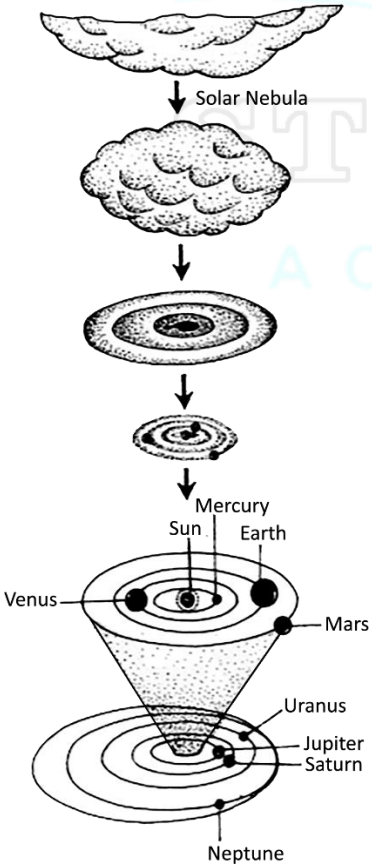
Chapter 6
Evolution

Evolutionary Biology

It is the study of history of life forms i.e. the changes in flora and fauna that have occurred over millions of years on earth.

What is Evolution?

- The word evolution means to unfold or unroll or to reveal hidden potentialities. Evolution simply means an orderly change from one condition to another.
- Evolution term was introduced by **Herbert Spencer.**
- It can be best explained by Darwin's concept of **'Descent with modification'.**
- This is the story of origin of life and evolution of life forms or biodiversity on Planet earth in the context of evolution of earth and against the background of evolution of universe itself.



What is Universe?

- The universe is a huge cluster of galaxies. Galaxies contain stars and clouds of gas and dust. Considering the size of universe, earth is indeed a speck i.e. The universe is vast.
- When we look at stars on a clear night sky we are, in a way, looking back in time. **Stellar distances are measured in light years.** The **Big Bang** theory attempts to explain to us the origin of universe.

Big Bang theory- Proposed by **Abbe Lemaitre.**

- According to it, the universe originated about 20 billion years ago due to a thermonuclear explosion of a dense entity. This single huge explosion which is unimaginable in physical terms, is called as **big bang.**
- The universe expanded and hence, the temperature came down.
- The gaseous clouds which were formed by big bang condensed under gravitation, and converted into many flat discs like structures called **nebula**, made up of atoms and small particles. **Solar nebula** was one of them, which formed our solar system.
- The very hot central part of solar nebula became still hotter and converted into the sun.
- Later on, due to condensation of atoms and dust particles moving around the sun other planets were formed.
- In the solar system of the Milky Way galaxy, earth was supposed to have been formed about 4.5 billion years back.
- There was no atmosphere on early earth. It was formed later.

Theories for origin of life :

1. **Theory of special creation -**

- The greatest supporter of this theory was **Father Suarez**. This is a mythology-based theory.
- This theory has three connotations-

(a) All living organisms that we see today were created as such.

(b) The diversity was always the same since creation and will be the same in future.

(c) The earth is about 4000 years old.

- All these ideas were strongly challenged during the nineteenth century based on observations of **Charles Darwin, Wallace** etc. They believed that life forms varied over the periods of time.
- From fossils records and their dating, we can conclude that earth is very old, not thousands of years as was thought earlier but billions of years old.

2. Cosmic panspermia theory

- Some scientists believe that life came from outer space.
- Early Greek thinkers thought units of life called **spores** were transferred to different planets including earth.
- **'Panspermia'** is still a favourite idea for some astronomers.

3. Theory of spontaneous generation (Abiogenesis/Autogenesis) -

- This hypothesis was supported by ancient Greek philosophers.
- According to this theory life came out of decaying and rotting matter like straw, mud, etc. spontaneously.
- They believed that the mud of **Nile** river could give rise to fishes, frogs, crocodiles etc. when warmed by light rays.

4. Theory of biogenesis – Proposed by **Harvey & Huxley**

- They stated "Omnis vivum ex ovo or vivo", which means "New life can be originated on earth only by pre-existing life."
- Experiments of **Francesco Redi, Lazzaro Spallanzani, and Louis Pasteur** etc. supported the theory of biogenesis and disproved the abiogenesis. Experiment of Louis Pasteur is most renowned among all of these.

Hence spontaneous generation theory was dismissed once and for all. However, this did not answer how the first life form came on earth.

Experiment of Louis Pasteur:

- His experiment is also known as **'Swan neck steam neck flask experiment.**
- He prepared sterilized syrup of sugar and killed yeast by boiling them in flasks.
- He took two flasks one of broken neck and another of curved neck (swan neck flask/ "S" shaped neck flask).
- He showed that in pre-sterilized swan neck flasks, life did not come from killed yeast because germ laden dust particles in the air were trapped by the curved neck which serves as filter while in another flask open to air (broken neck), new living, organisms arose

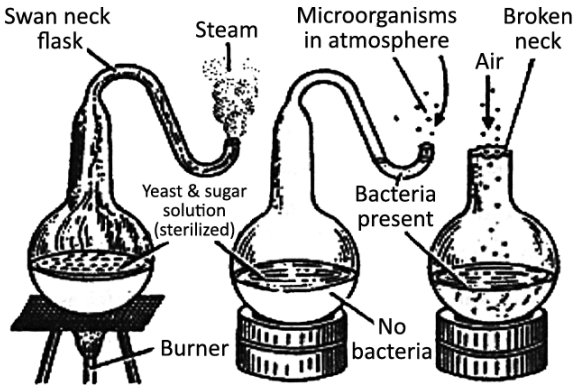


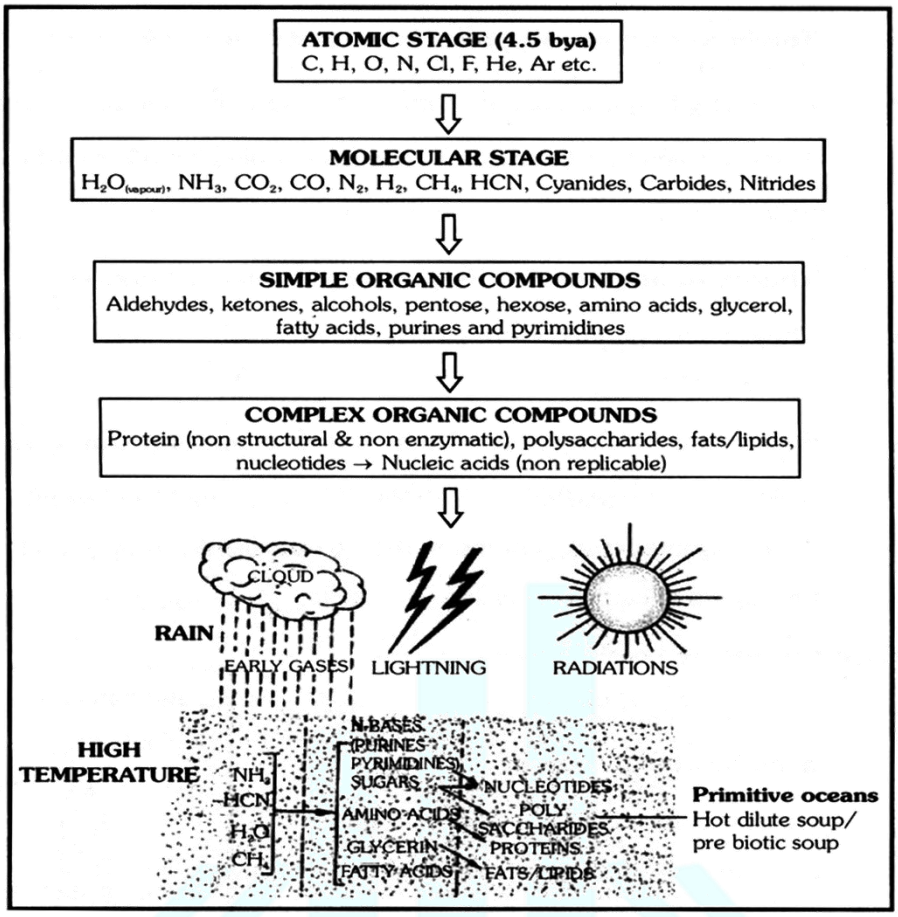
Fig. : Louis Pasteur's swan neck flask experiment

5. Oparin - Haldane theory (Modern theory) -

- Oparin of Russia and Haldane of England proposed that the first form of life could have come from **pre-existing non-living organic molecules (e.g. RNA, protein, etc.)** and that formation of life was preceded by chemical evolution, i.e., formation of diverse organic molecules from inorganic constituents.
- Oparin s theory was published in his book **'ORIGIN OF LIFE'.**
- First life originated in sea water, so water is essential for origin of life.

(A) CHEMICAL EVOLUTION (Chemogenic)

- The primitive conditions on earth were **high temperature, volcanic storms, lightening and reducing atmosphere**.
- Early earth had free atoms of all those elements which are essential for formation of protoplasm (C, H, O, N etc.).
- Hydrogen was maximum among all of them.
- Due to high temperature hydrogen reacted with oxygen to form **water** and no free oxygen was left, which made the atmosphere reducing.
- Hydrogen also reacted with nitrogen and formed **ammonia.**
- Hence **Water and ammonia** were probably the first inorganic compounds formed on earth.
- Methane **(CH_4)** was the first organic compound.
- As the earth cooled down, the water vapor fell as rain, to fill all the depressions and form primitive oceans. During this, molecules continued to react with each other and formed various simple and complex organic compounds.
- Now, the water of oceans became a rich mixture of macromolecules/ complex organic compounds. Haldane called it **Hot dilute soup/ pre biotic soup.**
- Hence the possibilities of life were established in the water of primitive oceans because these macromolecules **(Proteins, polysaccharides, fats/lipids, nucleic acids)** form the main components of protoplasm.



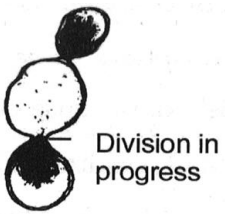
However, we have no clear idea about how the first self-replicating metabolic capsule of life arose, but many attempts were made to solve the mystery of arise of life on earth. From these macromolecules how first life was originated, will be studied in **biological evolution.**

(B) BIOLOGICAL EVOLUTION (Biogeny)

(a) Origin of photobionts-

- Macromolecules which were synthesized abiotically in primitive oceans later came together and formed large colloidal drop like structures named as **photobionts.**
- It is believed that they were the clusters of proteins, polysaccharides, lipids, nucleic acids etc.
- These photobionts were unable to reproduce but they could grow by absorbing molecules from their surroundings and can exhibit simple metabolism.

Photobionts were also synthesized artificially by some scientists in laboratory.



For example, **Oparin** prepared some photobionts without a lipid membrane and he called them **coacervates**.

Similarly, **Sydney Fox** synthesized some microscopic proteinoid bodies with a lipid coat and called them microspheres.

(b) Origin of protocells (Eobionts)-

- Nucleic acid developed the ability of self-duplication due to a sudden change called **mutation.**
- Nucleic acid and proteins combined to form **nucleoproteins.** Nucleoproteins were the first sign of life.
- Clusters of nucleoproteins surrounded by lipid coat called **protocell**, the **first form of life.**
- These **first non-cellular forms of life** could have originated **3 billion years ago.**
- They would have been giant molecules **(RNA, Protein, Polysaccharides, etc.).** These capsules reproduced their molecules perhaps.
- **Airman (1980)** discovered that some RNA molecules have enzymatic activity, called as **ribozymes**. It means at the time of origin of life, RNA molecule could carry out all the processes of life (replication, protein formation etc.) without the help of either protein or DNA. Hence this concept called as **RNA World.**

(c) Origin of first cellular form (Prokaryotes) -

- As a result of mutation protocells became more complex and efficient to use the materials available in the surrounding medium and evolved into **prokaryotic cells.**
- This first cellular form of life did not possibly originate till about 2000 million years ago.
- The **first living beings** were single celled bacteria like prokaryotes with naked DNA.
- They were probably **chemoheterotrophs** and **anaerobic.**
- Some of the chemoheterotrophic bacteria evolved into **chemoautotrophs.** They were anaerobic and synthesized organic food from inorganic material; this mode of nutrition is called as **chemosynthesis.**
  e.g. Iron bacteria, Nitrifying bacteria etc.
- When bacteriochlorophyll was developed in some chemoautotrophic bacteria, they started to convert light energy into chemical energy, this mode of nutrition is called as **photosynthesis.**
- They used H_2S as source of hydrogen instead of H_2O hence they were **non oxygenic photosynthetic** bacteria.
  e.g. Planktonic sulphur bacteria
- Some molecular changes occurred in bacteriochlorophyll, and it transformed into true chlorophyll. Such organisms used H_2O as source of hydrogen and released oxygen in the environment, they were **oxygenic photosynthetic** bacteria.
  e.g. Cyanobacteria (Blue green algae)

Oxygen revolution

Liberation of free oxygen by cyanobacteria was a revolutionary change in the history of earth. It includes some major changes like-

1. Atmosphere of earth changed from reducing to oxidizing, hence possibilities of further chemical evolution finished, because chemical evolution always takes place in reducing environment.
2. Free O_2 oxidized CH_2 and NH_3 to form gases like CO_2, N_2 and H_2O.
3. Accumulation of free oxygen formed a layer of ozone outside the atmosphere of earth which started to absorb most of the UV rays of sunlight.
4. Some prokaryotes adapted themselves for aerobic mode of respiration which provides approx. 20 times more energy than anaerobic respiration.

(d) Origin of Eukaryotic cell-

Nucleus, mitochondria and other cell organelles developed in the cell and metabolically it became more active.

Thus, free living eukaryotic cell like organisms originated about 1.5 billion years ago in the primitive ocean.

EVIDENCES IN FAVOUR OF CHEMICAL EVOLUTION

(1) Harold Urey & Stanley Miller Experiment:

- In 1953, S.L. Miller, an American scientist created similar conditions at laboratory scale which were thought to be on primitive earth.
- He took **NH_4, NH_3, H_2 (in ratio 2 : 1 : 2)** and water vapor at **800°C** in a large flask
- He created **electric discharge** by using two tungsten electrodes as source of energy.
- He observed the formation of simple amino acids like glycine, alanine, and aspartic acid.

In similar experiments other scientists observed, formation of sugars, nitrogen bases, pigment and fats.

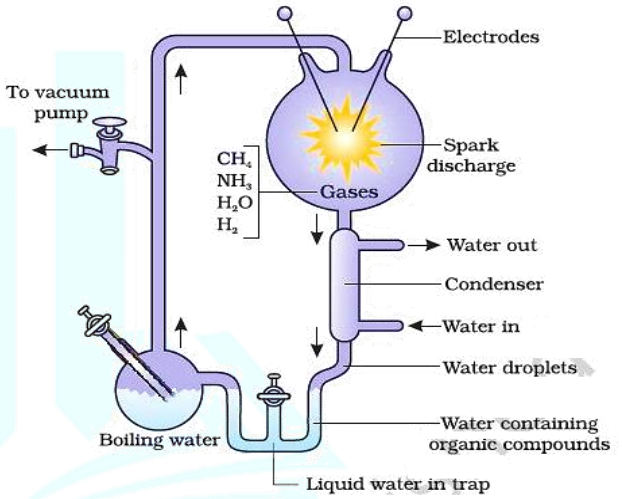


Fig. Diagrammatic representation of Miller's experiment

(2) Evidences from meteorites:

- Analysis of meteorite contents also revealed similar compounds indicating that similar processes are occurring elsewhere in space.

☞ With these limited evidences, the first part of the conjectured story, i.e., chemical evolution was more or less accepted.

☞ This version of abiogenesis, i.e., the first form of life arose slowly through evolutionary forces from non-living molecules is accepted by majority. However, once formed, how the first cellular forms of life could have evolved into the complex biodiversity of today is the fascinating story that will be discussed in **organic evolution**.

- Evolution up to formation of coacervates - Chemical evolution
- Evolution from coacervate to simple cell - Biological evolution

- Evolution from simple cell to recent - Organic evolution
- Oparin's theory is also known as primary abiogenesis and it is based on artificial synthesis, so also called as artificial synthetic theory.
- Louis Pasteur also proposed the **'Germ theory of diseases'** and he is famous for his **pasteurization technique.**
- From protocells or eobionts few core of nucleoproteins get separated free in oceans and became inactive but when they enter in another eobionts they became active so virus like structures were formed. This is an example of **retrogressive evolution.**
- **Universe** originated about **20 bya.**
- **Solar system** and **earth** were formed about **4.5 bya.**
- Life appeared about **4 bya.**
- First cellular form of life **- 2 bya.**

EVOLUTION (ORGIN AND EVOLUTION)

- Evolutionary biology is the study of history of life forms on earth.
- Universe originated about 20 billion years ago by thermo – nuclear explosion called Big – Bang.
- Earth originated about 4.5 billion years ago.
- Stellar distances are measured in light years.
- Life appeared 500 million years after the formation of earth i.e., almost four billion years back.

Ancient Theories of Origin of Life

1. **Theory of Special Creation :** Supported by **Father Suarez.**
2. **Theory of Spontaneous Generation (Abiogenesis or Autogenesis) :** This theory states that life originated from nonliving things in a spontaneous manner.

According to theory of spontaneous generation life came out of decaying and rotting matter like straw, mud etc.

Evidences against the Theory of Spontaneous Generation

a. **Spallanzani's Experiment :** Spallanzani disproved the spontaneous generation of microorganisms. He experimented that animal and vegetable broths boiled for several hours and soon after sealed, were never infested with microorganisms. From this experiment he concludes that high temperature had killed all living organisms in the broths and without them life could not appear. When the broths were left exposed to air, were soon invaded by microorganisms.

b. **Pasteur's Experiment :** Louis Pasteur took broths in a long-necked flask and then he bent the neck of the flask He boiled the broths in the flask to kill any microorganisms that might be present in them. The curved neck acted as a filter. If the flask with 'swan neck' (curved neck) is kept for months together, no life appeared, as the germ laden dust particles in the air were trapped by the curved neck which serves as filter. If the swan neck was broken off, the broths developed colonies of moulds and bacteria. Thus, he showed that the source of the micro-organisms for fermentation or putrefaction such as for milk, sugar and wine, etc., was the air and the organisms did not arise from the nutrient media.

Thus Louis Pasteur (famous for **"Germ Theory of Disease and Immunology")** finally disapproved abiogenesis and proved biogenesis.

3. **Modern Theory of Oparin-Haldane Theory of Origin of Life :** According to this theory life originated on early earth through physio-chemical processes atoms combining to form molecules, molecules in turn reacting to produce inorganic and organic compounds. Organic compounds interacting to produce all types of macromolecules which organized to form the first living system or cells.

1. Paleontological evidences-

- Study of **fossils** is called **paleontology**.
- Rocks form sediments and a cross-section of earth's crust indicates the arrangement of sediments one over the other during the long history of earth. Such types of rocks are called as **sedimentary rocks.**
- Mostly fossils are found in sedimentary rocks.
- Different-aged rock sediments contain fossils of different life-forms who probably died during the formation of the particular sediment.
- A study of fossils in different sedimentary layers indicates the geological period in which they existed.
- Some of them represent extinct organisms (e.g., Dinosaurs).
- The study shows that life-forms varied over time and certain life forms are restricted to certain geological time spans.
- New forms of life have arisen at different times in the history of earth, i.e. evolution has taken place.
- Generally, fossils found in older rocks are of simpler types and found in newer rocks are of complex type.
- By fossils we can study the evolutionary pedigree of animals like horse, elephants and man etc.
- **The geological history of earth closely correlates with the biological history of earth.**

Type of fossils-

(1) Unaltered fossils: Fossils which are preserved in their original or intact form in ice, amber etc.

e.g. (i) **Wooly mammoths** found frozen in ice (25000 years before extinct fossils were found from Siberian region).

(ii) Insects or plant parts trapped in amber

(2) Petrified fossils: Replacement of organic or soft parts of dead organisms by mineral deposits is called **petrifaction**. Here only the hard parts like bones, teeth, shells, and wood etc. get preserved, which are called petrified fossils. These are the most common types of fossils.

(3) Mould fossils: Only an impression of the external structure of body is preserved in wet soil and no body part is recovered of dead organisms.

(4) Cast fossils: Sometimes minerals are filled in the mould, resulting in cast fossils.

(5) Print fossils: Fossilized impressions of foot, wings, leaves, stem etc.

How the ages of the fossils are calculated?

Answer: To find out the correct age of fossils, we determine the age of rocks from which fossils are found.

Rocks contain some radioactive elements that decay and convert into their more stable forms. This radioactive decay takes place at a constant rate for each radioactive element irrespective of the environmental conditions.

It is already calculated that how long it will take for half the quantity of the element to change into its stable form, and this time is known as its **half-life.** After another half-life has passed, the element will have decayed to a quarter of its original amount and so on.

For example: half-life of carbon-14 is 5730 years; it means in 5730 years, half of the C-14 converts into its stable form N-14.

Thus, we can calculate the age of rocks by relative proportions of radioactive element and non-radioactive element in a sample of rock. This method is called **radioactive dating.**

There are several methods used to determine the age of fossils-

(1) Uranium Lead method

(2) Radio carbon method

(3) Potassium argon method - this method is more commonly used to determine the age of older hominid fossils.

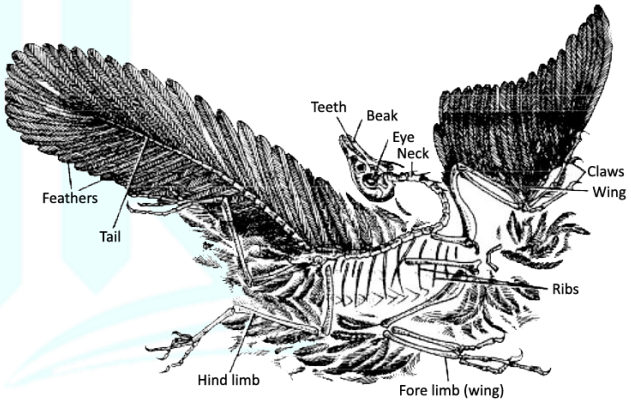
(4) Electron spin resonance (ESR) method - this is the modern and most accurate technique.

Archaeopteryx-

- It is a missing link between reptiles and birds.
- The connecting links which are not found in present times are called as **Missing links.**

Reptilian characters:

- Long lizard tail with free caudal vertebrae
- Non pneumatic bones
- Weak sternum
- Teeth present in jaw



Avian Characters :

- Feathers on body
- Jaws modified into beak
- Forelimbs modified into wings (reduced)

Hind limbs built in avian plan

Evolution (Pedigree) of Horse-

- Evolution of horse was described by C. Marsh.
- Many evolutionary changes were observed in horse-

(i) Increment in body height, length of neck & legs.

(ii) Reduction in number of toes or fingers and development of running habit.

(i) Development of high crown on teeth and formation of cement.

(ii) Enlargement in size of brain.

Epochs	Height (in cms)	Appearance	Horse	Bones of limbs	No. of toes
Pleistocene	160	Modern Horse	Equus		1 - toed (2 Splint bones)
Pliocene	120	Pony like	Pliohippus		1 - toed (2 Splint bones)
Miocene	100	Donkey like	Merychippus		3 - toed (No Splint bones)
Oligocene	60	Sheep like	Mesohippus		3- toed (1 Splint bones)
Eocene	40	Fox like	Eohippus		4- toed (1 Splint bones)

Geological time scale -

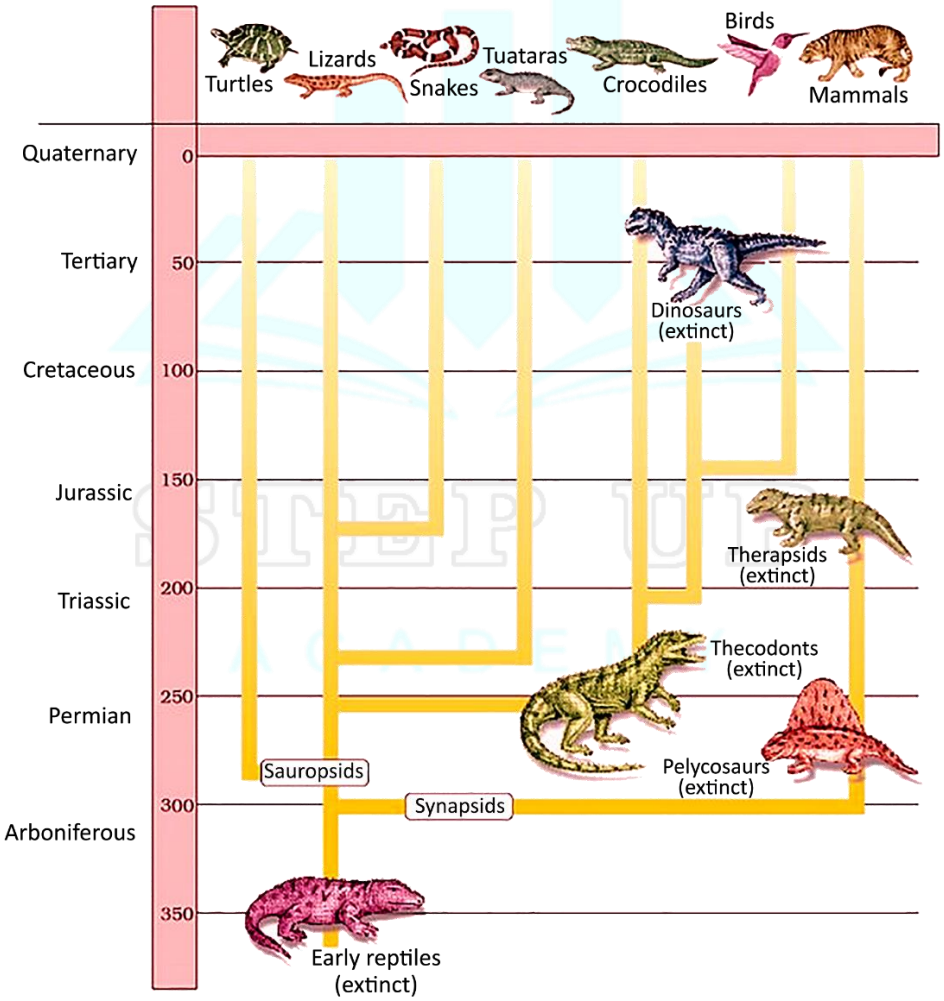
- It is the chronological order of the history of organic evolution on earth.
- The time after formation of the earth (4.5 billion years) is divided into 6 **Eras**, some Eras further divided into **Periods** and periods of recent era are divided into smaller time spans called **Epochs.**
- Intense geological disturbances have occurred on earth time to time, in which most of the pre-existing organisms perished out and the few remaining ones evolved into new and varied organisms. These disturbances are called as **revolution** or **cataclysm.**
- The time before palaeozoic era is also called as Precambrian era because the first period of palaeozoic is Cambrian.

GEOLOGICAL TIME SCALE			
Era	**Period**	**Epochs**	**Life forms**
COENOZOIC (Age of Birds Mammals and angiosperms)	**QUATERNARY**	Holocene (Age of Man)	Mental age, supremacy of man
		Pleistocene (ICE AGE)	Human appeared; social life of human started
	TERTIARY	Pliocene	Ape like ancestors of human appeared
		Oligocene	Anthropoid apes evolved from monkeys Rise of monocots
		Eocene	Eohippus appeared
		Palaeocene	Origin of primates
ROCKY MOUNTAIN REVOLUTION			
MESOZOIC (Age of Reptiles	**CRETACEOUS**		Extinction of Dinosaurs & archaeopteryx Origin of primitive placental mammals and Modern bird Angiosperms also appeared
	JURASSIC (Golden age of Dinosaurs)		Dominance of dinosaurs and origin of first toothed birds and marsupial mammals Gymnosperms and ferns also dominated
	TRIASSIC		Origin of dinosaurs and oviparous mammals
APPLACHIAN REVOLUTION			
PALAEOZOIC	**PERMIAN**		Origin mammal like reptiles, first Gymnosperm appeared
	CARBONIFEROUS Golden age of amphibians)		Amphibians were dominant and origin of reptiles (seymauria) First seed plant originated
	DEVONIAN (Golden age of fishes)		Fishes were dominant and origin of amphibians
	SILURIAN		Jawless fishes were dominant and Origin of true fishes
	ORDOVICIAN		Giant mollusks were dominant Origin of jawless fishes (1st vertebrates), origin of chordate
	CAMBRIAN		Trilobites (Extinct arthropods) were dominant

SECOND GREAT GEOLOGCAL REVOLUTION			
PROTEROZOIC			Origin of protozoa, sponges, coelenterate, annelid & Mollusca
FIRST GREAT GEOLOGCAL REVOLUTION			
ARCHAEOZOIC			Prokaryotes originated and dominated, Annelida & Mollusca
AZOIC			No life, only **chemical evolution** took place

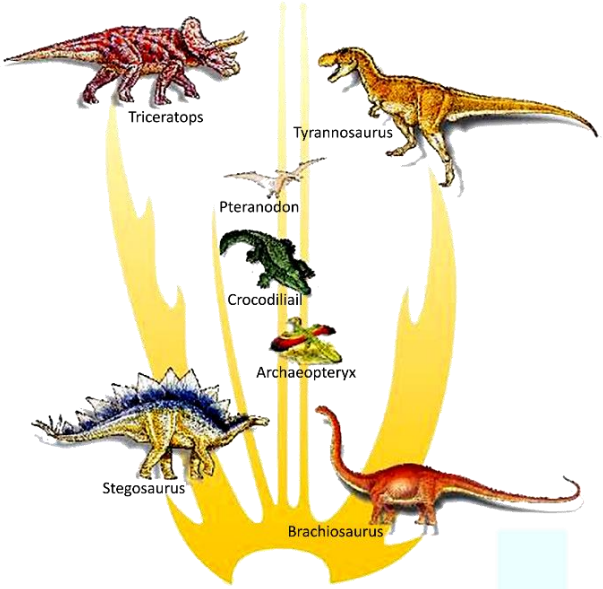
A brief account of evolution-

- About 2000 million years ago (mya) the first cellular forms of life appeared on earth.
- By the time of 500 mya, invertebrates were formed and became active.
- Jawless fishes probably evolved around 350 mya.
- Sea weeds and few plants existed probably around 320 mya.
- The first organisms that invaded land were plants. They were widespread on land when animals invaded land.



- Fish with stout strong fins could move on land and go back to water. This was about 350 mya. In 1938, a fish caught in South Africa happened to be a **Coelacanth** which was thought to be extinct.
- These **Coelacanth or lobefins** evolved into the first amphibians that lived on both land and water. There are no specimens of these left with us. However, these were ancestors of modern-day frogs and salamanders.
- The amphibians evolved into reptiles. They lay thick shelled eggs which do not dry up in sun unlike those of amphibians. Again, we only see their modern-day descendants, the turtles, tortoises and crocodiles.

- **Synapsids** were the mammal like early reptiles which gave rise to mammals.
- **Sauropsids** were the lizard like early reptiles which gave rise to different dinosaurs, modern reptiles and birds.



- In the next 200 million years or so, reptiles of different shapes and sizes dominated on earth.
- **Giant ferns (pteridophytes)** were present but they all fell to form coal deposits slowly.
- Some of the land reptiles went back into water to evolve into fish like reptiles probably 200 mya (e.g. **lchthyosaurs).**
- The land reptiles were, of course, the dinosaurs. The biggest of them, was **Tyrannosaurs rex** about 20 feet in height and had huge fearsome dagger like teeth.
- About 65 mya, the dinosaurs suddenly disappeared from the earth. We do not know the true reason. This may happen due to (i) Climatic changes killed them or (ii) Most of them evolved into birds or (iii) Meteorites collisions killed them. The truth is still unknown.
- Small sized reptiles of that era still exist today.
- The first mammals were like shrews. Their fossils are small sized.
- Mammals were **viviparous** and protected their unborn young inside the mother's body. Mammals were **more intelligent** in sensing and avoiding danger at least.
- When reptiles came down mammals took over this earth.

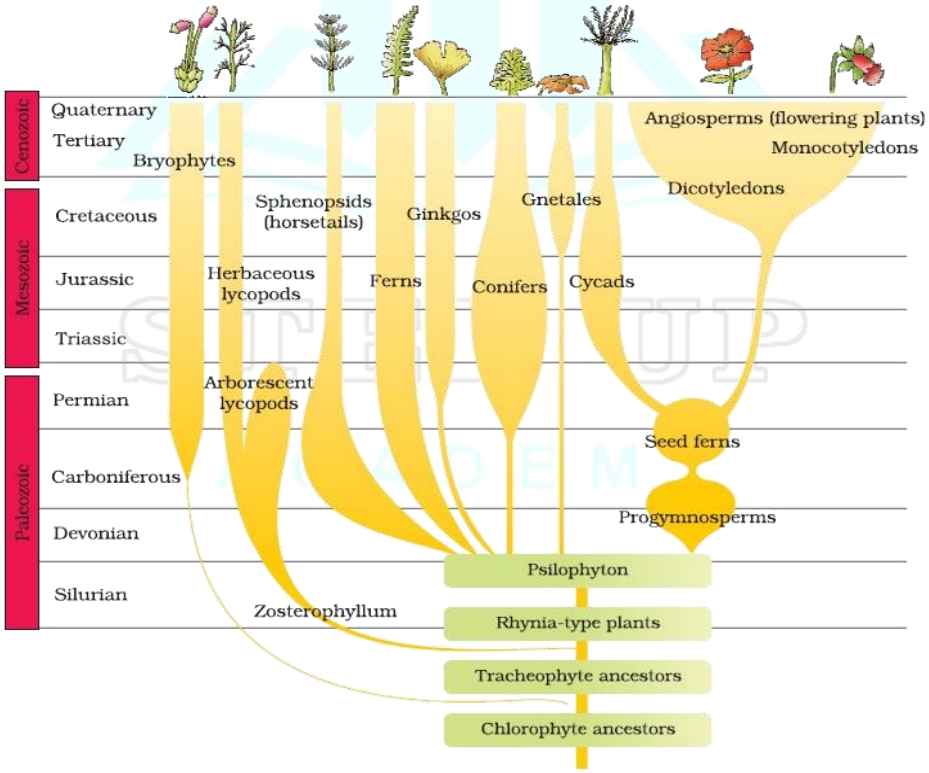


Fig. : A sketch of the evolution of plant forms through geological periods

Thus, according to this theory 'life' originated upon our earth spontaneously from nonliving matte. First inorganic compounds and then organic compound were formed in accordance with everchanging environmental conditions. This is called chemical evolution which cannot occur under present environmental conditions upon earth. Conditions suitable for origin of life existed only upon primitive earth. Oparin-Haldane theory is also called chemical theory.

- The first non-cellular forms of life could have originated 3 million years ago.
- Theory of special creation has three connotations –

- All living organisms that we see today were created as such,
- The diversity was always the same since creation and will be the same in future.
- The earth is about 4000 years old.

- According to 'Panspermia theory' unit of life called 'spores' were transferred to different planets including earth
- The geological history of earth closely correlates with the biological history of earth.
- Fossils are remains of hard parts of life forms found in Rocks.
- Different aged rock sediments contain fossils of different life forms who probably died during the formation of the particular sediment.
- **Homology present in organisms shows divergent evolution and analogy shows convergent evolution.**
- **The same structure developed along different divergent evolution and these structures are homologous. Homology indicates common ancestor.**

e.g., - Forelimbs of all mammals.

- Visceral organs of vertebrate like heart, brain,
- Thom and tendrils of Bougainvillea and Cucurbita.
- Mouth parts of some insects, the mouth parts of cockroach, honey bee, mosquito and butterfly have the same fundamental plan, but they have different functions to perform.

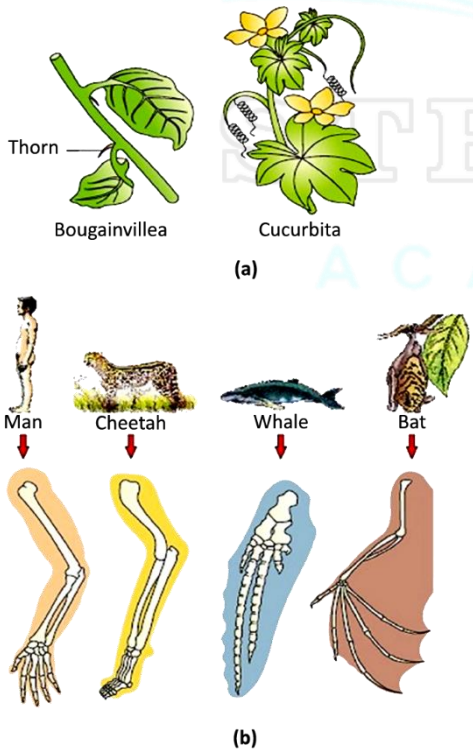


Fig. : Example of homologous organs in (a) Plants and (b) Animals

- **Analogous structures are a result of convergent, evolution. Different structures evolving for the same function.**
- Similar habitat has resulted in selection of similar adaptive features in different groups of organisms.

  e.g., - Eyes of octopus and mammals.
  - Flippers of Penguins and Dolphins
  - Wings of butterfly and birds.
  - Potato and Sweet potato.
  - Stings of honey bee and scorpion
  - Pectoral Fins of sharks and flippers of Dolphins
- Proteins and genes performing a given function among diverse organisms give clues to common ancestry.

Vestigial Organs is Human Body : Human body has been described to possess about 90 vestigial organs. Some of these are nictitating membrane, auricular muscles (muscles of pinna), segmental muscles of abdomen, panniculus carnosis (subcutaneous muscles), vermiform appendix, caudal vertebrae (also called coccyx or tail bone), third molars (wisdom teeth), hair on body, and nipples in male.

- According to industrial melanisation phenomenon in a mixed population, those that can better, adapt, survive and increase in a population size. No variant is completely wiped out.
- Evolution is not a direct process in the sense of determinism, it is a stochastic process (probable process) based on chance event is nature and chance mutation in the organisms.
- The process of evolution of different species in a given geographical area starting from a point and radiating to other areas of geography (habitats) is called as adaptive radiation. For e.g. Darwin's Finches and Marsupials of Australia.

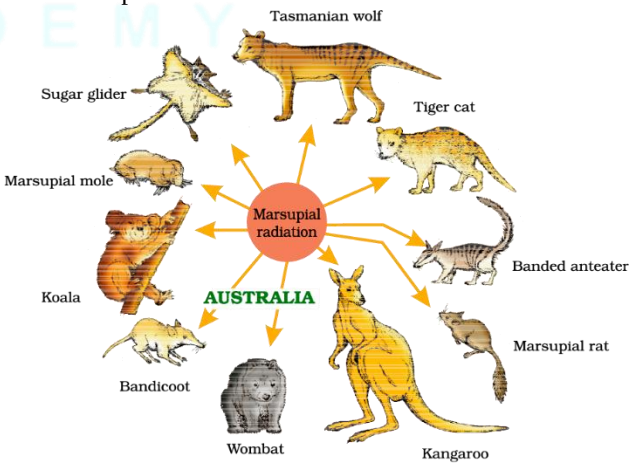


Fig. : Adaptive radiation of marsupials of Australia

When more than adaptive radiation appeared to have occurred in as isolated geographical area (representing different habitats). One can call this convergent evolution.

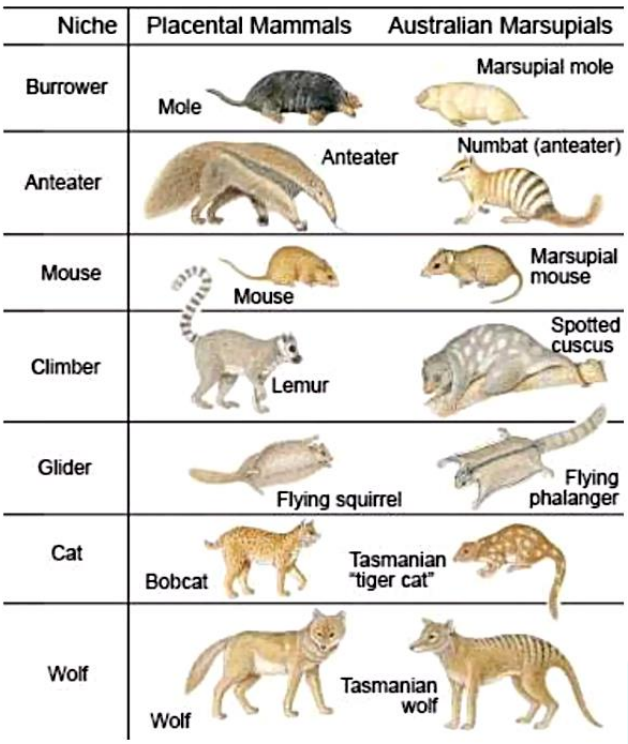


Fig. Picture Showing convergent evolution of Australian Marsupials and placental mammals

- According to Darwinism adaptive ability is inherited. It has a genetic basic. Fitness is the end result of the ability to adapt and get selected by nature.
- **Branching descent (**adaptive radiation) and **natural selection** are the two key concepts of Darwinism.
- According to Lamarckism evolution of forms has occurred but driven by use and disused of organs.
- When the original drifted population becomes founders and the effect is called founder effect.
- Mutations are random and directionless while Darwinian variations are small and directional.
- De – varies called mutation as saltation (single step large mutation).
- During evolution the first organism were plants.
- By the time of 500 million years ago, invertebrates were formed and active.
- Jawless fish probably evolved around 350 million years ago.
- Sea weeds and few plants existed probably around 320 million years ago.
- In 1938, a fish caught in South Africa happened to be a Coelacanth which was through to be extinct. These animals called lobe fins evolved into the first amphibians.
- Some of the land reptiles went back into water to evolve into fish like reptiles probably 200 million years ago (e.g. lchthyosaurs).
- The biggest dinosaur was Tyrannosaurus rex, about 20 feet.
- About 65 million years ago the dinosaurs suddenly disappeared from the earth.
- The first mammals were like shrews.

HARDY – WEINBERG PRINCIPLE

It was proposed by **G.H. Hardy** and **Weinberg** in 1908. This principle states that a population is said to be in **genetic equilibrium** if it is not undergoing any kind of evolutionary change. Genetic equilibrium means that the frequency of occurrence of alleles of a gene is supposed to remain fixed and even remain that same through generations. Hardy – Weinberg principle stated it using algebraic equations. Main concepts of this principle are :

(1) This principle says that allele frequencies in a population are stable and is constant from generation to generation.

(2) The gene pool (total genes and their alleles in a population) remains constant. This is called genetic equilibrium.

(3) Sum total of all the allelic frequencies is 1.

This can be explained as follows:

❖ The term allele is employed for any of the two forms of a gene, present on the same locus in the two homologous chromosomes and allelic frequency is the frequency with which a particular allele occurs in a population.

❖ For example, in a population of diploid organisms a gene has two alleles – A and a. Suppose the frequency of occurrence of allele A is p and of a is q. Then what is the probability that allele A will appear on both the chromosomes of a diploid individual? It will be simply the product of its probabilities, i.e., p x p = p^2. Hence, we can say that the frequency of AA individuals in this population is simply p^2.

Similarly, the frequency of aa individuals in this population is q^2 and the frequency of Aa individuals (with allele A on one chromosome and allele a on other chromosome) in this population is 2pq (or 2 x p x q).

We can see that the probability of occurrence of hybrid condition (Aa) is twice (2 x pq) than that of homozygous genotype (AA or aa) having p^2 and q^2 frequencies.

- Hardy – Weinberg principle says that the sum total of all the allelic frequencies of a gene is 1 and the possible frequencies of above-mentioned genotypes, i.e., AA, aa and Aa are p^2, q^2 and 2pq, respectively. So mathematically this statement can be written as

$$\mathbf{P^2 + 2pq + q^2 = 1}$$

We can see that this equation is a binomial expansion of $(p + q)^2$.

Interpretation : Hardy – Weinberg principle can be used to mathematically interpret whether evolution has occurred in a population or not. Disturbance in the genetic

equilibrium or Hardy – Weinberg equilibrium, i.e., change in frequency of alleles in a population, would be interpreted as resulting in evolution. When frequency measured, differs from expected values, the difference indicates the extent of evolutionary change.

Factors affection Hardy –Weinberg principle : Following five factors are known to affect Hardy – Weinberg equilibrium. Therefore, if any of the following phenomena occurs, change in frequency of alleles takes place that may result in the evolution.

(1) Gene migration as gene flow
(2) Genetic drift
(3) Mutation
(4) Genetic recombination
(5) Natural selection

(1) **Migration :** Migration defined in genetic terms as the movement of individuals from one population into another, can be a powerful force in upsetting the genetic stability of natural populations. The phenomenon of movement of alleles from one population to another is called gene migration. It can either occur by

(a) Migration of a section of population from one area to another, or by

(b) Interbreeding between members of two populations resulting in interchange of alleles. If the characteristics of the newly arrived animal differs from those already there, the genetic composition of the receiving population may be altered, if the newly individual or individuals can adapt to survive in the new area and mate successfully.

Gene pool : A total collection of all genes and its allele in a population is called gene pool. Thus, gene pool will have all genotypes i.e., genes of the organisms.

Gene flow : If genes are exchanged between two different populations of a species, it is gene flow.

(2) Genetic Drift / Sewall Wright Effect / Non – directional factor : Natural selection is not the only force responsible to bring about changes in gene frequencies. There is the role of chance or Genetic Drift also.

Genetic Drift causes the change in gene frequency by chance in a small population. In a small population, the individual alleles of a gene are represented by a few individuals in a population. These alleles will be lost if these individuals fail to reproduce. Allele frequencies appear to change randomly, as if the frequencies were drifting, thus, a random loss of alleles in small population is Genetic Drift. A series of small populations that are isolated from one another may come to differ strongly as a result of Genetic Drift. Genetic Drift has two ramifications are described below.

Founder's effect : When one or a few individuals are dispersed and become the founders of a few, isolated population at some distance from their place of origin, the alleles that they carry are of special significance. Even if these alleles are rare in the source population, they will be a significant fraction of the new population's genetic endowment. This effect by which rare alleles and combinations of alleles may be enhanced in new populations – is called the founder's effect. The founders effect is particularly important in the evolution of organisms on islands, such as Galapagos Islands which Darwin visited. Most of the kinds of organisms that occur in such areas were probably derived from one of a few initial founders.

Fixation of new mutations : Genetic drift fixes new alleles, genes that arise by mutation, from time to time and eliminate the original gene, thereby changing the genetic make-up of small population.

(3) Mutation : Replica Plate Experiment of Lederberg and Lederberg

(i) Mutations are random (indiscriminate) with respect to the adaptive needs of organisms.

(ii) Most mutations are harmful or with no effect (neutral) on their bearer.

(iii) Mutation rates are very slow.

The Lederberg Replica Plating Experiment, a beautiful example of the genetic basis of a particular adaptation was demonstrated in bacteria by an ingenious method devised by Joshua Lederberg and Esther Lederberg. E. coli bacteria are usually grown in the laboratory by plating dilute suspensions of bacterial cells on semi – solid agar plates. After a period of growth, discrete colonies appear on the agar plates. Each of these colonies originates from a single bacterium through a large number of cell divisions. The Lederberg's inoculated bacteria on an agar plate and obtained a 'master plate' containing several bacterial colonies. They, then created several replicas of this master plate did not grow on the replica plates. The few colonies that did grow were obviously resistant to penicillin. How did the bacteria acquire the ability to grow in a new environment (here, agar medium, containing penicillin) In other words, what was the origin of this adaptation?

A Lamarckian interpretation of this adaptation would have been that penicillin somehow induced a change in one or more bacteria, enabling them to grow in the presence of penicillin. A Darwinian view is that there were, in the original suspension of bacteria from which the master plates were prepared, a few bacteria carrying mutant genes which conferred on them the ability to survive the action of penicillin and form colonies. These mutations, which had arisen by chance, and not induced by penicillin, were present only is small numbers in the original suspension.

Lederberg's experiment provided evidence that mutations are actually pre-adaptive. These kinds of mutations are regarded as advantageous mutations. They appear without exposure to the environment.

Actually, the preadaptive mutations express themselves only after exposure to the new environment to which the organisms are to adapt themselves.

The new environment does not induce the mutations, if only selects the preadaptive mutations that occurred earlier.

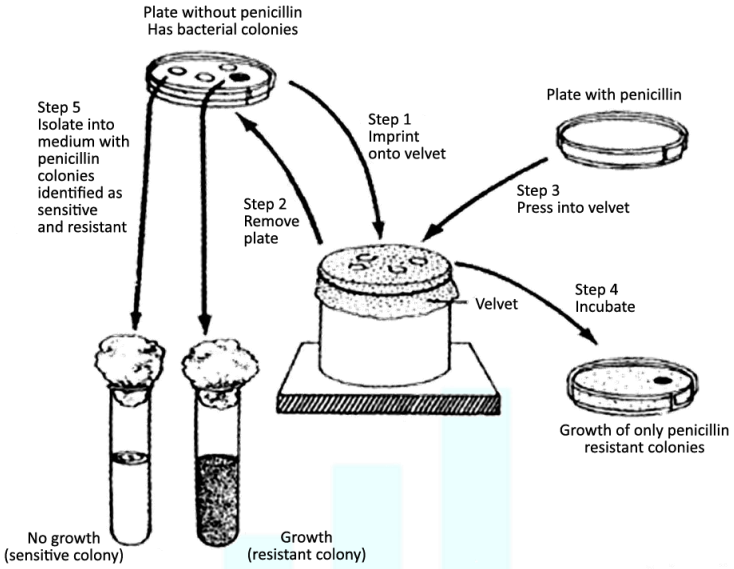
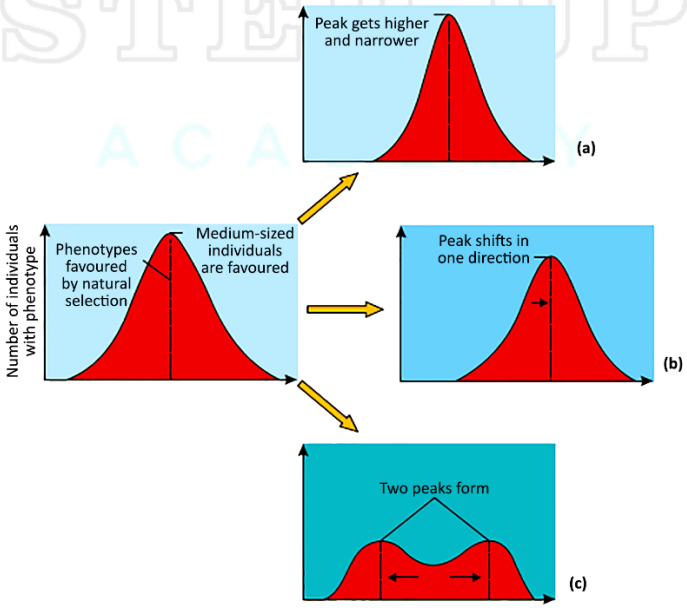


Fig. Lederberg's replica experiment

(4) **Genetic recombination :** During gamete (sperm or ovum) formation, the alleles present on the parental chromosomes separate and form new combinations. This results in the genetic recombination. The crossing over during meiosis is a major source of variations in a population. The offsprings produced from these gametes show 'new' combination of characters and are called recombinants.

(5) Natural selection : Nature selects those variations which are heritable (i.e., able to be inherited) and which make the survival better so that the individuals bearing these useful variations are enabled to reproduce and thence produce a greater number of progenies. Hence, natural selection also leads to change in allelic frequencies. However, the effects of natural selection on different traits can be **(a) Stabilising (b) Directional, or (c) Disruptive.**



Three types of natural selection

(a) Stabilising selection – The peak gets higher and narrower.

(b) Directional selection – Peak shifts in one direction.

(c) Disruptive selection – Two peaks form.

Darwin's Theory of Natural Selection

- During a sea voyage in a sail ship called H.M.S. Beagle round the world, Charles Darwin concluded that existing living forms share similarities to varying degrees not only among themselves but also with life forms that existed millions of years ago.
- Birds of Galapagos islands influenced Darwin to think about the evolutionary change. These birds were called finches. Finches were designated as **Darwin's Finches.**
- In 1798 T.R. Malthus, a British economist, put forward a theory of human population growth.

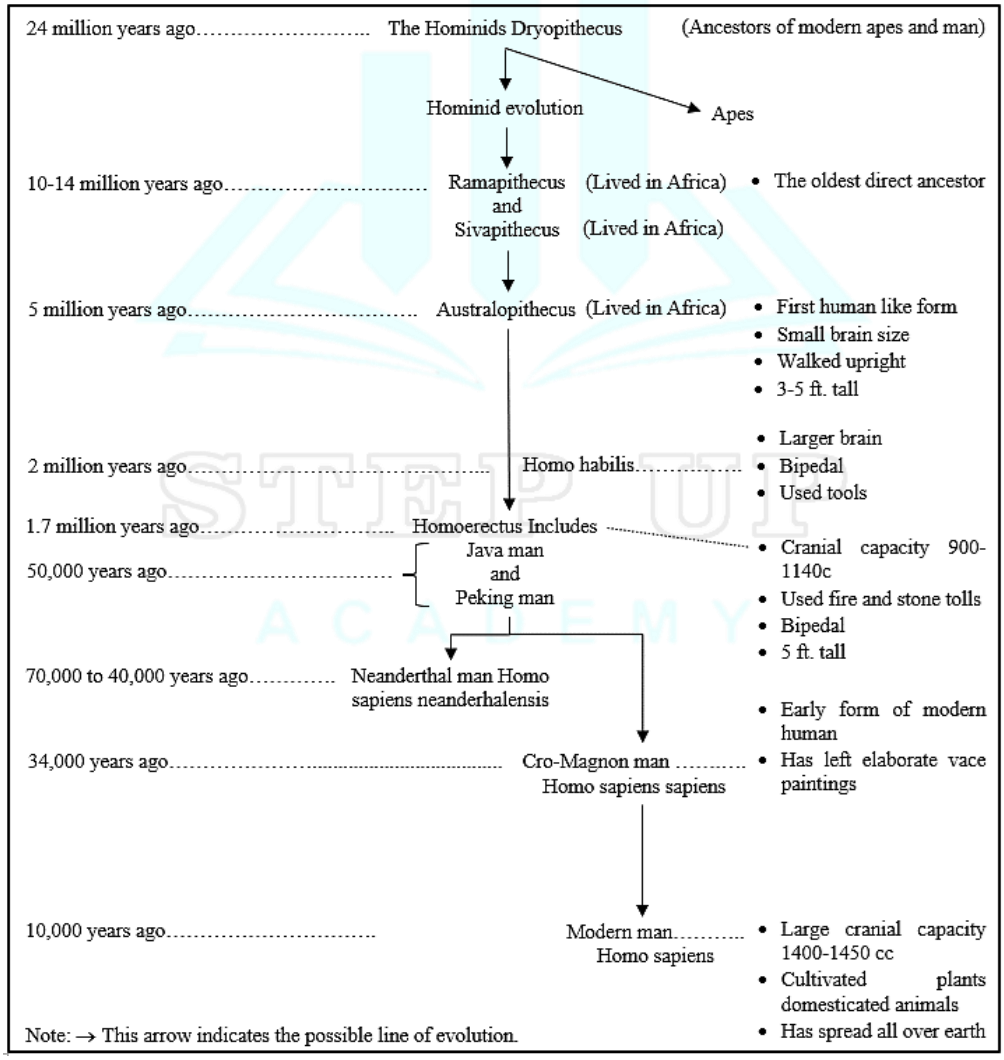
(i) He stated that population grows geometrically when unchecked, whereas the means of its substance like food grow only arithmetically.

(ii) Naturally, after some time an imbalance would occur in the population and the environment.

(iii) When the imbalance reaches a certain value, some factors like hunger, epidemics, floods, earthquakes, war. etc. will bring the population to a desired level. Such a population "crash" is called **catastrophic control of population.** These factors were called **"Positive checks"** by Malthus.

Human evolution

- Remapithecus was more man like while Dryopithecus was more ape like.
- During evolution first being was homo – habilis.
- Agriculture was started before 10,000 years.
- Cranial capacity-Homo habilis – 650 – 800 CC., Probably did not eat meat
  Homo erectus – 900 CC., Probably ate meat.
  Neanderthals – 1400 CC., Lived in near East and central Asia between 10,000 – 40,000 yrs. back.
- Pre – historic cave art developed about 18000 years ago.



Chapter 7
Human Health and Disease

HEALTH

The term health is very frequently used by everybody. Health does not simply mean 'absence of disease' or 'physical fitness'.

It could be defined as a state of complete **physical, mental and social well-being.** Of course, health is affected by

(i) **Genetic disorders :** Deficiencies with which a child is born and deficiencies/defects which the child inherits from parents from birth.

(ii) Infections and

(iii) Life style including food and water we take, rest and exercise we give to our bodies, habits that we have or lack etc.

Balanced diet, personal hygiene and regular exercise are very important to maintain good health. Yoga is being practiced to achieve physical and mental health. When people are healthy, they are more efficient at work, increasing productivity and thus bringing economic prosperity. Health also increases longevity of people and reduces infant and maternal mortality.

Disease

When the functioning of one or more organs or systems of the body is adversely affected, characterized by various signs and symptoms, we say, that we are not healthy, i.e., we have a **disease.** Disease can be broadly classified into two categories :

(A) Congenital Diseases : These diseases occur since birth and may result from metabolic disorder or defect in development.

(B) Acquired diseases : These diseases develop after birth and can be divided into two main categories :

(i) Infectious diseases/Communicable diseases : Diseases which are easily transmitted from one person to another are called as infectious diseases. Infectious diseases are very common and some of the infectious diseases like AIDS are fatal.

(ii) Non-infectious diseases/non-communicable diseases : These diseases are not spread to other persons. Among non-infectious diseases, cancer is the major cause of death. Drug and alcohol abuse also affect our health adversely.

Pathogen

A wide range of organisms could cause diseases in man. Such disease causing organisms are called **pathogens** e.g. bacteria, viruses, fungi, protozoans, helminthes etc.

Most parasites are therefore pathogens as they cause harm to the host body by living in or on them. The pathogens can enter our body by various means, multiply and interfere with normal vital activities, resulting in morphological and functional damage. Pathogens have to adapt to life within the environment of the host. For example, the pathogens that enter the gut must know a way of surviving in the stomach at low pH and resisting the various digestive enzymes.

COMMON DISEASES IN HUMANS

A. Bacterial Diseases

(i) Typhoid : (Enteric fever)

Pathogen : Salmonella typhi (A Gram-negative bacterium)

Mode of transmission : These pathogens generally enter the small intestine through contaminated food & water and migrate to other organs through blood.

Symptoms : Sustained high fever (39° to 40°C) weakness, stomach pain, constipation, headache may occur in severe cases.

Test : Typhoid fever could be confirmed by **Widal test.**

(ii) Pneumonia :

Pathogen : Streptococcus pneumonia and Haemophilus influenza.

Mode of transmission : A healthy person acquires the infection by inhaling the droplets/aerosols released by an infected person or even by sharing glasses and utensils with an infected person.

Symptoms : In pneumonia, infection occurs in alveoli of the lungs. As a result of the infection, the alveoli get filled with fluid leading to severe problems in

respiration. The symptoms of pneumonia are fever, chills, cough and headache. In severe cases, the lips and finger nails may turn gray to bluish in colour.

(iii) Plague / Bubonic plague : (Black death)

Pathogen : Yersinia pestis

Mode of infection : It is primarily a disease of rodents but can accidently affect man. It spreads from rat to rat through rat flea (Xenopsylla). But when the infected rats die, the fleas their body and can even bite man and inject plague germs into his blood.

Symptoms : It is characterized by high fever and a bubo (lump) in the groin or the armpit. Red patches appear on skin which turn black and ultimately leads to death (black death).

(iv) Tuberculosis (TB) : It is also called **Koch's disease.** It is caused by Mycobacterium tuberculosis. The bacteria damage the tissues and release toxin named **tuberculin** which produces the disease. It affects the lungs, lymph nodes, bones and joints. Incubation period is quite variable. Symptoms of pulmonary (lungs) tuberculosis are fever, cough, sputum containing blood, pain in the chest and loss of weight, excessive fatigue, loss of appetite, rise of temperature in the evening, hoarseness of throat, night sweating and rapid pulse. BVG vaccine gives considerable protection against tuberculosis.

(v) Leprosy (Hansen's Disease) : This disease is caused by mycobacterium leprae, which was discovered by **Hansen.** Symptoms of leprosy include appearance of light coloured patches on the skin, thickening of the nerves, partial or total loss of sensation in the affected parts of the body. These are accompanied by fever, pain, ulcers and skin eruptions. Deformities of toes and fingers may also develop. The bacilli leave the body in nasal discharge, from the throat during coughing, sneezing and even speaking and through broken skin lesions. The patient is treated with DDS (diamino diphenyl sulphone).

(vi) Cholera : This is an acute infectious disease caused by Vibrio cholerae. These may get into a healthy person with contaminated food and water. The patient starts passing stools frequently, which are white like rice-water, and gets repeated vomiting.

Since, a large quantity of fluid and salts are rapidly lost through stools and vomit, therefore, the most important treatment is to replace the lost fluid and salts equally by **oral rehydration-therapy.**

(vii) Diphtheria : This disease is caused by Corynebacterium diphtheria usually affecting children upto five years of age. It may start as sore throat, chills with mild fever, sometimes vomiting, headache. Throat and or tonsils show a grey membrane which may spread down and cause hoarseness and difficulty in breathing. The most important preventive measure – against this disease is that all babies should be, immunized within the first six weeks of birth using DPT vaccine.

(viii) Tetanus (Lock Jaw) : It is caused by Clostridium tetani which produces a neurotoxin-**tetanospasmin** which acts at neuromuscular junction. The first indications of this disease are irritability and restlessness, the neck becomes stiff and there is difficulty in chewing and swallowing. Subsequently spasms of muscles of the jaw and face take place and thus **"Lock Jaw"** occurs. There is a severe pain. It is often a fatal disease. The toxin affects **' voluntary muscles'** mainly. Tetanus organisms live in the intestine of horses and other animals without doing any harm. Anti tetanus serum (ATS) injection should be administered in case of an injury.

B. Viral Diseases

(i) Common cold/Rhinitis :

Pathogen : Rhino virus.

Mode of infection : It is one of the infectious human diseases, transmitted through inhalation of droplets resulting from cough or sneezes of an infected person, either inhaled directly or transmitted through contaminated objects such as pens, books, cups, door knobs, computer keyboard or mouse etc. (fomite borne).

Symptoms : Rhino virus infects the nose and respiratory passage but not the lungs. It is characterized by nasal congestion and discharge, sore throat, hoarseness, cough, headache and tiredness, etc., which usually last for 3-7 days.

(ii) Influenzas : It is commonly known as "Flu" and is highly infectious. The disease is caused by various types of influenza viruses (e.g., Myxovirus influenza). It causes fever and pain all over the body and affects the nose, throat and air passages as in common cold. It starts with fever, headache, sore throat, cold with sneezing and pair all over the body with restlessness. In neglected cases, complications like pneumonia, bronchitis and ear infections may develop.

(iii) Small Pox : This disease is caused by a small pox virus named Variola virus (ds DNA virus). The virus is present in the oral and nasal discharges of the patients and is ejected during the acts of coughing, sneezing, fomites etc., and infects the healthy people. It is highly infectious disease starting with high fever, chill, backache and headache, followed by appearance of rash on the third day of illness. The rash the appears first on the face, then on the rest of the body. It is more on the face and limbs and less on the trunk. The rash

starts as small reddish which change into papules. These in turn change into off by the third and develop discharge from the ear. Vaccination against small pox is one of the best preventive remedies available today. This was discovered by Edward Jenner in 1798. Small-pox has been eradicated from the world.

(iv) Chicken Pox : This disease is caused by a virus of chicken-pox named Varicella zoster (dsDNA virus) which is passed out in the discharges of the respiratory tract of an infected person directly as droplets or through contaminated articles used by the patient. It is a mild but highly infectious disease-causing slight fever and a rash which undergoes changes into vesicles, pustules and finally a dark brown scab which falls off leaving no scar unlike smallpox. The rash comes out in crops and with each fresh crop, there may be slight fever again. The rash first appears on the trunk and there are chicken-pox is now available. The most common late complication of chicken-pox is shingles caused by reactivation of Varicella zoster.

(v) Measles (Rubeola Disease) : It is caused by Rubeola virus (RNA virus) which is passed out in the secretions of nose and throat of the infected persons as droplets or in articles soiled by these secretions. The disease starts with catarrh of the nose and throat, and fever. It is a highly infectious disease causing, appears first on the back of the ear and face, and spreads downwards on the body. It attacks especially the children below the age of 5 years and those who have escaped may be attacked even in the later life. The eyes are red and watery, and the face is flushed.

(vi) Mumps (Infectious Parotitis) : It is infectious disease-causing fever, difficulty in opening the mouth and painful swelling of the parotid glands which lie just below the lobe of the ears. It is caused by Paramyxovirus (RNA virus), which comes out in the saliva of the infected person. The patient should take complete bed rest till the swelling subsides in order to avoid complications. Usually, there are no complications, but in some cases, there may be pain and swelling of the testes or pain in the abdomen.

(vii) Rabies (Hydrophobia) : It is caused by a virus named as Rhabdo virus. It is introduced in the body by the bite of rabid (mad) dogs usually. It can be injected by the bite of jackals, wolves, cats etc., incubation period is from 10 days to one year. Fear of water is the most important characteristic symptom of this disease. Other symptoms are excess salivation, severe headache, high fever, alternating periods of excitement and depression, inability to swallow even fluids due to choked throat. The virus destroys the brain and spinal cord. **Rabies is 100% fatal.** There should be compulsory immunization of dogs and cat population.

(viii) Poliomyelitis : This disease was called infantile paralysis. But is now known that the disease may occur at any age. This disease spreads mainly through intestinal discharge. It may also spread through contaminated food or drink and by flies or other insects they may contaminate food or drink. Polio virus (ss RNA) usually enters the body via alimentary canal where it multiplies and reaches the nervous system (spinal cord) through the blood stream. Its incubation period is 7-14 days. It produces inflammation of the nervous system. The earliest sign of this disease is involvement of the central nervous system causing inability to bend the head forward. Stiffness of the neck is an important sign. Paralysis starts following the weakness of particular skeletal muscles. The attack of paralysis begins with high fever, headache, chills, pain all over the body. If muscles of larynx and pharynx are involved it proves fatal. Within two to three days the paralysis reaches its maximum. There is no sure cure for polio. The patient should be given complete rest. An adequate arrangement for proper disposal of urine and faces of the patient must be provided because they contain polio virus. Overcrowding of children in schools, playgrounds and cinema should be avoided. Polio is preventable. Polio vaccine is safe and effective. Now-a-days multiple vaccines are used against polio, diphtheria, whooping cough and tetanus simultaneously.

(ix) Dengue fever : Dengue fever is caused by an RNA containing **Arbovirus** (Arthropod borne virus) of flavivirus group which also causes yellow fever (not found in India). This, the virus which causes dengue fever is a mosquito borne **flavi-ribo virus.** The virus of dengue fever is transmitted by the bite of female Aedes aegypti (tiger mosquito). Incubation period is 3-8 days. Two types : classical dengue fever and dengue haemorrhagic fever are known to occur.

Symptoms of Classical Dengue Fever are (i) Abrupt onset of high fever. (ii) Severe frontal headache. (iii) Pain behind the eyes which worsens with eye movement. (iv) Muscles and joint, (v) Loss of sense of taste and appetite. (vi) Measles like rash over chest and upper limbs. (vii) Nausea and vomiting.

Symptoms of Dengue Haemorrhagic Fever are symptoms similar to classical dengue fever except the following (i) Bleeding from the nose, mouth gums and skin bruising. (ii) Severe and continuous stomach pains. (iii) Frequent vomiting with or without blood. (iv) Pale cold or clammy skin. (v) Excessive thirst (dry mouth). (vi) Rapid weak pulse. (vii) Difficulty in breathing. (viii) Restlessness and constant crying.

If there is fever doctor should be consulted at once, paracetamol tablets should be taken on the advice of

doctor. **Aspirin (Disprin)** to be avoided. Cold sponging required if fever is high. Plenty of liquids to be given to the patient.

No vaccine for Dengue fever is available. Mosquito breeding places to be eliminated by covering small water containers, water tanks, changing the water of cooler every week, and where Aedes mosquito breed. Do not allow children to play in shorts and half sleeved clothes. Use mosquito repellents, repellent cream and sleep in mosquito-net.

(x) Chikungunya : It is caused by Chikungunya virus. This virus was first isolated from human patients and Aedes aegypti mosquitoes from Tanzania in 1952. The name 'Chikungunya' is derived from the native word for the disease in which patient walks "doubled up" due to severe joint pain. Its symptoms include sudden onset of fever, crippling joint pain, lymphadenopathy and conjunctivitis. Some show haemorrhagic manifestations. No vaccine is available.

C. Disease Caused by Protozoans

(i) Malaria :

Pathogen : Malaria is caused by Plasmodium, a tiny protozoan. Different species of Plasmodium which attack humans are P.vivax, P. malariae, P. ovale and P. falciparum.

Mode of transmission : Malarial parasite (Plasmodium) requires two hosts to complete its life cycle : (i) Human, (ii) Mosquito (female Anopheles) which is the vector/transmitting agent too. Plasmodium enters the human body as **sporozoites(**infectious form) through the bite of infected **female Anopheles.**

Life-history : When an infected female Anopheles bites a human to suck blood, it also injects the malarial parasites into the human blood with its saliva. **This infective stage of Plasmodium is a minute sickle-shaped sporozoite.** Sporozoites are inoculated in thousands into the human blood. In about half an hour the sporozoites disappear from the blood stream and enter the parenchymatous cells of the liver to escape from phagocytic white blood corpuscles and multiply their own number.

Schizogony : Each sporozoite grows in the liver cell to form a large and rounded **schizont,** which divides to form about 1,000 small spindle-shaped **merozoites.** The multiple fission is called schizogony. The schizont ruptures and merozoites are liberated into liver venous passages (sinusoids). This phase of reproduction is termed as the **pre-erythrocytic phase** and in this the merozoites are also called **cryptozoites:** they are immune to medicines and also to the resistance of the host.

The cryptozoites (merozoites) enter new liver cells, grow into schizonts which again divide to form merozoites. These merozoites of the second generation are termed **metacryptozoites** and their formation is called the **exo-erythrocytic phase.** The exo-erythrocytic cycle may continue in more liver cells to form a reservoir of parasites, or some merozoites after two or more cycles in the liver may re-enter the blood streams. Those merozoites which reach the blood stream attack healthy erythrocytes these are called **phanerozoites** or **metacryptomerozoites. Pre-patent period** is the duration between the initial sporozoite infection and the first appearance of the parasites in blood. It is 8 days in Plasmodium vivax. **Incubation period** is the duration between the initial sporozoite infection and the first appearance of malarial symptoms. It is about **14 days** in P. vivax, **30 days** in P. malariae, **14 days** in P. ovale and **12 days in** P. falciparum.

Erythrocytic Schizogony :

Inside the erythrocyte, another multiplication phase of schizogony follows, which is known as **erythrocytic phase** (also called **cycle of Golgi).** On entering the red blood corpuscles the merozoites begin to feed on the corpuscles. Soon a vacuole appears in the merozoite pushing its nucleus to one side, this stage is called a **ring-shaped trophozoite** which is 1/3 to ½ the size of an erythrocyte. The trophozoite grows further at the expense of the corpuscle to become somewhat rounded and amoeboid. This full grown trophozoite is called a **schizont** and it has yellowish-brown pigment granules of **haemozoin** derived from the **iron of haemoglobin** of the corpuscle. The corpuscle becomes much enlarged and acquires granules called **Schaffner's dots.**

The schizont undergoes repeated divisions to form about 12 to 24 merozoites. A certain amount of residual cytoplasm along with the haemozoin granules is left unshared. This phase of asexual multiplication is called **erythrocytic schizogony.** The merozoites so produced burst the weakened corpuscle and are liberated into blood plasma. These merozoites or **schizozoites** are short thick spindles and they enter new erythrocytes and repeat plasma. These merozoites of **schizozoites** are short thick spindles and they enter new erythrocytes and repeat the cycle of erythrocytic schizogony once every 48 hours, and this may go on for many generations. They may also go to the liver cells to undergo a **post-erythrocytic schizogony.**

Along with the merozoites, toxins are liberated into blood when corpuscles rupture, they are carried to all parts of the body and deposited in the spleen, liver and under the skin. Accumulation of toxins causes **benign tertiary malaria** fever, skin becomes sallow coloured, there is high temperature after every 48 hours with chill and shivering, followed by profuse sweating.

Sexual cycle or Gamogony : After several generations of schizogony some of the merozoites grow into large cells inside blood corpuscles. They become large slowly and produce much pigment. These merozoites form two types of cells called **gamonts** or **gametocytes :** these are male and female gametocytes. The female gametocytes or **mega gametocytes** are found with food- laden cytoplasm and a small excentric nucleus. Male gametocytes of **microgametocytes** have clear cytoplasm and a large central nucleus. Both contain large amounts of haemozoin.

If gametocytes are sucked up by an Anopheline mosquito along with blood, they reach its stomach, the corpuscles are dissolved but gametocytes are not digested. When removed from the warm human blood into the mosquito, the microgametocytes undergo a striking change called **Ex flagellation.** The nucleus divides into 6 to 8 nuclei around which cytoplasm collects to form long flagellated structures called **microgametes,** which break from the pigmented residual cytoplasm. They grow and begin to swim in the gut of the mosquito.

The mega gametocyte undergoes little change, nucleus divides into two, one of which moves out along with some cytoplasm and projects outward as a polar body; these changes it into a mature **mega gamete.** Soon the mature mega gamete puts out a small conical projection, the **cone of fertilization**. The gametes, being dissimilar, are called anisogametes.

One microgamete attaches itself to the cone of fertilization of the mega gamete and the two fuse to form a round **zygote**. (Anisogamous syngamy).

The sexual cycle is completed in any kind of mosquito which may feed on human blood, but further development of malarial parasite will occur only if the parasite is sucked along with blood by an appropriate species and variety of mosquito, i.e., only in certain species of Anopheles.

Sporogony : The round zygote may come to lie between the cells of the stomach of the mosquito.

Ookinete: The zygote elongates and becomes worm like motile organism called Ookinete.

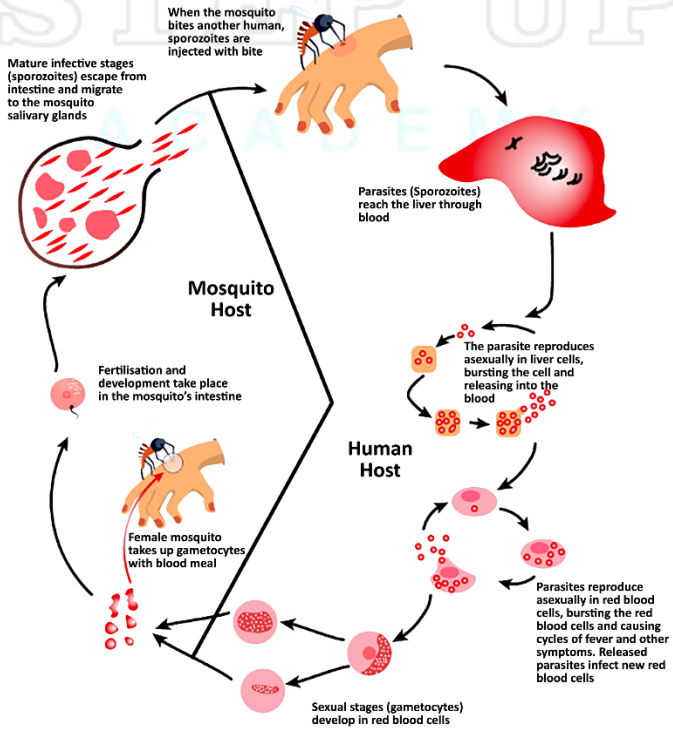
Penetration and Encystment :

The ookinete moves and bores through the wall of the stomach of female Anopheles mosquito. Here the ookinete becomes immobile, reassumes spherical shape and begins to encyst. The encysted zygote is called **oocyst**. Just one or two days after fertilization, several oocysts appear upon the surface of the stomach of an infected female Anopheles bulging as tiny nodules. The **cyst wall** of the oocyst is partly secreted by the zygote and partly secreted by the stomach of the mosquito. The encysted zygote in each oocyst is now called a sporont.

The nucleus of oocyst (sporont) divides first by meiosis and subsequently by mitosis, forming large number of small haploid nuclei. At the same time, the cytoplasm develops large vacuoles. The tiny nuclei and cytoplasmic masses form elongated and spindle shaped bodies called **sporozoites**.

When mature oocysts rupture, the sporozoites are liberated into the haemocoel (body cavity filled with hemolymph) of the mosquito. Being motile, the sporozoites move different organs in the body cavity of such mosquito, but many of them penetrate the salivary glands. The mosquito now becomes infective.

When such female Anopheles mosquito bites a healthy person, the **sporozoites** are injected in his/her blood along with saliva. These sporozoites start the cycle again in human body.



Four species of Plasmodium cause malaria in man but their life histories are very much alike with minor differences in the structure of stages and the time required for schizogony. These species are :

(i) Plasmodium ovale

(ii) Plasmodium vivax causes **benign tertian malaria**

(iii) Plasmodium malariae causes **quartan malaria**,

(iv) Plasmodium falciparum is very common in tropics and it causes malignant deadly **cerebral malaria** or sub-tertian malaria fever

TYPES OF MALARIA

Disease	Causative agent
Benign tertian malaria	Plasmodium vivax
Malignant tertian malaria	Plasmodium falciparum
Black water malaria	Plasmodium falciparum
Quotidian malaria	Mixed infections

(ii) Amoebiasis/Amoebic dysentery :

Pathogen : It is caused by a protozoan parasite, Entamoeba histolytica in the large intestine of human.

Mode of transmission : Housefly acts as mechanical carriers and serve to transmit the parasite from faeces of infected person to food and food products, thereby contaminating them. So intake of contaminated food and water are the main source of infection. Person to food and food products and water are the main source of infection. Adult Entamoeba is called trophozoite and is **monopodial.** It has two forms, **magna** or pathogenic form found in the mucosa and sub-mucosa of intestine forming ulcers and **minuta,** nonpathogenic form found in the lumen of intestine. Entamoeba has no contractile vacuole. Trophozoite of Entamoeba reproduces by binary fission. Minuta form encysts. A mature cyst is called **quadrinucleate** cyst. It has four nuclei and two **chromatid bodies.** The reserve food material in cyst of E.histolytica is **glycogen.** Quadri (tetra) nucleate cyst is the infective stage.

Symptoms : This disease is characterized by constipation, abdominal pain, cramps and stools with excess mucus and blood clots.

D. Diseases Caused by Helminthes

(i) Ascariasis :

Pathogen : It is caused by the common round worm, Ascaris lumbricoides, an intestinal parasite of small intestine of human beings.

Mode of transmission : A healthy person acquires this infection through contaminated water, vegetables, fruits, etc.

Symptoms : This disease is characterized by internal bleeding, muscular pain, fever, anemia and blockage of intestinal passage. The eggs of the parasite come out along with the faeces of infected persons which contaminate soil, water and plants, etc.

(ii) Filariasis :

Pathogen : This disease is caused **by Wuchereria (W. bancrofti and W. malayi)**, filarial worm.

Mode of transmission : The pathogens are transmitted to a healthy person through the bite of infected female Culex (mosquito).

Symptoms : The filarial worms cause a slowly developing chronic infection / inflammation of the organs in which they live for many years. They usually affect the lymphatic vessels. This disease is characterized by the swelling of the legs, scrotum and other parts of the body. This is commonly called Elephantiasis due to its resemblance to a leg of an elephant. The genital organs are also often affected, resulting in gross deformities.

E. Fungal Diseases

Ringworm :

Pathogen : Many fungi belonging to genera **Microsporum, Trichophyton and Epidermophyton** are responsible for ringworms, one of the most common infectious diseases in man.

Mode of transmission : Ringworms are generally transmitted from soil or by using infected towels, clothes or even the comb of infected individuals.

Symptoms : Appearance of dry, scaly lesions on various parts of the body such as skin, nails and scalp are the main symptoms of the disease. These lesions are accompanied by intense are accompanied by intense itching. Heat and moisture help these fungi to grow, which, makes them thrive in skin folds such as those in the groins or between the toes (athletes' foot). In Tinea cruris, the groin and perineum are involved. In Tinea barbae, the beard areas of the face and neck are involved. Tinea pedis or athletes' food affects foot.

Preventive Measures and Control of Diseases

Maintenance of personal and public hygiene is very important for prevention and control of many infectious diseases.

(i) Personal hygiene include keeping the body clean, consumption of clean drinking water, food, vegetables and fruits etc.

(ii) Public hygiene include proper disposal of waste and excreta, periodic cleaning and disinfection of water

reservoirs, pools and tanks. These measures are particularly essential where the infectious agents are transmitted through food and water such as typhoid, amoebiasis and ascariasis.

(iii) For diseases such as malaria and filariasis transmission occurs through insect vectors, the most important measure is to control or eliminate the vectors and their breeding places. This can be achieved by avoiding stagnation of water in and around residential areas, regular cleaning of household coolers, use of mosquito nets, introducing larvivorous fish like Gambusia in pond that feed on mosquito larvae spraying of insecticides like DDT in ditches, drainage area and swamps etc., doors and windows should be provided with wire mesh to prevent the entry of mosquitoes. Such precautions have become all the more important especially in the light of recent widespread incidences of vector-borne (Aedes mosquito) diseases like Dengue and Chikungunya in many parts of India.

(iv) The use of vaccines and immunization programmes have enabled us to **completely eradicate a deadly disease like small pox.** Large number of other infectious diseases like **polio, diphtheria, pneumonia, tetanus** have been controlled to a large extent by the use of vaccines. Many newer and safer vaccine are available and are synthesized by suing biotechnology.

IMMUNITY

Everyday we are exposed to a large number of infectious agents. However, only few of these exposures result in disease, as body is able to defend itself from most of these foreign agents. This overall ability of the host to fight the disease causing organisms, provided by, immune system is called **immunity.**

Immunity is two types :

1. Innate immunity
2. Acquired immunity

1. Innate Immunity

Innate immunity is accomplished by providing different types of barrier to the entry of foreign agent of any pathogen into our body. It is present at the time of birth so also called **inborn immunity.** This is non-specific type of defense.

Innate immunity consists of four types of barriers. These are :

(i) Physical barriers : It includes skin that is the main barrier which prevents entry of the micro-organisms and mucus coating of epithelium lining the respiratory, gastrointestinal and urogenital tracts also helps in trapping microbes entering our body.

(ii) Physiological barriers : It includes acid in stomach, saliva in mouth, tears from eyes. Tears and saliva contain lysozymes which can destroy bacterial pathogens.

(iii) Cellular barriers : It includes WBC like PMNL-neutrophils (polymorph-nuclear leukocytes), monocytes and natural killer cells. Natural Killer cells are a type of lymphocytes which will produce proteins called perforins that create pores on plasma membrane of tumor cells and virally infected cells through which water enters and cause lysis of cells. Later on macrophages in the tissues and body fluid phagocytes and destroy microbes.

(iv) Cytokine barriers: It includes interferons, a type of protein secreted by virus infected cells which protect non-infected cells from further viral infection. Interferons will act by inhibiting the viral replication as they stimulate synthesis of enzyme which inhibit the synthesis of proteins required for replication of virus. So they used to control viral diseases but they do not kill the virus.

2. Acquired Immunity

Acquired immunity is gained after birth and is pathogen specific. When our body encounters the pathogen for the first time, it initiates a response called primary response. It is of low intensity and person will feel sick. When a specific antigen enters into the body of person ,specific B and T cells start dividing to produce effectors B and T cells for controlling disease and also produce memory B and T cell. These are stored in the spleen and lymph nodes throughout the life. If the same pathogen enters our body again, memory B and T Cells will immediately start dividing to produce affector B and Cells. This is called as secondary or anamnestic response, which is highly intensified or is of high intensity. This is due to the fact that our body has memory of the encounter.

The primary and secondary immune responses are carried out with the help two types of lymphocytes, present in our blood i.e., B-lymphocytes and T-lymphocytes provide two types of acquired immunity in body.

(i) Humeral immunity or antibody mediated immunity (AMI)

(ii) Cell mediated immunity (CMI)

(i) Humoral immunity: The B-lymphocytes produce an army of proteins in response to pathogen. As any pathogen enter into the blood, B-lymphocytes get activated and differentiated into plasma calls that secretes a large number of antibodies in response to pathogens into our blood to fight which them. Undifferentiated B-lymphocytes remain as memory cells.

Antigens: The antigens are 'molecules' which when introduced into the body, stimulated the production of

antibody. The word 'antigen' is a shortened form of 'antibody generating' because they stimulate the production of antibodies in response to infection. Antigens are generally large molecules. The majority of them are made of proteins or polysaccharides found on the cell walls of bacteria and other cells or on the coats of viruses. All antigens are not the parts of microorganism. Other structures like pollen grains, white of an egg, shell fish, certain fruits and vegetables, chicken, feathers of birds, blood cells from other persons or animals, drugs, chemicals, etc. can also induce the immune system to produce antibodies.

Antigen which are present on the body's own cell are self-antigen (Antigen related to blood group).

Antibody: Antibodies are immunoglobulin's (lg) which are produced in response to antigenic stimulation. Each antibody molecule has four peptide chains, two long chains called heavy or H chain each of molecular weight 50,000 Da and two short chains called light or L chains each of approximate molecular weight 25000 Da. Hence, an antibody is represented as H_2L_2. The heavy and light chains contains consist of amino acid sequences. In the regions concerned with antigen binding, these regions are extremely variable, whereas in other regions of the molecule, they are relatively constant. Thus each heavy and each light chain possesses a variable and a constant region. The isotype of an Ig is determined by the constant region. Both chains are liked by disulphide links.

One end of the antibody binds to antigen (the Fab portion, so called because it is the fragment of the molecule which is antigen binding or paratope) , and the other and which is crystallisable and therefore called Fc, is responsible for effector function

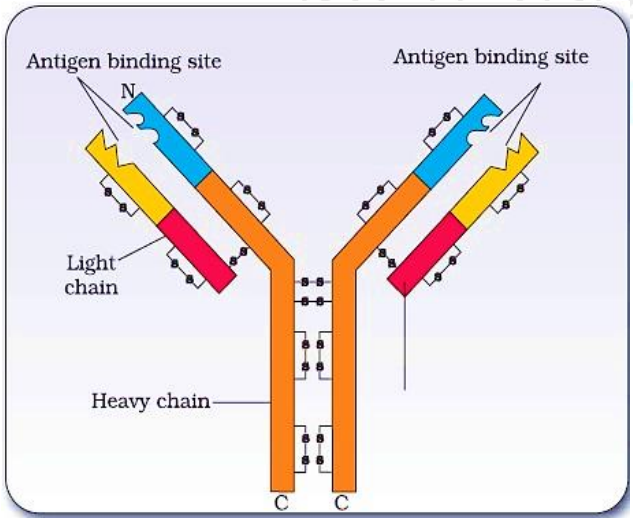


Fig. : Structure of an antibody molecule

Five Classes (isotypes) of Antibodies:

(a) **IgA forms 15% of total antibody count**. It is found in mucous secretions of the respiratory tract and the upper part of the digestive tract and the vagina. It is also found in colostrum's. Colostrum's is a golden liquid substance that a nursing mother expels from her breasts 24-48 hours after delivery. This substance is produced before the milk and is very important in the transfer of antibodies to a newborn infant. IgA given by the mother in the colostrum's will protect the body for about six months. Diametric IgA has four paratopes. The links between monomers are made by a J-chain of polypeptide.

(b) **IgD form less than 1%** of the total antibodies appears to have a role in activity found in large quantities in the cell membrane of many B-cells. IgD has two paratopes.

(c) **IgE is less the 1%** of total antibodies. Mediator in allergic responses. Most importantly activates histamine secreting cells. Also appears to play a role in parasitic infection. IgE has two paratopes.

(d) **IgG-composes 75% of our immunoglobulin pool.** IgG stimulates phagocyte cells, activates the complement system, binds neutrophils, opsonizes and can neturalise toxins. Most importantly, it is the only antibody that can cross the placenta and confer immunity on the foetus. IgG also has two paratopes.

(e) **IgM- Makes up 7-10% of total antibodies.** This is the predominant early antibody; the one that first activates in an initial attack of antigen. It exists as pen tamer where five molecules are linked by J-chain. Because of its high number of antigen biding sites (10) it is an effective agglutinator of antigen. This is important in the initial activation of B-cells, macrophages, and the complement system. It the largest antibody.

Because all these antibodies are found in the blood, the response is called **humoral (fluid) Immune response.**

Antigen-Antibody interaction:

(a) Agglutination: Clumping of micro-organism typically due to an antigen antibody reaction.

(b) Opsonisation or Adherence : Antibodies (especially IgG) called opsonins get attached over the surface of antigen pathogen. This enhances recognition of microbes by phagocytes .

(c) Precipitation : A soluble antigen is picked up by antibody making the complex heavier and insoluble so phagocyte cells ingest it more readily.

(d) Neutralisation : Antibodies cover the toxic sites of antigen. The converts virulent form into no virulent form. Some antibodies neutralise toxins by acting as antitoxin.

(e) Lysis : Some powerful antibodies attack plasma membrane of the cell and thereby causing rupture of plasma membrane and is called lyses.

(ii) **Cell mediated immunity (CMI):** It is T-lymphocytes mediated or CMI. T-cells themselves do not secrete antibodies but help B cells to produce them. Bone marrow gives rise to immature lymphocytes which migrate to thymus via blood. In the thymus these cells mature as T-lymphocytes/ T-cells and migrate to lymphoid tissue through the body and get differentiated into three types:

(a) **Helper T cell:** these cells stimulate B-cells to produce antibodies and killer T cells to destroy the non –self cells.

(b) **Cytotoxic/ Killer T cell :** These cells secrete perforins which create pores on the invader's cells membrane for water to enter into it thereby cell swells up and finally lyse.

(c) **Suppressor T cell :** These cells suppress the function of cytotoxic and helper T cell so that immune system does not attack the body's own cells.

(d) **Memory T cell:** These cells are formed by activated T cells and remain in the lymphatic tissues and recognize original invading or pathogen, even after many years of the encounter.

Active and Passive Immunity

Acquired immunity can also be classified into

(i) Active immunity: When a host is exposed to antigens (may be in the form of living or dead microbes or other proteins), antibodies are produced in the host body. This type immunity is called active immunity. Active immunity is slow and may be natural or artificial.

(a) **Natural active immunity:** It is acquired when antigens gain access into the body during natural infection. So a person who has recovered from attack of small pox or measles or mumps develops natural active immunity

(b) **Artificial active immunity:** it is the resistance induced by vaccines.

(ii) **Passive immunity :** When readymade antibodies antibodies are directly given to protect the body against foreign agent. It is called passive immunity.

(a) Natural Passive immunity: The yellowish fluid colostrum's secreted by mother during initial days of lactation has abundant antibodies (IgA) to protect the infant. The foetus also receives some antibodies IgG form their mother, through the placenta during pregnancy.

(b) Artificial passive immunity: When preformed antibodies are directly injected into the body. e.g., Anti-venom after snake bite, Anti-tetanus serum (ATS)

Vaccination and immunization

The principal of immunization or vaccination is based on the property of memory of the immune system.

Vaccination

In the preparation of vaccine inactivated/ weakened pathogens or antigenic protein are introduced in the body. The antibodies produced in the body against these antigens would neutralise the pathogenic agents during actual infection. The vaccines also memory B and T-cells that recognise the pathogen quickly on subsequent exposure and overwhelm the invaders with a massive production of antibodies.

Vaccines are classified as follows:

(a) **First generation vaccines:** These are whole organism vaccines, either live and weakened (attenuated or killed forms. e.g. Small pox, oral polio vaccine (OPV), BCG (Bacillus, Chalmette, Guerin), Influenza vaccines are attenuated. Vaccines against typhoid, rabies, cholera and Salk's polio vaccines are killed types.

(b) **Second generation vaccines:** These are subunit vaccines, consisting of defined protein antigens (such as tetanus or diphtheria toxoid) or recombinant protein components (such as hepatitis B surface antigen produced from yeast, Herpes vaccine).

Passive Immunisation

When preformed antibodies are injected to provide quick immune response it is called as passive immunisation. e.g., if a person is injected with some deadly microbes as in tetanus, to which quick immune response is required we need to directly inject the preformed antibodies or antitoxin (a preparation containing antibodies to the toxin). Even in case of snake bites, the injection which is given to the patients, contains preformed antibodies against snake venom. This type of immunisation is passive immunisation.

Disorders of immune System

(i) Allergy or Hypersensitivity

It is the hypersensitiveness of a person to some foreign substance called allergens coming in contact with or entering the body. It is the exaggerated response of the immune system to certain antigens present in the environment. The substance to which such an immune response is produced are called allergens. It may be mites, dust, pollens, animal danders fabrics, feather, mould, heat, cold, sunlight, etc.

Cause: The antibodies produced against these allergens are **IgE** type. Allergic reaction is due to the release of chemicals like histamine and serotonin from the mast cells. During allergic reaction there is increased release of **histamine** from mast cells. It causes marked dilation of all the peripheral blood vessels and the capillaries become highly permeable so that large amounts of fluid leak out from the blood

into the tissues. The blood pressure decreases drastically often resulting in the death of the individual within a short time. The exact nature of the substance of which a person is hypersensitive must be known before he can be properly treated.

Types of allergies :

(i) Hay fever : In this allergic form, there is swollen, reddened, running eyes and nose. The drug's called **antihistamines** are of major importance in the treatment of this allergic disorder.

(ii) Asthma : It is the sudden spasm of tissue surrounding respiratory tract causing narrowing of respiratory tract. The tissue surrounding the respiratory tubes in the lungs swell up and compress the tubes. Hence there is difficulty in breathing.

(iii) Anaphylactic shock : It is an allergic reaction involving all the tissues of the body and occurs in a few minutes after the injection of an antigen such as **penicillin**. Such a reaction is very serious. Histamine released from ruptured mast cells causes marked dilation of all the arteries so that a large amount of fluids passed from the blood to the tissues and there is a drastic fall in blood pressure. The affected person may become unconscious and the individual may die within a short time.

(iv) In eczema the skin becomes red, followed by the appearance of minute blisters.

Symptoms: Symptoms of allergic reaction includes sneezing, watery eyes, running nose and difficulty in breathing.

Treatment: For determining the cause of allergy, patient is exposed to or injected with a very small doses of possible allergen and after that the reactions on the body are studied. The use of drugs like antihistamine, adrenaline and steroids quickly reduce the symptoms of allergy.

(ii) Autoimmunity

Higher vertebrates have memory-based acquired immunity. The uniqueness of the immune system is that it always destroys the foreign particles/proteins but never attacks the body's own protein as it has the ability to differentiate foreign organisms (e.g. pathogens) from self-cells. But sometimes, due to genetic and other unknown reasons, the body attacks self-cells. This results in damage to the body and is called as Autoimmune Disease.

So autoimmune diseases occur if the body's immune system fails to recognize 'self from 'non-self and starts destroying the body's own cells.

For example, If the autoantigens are RBCs, then the body destroys its own RBC resulting in chronic **anaemia**; if the autoantigen are muscle cells, then it results in destruction of its own muscles resulting is severe weakness : **(Myasthenia gravis)**; if the autoantigens are liver cells, then it results in **chronic hepatitis** etc. Other autoimmune diseases are insulin-dependent diabetes, **Addison's disease, ulcerative colitis and rheumatoid arthritis.**

Immune System in the Body

The human immune system consists of lymphoid organs, tissues, cells and soluble molecules like antibodies. Immune system recognise, respond to foreign antigens and remembers them (memory). Human immune system also plays an important role in allergic reactions; auto immune diseases and organ transplantation.

Lymphoid Organs

These are the. organs where origin, maturation and proliferation of lymphocytes occur. There are two types of lymphoid organs

(i) Primary lymphoid organs

(ii) Secondary lymphoid organs

(i) Primary lymphoid organs : Primary lymphoid organs are those where immature lymphocytes differentiate into antigen specific lymphocytes, e.g., bone marrow and thymus.

Bone marrow is the main lymphoid organ where all blood cells including lymphocytes are produced and is considered equivalent to Avian Bursa of Fabricius. It is the site where B-lymphocytes mature.

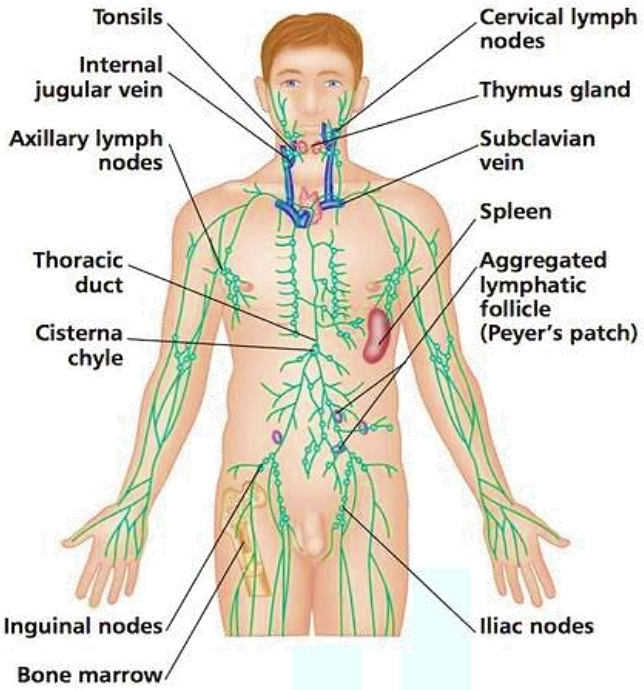
Thymus is a lobed organ located near the heart and beneath the breast bone (sternum). It is quite large at the time of birth but keeps on reducing in size with age and by the time puberty is attained, it reduces to a very small size. It is the site where T-lymphocytes mature.

Primary lymphoid organs (bone marrow, thymus) provide micro environment for the development and maturation of lymphocytes.

(ii) Secondary lymphoid organs: After maturation, B-lymphocytes and T-lymphocytes migrate through blood vascular and lymphatic system to secondary lymphoid organs which provide the site for interaction of lymph nodes, tonsils, Peyer's patches of small intestine and appendix.

Speen : It is a large bean shaped organ which mainly contains lymphocytes, phagocytes and large number of erythrocytes. It acts as a filter of blood by trapping blood borne micro-organisms. Spleen also has a large reservoir of RBC and is commonly called as **"Graveyard of RBC".**

Lymph nodes : These are the small solid structures located at different points along the lymphatic system. Lymph nodes serve to trap the micro-organisms or other antigens which are present in lymph and tissue fluid. Antigens trapped in lymph nodes are responsible for the activation of lymphocytes present there and cause the immune response.



AIDS

AIDS stands for Acquired Immuno Deficiency Syndrome. It is a deficiency of immune system, acquired during the life time of an individual indicating that it is not a **congenital disease.**

Pathogen

AIDS is caused by Human Immuno deficiency Virus (HIV), a name given in 1986 by the International committee on viral Nomenclature. This virus belongs to group of viruses called **Retrovirus** which have an envelope consisting of a lipid bilayer derived from host cell membrane and projecting knob like glycoprotein spikes with pedicel formed of virus coded glycoprotein. This envelops encloses the RNA genome (single stranded RNA filament is segmented into two identical filaments and associated with a reverse transcriptase enzyme). HIV consists of a core **RNA** with **Reverse Transcriptase** surrounded by a protein coat. The protein coat around the core consists of a protein called **P24.** Outside this protein coat is a layer composed of another protein called **P17.**

The outermost envelope consists of a phospholipids bilayer studded with glycoproteins (**GP120 and GP41).** HIV is a **retrovirus;** using the enzyme reverse transcriptase, it can synthesize DNA from RNA. Once HIV produce DNA from its RNA, the DNA is integrated into the host cell's DNA. There it can remain dormant, giving no sign of its presence, or it can take over the host cell's genetic machinery to produce more viruses. The major cell infected by HIV is the **Helper T-Lymphocyte** that bears the **CD4 receptors site. The attachment of virus to CD4 receptor site is by the help of GP120 on the protein coat of the virus.**

Mode of Transmission

Transmission of HIV infection generally occurs by

(a) Sexual contact with infected person.
(b) By transfusion of contaminated blood and blood products.
(c) By sharing infected needles as in the case of intravenous drug abusers.
(d) From infected mother to her child through placenta.

So, people who are at high risk of getting AIDS infection include

(a) Individuals who have multiple sexual partners.
(b) Drug addicts, who take drugs intravenously.
(c) Individuals who require repeated blood transfusions.
(d) Children born to an HIV infected mother.

Mode of Action of AIDS Virus

After the entry of virus into the body through body fluids or blood, the virus enters into the macrophages where RNA genome of the virus replicates to form viral DNA with the help of enzyme reverse transcriptase. This viral DNA gets incorporated into host cell's DNA and directs the infected' cells to produce virus particles. The macrophages .continue to produce virus' and in this way **'Macrophage acts like an HIV Factory'.**

Simultaneously, HIV enters into Helper T-lymphocytes below 200/mm^3 (Having CD_4 receptors), replicates and produces progeny viruses which destruct the helper T-cell. The progeny virus released-in the blood' attacks the other helper T cells. This is repeated leading to progressive decrease in the number of helper T-lymphocytes in the body of the infected person. During this period, the infected person suffers from fever, diarrhea and weight loss.

Due to decrease in the number of helper T-lymphocytes below' 200/mm3 , the person starts suffering from opportunistic infections of bacteria especially *Mycobacterium*, viruses, fungi and even parasites like Toxoplasma. The patient becomes so immunodeficient that he/she is unable to-protect himself/herself against these infections. Besides Pneumocystis carinii (Pneumonia), AIDS victims have persistent diarrhea and are especially susceptible to Toxoplasma infections (tuberculosis)", leukoplakia (whitish patches on mucus membranes primarily due to yeast infections), Cytomegalovirus (leading to blindness 0nd .dementia). Herpes simplex, among many other bacterial fungal infections, and Kaposi's sarcoma.

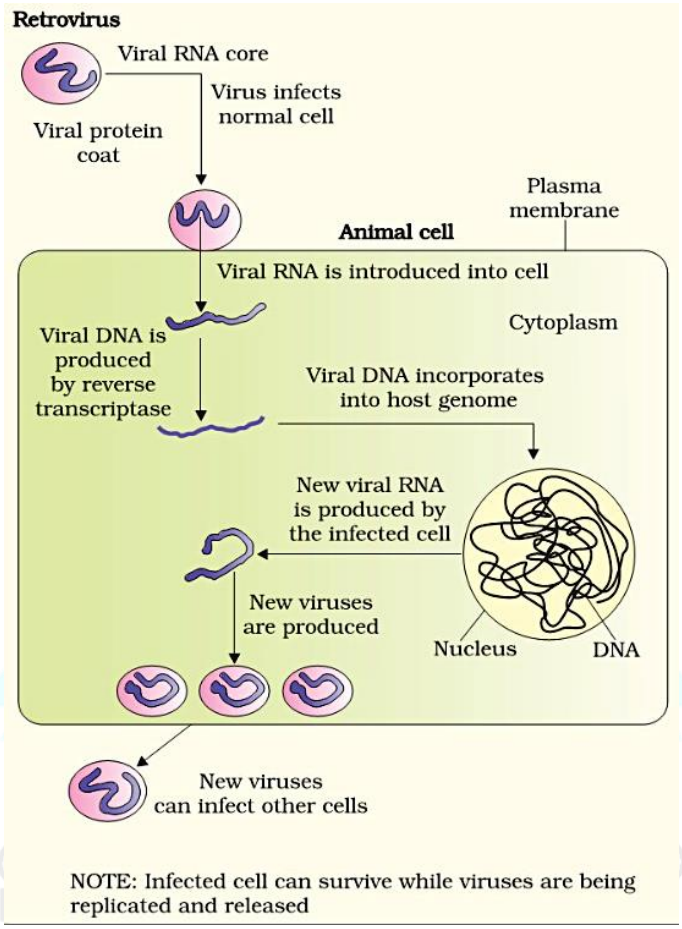


Fig. : Replication of retrovirus

Diagnosis

A widely used diagnostic test for AIDS is Enzyme Linked Immuno-Sorbent Assay (**ELISA). Western blotting** test is employed for confirmation of ELISA positive cases.

Treatment

Treatment of AIDS with antiretroviral drugs is only effective because it can only prolong the life of the patient but cannot prevent death. **Zidovudine or AZT (Azidothymidine)** was first and still continues to be the drug of choice for the treatment of AIDS.

Prevention of AIDS :

As AIDS has no cure, prevention is the best option. No vaccine has been prepared so far against AIDS virus. WHO (World Health Organisation) has started number of programmes to prevent the spreading of HIV infections. The following steps may help in preventing the AIDS.

(i) Proper blood testing to make blood safe from HIV.

(ii) Ensuring the use of only disposable needles and syringes in public/private hospital? and clinics.

(iii) Free distribution of condoms and advocating safe sex.

(iv) Controlling drug abuse.

(iii) Promoting regular checkup for HIV in susceptible population.

(vi) People should be educated about AIDS.

Every year **1st December is commemorated as World AIDS Day**. **NACO** (National AIDS Control Organisation) and **NGOs** (Non-governmental Organisations) are doing a lot of work to educate people about AIDS.

AIDS can only be tackled by the society and medical fraternity working together by preventing its spread and finding new ways to cure the disease.

CANCER

It is one of the most dreaded diseases of human beings and is a major cause of death all over the world. More than a million Indians suffer from cancer and a large number of them die annually.

In our body, cell growth and differentiation is highly controlled and regulated process. But in cancer cells, there is an abnormal and uncontrolled division of cells due to breakdown of these regulatory mechanisms.

Normal cells show a property called **contact inhibition**, in which, the dividing cells when in contact with other cells, inhibit their uncontrolled growth but cancer cells do not have this property. Therefore, cancerous cells continue to divide giving rise to mass of cells called **neoplasm** or **tumors**.

Types of Tumors:

(i) Benign Tumors : It remains confined to their original location and do not spread to other parts of the body and hence causes little damage.

(ii) Malignant Tumors : It is a tumor when neoplastic or tumor cells divide and grow very rapidly, invading and damaging surrounding normal tissues, starving the normal cells by competing for vital nutrients. Cells sloughed from such tumors reach distant site through blood and whenever they get lodged in the body, they start a new tumor formation. This property is called **metastasis** and is the most feared property of malignant tumors.

Causes of Cancer/Carcinogenic agents

(i) Physical agents

Ionising radiations like X-rays and gamma rays and non-ionizing radiations like UV rays cause DNA damage which leads to neoplastic transformation.

(ii) Chemical agents

Chemical carcinogens are present in tobacco smoke which have been identified as a major cause of lung cancer.

(iii) Biological agents

Cancer causing viruses called **oncogenic viruses** have genes called viral **oncogenes** which can cause cancer. Several genes called cellular oncogenes (c-onc) or protooncogenes have been identified in normal cell. Activation of these genes under certain conditions could lead to oncogenic/carcinogenic transformation of the cells.

Types of cancer :

(a) Carcinomas are malignant growths of the epithelial (ectodermal) tissues that cover or line the body organs, e.g., skin cancer, breast cancer, lung cancer, cancer of the stomach and pancreas (about 85 percent of all tumors are carcinoma).

(b) Sarcomas are malignant growths arising in tissues derived from primitive mesoderm. e.g., bone tumors, cancer of lymph nodes.

(c) Leukemias result from unchecked proliferation of cell types present in blood and their precursors in the bone marrow.

Other types of cancers are :

Melanoma	:	Cancer of pigment cells of the skin
Adenoma	:	Cancer of glands
Lymphoma	:	Cancer of lymphatic tissue
Glioma	:	Cancer of glial cells of CNS

Selection and Diagnosis

An early detection of cancer is life saving, but if it spreads to various parts and organs, the treatment becomes ineffective. The diagnosis of cancer is usually done by

(a) **Biopsy and histopathological studies of the suspected tissue.** In this a piece of suspected tissue is cut into thin sections, stained and examined under a microscope, by a pathologist for detection of cancerous cells.

(b) **Blood and bone marrow tests** for increased cell counts in the case of leukaemia.

(c) Techniques like radiography (use of X-rays).

CT (Computed tomography) and **MRI** (Magnetic Resonance Imaging) are useful to detect cancers of the internal organs. CT scan uses X-rays to generate a three dimensional image of internal organs while MRI uses strong magnetic fields and non-ionising radiations to accurately detect pathological and physiological changes in the living tissue.

(d) against cancer specific antigens are also used for detection of certain cancers, e.g., Herceptin.

(e) Techniques of molecular biology can be used to detect genes in individuals with inherited susceptibility for certain cancers. After detection or identification of these genes in any individual, they may be advised to avoid exposure to particular carcinogens to which they are susceptible {e.g. Tobacco smoke in case of lung cancer)

Treatment of Cancer

Common treatment prescribed for different types of cancers are :

(i) Surgery

Generally a tumor is surgically removed wherever possible.

(ii) Radiotherapy/Radiation therapy

Tumor cells are irradiated lethally by gamma radiations taking proper care of the normal tissues surrounding the tumor mass or neoplasm, e.g., use of I131 for thyroid cancer.

(iii) Chemotherapy

Several chemotherapeutic drugs are used to kill cancerous cell. Some of these are specific for particular tumor e.g. two anticancer drugs, vincristine and vinblastine, used in the treatment of leukaemia are obtained from common weed Catharanthus roseus. Majority of drugs have side effects like hair loss, anemia etc.

(iv) Immunotherapy

Sometimes tumor cells are seen to avoid detection and destruction by immune system. So, the patients are given substances called **biological response modifiers** such as **α-interferon** which activate their immune system and help in destroying the tumor.

DRUGS AND ALCOHOL ABUSE

It has been observed that the use of drugs has increased especially among youth. This is a matter of concern, as it could cause harmful effects. Proper education and guidance would enable youth to safeguard themselves against dangerous behavioural patterns and follow life styles.

Major Categories of Psychoactive Drugs, Their Effects and Clinical Uses

Type of Drug	Example	Effects	Clinical Uses
Sedatives and tranquillisers (depressants)	Barbiturates Benzodiazepines (e.g., Valium)	Depress brain activity and produce feelings of calmness, relaxation, drowsiness and deep sleep (high doses)	Hypnotic antianxiety
Opiate narcotics	Opium, morphine, heroin, pethidine, methadone	Suppress brain function, relieve intense pain (physical and mental), produce temporary euphoria	Analgesic
Stimulants	Caffeine (very mild), amphetamines (including dexamphetamine), cocaine and its derivative Novacaine	Stimulate the nervous system; make a person more wakeful, increase alertness and activity, produce excitement	Attention deficit, Narcolepsy, weight control
Hallucinogens	LSD, mescaline, psilocybin, (charas, hashish, marijuana bhang) Cannabinoids	Alter thought, feelings and perceptions ; hallucinations	None

Commonly abused drugs are **opioids, cannabinoids and coca alkaloids.**

(i) Opioids :

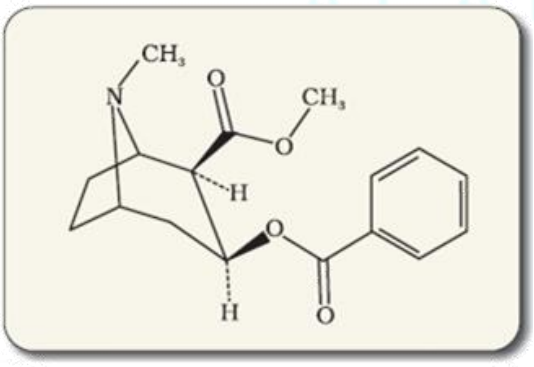


Fig. : Chemical structure of Morphine

Fig. : Opium Poppy

These drugs bind to specific receptors present in our central nervous system and gastrointestinal tract and relieve pain, so they are also called as analgesic (pain killers).

Morphine is extracted from the latex of poppy plant Papaver somniferum. It is very effective sedative and painkiller and is very useful for patients who have undergone surgery.

Heroin is obtained by acetylation of morphine (diacetyl

morphine) commonly called as smack which is white, odorless and bitter crystalline compound. Generally, it is taken by snorting or injection. It is a depressant and slows down body functions.

(ii) Cannabinoids :

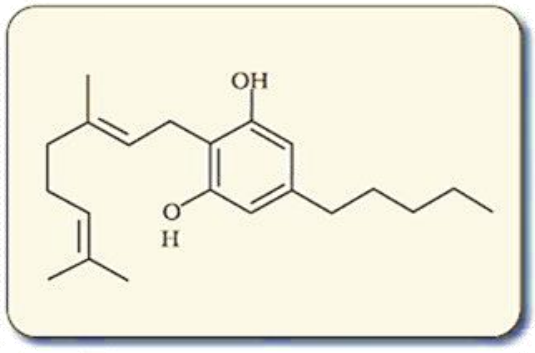


Fig. : Skeletal structure of cannabinoid molecule

Fig. : Leaves of *Cannabis sativa*

These are a group of chemicals, which interact with cannabinoid receptors present principally in brain.

- Natural cannabinoids are obtained from the inflorescences of the plant Cannabis sativa.
- The flower tops, leaves and their resin of Cannabis plant are used in various combinations to produce marijuana, hashish, charas and ganja.
- Generally taken by inhalation and oral ingestion and are known for their effects on cardiovascular system of body.
- These days cannabinoids are also being abused by some sports persons.

(iii) Cocaine :

Coca alkaloid or cocaine is obtained from coca plant, Erythroxylum coca, a native to south America-

- Cocaine, commonly called **coke** or **crack** is usually snorted.
- It interferes with the transport of the neuro-transmitter **dopamine.**
- It has a potent stimulating action on central nervous system, producing a sense of euphoria (feeling of well-being) and increased energy.
- Excessive dosage of cocaine causes hallucinations.

(iv) Seeds of Datura and aerial parts of Atropa belladona are misused for their hallucinogenic properties.

(v) Tobacco addiction:

- Tobacco has been used by human being for more than 400 years
- It is smoked, chewed or snuffed.
- Tobacco contains large number of chemical substances including **nicotine** (an alkaloid).
- Nicotine stimulates adrenal gland to release **adrenaline** and **nor-adrenaline** into blood circulation, both of which increase blood pressure and heart rate.
- **Tobacco chewing** is associated with increased risk of oral cavity cancer.
- **Smoking increases carbon monoxide (CO)** content in blood and reduces the concentration of haembound oxygen so it causes oxygen deficiency in body and is associated with increased incidence of cancer of lungs, urinary bladder and throat, bronchitis, emphysema, coronary heart diseases, gastric ulcer etc.

Adolescence and Drug/Alcohol Abuse

Adolescence is the period of rapid growth (physical, mental and psychological development) and is a bridge linking childhood with adulthood (the period between 12-18 years). It is also marked by several biological and behavioural changes.

Curiosity, excitement, need for adventure and experimentation are common causes which motivates youngsters towards drug and alcohol. The first use of drugs or alcohol may be due to curiosity or experimentation but later on child starts using it to escape facing problems such as academics or examinations etc. The thought amongst youngsters that it is 'cool' or progressive to smoke, use drugs or alcohol is also a major cause for youth to start these habits. Television, movies, newspapers, internet etc. also promote this perception. Unstable or unsupportive family structures have been seen to be associated with drug and alcohol abuse among adolescents.

Alcohol Abuse

The use of alcohol during adolescence may also have long term effects. It could lead to heavy drinking in adulthood. The chronic use of alcohol damages the nervous system and liver **(Liver cirrhosis).**

Effects of Alcohol on an Individual

1. **Effect on liver :** Absorbed alcohol is carried directly to the liver, where it becomes the preferred fuel. Use of moderate amounts of alcohol does not cause liver damage, provided adequate nutrition is maintained. However, chronic alcoholism causes the following diseases, **(i) Alcoholic fatty liver**. The liver becomes enlarged, yellow, greasy and firm. Hepatocytes (cells of liver) are distended by large fat globules which push

the hepatocyte nucleus against the cell membrane. There is increase in the fat synthesis in the liver **(ii) Alcoholic hepatitis**. It is characterised by degeneration of hepatocytes. The damaged (degenerated) hepatocytes are surrounded by polymorphonuclear leucocytes. These hepatocytes may be pale and swollen and some contain dense eosinophilic masses called **Mallory's hyaline. (iii) Alcoholic cirrhosis.** With continued alcohol intake, there is destruction of hepatocytes and fibroblasts (cells which form fibres) and stimulation of collagen protein formation, **(iv) Cholestasis** (Gr. Chole - bile, stasis - a standing still). It is stoppage in the flow of bile. It is characterized by jaundice, abdominal pain and hepatomegaly (enlargement of liver).

2. **Effect on nervous system :** These are characterised as:
   (i) Will power, judgement power and self-control become reduced.
   (ii) Control on emotion becomes reduced.
   (iii) Moral sense becomes reduced.
   (iv) Cerebellum becomes affected which results the loss of muscle co-ordination so affected person shows **staggering gait** and incoherent speech.
   (v) Inflammation of **axons** of neurons leads to **neuritis**.
3. **Effects on stomach :** High doses of alcohol cause ill effect on gastric glands of stomach, these glands secrete gastric juices in excess which cause the inflammation to gastric mucosa. This condition is known as **gastritis**. It may also result **gastric carcinoma,** peptic ulcer. Dilute alcohol (optimum 10%) stimulates gastric secretion (especially acid). Acute alcoholic intake can result in inflammation of the oesophagus **(oesophagitis)** and stomach **(gastritis).**
4. **Effect on heart :** Due to deposition of alcoholic fat on the wall of blood vessels the lumen of blood vessels becomes reduced, this increases the blood pressure and hence, the activity of heart.

   Size of RBCs becomes increased but the number of RBCs, WBCs and platelets is reduced.
5. **Effect on kidneys :** Alcohol reduces the release of hormone ADH (Antidiuretic hormone) due to which the excess amount of water is released from the body. So, alcoholism greatly causes dehydration condition.

Addiction and Dependence

The drug are normally used as medicines to help the patients cope with illnesses. But when these drugs are taken for purpose other than their normal clinical use, it is called drug abuse/addiction. Addiction is a habitual, physiological and psychological dependence of a substance which is beyond voluntary control. A person who is habituated to a substance/drug is called an **addict.** Repeated use of drugs increases the tolerance level of the receptors present in our body. consequently, the receptors respond only to higher doses of drugs or alcohol leading intake. So, addiction is a psychological attachment to certain effects such as euphoria and a temporary feeling of well-being associated with drugs and alcohol.

In the absence of any guidance or counseling, the person gets addicted and becomes dependent on drug use. **WHO (1964) has introduced the terms drug dependence in place of drug addiction.**

Dependence is the tendency of the drug addict's body to manifest a characteristic and unpleasant withdrawal syndrome symptoms, if regular dose of drugs/alcohol is discontinued. This is characterized by anxiety, shakiness, nausea and sweating, which may be relieved when drug use in resumed.

In some cases, withdrawal symptoms can be severe and even life threatening and the person may need medical supervision.

Effects of Drug/Alcohol Abuse

(i) Behavioural and psychological changes: The most common warning signs of drug and alcohol abuse among youth include drop in academic performance, unexplained absence from school/college lack of interest, withdrawal, isolation, depression, fatigue, aggressive and deteriorating relationship with family and friends.

(ii) Social problems : If an abuser is unable to get money to buy drugs/alcohol, he/she may turn to stealing. Adverse effects are just not restricted to the addicts only but cause mental and financial agony to their entire family and friends.

(iii) AIDS and Hepatitis: Those who take drugs intravenously (direct injection into the veins using a needle or syringe) are much more likely to acquire serious infections like AIDS and hepatitis B because the viruses which are responsible for these diseases are transferred from one person to another by sharing of infected needles and syringes.

(iv) Misuse of drugs by certain sports persons: Some drugs like **narcotic/analgesics, anabolic steroids, diuretics and certain hormones** are misused by certain sports person to enhance their performance because these drugs stimulate or increase muscle strength / bulk and promote aggressiveness thereby increasing the athletic performance.

The side effects of the use of anabolic steroids in **females** include masculinisation (features like males), increased aggressiveness, mood swings, depression, abnormal menstrual cycle, excessive hair growth on the face and body, enlargement of clitoris and deepening of voice etc. In males, it includes acne, increased aggressiveness, mood swings, depression, reduction of size of testicles, decreased sperm production, potential for kidney and liver dysfunction, breast enlargement, premature baldness, enlargement of prostate gland etc. **In adolescent male or female, premature closure of the growth centers of the long bones occur which may result in stunted growth.**

(v) The use of drugs and alcohol during pregnancy adversely affects the foetus.

(vi) The chronic use of drugs and alcohol damages the nervous system and liver (Cirrhosis).

Prevention and Control

'**Prevention is better than cure'.** Tobacco, drug/alcohol abuse are more during young adolescent age. Thus remedial measures should be taken well in time. In this regard, parents and teachers have a special responsibility. The following measures would be particularly useful for prevention and control of alcohol and drug abuse in adolescents.

(i) Avoid pressure: Every child has his/her own choice and personality, which should be kept in mind. So a child should not be pressurized unduly to achieve beyond his/her capacities be it in studies, sports etc.

(ii) Education and counseling: Education and counseling are very important to face problems, stresses disappointments and failure in life. These should be taken as a part of life. One should utilize child's energy in some positive activities like sports, music, reading, yoga and other extracurricular activities.

(iii) Seeking help from parents and peers: Whenever there is any problem, one should seek help and guidance from parents and peers. Help should be taken from close and trusted friends. This would help young to share their feelings of anxiety and inner thoughts.

(iv) Looking for danger signs: If friends find someone using drugs or alcohol, they should bring this to the notice of parents or teachers so that appropriate measures are taken to diagnose the illness and the causes.

This would help in taking proper remedial steps or treatment.

(v) Seeking professional and medical helps.

Chapter 8
Microbes in Human Welfare

Introduction:

Microbes are diverse-protozoa, bacteria, fungi and microscopic plants viruses, viroid and also prions that are proteinaceous infectious agents. They are found everywhere on earth ranging from soil, air water and some inhabitable places.

Bacteria and fungi can be grown on nutritive media to form colonies, which can be seen by necked eyes and very useful in study of microorganisms.

Microbes cause many diseases in human beings, plants and animals. Several microorganisms are useful to man in diverse ways.

Microbes in household products

a. Microorganisms like Lactobacillus and other commonly called lactic acid bacteria (LAB) grow in milk and convert it to curd. The LAB produces acids that coagulate and partially digest the milk proteins. It also improves its nutritional quality by increasing vitamin B12. In our stomach too, the LAB play very beneficial role in checking disease causing microbes.

b. The dough is used for making foods such as dosa and idli is fermented by bacteria. The puffed-up appearance of dough is due to the production of CO_2 gas. The dough used for making bread is fermented using baker's yeast (Saccharomyces cervisiae).

c. Cheese, is one of the oldest food items in which microbes were used. The large holes in 'Swiss cheese' are due to production of a large amount of CO2 by a bacterium named Propionibacterium sharmanii. The 'Roquefort cheese' is ripened by growing a specific fungus on them for a particular flavour.

Microbes in industrial production

A number of products like beverages and antibiotics involve uses of microbes. Production on large scale requires growing microbes in very large vessels called fermenters.

a. **Fermented Beverages -** Saccharomyces cerevisiae used for bread-making and commonly called brewer's yeast, is used for fermenting malted cereals and fruit juices, to produce beverages like wine, bear, whisky and rum.. Wine and bear are produced without distillation whereas whisky, brandy and rum are produced by distillation of the fermented broth.

1.	Bear	Barley	4-6 %
2.	Wine	Grapes	10-20 %
3.	Brandy	Distillation of wine	55-60 %
4.	Rum	Molasses	40-45 %
5.	Whisky	Cereal	20-40 %
6.	Gin	Secale cereal	40 %

b. **Antibiotics -** they are chemical substances produced by some microbes and can kill or retard the growth of other microbes. Penicillin was first antibiotic to be discovered.

Antibiotics have great improved our capacity to treat deadly diseases such as plague, whooping cough, diphtheria and leprosy.

c. Chemical, Organic acids, Enzymes and other Bioactive Molecules are commercially produced by microbes.

Chemicals :

Aspergillus niger (fungus) – Citric acid

Acetobacter aceti (bacterium) – Acetic acid

Clostridium butylicum (bacterium) – Butyric acid

Lactobacillus (bacterium) – Lactic acid

Saccharomyces cerevisiae – Ethanol

Enzymes:

Lipase – used in laundry detergents

Pectinase and protease – used in bottled juices

Streptokinase (Streptococcus bacterium) – used as clot buster (to remove clots)

Bioactive molecules:

Cyclosporin A (Trichoderma polysporum fungi) – used as immunosuppressive agent (for organ transplant patients).

Statins (Monascus purpureus yeast) – used as blood cholesterol lowering agents.

Microbes in sewage Treatment

Municipal waste water (sewage) contains large amount of organic matter and microbes which are pathogenic and cannot be discharged into natural water bodies like rivers and streams.

Sewage is treated in sewage treatment plant to make it less polluting by using heterotrophic microbes naturally present in sewage. Sewage treatment is done in two stages:

Process of sewage treatment in STP

- Primary treatment (Physical)
  - Filtration & sedimentation
- Secondary treatment (Biological)
  - Filtration & sedimentation
  - Alison & rapid growth of serobic microbes (Floces)
  - Consumes organic matter, reduces BOO
  - Effluent passed to setting tank
  - Floces sediments form - activated sludge
  - Anaerobic Sludge Digester
    - Form Biogas
    - Water released into rivers and streams

In primary treatment, floating debris is removed by sequential filtration. Grit (soil and small pebbles) are removed by sedimentation.

Secondary treatment or biological treatment involves passing of primary effluents in large aeration tank to help the growth of aerobic microbes into flocs (masses of bacteria associated with fungal filaments to form mesh like structures). These microbes increase the consumption of organic wastes and decrease the BOD (biological oxygen demand)of the effluents.

BOD is the amount of oxygen that would be consumed if all the organic matter in one litre of water were oxidised by bacteria. It measures the amount of organic matter present in the water. Greater the BOD of water more it is polluted.

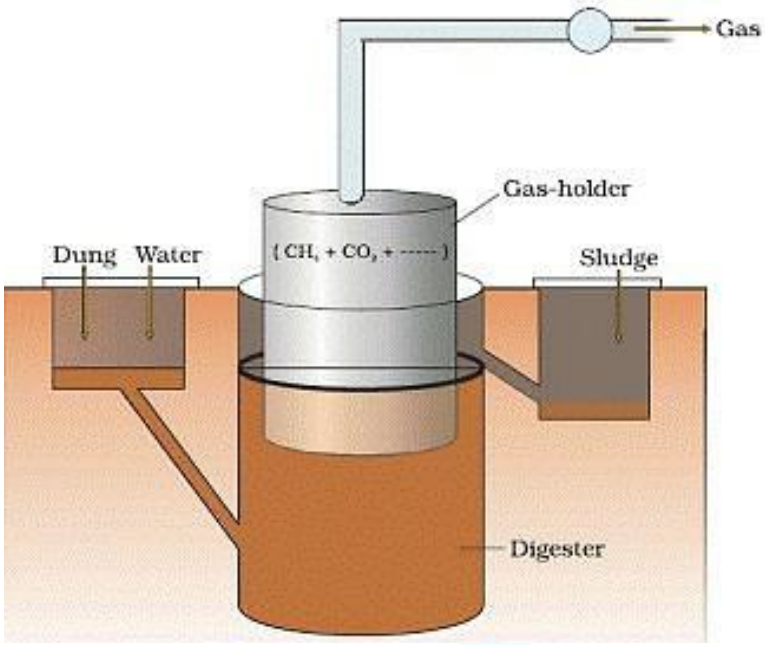
Once the BOD of sewage or waste water is reduced, the effluent is then passed into a settling tank where the bacterial 'flocs' are allowed to sediment. This sediment is called activated sludge.

Sludge is passed into large tanks called anaerobic sludge digesters in which anaerobic bacteria digest the bacteria and fungi in the sludge and produce mixture of gas called biogas, which is a mixture of methane, hydrogen sulphide and carbon dioxide.

The effluents from the secondary treatment plant are released into water bodies.

Microbes in Production of Biogas

Biogas is a mixture of gases produced by the microbial activity that can be used as fuel. Certain bacteria that grows anaerobically on cellulosic material produce large amount of methane along with CO2and H2. These bacteria are collectively called methanogens (Methanobacterium).



Biogas Plant – the excreta of cattle (gobar) is rich in methanogens bacteria and is used for generation of biogas also called as gobar gas.

The technology of biogas production was developed in India mainly due to the efforts

of Indian Agricultural Research Institute (IARI) and Khadi and Village Industries Commission (KVIC).

Biogas plant consists of a concrete tank in which bio-wastes are collected and slurry of dung is fed.

A floating cover is placed over digester that moves upward when gas is produced. The gas produced is removed and supplied through an outlet pipe for consumption.

The spent slurry is removed through another outlet and used as fertilisers. Biogas plant is more often build in rural areas as large amount of cattle dug is available easily.

Microbes as Biocontrol agent

Biocontrol means use of biochemical method for controlling plant disease and pests. The chemical used as pesticides and insecticides are harmful to human beings and animals.

Biological control of pests and disease is a method of controlling pest on natural prediction rather than chemicals. The organic farmer creates a system where the pests are not eradicated but kept at manageable level by complex system of check and balance within the living and vibrant ecosystem. For example, the Ladybird and Dragonflies are used to get rid of aphids and mosquitoes respectively. On brassicas and fruit tree, to control butterfly caterpillars bacteria Bacillus thuringiensis is used.

Biological control developed for use in the treatment of plant disease is the fungus Trichoderma. Trichoderma are free-living fungi that are very common in the root systems that control several plant pathogens.

Baculoviruses are pathogens that attack insects and other arthropods. The majority of baculoviruses used as biological control agents are in the genus Nucleopolyhedrovirus. These viruses are excellent candidates for species-specific, narrow spectrum insecticidal applications.

	Biological control agent	**Used to control**
1.	Ladybird Beetle	Aphids
2.	Dragonflies	Mosquitoes
3.	Bacteria (Bacillus thuriengiensis) (Available in sachets as dry spores)	Butterfly caterpillars
4.	Fungus (Trichoderma)	Several plant pathogens
5.	Baculoviruses (NPV) (They are species specific and narrow spectrum)	Insects and other arthropods

Microbes as Bio fertilisers

Bio fertilisers are organisms that enrich the nutrient quality of the soil. The main sources includes bacteria, fungi and cyanobacteria.

The root nodule formed by Rhizobium bacteria on root of leguminous plants increase the nitrogen level of soil,

necessary for various metabolic processes. Azotobacter and Azospirillum are free living bacteria that live in soil and fix atmospheric nitrogen into organic forms.

Symbiotic association of fungi with angiosperm plants (mycorrhiza) also increase the fertility of soil. Glomus form mycorrhiza that absorbs phosphorus from the soil and passes it to the plant. These microbes also provide benefits like resistance to root-borne pathogens, tolerance to salinity and drought.

Cyanobacteria (Nostoc, Anabaena), an autotrophic microbes found in aquatic and terrestrial environment fix atmospheric nitrogen. In paddy field this acts as important bio-fertiliser. Blue green algae also add organic matter to the soil and increase its fertility.

Chapter 9
Biotechnology Principle and Processes

Introduction:

This chapter deals with basic principles of biotechnology, the components central to the process of gene cloning such as DNA manipulative enzymes and vectors which transport the desired gene into host cell. Latter part of the chapter turns our focus to PCR process and applications along with obtaining the desired product on large scale using bioreactors.

In its purest form, the term biotechnology refers to the use of living organisms or their products to modify human health and the human environment. However, it is used in a restricted sense today, to refer to such of those processes which use genetically modified organisms to achieve the same on a larger scale. Further, many other processes/techniques are also included under biotechnology. For example, in-vitro fertilisation leading to a 'test-tube' baby, synthesising a gene and using it, developing a DNA vaccine or correcting a defective gene, are all part of biotechnology.

PRINCIPLES OF BIOTECHNOLOGY

(i) **Genetic Engineering :** Techniques to alter the chemistry of genetic material (DNA and RNA), to introduce these into host organisms and thus change the phenotype of the host organism.

(ii) **Maintenance of Sterile Conditions :** Microbial contamination-free ambience in chemical – engineering processes to enable growth of only the desired microbe/eukaryotic cell in large quantities for the manufacture of biotechnological products like antibiotics, vaccines, enzymes, etc.

In these techniques functioning lengths of DNA can be taken from one organism and placed into the cells of another organism. As a result, for example, we can cause bacterial cells to produce human molecules. Cows can produce more milk for the same amount of feed. And we can synthesize therapeutic molecules that have never before existed.

Let us now understand the conceptual development of the principles of genetic engineering.

Traditional hybridisation procedures used in plant and animal breeding, very often lead to inclusion and multiplication of undesirable genes along with desired genes. The techniques of genetic engineering which include creation of recombinant DNA, use of gene cloning and gene transfer overcome this limitation and allow us to isolate and introduce only one or a set of desirable genes without introducing undesirable genes into the target organism.

Let us now focus on the first instance of the construction of an artificial recombinant DNA molecule. The construction of the first recombinant DNA emerged from the possibility of linking a gene-encoding antibiotic resistance with a native plasmid (autonomously replicating circular extra-chromosomal DNA) of **Salmonella typhimurium**. **Stanley Cohen** and **Herbert Boyer** accomplished this in 1972 by isolating the antibiotic resistance gene by cutting out a piece of DNA from a plasmid which was responsible for conferring antibiotic resistance. The cutting of DNA at specific locations became possible with the discovery of the so-called 'molecular scissors' - **restriction enzymes**. The cut piece of DNA was then linked with the plasmid DNA. These plasmid DNA act as **vectors** to transfer the piece of DNA attached to it into the host organism. The linking of antibiotic resistance gene with the plasmid vector became possible with the enzyme **DNA ligase**, which acts on cut DNA molecules and join their ends. This makes a new combination of circular autonomously replicating DNA created in-vitro and is known as **recombinant DNA**. When this DNA is transferred into Escherichia coli, a bacterium closely related to Salmonella, it could replicate using the new host's DNA polymerase enzyme and make multiple copies. The ability to multiply copies of antibiotic resistance gene in E. coli, was called **cloning** of antibiotic-resistance gene in E.coli.

Thus, there are three basic steps in genetically modifying an organism.

(i) Identification of DNA with desirable genes.

(ii) Introduction of the identified DNA into the host.

(iii) Maintenance of introduced DNA in the host and transfer of the DNA to its progeny.

TOOLS OF RECOMBINANT DNA TECHNOLOGY

Tools required to accomplish genetic engineering include

1. DNA manipulative enzymes
   (i) Restriction enzymes
   (ii) Polymerase enzymes
   (iii) Ligases enzymes
2. Vectors
3. Host organism

Restriction Enzymes (RE)

In the year 1963, the two enzymes responsible for restricting the growth of bacteriophage in E.coli were isolated. One of these added methyl groups to DNA (methylase), while the other cut DNA. The later was called **restriction endonuclease.**

Restriction enzymes serve as chemical knives to cut genes (DNA) into defined fragments. These may then be used

(i) To determine the order of genes on chromosomes.

(ii) To analyze the chemical structure of genes and of regions of DNA which regulate the functions of gene.

(iii) To create new combinations of genes.

The first restriction endonuclease - Hind II, whose functioning depended on a specific DNA nucleotide sequence was isolated and characterized five years later. It was found that Hind II always cut DNA molecules at a particular point by recognizing a specific sequence of six base pairs. This specific base sequence is known as the recognition sequence for Hind II

Besides Hind II, we know more than 900 restriction enzymes that have been isolated from over 230 strains of bacteria each of which recognize different recognition sequences.

The convention for naming these enzymes is the first letter comes from the name of the genus and second two letters come from the species of the prokaryotic cell from which they were isolated for example, EcoRI comes from Escherichia coli RY13. In EcoRI, the letter 'R' is derived from the name of strain "Rough". Roman number following the name indicated the order in which the enzymes were isolated from that strain of bacteria.

Restriction enzymes belong to a larger class of enzymes called **Nucleases.**

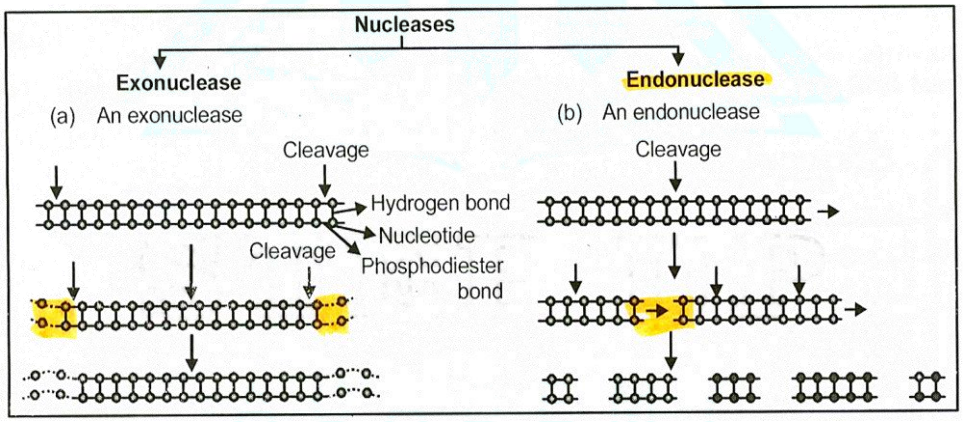


Fig.: The reactions catalyzed by the two different kinds of nuclease. (a) An exonuclease, which removes nucleotides from the end of a DNA molecule, (b) An endonuclease, which breaks internal phosphodiester bonds.

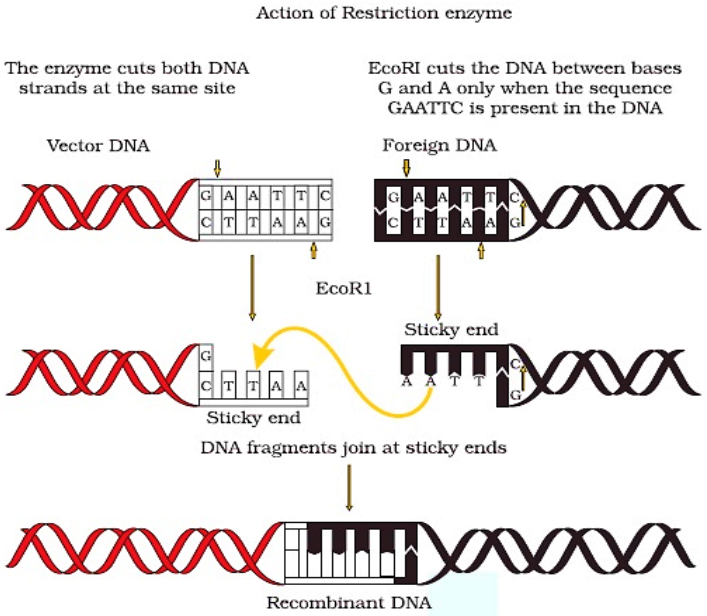
Each restriction enzyme recognises a specific palindromic nucleotide sequence in DNA. A palindrome is a word, phrase, number or other sequence of units that can be read the same way in either direction.

Palindrome in DNA is a sequence of base pairs that reads same on the two strands when orientation of reading is kept same. These sequences may range from 4-8 nucleotides in length.

Working of Restriction Enzymes

Each restriction endonuclease functions by **'inspecting'** the length of a DNA sequence. Once it **finds** its specific recognition sequence, it will bind to the DNA and cut each of the two strands of the double helix at specific points in their sugar-phosphate backbones.

Some restriction enzymes (RE) such as EcoRI cut the strand of DNA a little away from the centre of the palindrome sites, but between the same two bases on the opposite strands. This leaves single- stranded portions at the ends. There are overhanging stretches called sticky ends or cohesive ends on each strand (shown in figure below). These are named so because they form hydrogen bonds with their complementary cut counterparts. This stickiness of the ends facilitates the action of the enzyme DNA ligase. Some restriction enzymes cut the strand of DNA in the centre of palindrome. Such ends are called blunt ends or **flush ends.**

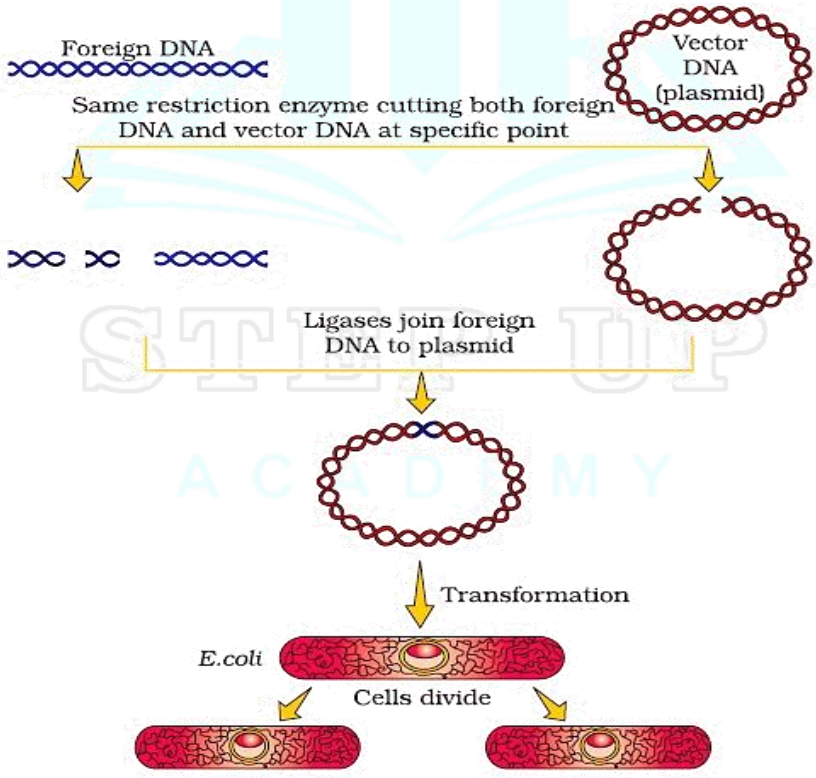


Restriction endonucleases are used in genetic engineering to form 'recombinant' molecules of DNA(rDNA), which are composed of DNA from different sources/genomes.

When cut by the same restriction enzyme, the resultant DNA fragments have the same kind of 'sticky-ends' and, these can be joined together (end-to-end) using **DNA ligases.**

5'———— GAATTC ————3'

3'———— CTTAAG ————5'



DNA Ligases

This enzyme forms phosphodiester bonds between adjacent nucleotides and covalently links two individual fragments of double-stranded DNA by utilising energy from cell. The enzyme used most often in rDNA technology is T_4 DNA ligase, which is encoded by phage T_4.

DNA Polymerases

These enzymes synthesize a new strand of DNA complementary to an existing DNA template in 5' to 3' direction.

Usually DNA polymerase I is employed in genetic engineering.

Cloning Vectors

Vector is carrier/vehicle that delivers a foreign piece of DNA into the host organism and they are engineered in such a way that help in following :

(i) Easy linking foreign DNA.

(ii) Selection of recombinants from non-recombinants.

Essential features of a vector are :

(i) Origin of replication (ori) : This is a sequence from where replication starts and any piece of DNA when linked to this sequence can be made to replicate within the host cells. If a piece of DNA, is somehow transferred into an alien organism, most likely, this piece of DNA would not be able to multiply itself in the progeny cells of the organism. But when it gets integrated into the genome of the recipient, it may multiply and be inherited along with the host DNA. This is because the alien piece of DNA has become a part of a chromosome, which has the ability to replicate. In a chromosome, there is a specific DNA sequence called the 'origin of replication'. **Thus, an alien DNA is linked with the origin of replication, so that, this alien piece of DNA can replicate and multiply itself' in the host organism.** This can also be called cloning or making multiple identical copies of any template DNA. This can responsible for controlling the copy number of the linked DNA. So, if one wants to recover many copies of the target DNA it should be cloned in a vector whose origin support high copy number.

(ii) Selectable marker : Helps in identifying and eliminating non-transformants and selectively permitting the growth of the transformants. Normally, the genes encoding resistance to antibiotics such as ampicillin, chloramphenicol, tetracycline or kanamycin, etc., are considered useful selectable markers for E.coli **The normal E.coli do not carry resistance against any of these antibiotics.** Also, the gene lac Z coding for β-galactosidase enzyme may utilizes its substrate to produce a blue-coloured product.

(iii) Cloning sites : In order to link the alien DNA, the vector needs to have very few, preferably single, recognition sites for the commonly used restriction enzymes. Presence of more than one recognition site within the vector will generate several fragments, which will complicate gene cloning. The gene of interest is inserted at restriction enzyme site located in any one of the antibiotic resistance genes or selectable marker genes, such that, recombinant vector will lose antibiotic resistance due to insertion of foreign DNA to one selectable marker, but can still be selected out from non-recombinant ones by presence of intact antibiotic resistance for another selectable marker.

(iv) Size of vector : Should be small as large molecules have tendency to breakdown during purification.

Examples of Vectors commonly used in RDT :

(A) Plasmids : These are extra chromosomal, circular, non-essential, double-stranded, autonomous, self-replicating pieces of DNA in bacterial and some yeast cells. They may confer the property of antibiotic resistance (bacteria) and virulence (Ti plasmid in Agrobacterium tumefaciens).

Table : Comparison of Plasmid DNA versus Chromosomal DNA

Plasmid DNA	Chromosomal DNA
1. It is always double stranded.	1. It may be single stranded or double stranded.
2. It is circular.	2. It is linear or circular.
3. It is naked without histone protein.	3. It is coated with histone protein.
4. It does not carry any vital gene necessary for cell.	4. It carrier vital genes necessary for cell.
5. It can replicate independent of main genome.	5. It replicates with genome.
6. Introns are absent.	6. Both exons and introns are present.

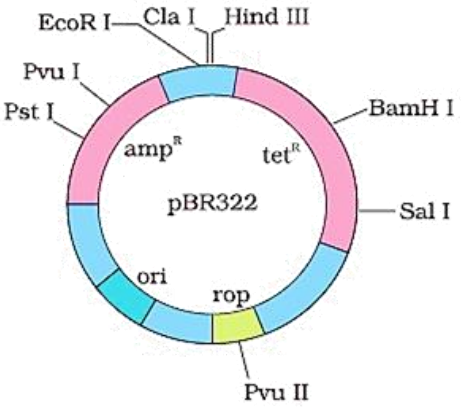
Characteristics of pBR322 :

(1) Two sets of antibiotic resistance gene. Therefore, selection is a two-step process.

(2) Reasonably high copy number.

Selection using Antibiotic resistance : The ligation of alien DNA is carried out at a restriction site present in one of the two antibiotic resistance genes. For example, you can ligate a foreign DNA at the BamH I site of tetracycline resistance gene in the vector pBR322. The recombinant plasmids will lose tetracycline resistance due to insertion of foreign DNA but can still be selected out from non-recombinant ones by plating the transformant on ampicillin-containing medium. The transformant growing on ampicillin-containing medium are then transferred on a medium containing tetracycline. The recombinants will grow in ampicillin-containing medium but not on that containing tetracycline. But, non-recombinants will grow on the medium containing both the antibiotics. **In this case, one antibiotic resistance gene helps in selecting the**

transformants, whereas the antibiotic resistance gene gets "inactivated due to insertion" of alien DNA and helps in selection of recombinants.



Disadvantage of pBR322 : Selection of recombinants due to inactivation of antibiotics is a cumbersome procedure because it requires simultaneous plating on two plates having different antibiotics.

Blue/white selection : Alternative selectable marker in pUC8 plasmid differentiates recombinants from non-recombinants on the basis of their ability to produce colour in the presence of a chromogenic substrate. In this, a recombinant DNA is inserted within the coding sequence of an enzyme, β -galactosidase. This results into inactivation of the enzyme, which is referred to as insertional inactivation. **The presence of a chromogenic substrate gives blue-coloured colonies if the plasmid in the bacteria does not have an insert**. Presence of insert results into insertional inactivation of the β-galactosidase and the colonies do not produce any colour , these are identified as recombinant colonies.

(B) Bacteriophage : It is a virus that infects bacteria and has the ability to replicate within bacterial cells independent of the control of chromosomal DNA.

Bacteriophages, because of their high number per cell, have very high copy number of their genome within the bacterial cell.

If we are able to link an alien piece of DNA with bacteriophage, we can multiply its Numbers equal to the copy number of the bacteriophage.

(C) **Cosmid** (capacity = 30-45 kbp)

(D) BAC (capacity = 50-300 kbp)

(E) YAC (capacity = 1000-2500 kbp)

(H) Vectors for cloning genes in plants :

Ti plasmid of Agrobacterium tumefaciens : Agrobacterium tumefaciens, a pathogen of several dicot plants is able to deliver a piece of DNA known as 'T-DNA' to transform normal plant cells into a tumor and direct these tumor cells to produce the chemicals required by the pathogen. This bacterium invades plants at the site of wound, transforming them and nearby cells to form a tumor called crown gall.

When the bacterium contacts a damaged plant cell, it delivers a T-DNA (transferred DNA) fragment from plasmid into host cell that integrates at a random position in plant cells chromosome. The tumor inducing (Ti) plasmid of A. tumefaciens has now been modified into a cloning vector which is no more pathogenic to the plants but is still able to use the mechanism to deliver genes of our interest into a variety of plants as shown in figure below.

(I) Viruses used to clone genes in animals :

Retroviruses : These viruses in animals have the ability to transform normal cells into cancerous cells. Similarly retroviruses have also been disarmed and are now used to deliver desirable genes into animal cells.

So, once a gene or a DNA fragment has been ligated into a suitable vector it is transferred into a bacterial plant or animal host (where it multiplies).

PROCESSES OF RECOMBINANT DNA TECHNOLOGIES

Recombinant DNA technology (RDT) involves several steps in specific sequence such as

(1) Isolation of DNA (total cell DNA or plasmid)

(2) Fragmentation of DNA by RE

(3) Separation and isolation of a desired DNA fragment

(4) Amplification of gene of interest using PCR

(5) Ligation of the DNA fragment into a vector

(6) Transferring the recombinant DNA into the host

(7) Culturing the host cells in a nutrient medium at a large scale

(8) Extraction of the desired product

Let us examine each of these steps in some details :

(1) Isolation of the Genetic Material (DNA)

The procedure for total DNA preparation from a culture of bacterial cells can be divided into four stages (shown in figure above):

1. A culture of bacteria is grown and then harvested.
2. The cells are broken open to release their contents according to cell type :

   Bacterial – Lysozyme

   Fungal – Chitinase

   Plant cell – Cellulase
3. This cell extract is treated to remove all components except the DNA.

   You know that genes are located on long molecules of DNA interwind with proteins such as histones. The

RNA can be removed by treatment with ribonuclease whereas proteins can be removed by treatment with protease.

4. The resulting DNA solution is concentrated.

   Other molecules can be removed by appropriate treatments and purified DNA ultimately precipitates out after the addition of chilled ethanol. This can be seen as collection of fine threads in the suspension (shown in figure below).

(2) Fragmentation of DNA at Specific Locations by RE:

Restriction enzyme digestions are performed by incubating purified DNA molecules with the restriction enzyme. Agarose gel electrophoresis is employed to check the progression of a restriction enzyme digestion. Both vector and insert should be digested with compatible, i.e., the same restriction enzyme.

(3) Separation and Isolation of DNA Fragments

A. Separation of DNA fragments by gel electrophoresis

- The cutting of DNA by restriction endonucleases results in the fragments of DNA. These fragments can be separated by a technique known as gel electrophoresis.
- Since DNA fragments are negatively charged molecules they can be separated by forcing them to move towards the positive electrode anode under an electric field through a medium/matrix.
- Nowadays, the most commonly used matrix is agarose which is a natural polymer extracted from sea weeds.
- The DNA fragments separate (resolve) according to their size through sieving effect provided by the agarose gel. **Hence, the smaller the fragment size, the farther it moves.**
- Agarose gel electrophoresis is employed to check the progression of a restriction enzyme digestion. This process is repeated with the vector DNA also.
- The separated DNA fragments can be visualised only after staining the DNA with a compound known as ethidium bromide followed by exposure to UV radiation (you cannot see pure DNA fragments in the visible light and without staining). You can see bright orange coloured bands of DNA in a ethidium bromide stained gel exposed to UV light (shown in figure above).

B. Isolation of desired DNA fragment : The separated bands of DNA are cut out from the agarose gel and extracted from the gel piece. This step is known as elution. The DNA fragments purified in this way are used in constructing recombinant DNA by joining them with cloning vectors.

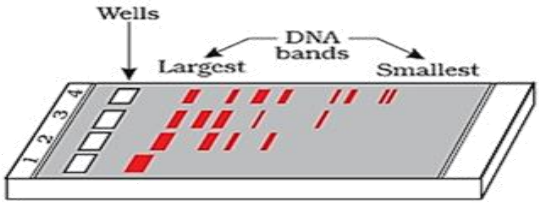


Fig. : A typical agarose gel electrophoresis showing migration of undigested (lane 1) and digested set of DNA fragments (lane 2 to 4)

4. Amplification of Gene of Interest using PCR:

It is important to note that another technique that revolutionised the field of biotechnology called PCR. PCR stands for polymerase chain reaction. Arguably, the PCR machine has recently become indispensable to biological research as the light microscope was some 100 years ago. The idea of PCR was born only in the early 1970's but it took more than a decade before American biochemist **Kary Mullis** turned the idea into reality.

PCR enables the production, or amplification of billions of copies of an original piece of DNA in a tube within minutes or hours not days.

How PCR Works?

Basically, PCR is DNA replication on a grander scale. The polymerase chain reaction relies on the use of several essential chemical ingredients, including the following :

- **A DNA polymerase ;** A major limitation of early PCR method was that fresh DNA polymerase had to be added during every cycle. This repetitive step was not just tedious, but it also greatly increased the likelihood of error. Mullis and colleagues addressed this deficiency just a year later when they demonstrated how a particular type of thermostable **DNA polymerase** often referred to by its popular nickname 'Taq polymerase', a heat-resistant enzyme isolated from the bacterium Thermus aquaticus, eliminated the need to add fresh polymerase during life cycle. **Thermus aquaticus** is a thermophilic bacterium that can survive temperatures up to 95°C. In fact, its natural habitat is the hot spring ecosystem of Yellowstone National Park. This innovation greatly improved the quantity and quality of PCR products.
- A small amount of DNA to serve as the initial template (nanograms).
- The four deoxyribonucleotides to serve as the substrates for the DNA polymerase and the raw ingredients of the new DNA molecules.
- A few necessary ions and salts

- A pair of primers (small chemically synthesized oligonucleotides that are complementary to the regions of DNA) with exposed 3'-OH groups that will bind to the particular sequence of interest in the DNA template.

The DNA polymerases can only add new nucleotides to the 3'-OH end of a growing strand. **They, therefore, require the presence of a primer to get started, because they cannot begin synthesis do novo. In fact, two primers are required - one to initiate replication of each of the two DNA strands.**

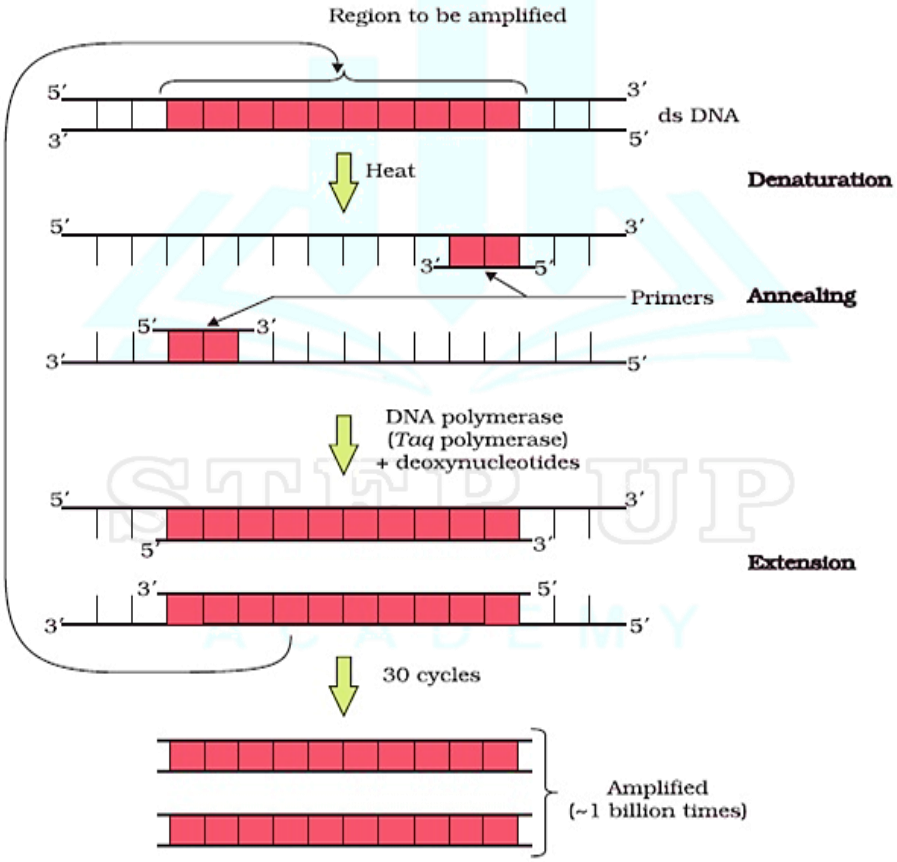
A single PCR reaction involves three temperature-dependent steps, described as follows :

(i) Denaturation : The starting solution is heated, usually to 94°C. The high temperatures break the hydrogen bonds between the two strands of the original DNA double helix, providing the necessary single-stranded templates.

(ii) Annealing : After just a couple of minutes at that temperature, the reaction mixture is quickly cooled, usually to somewhere between 50°C and 60°C. It is then held for less than a minute at this lower temperature - which is enough time for the primers to bind to their complementary sequences on the single-stranded templates.

(iii) Primer extension (polymerization) : The sample in next heated to 72°C for some time, during which time the DNA polymerase adds nucleotides to the primer, synthesizing a new DNA using only the template sequences that bind the primers (shown in figure below).

If The process of replication of DNA is repeated many times, the segment of DNA can be amplified to approximately billion times, i.e., 1 billion copies are made at the end of 30 PCR cycles. It is possible to generate '2^n' molecules after 'n' number of cycles.



5. Ligation of the DNA fragment into a vectors

The amplified fragment if desired can now be used to ligate with a vector for further cloning.

Applications of PCR

(i) Diagnosis of pathogens.

(ii) Diagnosis of specific mutation :.

(iii) DNA Fingerprinting :

(iv) Detection of Specific Microorganisms :.

(v) In Prenatal Diagnosis :.

(vi) Diagnosis of Plant Pathogens :

(vii) In Paleontology

6. Insertion of Recombinant DNA into the Host Cell/Organism

There are several methods of introducing the ligated DNA into recipient cells. Recipient cells after making them 'competent' to receive, take up DNA present in its surrounding.

Since DNA is a hydrophilic molecule, it cannot pass through cell membranes. In order to force bacteria to take up the plasmid, the bacterial cells must first be made 'competent' to take up DNA This is done by treating them with a **specific concentration of a divalent cation, such as calcium, which increases the efficiency with which DNA enters the bacterium through pores in its cell wall.** Possibly calcium chloride causes the DNA to precipitate onto the outside of the cells or it may improve DNA binding.

(i) Transformation : Recombinant DNA can then be forced into such cells by incubating the cells with recombinant DNA on ice. Followed by placing them briefly at **42ºC (heat shock**), and then putting them back on ice. This enables the bacteria to take up the recombinant DNA.

This method is transformation i.e., a procedure through which a piece of DNA is introduced into a host bacterium.

So, if a recombinant DNA bearing gene for resistance to an antibiotic (e.g., ampicillin) is transferred into E.coli cells, the host cells become transformed into ampicillin-resistant cells. If we spread the transformed cells on agar plates containing ampicillin, only transformants will grow, untransformed recipient cells will die. Since, one is able to select a transformed cell in the presence of ampicillin, the ampicillin-resistance gene in this case is called as selectable marker.

(ii) In another method known as **microinjection**, recombinant DNA is directly injected into the nucleus of an animal cell.

(iii) In another method, suitable for plants, cells are bombarded with high-velocity microparticles of gold or tungsten coated with DNA in a method known as **biolistic** or **gene gun.**

(iv) And the last method uses 'disarmed pathogen' vectors, which when allowed to infect the cell, transfer the recombinant DNA into the host. For example, Ti plasmid of Agrobacterium and Retroviruses.

(v) Electroporation : Short electrical impulses of high field strength are given. These increase the permeability of protoplast membrane by creating transient microscopic pores, thus making entry of DNA molecules into the cells much easier.

7. Culturing the Host Cells in a Nutrient Medium at a Large Scale for Obtaining the Foreign Gene Product

The ultimate aim of RDT is to produce a desirable protein in large quantities. For this, expression of protein is essential and to enhance the production of desired protein, inducers are employed that can increase the production of targeted protein or gene of interest.

If any protein encoding gene is expressed in a heterologous host it is called a recombinant protein. Expression of human insulin gene in a vector in E.Coli is a suitable example when the bacteria serve as a heterologous host. Moving from a laboratory scale to an industrial scale presents new problems for biotechnologists. For any new biotechnological manufacturing processes, it must be tried out on a laboratory scale. The cells harbouring cloned genes of interest may be grown on a small scale in the laboratory. The culture may be used for extracting the desired protein and then purifying it by using different separation techniques.

After initial investigations using ordinary laboratory apparatus it is usual to make a 'pilot plant' that involves use of a small 'fermenter' such as large shake flasks in laboratories. **Fermenter is the tank or vessel in which the process will be carried out.** Optimum nutrient and physical conditions for maximum yield must be determined.

New factors come into play when the process has to scaled up from pilot production to full-scale (100-1000 L).

Some of the important factors are as follows :

- Maintaining aseptic conditions. It is easy to contaminate both inputs and outputs to the main fermenter.
- Physical factors, such as mixing and aerating the media and getting rid of waste heat, create the biggest problems in moving from one scale to another.
- To supply enough oxygen in large-scale cultures, air must be forced through the medium because the simple agitation used at the laboratory scale is inadequate. Small bubbles are more effective than large bubbles, so a sparger is used (a tube with small holes). The mixture may also be stirred.
- Anti-foaming agents are required to reduce the foaming caused by stirring and aeration.
- Heat is produced by the activity of microorganisms and large-scale production. Cooling water must be circulated around the fermenter.
- To keep conditions constant, such as supply of nutrients, pH and oxygen concentration, throughout the medium on a large scale. Sophisticated monitoring devices and control processes are needed.

Fermenters, also known as **bioreactors**, are chambers in which microorganisms are cultured in a liquid/solid medium. To produce the desired product in large quantities, bioreactors were developed where large volumes (100-1000 L) of culture can be processed.

Bioreactors are vessels in which raw materials are biologically converted into specific products, individual enzymes, etc., using microbial plant, animal or human

cells. A bioreactor provides the optimal conditions for achieving the desired product by providing optimum growth conditions (temperature, pH, substrate, salts, vitamins, oxygen).

Fermenter design and use : The most commonly used bioreactors are of stirring type, which are shown in figure given below.

A stirred-tank reactor is usually cylindrical or with a curved base to facilitate the mixing of the reactor contents. The stirrer facilitates even mixing and oxygen availability throughout the bioreactor. Alternatively air can be bubbled through the reactor. If you look at the figure closely you will see that the bioreactor has an agitator system, an oxygen delivery system and a foam control system, a temperature control system, pH control system and sampling ports so that small volumes of the culture can be withdrawn periodically.

The processes which take place in fermenters are referred to as **fermentations**. The term fermentation originally applied only to anaerobic processes but is now used more broadly to include all the processes, whether aerobic or anaerobic. **The product is either the cells themselves (biomass) or some useful cell product. All operations must be carried out under sterile conditions to avoid contamination of the culture.**

In continuous culture system, the used medium is drained out from one side, while fresh medium is added from other to maintain the cells in their physiologically most active log/exponential phase. This type of culture method produces a larger biomass leading to higher yields of desired protein.

Small volume cultures (in shake flasks) cannot yield appreciable quantities of products.

Continuous culture involves continuous, long-term operation over many weeks, during which nutrient medium is added as fast as it is used, and the overflow is harvested.

8. Extraction of the Desired Product Utilises Downstream Processing

Downstream processing is the name given to the stage after fermentation when the desired product is recovered and purified. After completion of the biosynthetic stage, the product has to be subjected through a series of processes before it is ready for marketing as a finished product. These downstream processes include separation and purification of the desired product.

Usually the contents of the fermenter are first separated into a liquid component and a solid component which contains the cells. This is usually done by filtration or centrifugation. The liquid may contain the desired product in solution or it may be the cells or some product inside the cells that is needed.

A whole range of biochemical separation and purification techniques is available, such as drying, chromatography, solvent extraction and distillation. As an indication of the importance of downstream processing, it involves over 90 of the 200 staff employed by Eli Lilly in their human insulin plant.

After purification the product has to be formulated with suitable preservatives. Such formulation has to undergo through clinical trials as in case of drugs. Strict quality-control testing for each product is also required. The downstream processing and quality-control testing vary from product to product.

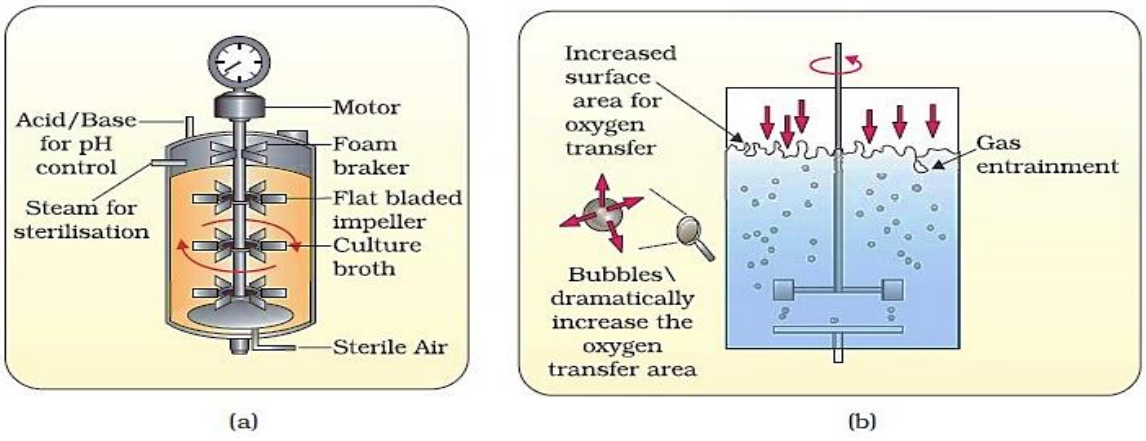


Fig. : (a) Simple stirred-tank bioreactor;
(b) Sparged stirred-tank bioreactor through which sterile air bubbles are sparged

Chapter 10
Biotechnology and Its Application

Introduction:

Biotechnology essentially deals with industrial scale production of Applications in Agriculture biopharmaceuticals and biologicals using genetically modified microbes, fungi, plants and animals. It finds application in medicine, therapeutics, diagnostics, bioremediation, agriculture, waste Applications in medicine , treatment, food science (processed food) and energy production.

The thrust areas of biotechnologies Include :

1. Improved organism mostly a microbe or pure enzyme acting as the best catalyst.
2. Providing optimum conditions through engineering for catalyst to act.
3. Downstream processing technologies to purity the protein/organic compound.

This chapter explains/describes the applications of gene cloning, PCR and other DNA analysis techniques in biotechnology, medicine and agriculture. It also sheds light onto the ethical implications of manipulating the genomes of microbes, plants and animals.

Biotechnology can be defined as the use of biological processes in industry and technology, one of the reasons why biotechnology has received so much attention during the past three decades is because of gene cloning.

Although many useful products can be obtained from microbial culture, the list in the past has been limited to those compounds naturally synthesized by microorganisms. Many important pharmaceuticals, which are produced not by microbes, but by higher organisms, could not be obtained in this way. This has been changed by the application of gene cloning to biotechnology. The ability to clone genes means that a gene for an important animal or plant protein cannot be taken from its normal host, inserted into a cloning vector, and introduced into a bacterium.

If manipulations are performed correctly the gene will be expressed and the recombinant protein synthesized by the bacterial cell. It may then be possible to obtain large amount of the protein through batch cultures or continuous cultures.

BIOTECHNOLOGICAL APPLICATIONS IN AGRICULTURE

In the last century, an all-round development in various fields significantly improved the quality of life of the people that consequently had an explosive impact on the growth of population.

Such increase in growth rate would lead to an absolute scarcity of basic requirement such as food. Many people had sensed this imminent calamity/disaster and were trying to work towards finding a solution.

Three options were considered for increasing food production:

(i) Agro-chemical based agriculture

This involves the use of agrochemicals that is chemical products used in agriculture. It includes broad range of pesticides, including insecticides, herbicides and fungicides. They also include synthetic fertilizers, hormones and other chemical growth agents and concentrated stores of raw animal manure.

(ii) Organic agriculture/farming

In this, farmers use manure, biofertilizers, biopesticides and biocontrols to increase the crop production instead of using artificial fertilizers and pesticides. Organic farming works in harmony with nature rather than against it. This involves using techniques to achieve good crop yields without harming the natural environment or people who live and work in it.

(iii) Genetically engineered crop-based agriculture

Among the pioneers, was Norman E. Borlaug recognised as father of Green Revolution.

What is Green Revolution?

Period in which significant increase in agricultural productivity of grains (particularly wheat and rice) was observed in 20th century, resulting from

(i) Introduction of improved crop varieties i.e., high-yielding varieties.

(ii) Better management practices (irrigation and soil conservation technique).

(iii) Use of agrochemicals (fertilizers or pesticides).

Its early dramatic success was in Mexico and the Indian subcontinent.

The green revolution succeeded in **tripling** the food supply but yet it was not enough to feed the growing human population.

Hinderances: Green Revolution was not a complete success in developing world due to various factors:

→ For farmers in developing world, agrochemicals are often too expensive.

→ Further increases in yield with existing varieties are not possible using conventional breeding.

→ The new varieties required large amounts of fertilizers, and pesticides to produce high yields, raising concern about cost and potentially harmful effects.

→ Minimising the use of fertilizers and chemicals so that their harmful effects on the environment are reduced.

For overcoming the above stated problems

Use of genetically modified crops is a possible solution

Gene cloning provides a new dimension to crop breeding by enabling directed changes to be made to the genotype of a plant, circumventing the random processes inherent in conventional breeding. Two general strategies have been used :

(a) Gene addition : in which cloning is used to alter the characteristics of a plant by providing it with one or more new genes.

(b) Gene Subtraction : in which genetic engineering techniques are used to inactivate one or more of the plant's existing genes.

Plants, bacteria, fungi and animals whose genes have been altered by manipulation are called **genetically modified organism (GMO) or transgenic organisms.**

Transgenic plants have been useful in many ways. For instance genetic modification has:

(i) Made crops more tolerant to abiotic stresses (cold, drought, salt, heat).

(ii) Reduced reliance on chemical pesticides (pest-resistant crops).

(iii) Helped to reduce post-harvest losses.

(iv) Increased efficiency of mineral usage by plants (this prevents early exhaustion of fertility of soil).

(v) Enhanced nutritional value of food, e.g., Vitamin 'A' enriched rice.

The Science behind Golden Rice : Golden rice was developed to combat vitamin A and Iron deficiency as this could accumulate more β (beta) - carotene.

GM plants have been used to create tailor-made plants to supply :

(i) Pharmaceuticals : Production of Hirudin by Brassica napus.

(ii) Alternative resources to industries in the form of fuel : Oil seed is- at present used mainly for margarine and other food oils, but it can be modified to produce fatty acids for detergent, substitute fuels and petrochemicals.

Plants that make their own insecticides

Plants are subject to predation by virtually all other types of organism - viruses, bacteria, fungi and animals but in agricultural settings the greatest problems are caused by insects. To reduce losses, crops are regularly sprayed with insecticides. Application of biotechnology led to development of pest resistant plants which could decrease the amount of pesticide used as discussed below

The endotoxin of Bacillus thuringiensis

Bt cotton :

Some strains of **Bacillus thurigiencies (**Bt) produce proteins that kill certain insects such as

Lepidopterans (Tobacco budworm, armyworm)

Coleopterans (Beetles)

Dipterans (Flis, mosquitoes)

Bt forms protein crystals, (Toxic insecticidal protein) during a particular phase of their growth.

This toxin exist as inactive protoxins but once ingested by insect it is converted into an active form of toxin due to alkaline pH of the gut. The activated toxin binds to surface of midgut epithelial cells and create pores that causes death of the insect.

Specific Bt toxin genes were isolated from Bt and incorporated into several crop plants (e.g. Cotton, Corn, etc.)

The choice of genes depends upon the crop and the targeted pest as most Bt toxins are insect group specific. The toxin is coded by cry gene.

Protein encoded by gene cry lAc & IIab control cotton bollworm and cry l Ab controls corn borer.

Gene Subtraction

This is another method of changing the-genotype of a plant by either inactivation of the gene or sometimes knockout of the gene. There are several possible strategies for inactivating a chosen gene in a living plant the most successful so far in practical terms being the use of anti-sense RNA.

Use of anti-sense RNA in creating pest resistant plants

Several nematodes parasite a wide variety of plants and animals including human beings. They attack nearly every food and fibre crop grown by invading the plants roots. It feeds on the roots cells causing roots to grow into large galls/knots, damaging the crop and reducing its yield. Hence, called root-knot nematode.

A nematode **Meloidogyne incognita** infect the roots of tobacco plants causing a great reduction in yield. The most cost effective and sustainable management tactic for preventing such damage was to bioengineer resistant plants that prevent the nematode from feeding on the roots.

The strategy adopted to prevent this infestation is based on the process of RNA interference (RNAi)

RNAi is a naturally occurring mechanism that leads to the "silencing" of genes. In consequence, the respective protein is no longer synthesized. In nature, this mechanism is used for the regulation of specific genes and is also applied as a defense against viruses. In research, this technique has been used for loss of function studies where a gene (responsible for parasitism) is specifically silenced. It takes place in all eukaryotic **organisms as a method of cellular defense.** This method involves silencing of a specific mRNA due to formation of dsRNA molecule formed by binding of complementary RNA (anti-sense RNA) molecule to original, thereby preventing translation of the original mRNA (silencing). The source of this complementary RNA could be from infection by viruses having RNA genomes or mobile genetic elements (transposons) that replicate via an RNA intermediate.

Using Agrobacterium vectors, nematode specific genes (responsible for parasitism) were introduced into the host plant. The introduction of DNA was such that it produced both sense anti-sense RNA in the host cells forming dsRNA. These two RNA's being complementary to each other formed a dsRNA that was taken upby the parasitic nematode and initiated RNAi, thus silenced the specific mRNA of the nematode. The consequence was that the parasite could not survive in a transgenic host expressing specific interfering RNA. The transgenic plant therefore got itself protected from the parasite.

BIOTECHNOLOGICAL APPICATIONS IN MEDICINE

The recombinant DNA technologies processes have made immense impact in the area of healthcare

Advantages of recombinant therapeutics :

1. Enables mass production
2. Safe and more effective drugs
3. Do not induce unwanted immunological responses as is common in case of similar products isolated from non-human sources.

Biopharmaceuticals : Biopharmaceuticals are medical drugs produced using biotechnology. They include proteins (including antibodies), nucleic acids (DNA, RNA or anti-sense oligonucleotides) etc.

Recombinant Insulin/Genetically Engineered Insulin

Insulin, synthesized by β cells islets of Langerhans in the pancreas, controls the level of glucose in the blood. An insulin deficiency manifests itself as diabetes mellitus whose symptoms can be alleviated by a continuing program of insulin injections, thereby supplementing the limited amount of hormone synthesized by the patient's pancreas. Insulin used for diabetes was earlier extracted form pancreas of slaughtered cattle and pigs. Animal insulin is generally satisfactory, though caused allergy in some patients.

Insulin cannot be orally administered to diabetic patient because it degrades in alimentary canal.

Two features that facilitated production of insulin by recombinant DNA techniques.

- It is not modified after translation by the addition of sugar molecules
- Insulin is a relatively small protein, comprising two polypeptides, one of 21 amino acids (the A chain)and the other of 30 amino acids (the B chain) that are linked together by disulphide bonds/bridges.

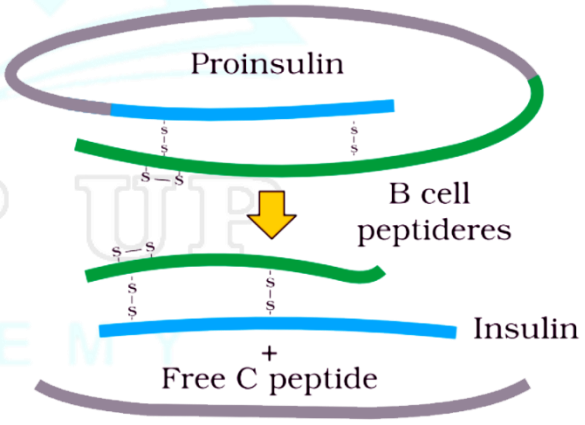


Fig. : Maturation of pro-insulin into insulin after removal of C-peptide (to be simplified)

In mammals including humans, insulin is synthesized as a pro-hormone (like a pro-enzyme, the pro-hormone also needs to be processed before it becomes a fully mature and functional hormone) and the gene for this protein synthesis is located on **chromosome 11**, this prohormone which contains an extra stretch called the **C peptide**. This C peptide is not present in the mature insulin and is removed during maturation into insulin The main challenge for production of insulin using rDNA techniques was getting insulin assembled into a mature form. In 1983, **Eli Lilly an American company prepared two DNA sequences** corresponding to A and B chains of human insulin and

introduced them in plasmids pBR322 and then transformed E.coli to produce insulin chains. Sequences for A and B chains were linked with lac z gene and introduced into pBR322. The transformed E.coli could be selected by blue-white screening. **Chains A and B were produced separately, extracted and combined by creating disulfide bonds to form human insulin.**

Gene Therapy

This is the name originally given to methods that aim to cure an inherited disease by providing the patient with a correct copy of the defective gene. Gene therapy has now been extended to include attempts to cure any disease by introduction of a cloned gene into the patient-If a person is born with a hereditary disease, then gene therapy can be a corrective therapy for such a disease.

Gene therapy is a collection of methods that allows correction of a gene defect that has been diagnosed in a child/embryo. Here genes are inserted into a person's cells and tissues to treat a disease. Correction of a genetic defect involves delivery of a normal gene into the individual or embryo to take over the function and compensate for the non-functional gene.

The first clinical gene therapy was given in 1990 to a four-year old girl with adenosine deaminase **(ADA deficiency**). This enzyme is crucial for the immune system to function because in its absence lymphocyte proliferation is inhibited. Without T cells, ADA-deficient children are wide open to the attacks of viruses and bacteria. The SCID (Severe Combined Immunol deficiency Disorder) is caused due to deletion of gene for adenosine deaminase.

In some children, ADA deficiency can be treated by bone marrow transplantation in others it can be treated by enzyme replacement therapy (ERT). In ERT, patient is given an intravenous injection of ADA or enzyme lacking in the content. But the problem with both approaches is that they are not completely curative.

As a first step towards gene therapy, lymphocytes from the blood of the patient are grown in a culture outside the body. A functional ADA cDNA (using a retroviral vector) is then introduced into these lymphocytes, which are subsequently returned to the patient. However, as these cells are not immortal, the patient requires periodic infusion of such genetically engineered lymphocytes. However, if the gene isolated from marrow cells producing ADA is introduced into cells at early embryonic stages, **it could be a permanent cure.**

Molecular Diagnosis

For effective treatment of a disease, early diagnosis and understanding the pathophysiology is very important. The term diagnosis refer to the act or process of determining the nature and cause of a disease through evaluation of patient history, examination and review of laboratory data. In short, diagnosis is what the disease is e.g., you can have a diagnosis of asthma.

Pathophysiology is the study of changes in normal, mechanical, physical and biochemical functions caused by a disease.

Presence of a pathogen (bacteria viruses) is normally suspected only when the pathogen has produced a disease symptoms. By this time, the concentration of pathogen is already very high in the body. Methods of detection fall under two categories :

Method of Diagnosis	
Traditional	**Modern**
1. Conventional method	1. Recombinant DNA technology (RDT)
2. Known biology or association of single analysis (Glucose, cholesterol etc.)	2. Polymerase chain reaction (PCR)
3. Early detection is not possible	3. Enzyme Linked Immunol Sorbent Assay (ELISA)
4. Involves serum and urine analysis	4. Serve purpose of early diagnosis

Details of modern methods of diagnosis :

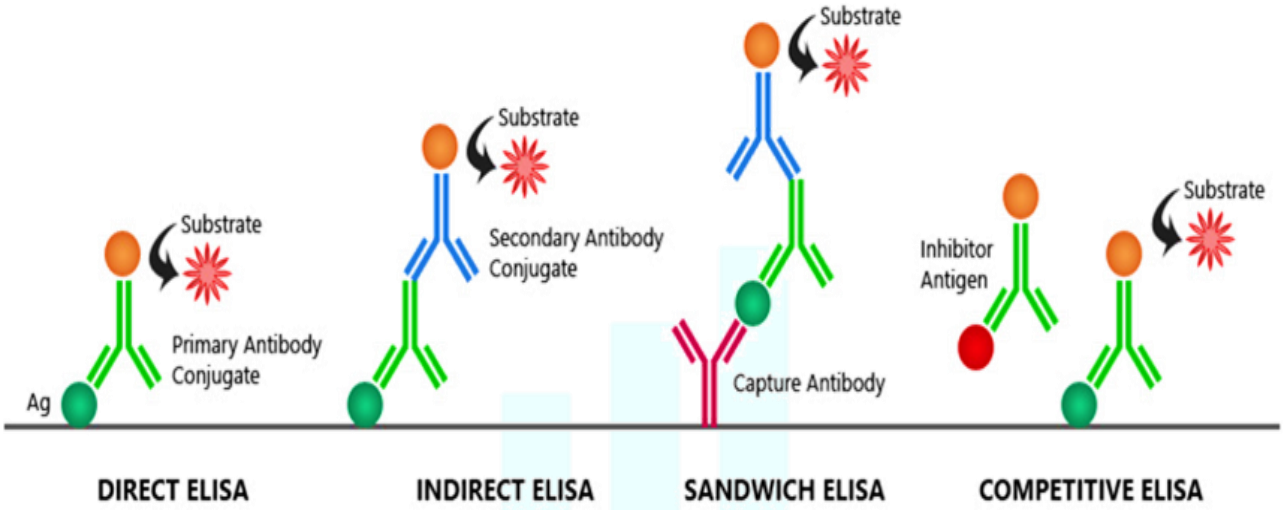
1. **PCR**
   - It helps to detect very low concentration of bacteria or virus at the time when the symptoms of the disease are not visible by amplification of their nucleic acid.
   - PCR is now routinely used to detect HIV in suspected AIDS patient.
   - It is being used to detect mutations in genes in suspected cancer patients too.
   - It is a powerful technique to identify many other genetic disorders.
2. **ELISA -**
   - Based on the principle of Antigen-Antibody interactions.
   - Infection by pathogen can be detected by the presence of antigens (proteins, glycoproteins) or by detecting the antibodies synthesized against the pathogen.

ELISA : ELISA is an extremely sensitive test that is used to detect antibodies (Ab) or specific antigens (Ag). It is carried out in a 96 well microtiter plate. The direct ELISA is a test for the presence of antigen. In this procedure, a known antibody is adsorbed to the inside of the well in a

microtiter plate. After rinsing to remove excess antibody, the sample suspected of containing the antigen is added. Next, an enzyme linked Ab that can react with Ag is added. If Ag is put in the well, the enzyme linked Ab binds to it and is retained. The colourless substrate for the enzyme is added. Development of colour indicates the presence of the Ag.

In indirect ELISA, Ag is added to the microtiter plate well and the Ag attaches to the walls of the micrometer plate. After rinsing, to remove excess Ag the serum suspected of containing the Abs is added. Enzyme linked antibody capable of reacting with the constant region of other Abs is then added, followed by addition of the colourless substrate. Development of colour indicates the presence of Ab being identified.

3. Many techniques used for early diagnosis based on RDT include RFLP, VNTR, DNA sequencing, etc.



TRANSGENIC ANIMALS

Animals that have had their DNA manipulated to possess and express an extra (foreign) gene are known as transgenic animals. The genome of their animals has been changed and they can carry genes from other species. Examples of transgenic animals include rats, rabbits, pigs, sheep, cow, monkey and fish although over **95% transgenic animals are mice.**

Transgenic Monkey : ANDI was the first genetically modified monkey. The GFP (green fluorescent protein) gene was inserted into the monkey's chromosome.

Why are these transgenic animals being produced?

The two most common reasons are :

1. Some transgenic animals are produced from specific economic trait.
2. Other transgenic animals are produced as disease models (animals genetically manipulated to exhibit disease symptoms so that effective treatment can be studied).

Benefits from transgenic animals can be studied broadly under 3 heads :

Medicine	Agriculture	Industry
Cows that produce human protein enriched milk.	Larger sheep that grow more wool.	Goats that produce spider silk for materials production.

1. Medicine

(i) Normal physiology and development: Transgenic animals can be specifically designed to allow the study of how genes are regulated, and how they affect the normal functions of the body and its development, e.g., study of complex factors involved in growth such as insulin-like growth factor. By introducing genes from other species that alter the formation of this factor and studying the biological effects that result, information is obtained about the biological role of the factor in the body.

(ii) Study of disease: Many transgenic animals are designed to increase our understanding of how genes contribute to the development of disease. These are specially made to serve as models for human diseases so that investigation of new treatments for diseases is made possible. Today transgenic models exist for many human diseases such as cancer, cystic fibrosis, rheumatoid arthritis and Alzheimer's disease.

(iii) Biological products: Medicines required to treat certain human diseases can contain biological products, but such products are often expensive to make. Transgenic animals that produce useful biological products can be created by the introduction of the portion of DNA (or genes) which codes for a particular product such as human protein (α-1-antitrypsin**) used to treat emphysema.** Similar attempts are being made for treatment of

phenylketonuria (PKU) and cystic fibrosis. In 1997, the **first transgenic cow Rosie,** produced human protein-enriched milk (2.4 grams per liter). The milk contained the human **alpha lactalbumin** and was nutritionally a more balanced product for human babies than natural cow-milk.

(iv) Vaccine safety: Transgenic mice are being developed for use in testing the safety of vaccines before they are used on humans. **Transgenic mice are being used to test the safety of the polio vaccine.** If successful and found to be reliable, they could replace the use of monkeys to test the safety of batches of the vaccine.

(v) Chemical safety testing: This is known as toxicity/safety testing. The procedure is the same as that used for testing toxicity of drugs. Transgenic animals are made that carry genes which make them more sensitive to toxic substances than non-transgenic animals. They are then exposed to the toxic substances and the effects studied. Toxicity testing in such animals will allow us to obtain results in less time.

2. Agriculture

(i) Breeding : Farmers have always used selective breeding to produce animals that exhibit desired traits (e.g., increased milk production, high growth rate). Traditional breeding is a time-consuming, difficult task. When technology using molecular biology was developed, it became possible to develop traits in animals in a shorter time and with more precision. In addition, it offers the farmer an easy way to increase yields.

(ii) Quality : Transgenic cows exist that produce more milk or milk with less lactose or cholesterol, pigs and cattle that have more meat on them, and sheep that grow more wool. In the past, farmers used growth hormones to spur the development of animals but this technique was problematic, especially since residue of the hormones remained in the animal product.

(iii) Disease resistance : Scientists are attempting to produce disease-resistant animals, such as influenza-resistant pigs, but a very limited number of genes are currently known to be responsible for resistance to diseases in farm animals.

3. Industrial Applications

Toxicity-sensitive transgenic animals have been produced for chemical safety testing. Microorganisms have been engineered to produce a wide variety of proteins, which in turn can produce enzymes that can speed up industrial chemical reactions.

ETHICAL ISSUES

Bioethics

Ethics includes a setoff standard by which a community regulates its behavior and decides as to which activity is legitimate and which is not. Therefore, **bioethics** may be viewed as a set of standards that maybe used to regulate our activities in relation to the biological world.

The manipulation of living organisms by the human race cannot go on any further, without regulation. Some ethical standards are required to evaluate the morality of all human activities that might help or harm living organisms. Going beyond the morality of such issues, the biological significance of such things is also important. Genetic modification of organisms can have unpredictable results when such organisms are introduced into the ecosystem.

Therefore, the Indian Government has set up organizations such as GEAC (Genetic Engineering Approval Committee), which will make decisions regarding the validity of GM research and the safety of introducing GM-organisms for public services.

The major bioethical concerns pertaining to biotechnology are summarised below :

(i) Use of animals in biotechnology causes great suffering to them.

(ii) When animals are used for production of pharmaceutical proteins, they are virtually reduced to the status of a 'factory'.

(iii) Introduction of a transgene from one species into another species violates the 'integrity of species'.

(iv) Transfer of human genes into animals (and vice-versa) dilutes the concept of humanness'

(v) Biotechnology is disrespectful to living beings, and only exploits them for the benefit of human beings.

(vi) Biotechnology may pose unforeseen risks to the environment, including risk to biodiversity.

The modification/usage of living organisms for public services (as food and medicine sources, for example)has also created problems with patents granted for the same.

Patent

A set of exclusive right granted by a state (national government) to an inventors or their assignee for a limited period of time in exchange for public disclosure of an invention. Patents are supposed to satisfy three criteria of – Novelty, non-obviousness and utility.

Novelty implies that the innovation must be new. It cannot be part of ‘prior art’ or existing knowledge. Non-obviousness implies that it may not be documented but is otherwise well-known. The discored fact or product should be of a particular use for the human beings.

A patent is granted for (a) an invention (including a product), (b) an improvement in an earlier invention, (c) the process of generating a product, and (d) a concept or design. Initially, patents were granted for industrial inventions, etc. But at present, patents are being granted for biological entities and for products derived from them. these patents are called **bio patents.** Primarily, industrialized countries, like U.S.A., Japan and members of European Union, are awarding Bio patents.

There is growing public anger that certain companies are being granted patents for products and technologies that make use of the genetic materials, plants and other biological resources that have long been identified, developed and used by farmers and indigenous people of a specific region/country.

Rice is an important food grain, the presence of which goes back thousands of years in Asia's agricultural history. There are an estimated **200,000 varieties of rice** in India alone. The diversity of rice in India is one of the richest in the world. Basmati rice is distinct for its unique aroma and flavour and **27 documented varieties of Basmati** are grown in India. There is reference to Basmati in ancient texts, folklore and poetry, as it has been grown for centuries.

Controversies in India regarding patent and biopiracy:

(i) Basmati rice: In September 1997, a Texas company called Rice Tec won a patent on "basmati rice lines and grains". The patent secured lines of basmati and basmati-like rice and ways of selecting that rice for breeding. Rice Tec, owned by Prince Hans-Adam of Liechtenstein, international outrage over allegations of biopiracy. It has also caused a brief diplomatic crisis between India and United States with India threatening to take the matter to WTO (World Trade Organisation) as a violation of TRIPS (trade- related aspects of intellectual property rights) which could have resulted in a major embarrassment for the United States. Both voluntarily, and due to review decisions by the United States patent Office, Rice Tec lost most of the claims of the patent.

(ii) Turmeric

(iii) Neem

Biopiracy is the term used to refer to the use of bio-resources by multinational companies and other organizations without proper authorisation from the countries and people concerned without compensatory payment.

Most of the industrialised nations are rich financially but poor in biodiversity and traditional knowledge. In contrast the developing and the underdeveloped world is rich in biodiversity and traditional knowledge related to bio-resources. Traditional resources include all those organisms that can be used to derive commercial benefits. Traditional knowledge related to bio resources is the knowledge developed by various communities over long periods of history regarding the utilisation of bio resources e.g., use of herbs, drugs etc. Traditional knowledge related to bio-resources can be exploited to develop modern applications and can also be used to save time, effort and expenditure during their commercialization.

There has been growing realisation of the injustice, inadequate compensation and benefit sharing between developed and developing countries. Therefore some nations are developing laws to prevent such unauthorized exploitation of their bio-resources and traditional knowledge. A west African plant, Pentadiplandra brazzeana produces a protein called brazzein, which is approximately 2,000 times as sweet as sugar. In addition, brazzein is a low-calorie sweetener. Local people have known and used the super-sweet berries of this plant for centuries. But the protein brazzein was patented in U.S.A. Subsequently, the gene encoding brazzein was also isolated, sequenced and patented in U.S.A. It is proposed to transfer the brazzein gene into maize and express it in maize kernels. These kernels will then be used for the extraction of brazzein. This development could have serious implications for countries exporting large quantities of sugar.

Chapter 11
Organisms and Populations

1. Term ecology was first used by **Reiter** in 1868.
2. **Ernst Haeckel** (1886) first correctly defined ecology as the science dealing with reciprocal relationship or organisms and the external would.
3. **Prof. R. Mishra** is known as father of ecology in India. **Warming** is known as father of plant ecology.
4. **E.P. Odum –** defined it as the "study of structure and function of nature".

LEVELS OF ORGANISATION

The various levels of ecological organization are organism, population, community, ecosystem landscape, biome and biosphere.

1. Organisms

They are basic unit of study in ecology. At the level of organism, we intend to understand the form, physiology, behavior, distribution and adaptations in relation to the environmental conditions.

2. Population

It is a grouping of individuals of the same species inhabiting a given area.

3. Biological community

It is an assemblage of population of different species present in an area which show interdependence and interaction like competition, predation, host-parasite interaction, amensalism etc.

4. Ecosystem

It is composed of a biological community, integrated with its physical environment through the exchange of energy and recycling of the nutrients.

5. Landscape

A unit of land with a natural boundary having mosaic of patches which generally represent different ecosystems.

6. Biome

A large regional unit characterized by a major vegetation type and associated fauna in a specific climatic zone.

7. Biosphere

It is life supporting zone which comprises all the earth's terrestrial and aquatic biomes.

Ecology is basically concerned with four levels of biological organization – organisms, population, communities and biomes.

Terrestrial Biomes

1. Tundra
2. Taiga (coniferous forest)
3. Temperature forest
4. Grasslands
(i) Temperate grassland
(ii) Tropical savanna grassland (grass cover with scattered trees)
5. Desert
6. Tropical rain forest.

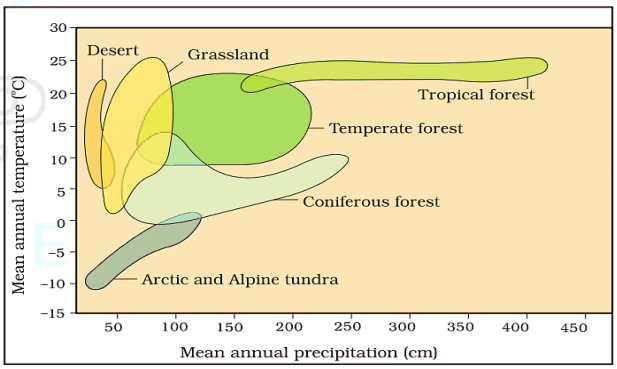


Fig. : Biome distribution w.r.t. annual temperature and precipitation

Amongst these temperature, water, light and soil are some of the key components that lead to so much variations in the physical and chemical conditions of different habitats.

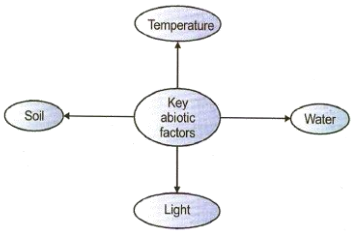


Fig. : Key abiotic factors

Responses to abiotic factors

Based upon thermal tolerance, organisms are classified into two categories

Stenothermal	Eurythermal
Such organisms live in areas where the temperature is uniform throughout the year. These organisms cannot tolerate large temperature variations and thus, restricted to narrow range of temperatures. Vast majority of organisms belong to this category. e.g., polar bears, lizards Abides.	A few organisms can tolerate and thrive in a wide range of temperatures. They are called eurythermal. Such organisms can tolerate large changes in temperature. e.g., most of the mammals and birds.

The salt concentration (measured as salinity in parts per thousand) for water bodies is given below:

Water bodies	Salinity (parts per thousand)
Inland water	< 5
Sea	30-35
Hyper saline lagoons	> 100

Based up on salinity tolerance, organisms are classified into two categories.

Euryhaline	Stenohaline
Euryhaline are organisms which tolerate a wide range of salinities (e.g., Salmon)	Stenohaline are those which are restricted to a narrow range of salinities (e.g., Shark). Due to osmotic problems and lack of adaptations, many fresh water animals cannot live for long in sea water and vice versa.

Light

Light is the visible part of electromagnetic spectrum (390-760 nm). Photosynthetically active radiations (PAR) have a range of 400-700 nm. Radiations below the visible light are ultraviolet (UV) radiations while those above the visible light are infra-red or heat waves. Amount of light and its intensity vary with latitude and season.

Important of light for plants: Importance of light can be understood by its role in the process of photosynthesis production of organic food by autotrophs. Depending upon requirement of light intensity, plants are of two types (a) heliophytes, require high intensity light and (b) Schizophytes, require low intensity light and grow in shaded area. Many species of small plants (herbs and shrubs) growing in forests are adapted to photosynthesise optimally under very low light condition (sciophytes), because they are constantly overshadowed by tall, canopied trees (heliophytes). Many plants are also dependent on sunlight to meet their photoperiodic requirement for flowering. Light also affects other processes like growth, reproduction, movements and phenology in plants.

Importance of light for animals: Many animals use the delusional and seasonal variations in light intensity and duration (photoperiod) as cues for timing their foraging, reproductive and migratory activities.

The availability of light on land is closely linked with that of temperature since, the sun is the source for both (sun is the ultimate source of all type of energy). Deep in the oceans (>500 m), the environment is perpetually dark and its inhabitants are not aware of the existence of a celestial source of energy called Sun. Such organisms, in majority are decomposers/consumers and they are dependent upon food material derived from producers found in upper light regime of oceans.

UV-C and about half of UV-B radiations are absorbed by ozone layer of stratosphere. A large amount of the rest is dissipated by particles of troposphere, only a small amount reaches on the earth.

Light Zonation of Lakes:

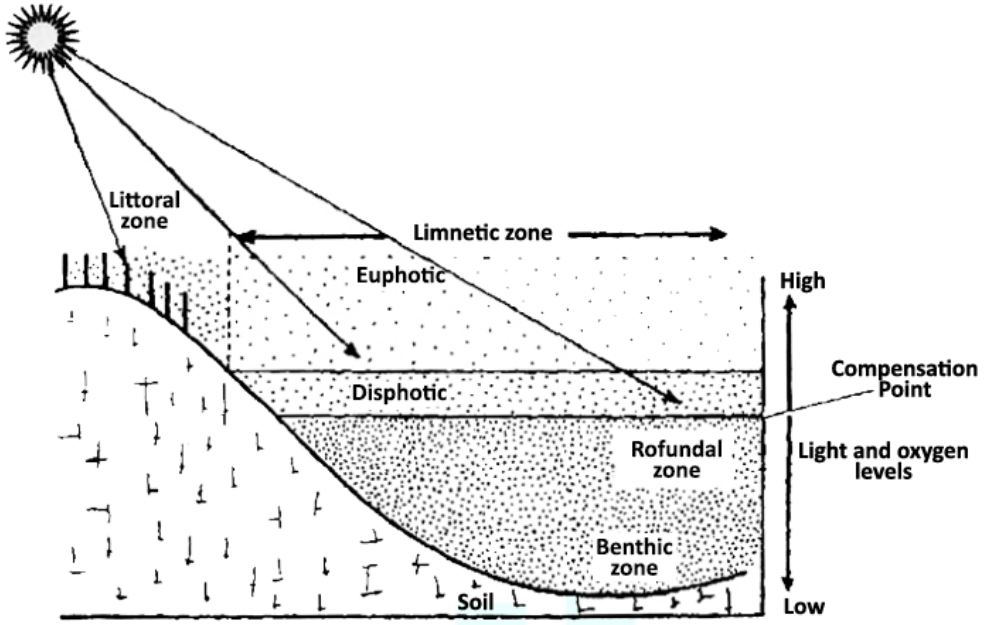


Fig.: Zonation in deep lake showing gradient of light and oxygen

Littoral zone Exposed to wave action and is highly productive

Limnetic zone Open water body, rich in planktons.

Euphotic zone Receives maximum light above light compensation point.

Disphotic zone Receives diffuse light at or below light compensation point. Also known as twilight zone.

Profundal (Dark) zone No light

Benthic zone : It is the bottom zone of perpetual darkness.

Responses to Abiotic Factors

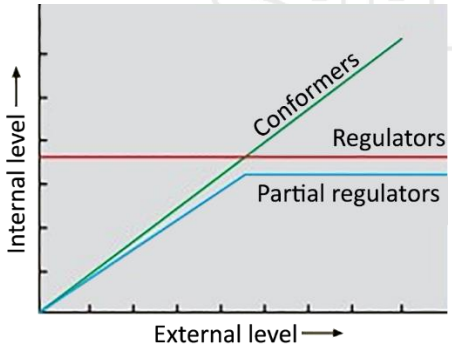


Fig.: Diagrammatic representation of organismic response

There appears various possibilities of responses of organisms towards fluctuating environmental conditions such

(1) Regulate : Some organisms like **all birds and mammals and a very few lower vertebrate and invertebrate species.**

(2) Conform : majority (99 percent) of animals and nearly all plants

(3) Migrate :

(4) Suspend :

(1) Regulate : Some organisms like **all birds and mammals and a very few lower vertebrate and invertebrate species,** are able to maintain homeostasis by physiological as well as behavioural means also which ensures constant body temperature (thermoregulation), constant osmotic concentration (osmoregulations) etc. plants do not have mechanisms to maintain internal temperatures.

Evolutionary biologists believe that the "success" of mammals is largely due to their similar to those of humans. Humans have a constant body temperature i.e., 37°C. The evaporative cooling by profuse sweating from body during summer brings down the body temperature. In winter, when the temperature of body is much lower than 37°C, we start shivering, a kind of exercise which produces heat and raises the body temperature.

(2) Conform : An overwhelming **majority (99 percent) of animals** and **nearly all plants** cannot maintain a constant internal environment. Their body temperature changes with the ambient temperature. In aquatic animals, the osmotic concentration of body fluids changes with that of the ambient water osmotic concentration. These animals and plants are simply **conformers.**

Considering the benefits of a constant internal environment to the organism, a question might be budding in your mind **"Why the conformers had not evolved to become regulators?".** Conformers had not

evolved mechanisms like regulators because, **thermoregulation and osmoregulation mechanisms are energetically expensive for many organisms particularly for small animals like shrews and humming birds.** Heat loss or heat gain is a function of surface area. Since small animals have a larger surface area relative to their volume, they tend to lose body heat very fast when it is cold outside, therefore, they have to spend much energy to generate body heat through metabolism. This is the main reason why very small animals are rarely found in polar regions. Thus, the **costs** and **benefits** of maintaining a constant internal environment are taken into consideration during course of evolution.

Some species have evolved the ability to regulate but only over a limited range of environmental conditions beyond which they simply conform. They are known as **partial or limited regulators.**

Organisms follow two other alternatives (migrate and suspend) under localized or short duration stressful external conditions.

(3) Migrate : The organisms can move away temporarily from the stressful habitat to a more hospitable area and return when stressful period over. Many animals, particularly birds, during winter undertake long distance migrations to move to hospitable areas. Every winter the famous **Keolado National Park (Bharatpur) in Rajasthan** hosts thousands of migratory birds coming from Siberia and other extremely cold northern regions.

(4) Suspend : It is the stage in life cycle where an organism changes its developmental, physiological, structural, and biochemical behavior to pass through unfavourable conditions. Different organisms produce different types of perennating structures to overcome adverse environmental conditions.

Thick-walled spores are formed in bacteria, fungi and lower plants (algae) to overcome unfavourable conditions. Higher plants produce dormant structures like seeds and other vegetative propagules (structures) as means to overcome unfavourable conditions. These structures germinate and produce new organisms when conditions become favourable.

Some organisms avoid the stress by escaping in time. For example, bear escapes in time during winter by a process called **hibernation** (over wintering) whereas, snails and fish escape in time by another mechanism called **aestivation** (over summer). A stage of suspended development **(diapauses)** is found in many zooplanktons species in lakes and ponds.

ADAPTATIONS

(i) Kangaroo rat, for example, in North American deserts is capable of meeting all of its water requirements through its internal fat oxidation where water is released as by- product. It can also concentrate its urine so that minimal volume of water is used to remove excretory products.

(ii) Many desert plants (xerophytes) have a thick cuticle on their leaf surface and have their stomata arranged in deep pits (sunken) to minimize water loss through transpiration. They also have a special photosynthetic pathway called CAM (Crassulacrean Acid Metabolism) that enables their stomata to remain closed during day time (Scot active stomata open at night only). In some desert plants like Opuntia, leaves are stem becomes flattened, green-coloured (phylloclade) and performs photosynthetic activity of plant.

(iii) Mammals from colder climates generally have shorter ears and limbs (extremities) to minimize heat loss. This is called as Allen's rule.

(iv) In the polar sea, a thick layer of fat (blubber) is found in aquatic mammals like seals below their skin. This acts as an insulator and reduces loss of body heat.

(v) Altitude sickness can be experienced at high altitude where body not get enough oxygen due to low atmospheric pressure and causes nausea, fatigue and heart palpitations. Under these conditions, body increases RBCs production, decreases binding capacity of haemoglobin and increases breathing rate. These physiological adaptations allow organisms to respond quickly to stressful conditions.

(vi) In most of animals, the metabolic reactions of entire physiological functions proceed optimally in a narrow temperature range. In humans, it occurs at 37°C temperature. However, there are microbes like Archaebacteria that flourish in hot springs and deep-sea hydrothermal vents where temperatures far exceed 100°C.

(vii) Antarctic fishes can survive below O°C. The body fluid of these fishes contains antifreeze solutes by which they can manage to keep their body fluids from freezing.

(viii) A large variety of marine invertebrates and fishes are adapted biochemically to survive great depths in the ocean where the pressure (called crushing pressure) could be > 100 times than the normal atmospheric pressure.

(ix) Some organisms like desert lizards lack the physiological ability to cope with extreme temperature but manage the body temperature by behavioral means. They bask in the sun and absorb when their body temperature drops below the comfort zone. But move into shade when the ambient temperature starts increasing.

POPULATIONS

Population Attributes

A population has certain attributes that an individual organism does not possess. Following are some important characteristics of a population:

(i) Birth and death rates: These rates express the change in numbers (increase or decrease) w.r.t. members of population . These rates refer to **per capita** births and deaths, respectively. A population has both birth and death rates but an individual may have birth and death only, not the birth and death rates. This can be illustrated by some examples.

(a) If in a pond there are 20 lotus plants last year and through reproduction, eight new plants are added, taking the current population to 28, we calculate the birth rate as 8/20 = 0.4 offspring per lotus per year.

(b) Similarly, we can understand death rates by taking an example. If 4 individuals in a laboratory population of 40 fructifies died during a specified time interval, say a week, the death rate in the population during that period is 4/40 = 0.1 individuals per fruit fly per week.

(ii) Sex ratio: Another attribute characteristic of a population is sex ratio. An individual is either a male or female but a population has a sex ratio *i.e.*, a population has both males and females. For example, if in a population of 1000 individuals, 600 individuals are females and rest of them are males than there is 60:40 sex ratio for females to males.

(iii) Population age: A population at any given time is composed of individuals of different ages: pre-reproductive, reproductive and post-reproductive. The representation of population age is carried out by construction of age pyramids. An **age pyramid** is a graphic representation of proportion of various age group of a population. In human populations, the age pyramids generally show age distribution of males and females in a combined diagram. The shape of the pyramids reflects the growth status of the population. There are three types of age pyramids:

(a) Triangular:

(b) Bell-shaped:

(c) Urn-shaped:

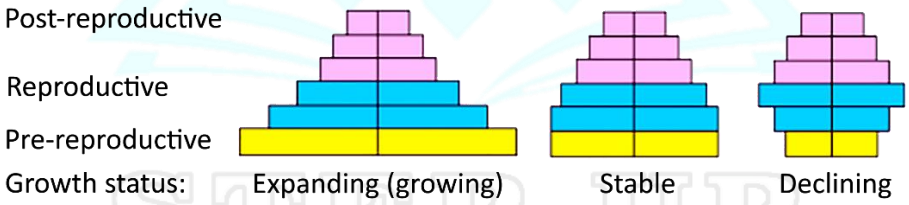
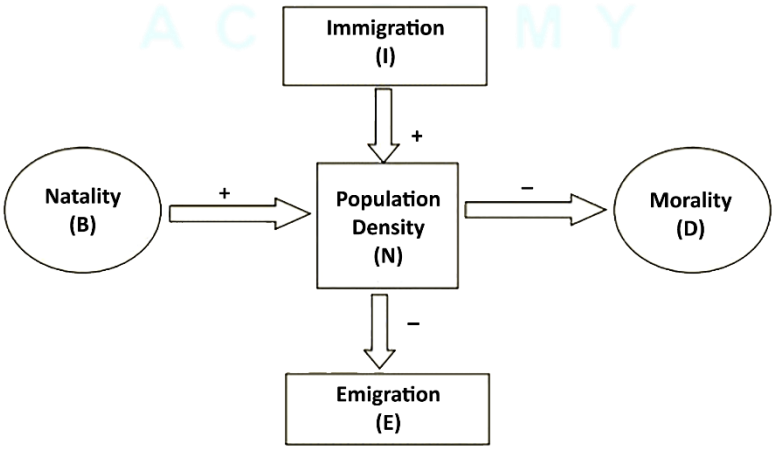


Fig. : Pepresentation of age pyramids for human population

POPULATIONS GROWTH



$N_{(t+1)} = N_t + [(B + I) – (D + E)]$

Where B – Number of births, I – Number of immigrants

D – Number of deaths. E – Number of emigrants

GROWTH MODELS

- Some organisms breed only once in their lifetime **(Pacific salmon fish, bamboo)** while others breed many times during their lifetime (most birds and mammals). Some produce a large number small-sized offsprings (Oysters, pelagic fishes) while others produce a small number of large-sized offsprings (birds, mammals.

1. **Exponential growth :** Also called as geometric fashion of growth occur when resources (food and space) are unlimited. According to Darwin, when resources in the habitat are unlimited, each species has the ability to realize fully its innate potential to grow in number. **This intrinsic rate of natural increase is called as biotic potential (r).** The value of r is an important parameter to assess impact of environmental factors on population growth. Following are the important aspects of exponential growth.

(i) Any increase or decrease in a population (N_o) during time t will be

$\frac{dN}{dt} = (b - d) \times N$,

Where, b = per capita birth rate.

d = per capita death rate,

if $(b - d) = r$, then $\frac{dN}{dt} = rN$

(r =intrinsic rate of natural increase)

(ii) The integral form exponential growth equation will be

$N_t = N_o e^{rt}$,

Were,

N_t = Population density after t time

N_o = Population density at time zero

e = The base of natural logarithms (2.71828)

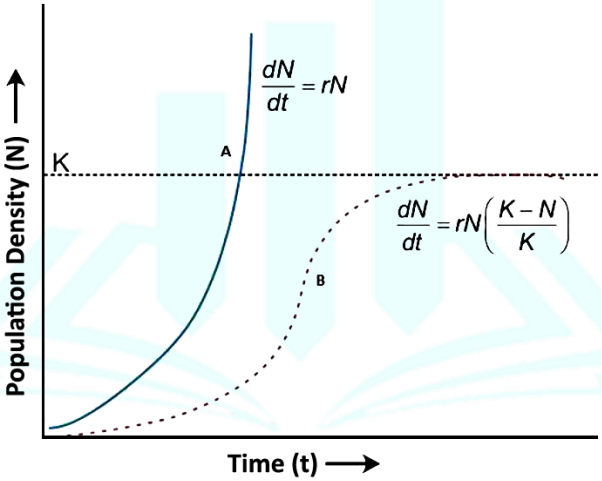


Fig. : Population growth curve (a) when responses are not limiting the growth, plot is exponential. (b) When responses are limiting K is carrying capacity.

(2) Logistic growth : It describes a situation in which resources present in the environment are limiting. In nature, a given habitat has enough resources to support a maximum possible number, beyond which no further growth is possible. This natural limit for that species in that species in that habitat is called carrying capacity (K). Thus, nature does not dispose/provide unlimited resources availability to permit exponential growth to population of any species. This leads to competition between individuals for limited resources and hence, the fittest individual will survive and reproduce.

The sum of environmental factors that limits the population size is called environmental resistance. Environmental resistance rises with the rise in population size. The influence of environmental resistance over the biotic potential is denoted by $\left(\frac{K-N}{K}\right)$.

Following are important aspects of logistic growth :

(i) A population growing in a habitat with limited resources shows initially a lag phase, followed by phases of acceleration then deceleration and finally an asymptole, when the population density reaches the carrying capacity.

(ii) Such a population growth is represented by a sigmoid curve.

(iii) This type of population growth is called **Verhulst Pearl Logistic Growth** (figure) and is described by the equation $\frac{dN}{dt} = rN\left(\frac{K-N}{K}\right)$.

Where, N = Population density at time t; r = Intrinsic rate of natural increase; K = Carrying capacity Since, resources for growth for most animal population are finite and become limiting sooner or later, the logistic growth model is considered a more realistic one.

Population Interactions

S.No.	Species A	Species B	Name of Interaction
(i)	+	+	Mutualism
(ii)	+	+	Protocooperation
(iii)	+	0	Commensalism
(iv)	-	-	Competition
(v)	+	-	Predation
(vi)	+	-	Parasitism
(vii)	-	0	Amensalism

'+'→Beneficial interaction, '-'→ Detrimental interaction, '0'→Neutral interaction

The details of all these types of interaction are as follows :

(1) Mutualism

It confers benefits on both the interacting species. It is an obligate association of two organisms where two organisms often live together and cannot live separately. Some of the examples of mutualism are :

(a) Plant and fungal mutualism. It includes :

(i) **Lichens :** It is a mutualistic relationship between a fungus and photosynthesizing green algae or cyanobacteria (blue-green algae). Algae (phycobiont) produced food through photosynthesis whereas fungi (mycobiont) absorb nutrients from soil.

(ii) **Mycorrhizae :** This is a mutualistic relationship between funi and roots or higher plants. Fungi absorb essential nutrients from soil while the plant in turn provide energy – yielding carbohydrates to the fungi.

(b) Plant – animal mutualism : Plants need help of animals for pollination their flowers (zoophily) and dispersing their seeds (Zoochory). Plant offers rewards in the form of pollen and nectar for pollinators and juicy and nutritious fruits for seed dispersers.

Plant-animal interactions often involve co-evolution of the mutualists. Co-evolution is an evolutionary mechanism where the change of a biological object is triggered by the change of the related object. The evolution of the flower and its pollinator species are tightly linked with one another.

(i) Fig and Fig wasp : These is a tight one-to-one relationship with the pollinator species of wasp and many species of fig trees. It means, that for that for each fig species there occur a partner wasp species without which pollination cannot occur. Female wasp uses the fig fruit as an oviposition (egg-laying site and developing seeds for nourishing its larvae. The wasp pollinates the fig inflorescence while searching for suitable egg-laying (oviposition) sites.

Fig. : Mutual relationship between fig tree and wasp : (a) Fig flower is pollinated by wasp; (b) Wasp laying eggs in a fig fruit

(ii) Orchids and bees : To ensure guaranteed pollination while attracting the right pollinator insect (bees and bumble bees), orchids have evolved a bewildering diversity of floral patterns.

Like fig, all orchids do not offer rewards to their insect pollinators. For example, the Mediterranean orchid Ophrys employs '**sexual deceit**' to get pollination done by a species of bee. One petal of its flower bears an uncanny resemblance to the female of the bee in size, colour and markings. The male bee perceives it as female and gets dusted with pollen from the flower during the process of pseudocopulation. When same bee pseudo-copulates with another flower, it transfers pollen to it and thus pollinates the flower.

The significance of co-evolution process can be understood here. If female bee changes its colour pattern ever slightly, the success of pollination will be reduced unless orchid flower co-evolves to maintain resemblance of its petal to female bee.

Fig. : Showing bee-a pollinator on orchid flower

(2) Protocooperation

Protocooperation is an association between individuals of two species, each of which is benefited by the presence of the other but can live equally well without association. Some examples of protocooperation are as follows :

(i) The crocodile bird (a plover) enters the mouth of a crocodile to feed on the parasitic leeches. The bird gets food and the crocodile gets rid of the blood-sucking parasites.

(ii) Sea anemone attached to the body of hermit crab protects it from the enemies with its nematocysts. In return, the anemone receives pieces of food dropped by the crab and is carried to new places by it.

(3) Commensalism

This is the interaction in which one species is benefited and other is nether harmed nor benefits. The species which is benefited is termed commensal and the other species is called host. Commensalism is observed in diverse type of animals and even in plants also. Some examples of commensalism are as follow.

(i) Epiphytes (orchids) growing on other plants like mango (host) gets benefit in the form of shelter but host neither drives any apparent benefit nor it gets harmed.

(ii) Barnacles growing on the back of a whale benefits in the form of shelter. Whale remains unaffected.

(iii) Clown fish that lives among sea anemone gets protection from predators which stay away from the stinging tentacles of sea anemone.

(iv) Cattle egrets (birds) forage close to where cattle are grazing because the cattle's as they move, stir up and flush out from the vegetation, insects that otherwise, might be difficult for the egrets to find and catch.

(4) Competition

Competition in the broadest sense refers to the interaction of two organisms for the same resource. Competition amongst the individuals of same species for one or more common resources is intraspecific competition, and between the organisms of different species is interspecific competition. Surely, intraspecific competition is more acute because all organisms of the same species have similar requirements for food, space, light, water, shelter, mate etc. When Darwin spoke of the struggle for existence and "survival of the fittest in nature", he was convinced that interspecific is a potent force in organic evolution.

Competition can take two forms.

(a) Competitive exclusion

(b) Competitive co-existence

Let us try to understand this in detail by taking suitable examples

(a) Competitive exclusion : (By Gause) It states that two closely related species competing for the same resources cannot co-exist indefinitely and the completely inferior one will be eventually eliminated.

Example 1 : Let's discuss one of the Gause original experiment with ciliates, which is a classic example of this. Paramecium caudatum and Paramecium Aurelia, two closely related ciliate protozoans, were placed in the same culture, P. Aurelia alone survived after 16 days because it had a more rapid growth rate and thus 'out competed'/excluded' P. caudatum for the limited amount of food.

Example 2 : Introduction of goats, resulted in exclusion of Abingdon tortoise from Galapagos islands because goats are better browsers.

Example 3 : On the rocky coasts of Scotland, the larger and competitively superior barnacle Balanus dominates the intertidal area and excludes the smaller barnacle Chathamulus from that zone.

(b) Competitive co-existence : Recent studies do not support such gross generalization of about competitions where one species is eliminated. They point out that species facing competition might evolve mechanisms that promote co-existence rather than exclusion. One such mechanism is resource partitioning or resource sharing by choosing different times for feeding or different foraging patterns.

Example 1 : Five closely related species of warblers avoid competition by changing foraging pattern (Mac Arthur).

Example 2 : Darwin found that fourteen species of finches co-exist in Galapagos islands due to development of different feeding habits.

From the foregoing discussion, it is quite clear that competition can occur between **closely related species** for the same resources that are limiting but also, the **totally unrelated species.** One best example for this is South American lakes, where visiting flamingoes and resident

fishes compete for their common food, the zooplankton in the lake.

Evidence for occurrence of competition

Evidence for competitive exclusion is easy to demonstrate in laboratory experiment as done by Gause but evidence for such competitive exclusion occurring in nature is not always conclusive. Evidence for the occurrence of competition in nature comes from what is called **"Competition release".** i.e., there occurs a dramatical increase in population of a less distributed species in a geographical area when its superior competitor is removed experimentally from that area.

"Competition will occur only when resources present in the environment are limiting". **Is this true?** No, resource need not to be limiting for competition to occur as feeding efficiency of one species might be reduced due to inhibitory presence of the other species. This is called **"interference completion".**

So, competition is best defined as a process in which fitness of one species (measured in terms of its 'r' the intrinsic rate of increase) is significantly lower in the presence of another species. In general, herbivores and plants appear to be more adversely affected by competition than carnivores.

Note : Competition can occur/leads to

1. Between closely and unrelated species
2. Exclusion and co-existence
3. When resource is limited or unlimited

(5) Predations

In predation, only one species (**predator)** benefits and the interaction are detrimental to the other species (**prey).** Predation is a natural way of transferring energy fixed to higher levels. Some examples of predator and prey are as follows :

Predator	Prey
Tiger	Deer
Sparrow	Seeds
Animals (herbivores)	Plants

Predators play important roles in ecosystem, such as :

(a) Transfer of energy across trophic levels

(b) Keep prey population under control : Exotic species become invasive and spread fast because the invaded land does not have its natural predators. For example, the prickly pear introduced into Australia in the early 1920's caused havoc by spreading rapidly into millions of hectares of rangeland. The invasive cactus was brought under control by introduction of a cactus-feeding predator (a moth) from its natural habitat into the country.

Biological pest control methods used in agriculture are based on the ability of predator to regulate prey population.

(c) Maintain species diversity in a community: Predators help in maintaining species diversity in a community by reducing the intensity of competition among completion prey species. For example, in the rocky intertidal communities of the American Pacific Coast the star fish Pisaster is an important predator. When all the star fish (Pisaster) were removed experimentally, from an enclosed intertidal area more than 10 species of invertebrates became extinct within a year, because of interspecific competition.

Some facts related to predation are as follow :

(i) Prudent-predator : Predators in nature are 'Prudent'. The existence of prudent predator is because if a too efficient predator overexploits its prey, they might become extinct and it will result in extinction of predator also due to lack of food.

(ii) Evolution of various defenses (adaptations) in prey : Prey species have evolved various defenses to minimize or challenge the impact of predation. Following are some examples:

(a) In Animals :

1. Camouflage : Cryptic appearance of an organism similar to objects found in the surrounding environment to protect itself from predators. It occurs in some species of insects and frogs to avoid their easy detection by the predator.

2. Chemical defense : Some prey species are poisonous and are therefore avoided by the predators. For example, Monarch butterfly is avoided by its predators (birds) due to the presence of a special chemical in its body which makes it highly distasteful. This chemical is acquired by monarch butterfly from a poisonous weed during its caterpillar stage.

3. Mimicry : It refers to the resemblance of one organism to another or the natural objects among which it lives, that secures its concealment, protection or some other advantage. During mimicry the subject is known as mimic or mimictic and the object it copies is called the model.

4. Warning colouration : Concealing form as well as colouration enables the animal species to avoid its natural predator.

(b) **In Plant :** They have evolved an astonishing variety of morphological and chemical defenses against their herbivore predators which includes **nearly 25 per cent of all insects known as phytophagous** (feeding on plant sap and other parts of plants) and other animals (cattle like goats etc.).

Presence of thorns (most common morphological

means of defense as found in Acacia, Cactus) as well as production and storage of highly toxic or poisonous chemicals like **cardiac glycosides** (Calotropis) are some of the protective/defense mechanisms which plants have evolved. Such mechanisms reduce the impact of herbivore predators on plants. Inhibition of feeding or digesting, disruption of reproduction, sickness and even killing of herbivore may occur when such poisonous plants are eaten by predator herbivores.

A wide variety of other chemicals like nicotine, caffeing, quinine, strychnine, opium etc., (also extracted at commercial level from plants) are produced by plants actually as defenses against grazers and browsers.

(6) Parasitism

Like predation, in parasitism only one species benefit (parasite) and the interaction are detrimental to the other species (host). Parasitism is a relationship between two organisms, where one organism spends a part or whole of its life, on or in the body of other organism and gets nourishment and shelter from the second organism. The former organism is termed as parasite and the latter as host. Parasitism has evolved in so many taxonomic groups from plants to higher vertebrates.

Many parasites are host-specific (they can parasitized only a single species of host). Both parasite and host tend to co-evolve i.e., if the host evolves special mechanisms for resistance against parasite, the parasite has also to evolve mechanisms to counteract and neutralise them in order to be successful with the same host species.

Adaptation shown by parasites : In accordance with life styles, parasites, have evolved a number of special adaptations. Some of these are as follows :

(a) Loss of unnecessary sense organs

(b) Presence of adhesive organs or suckers to cling onto the host

(c) Loss of digestive system

(d) High reproductive capacity

(e) Complex life cycle involving one or two intermediate hosts or vectors to facilitate parasitisation of its of its primary hosts e.g., the human liver fluke (a trematode parasite) depends on two intermediate hosts (a snail and a fish) to complete its life cycle. The malarial parasite Plasmodium needs a vector (mosquito) to spread to other hosts.

Impact of parasite of host :

(i) They may reduce the survival of the host.

(ii) They may also reduce the growth and reproduction of host.

(iii) Parasites also reduce host population density.

(iv) They render host more vulnerable to predation by making it physically weak.

Depending upon their occurrence with the host body, parasites are of two major types:

(i) Ectoparasites : They feed on the external surface of the organism e.g. lice on human, ticks on dogs, copepods on marine fishes, Cuscuta (a non-green plant) on hedge plants. It has lost its chlorophyll and leaves in the course of evolution.

Note : Female mosquito needs (for blood proteins) for its reproduction. It is not considered as a parasite because it never spends even a short duration as other parasites do.

(ii) **Endoparasites :** Parasites that live inside the host body at different sites (liver, kidney, lungs, red blood cells etc.). **Life cycle is more complex** due to their extreme specialization. They have highly simplified morphological and anatomical features. Endoparasites mainly emphasized on their reproductive potential.

Hyperparasites : Bacteriophages which are over parasitic bacteria. Pasteurella pestis is parasite on rate flea which is a rat parasite.

Brood Parasitism : it is a kind of parasitism which is found in birds where parasitic bird lays its eggs in the nest of its host (other birds) and lets the host incubate them. For example, cuckoo (parasite) lays its eggs in crow's (host) nest. During the course of evolution, the eggs of parasitic bird have evolved to resemble the host's egg in size and colour to reduce the chances of the host bird detecting the foreign eggs and ejection them from the nest.

(7) Amensalism

It is an interaction between two organisms of different species in which one species inhibits the growth of other species by secreting certain chemicals. The organism which inhibits the growth of another organism is called **amensal.** This phenomenon of inhibition of growth of one species by the other species through secretion of certain chemicals is also termed **allelopathy (in plants), or antibiosis or biological antagonism.** Following are some examples of amensalism.

❖ Penicillium secretes penicillin, that inhibits the growth of large number of bacteria.

❖❖

Chapter 12
Ecosystem

Introduction:

Wide range of living organisms are present on earth surface. All living organisms such as plants, animals and microorganisms interact among themselves and also with the surrounding physical environment and maintains a balance in nature. This forms a self-sustaining or functional unit of the living world called as ecosystem. Thus, an ecosystem consists of interaction of biotic component comprising living organisms and abiotic components comprising physical factors like temperature, rainfall, wind, soil and minerals. This chapter gives overview of different types of ecosystems, structural as well as functional aspects related to productivity, energy flow, decomposition, ecological efficiencies and nutrient, cycling.

TYPES OF ECOSYSTEMS

Ecosystem varies greatly in size from a small pond to a large forest or a sea. Many ecologists regard the entire biosphere as a global ecosystem as a composite of all local ecosystems on earth. Since this system is too much big and complex to be studied at one time, so to make the study easier, it is broadly divided into two basic categories :

(i) **Terrestrial Ecosystem:** It occurs over land e.g., forest, grassland, desert.

(ii) **Aquatic Ecosystem:** It occurs in water bodies e.g., pond, lake, river (fresh water), wet land, sea, estuaries (salt water).

Similarly, w.r.t, human interference the ecosystems are of two types:

(i) **Natural ecosystem :** It develops in nature without human support or interference e.g., forests, marine ecosystem.

(ii) Anthropogenic ecosystem or man-made ecosystem: It is the one which is created and maintained by human beings e.g., crop fields, garden, aquarium. **Agroecosystem or agriculture is the largest man-made ecosystem.**

Characteristics of anthropogenic ecosystem :

(i) Do not possess self-regulatory mechanism

(ii) Have little diversity

(iii) Simple food chain

(iv) High productivity

(v) Little cycling of nutrients

ECOSYSTEM – COMPONENTS

An ecosystem is made up of two components i.e., abiotic and biotic.

I. **Abiotic Components**

The non-living factors or the physical environmental factors prevailing in an ecosystem constitute the abiotic components. They are mainly of three types I.e., climatic, edaphic, topographic factors which you have studied in detail in previous chapter. So, here only main points are mentioned below.

(i) Climatic factors: It includes temperature, water, light, wind, humidity, air currents.

(ii) Edaphic factors: It includes factors related to the structure and composition of soil including its physical and chemical properties.

(iii) Topographic factors: It includes factors related to physical features of earth like slope valley mountain and plain etc.

II. **Biotic Components**

All living organisms i.e., plants, animals and microorganisms that are present in environment constitute the biotic components of the ecosystem. On the basis of their role in the ecosystem, these can be classified into three main groups:

(i) Producers : They are green photosynthetic plants that trap solar energy through chlorophyll to synthesise organic food from inorganic raw materials. So, they are called autotrophs (self-nourishing).

In **terrestrial ecosystem**, major producers are herbaceous and woody plants.

In **aquatic ecosystem**, chief producers are

phytoplankton's, algae and the floating, submerged and marginal plants found at the edges.

Producers are also known as **"converters"** or **"transducers"** because they convert solar energy into chemical energy stored in the bonds of sugars.

(ii) Consumers : They are the animals that are not capable of synthesizing the food materials. They are dependent on producers directly or indirectly for their survival. Thus, they are called heterotrophs.

Consumers are of following types :

(a) Primary Consumers (PC) or First Order Consumers : These animals directly feed on producers. They are also called herbivores or key industry animals. (Convert plant matter into animal matter)

Terrestrial ecosystem : Common herbivores are grasshopper, cow and deer.

Aquatic ecosystem : Common herbivores are mollusks, tadpole and mosquito larvae.

(b) Secondary Consumers (SC) or Second Order Consumers or Primary Carnivores : They are animals which feed on herbivores.

Terrestrial ecosystem : Toad, spiders, lizards, centipedes and insectivorous birds.

Aquatic ecosystem : Hydra, frog and some fishes.

(c) Tertiary Consumers (TC) or Third Order Consumers or Secondary Carnivores : Carnivores which feed upon secondary consumers e.g., large fishes (aquatic ecosystem), snake (terrestrial ecosystem). There may be quaternary or fourth order consumers which prey upon secondary carnivores.

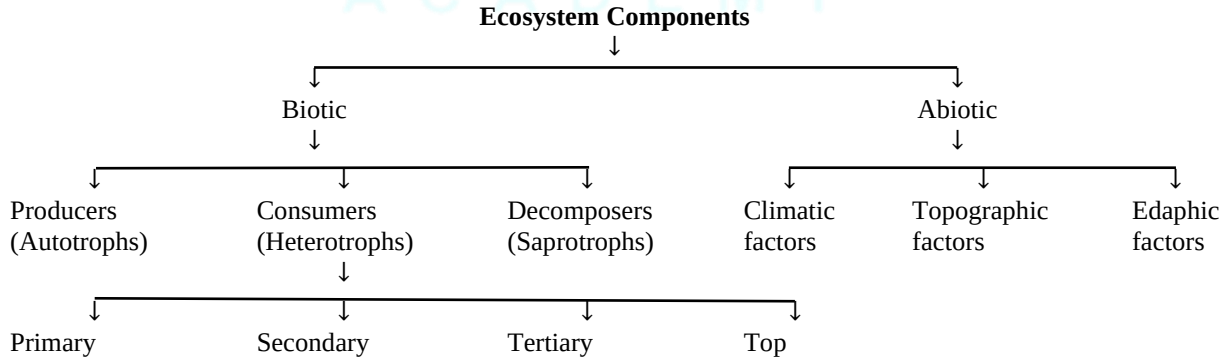
(d) Top Carnivores: The carnivores which are not eaten by others are called top carnivores. They may belong to the category of primary, secondary, tertiary carnivores, e.g., tiger, lion, panthers and falcon, peacock.

(iii) Decomposers : They are saprophytic microorganisms deriving their food material from organic matter present in dead remains of plants and animals. They secrete digestive enzymes which convert complex organic substances into simpler ones. A part of the digested organic matter is assimilated by the microorganisms and the rest is broken down into simpler inorganic compounds for recycling. They bring about cyclic exchange of materials between biotic community and the environment. They are thus, very essential components of an ecosystem. They are also called jeducers, as they are capable of degrading the dead organisms. These are the fungi, bacteria and flagellates especially abundant in the bottom of the pond. Decomposition involves the following conversions :

Complex organic substances → Simple organic substances → Inorganic compounds

Functions of decomposers in ecosystem :

1. They are natural scavengers as they reduce organic remains of earth.
2. Replenish the soil naturally with minerals that are essential for growth of plants and hence, maintenance of ecosystem. Some workers differentiated few other categories of living beings amongst the biotic components of an ecosystem. They are scavengers, detrivores and parasites. Parasites belong to diverse groups, e.g., bacteria, fungi, protozoans, worms etc. Detrevosours are animals which feed on detritus e.g., termites, earthworm etc. They are helpful in quick disposal of the dead bodies. Scavenger are animals that feed on dead or injured animals and they clean the earth of organic garbage's e.g., carrion beetles, crow and vultures (full-time scavengers).



ECOSYSTEM - STRUCTURE

Interaction of biotic and abiotic components result in a physical structure that is characteristic for each type of ecosystem. Important structural features include :

(i) Species Composition :

It is the identification and enumeration of plant and animal species of an ecosystem. For example, tropical rain forest is dense with amazing number of biological species. On the other hand, vegetation is **sparse** in the desert ecosystem.

(ii) Stratification :

Vertical distribution of different species occupying different levels. It is the structure as recognizable pattern in spatial arrangement of the members of the communities. For example, in a forest following vertical subdivisions are present.

(a) Top layer - Trees.

(b) Second layer - Shrubs.

(c) Bottom layer - Grasses and herbs.

ECOSYSTEM – FUNCTION

Ecosystem possesses a natural tendency to persist. This is made possible by a variety of functions (activities undertaken to ensure persistence) performed by the structural components. The components of the ecosystem are seen to function as a unit to ensure its persistence. The key functional aspects of the ecosystem are:

(I) Productivity

(II) Decomposition

(III) Energy flow

(IV) Nutrient cycling

I. Productivity

It is the rate of biomass production. Productivity in ecosystem is of two types - Primary and Secondary productivity.

1. **Most productive ecosystem** are coral reefs, tropical rain forest, sugarcane field.
2. **Least productive ecosystem** - Desert and deep sea.

(i) Primary Productivity : It is the rate at which biomass or organic matter is produced by plants or producers during photosynthesis per unit area over a time period or it refers to rate at which sunlight is captured by producers for the synthesis of energy-rich organic compounds through photosynthesis.

It is expressed in terms of weight as (gm m^{-2}) or energy as (Kcalm^{-2}) yr^{-1} to compare the productivity of different ecosystems. It can be further divided into two categories :

(a) Gross Primary Productivity (GPP) : Rate of production or synthesis of organic matter by producers during photosynthesis per unit time and area. Energy-captured process is operating in the green tissues; these as well as other tissues are consuming photosynthates in respiration. So, considerable amount of GPP is utilised by plants in respiration.

(b) Net Primary Productivity (NPP) : Gross primary productivity minus respiratory losses (R). So, you can say, it is rate of organic matter build up or stored by producers in excess of respiratory utilization per unit time and area.

$$\textbf{NPP = GPP - R}$$

Net primary productivity is the available biomass for the consumption to heterotrophs i.e., both herbivores and decomposers.

(i) Factors affecting primary productivity : Several biotic and abiotic factors given below affects magnitude of primary productivity,

(a) Photosynthetic capacity of producers which means the ability to utilise incident solar radiation to raise gross primary productivity.

(b) Solar radiations available

(c) Temperature

(d) Soil moisture

(e) Availability of nutrients

Productivity of biosphere : Annual NPP of whole biosphere is approximately 170 billion tons (dry weight) of organic matter. Of this, despite occupying about 70% of the surface, the productivity of oceans is only 55billion tons and for terrestrial ecosystem is 115 billion tons.

Reasons for the low productivity of oceans : In deep marine habitats two main limiting factors are there.

(a) **Light :** It decreases with depth.

(b) **Nutrients : Most limiting nutrient** of marine ecosystem is **nitrogen.**

(ii) Secondary productivity : It is the rate of formation of new organic matter by consumers.

(iii) Community productivity : It is the rate of net synthesis and buildup of organic matter by a community per unit time and area.

Ecological efficiency/Trophic level efficiency : The percentage of energy converted into biomass by a higher trophic level over the energy of food resources available at the lower trophic level is called ecological efficiency.

$$\textbf{Ecological efficiency} = \frac{\text{Energy converted into biomass at a trophic level}}{\text{Energy present in biomass at lower trophic level}} \times 100$$

$$\textbf{Photosynthetic efficiency} = \frac{\text{Gross primary productivity}}{\text{Incident total solar radiation}} \times 100$$

$$\textbf{Net production efficiency} = \frac{\text{Net primary productivity}}{\text{Gross primary productivity}} \times 100$$

II. Decomposition

While productivity involves synthesis and building processes, decomposition is equally important, which concerns with breakdown of complex organic matter to inorganic raw materials like CO_2, H_2O, and various nutrients by decomposers. The major site of decomposition is the upper layer of soil in terrestrial habitats and bottom of water bodies. Dead remains such as leaves, bark, flowers and dead remain of animals including faecal matter constitute detritus which is the raw material for decomposition.

(i) Decomposition Processes : There are three important steps in the process of decomposition, viz., fragmentation, leaching and catabolism. These processes occur simultaneously.

(A) Fragmentation of Detritus : Small invertebrate animals called detrivores feed on detritus, e.g., earthworm, termites. They bring about its fragmentation. A part of detritus eaten by detrivores comes out in highly pulverised state in their faces. Due to fragmentation during eating and pulverisation in digestive tracts, detritus is changed into fine particles which have a large surface area and can be easily acted upon by enzymes.

(B) Leaching : Part of water-soluble substances present in the fragmented and decomposing detritus (e.g., sugars, inorganic nutrients) go down into the soil horizon by percolating water and get precipitated as unavailable salts.

(C) Catabolism : It is carried out by **saprotrophic bacteria and fungi**. They secrete digestive enzymes over the fragmented detritus. The enzymes change complex compounds into, simple compounds; `compounds: inorganic substances are released this process. They act as **"nature's scavengers"**.

The rate of catabolic action or breakdown of different complex substances is different. This differential decomposition produces two substances, humus and inorganic nutrients, by process called humification and mineralisation respectively.

(a) Humification. It is the process of decomposition of detritus to form humus. Humus is a dark-coloured, amorphous, more or less decomposed organic matter rich in cellulose, lignin, tannins, resin, etc. and is highly resistant to microbial action. It undergoes decomposition at an extremely slow rate. Humus is slightly acidic, colloidal and functions as reservoir of nutrients.

(b) Mineralisation. It is the release of inorganic substances (e.g., CO_2, H_2O, minerals) from organic matter or humus during the process of decomposition. They are formed along with (simple and soluble organic substances when digestive enzymes are poured over organic matter by saprotrophic microbes.

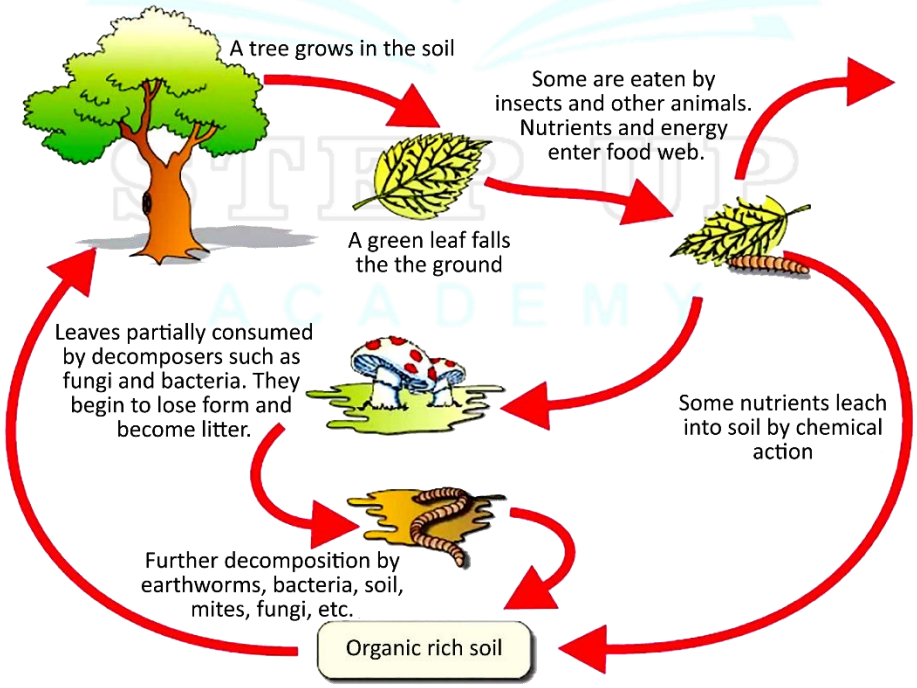


Fig. : Diagrammatic representation of decomposition cycle in a terrestrial ecosystem

(ii) **Factors Affecting Decomposition :** The rate of decomposition of detritus is controlled by chemical nature of detritus and a number of climatic factors given below :

(a) Chemical nature of detritus. Decomposition of detritus is slow if it contains lignin, chitin, tannins (phenolics) and cellulose. It is rapid, if detritus possesses more of nitrogenous compounds (like

proteins, nucleic acids) and water-soluble reserve carbohydrates or sugars.

(b) Temperature. At a temperature of more than 25°C, decomposers are very active in soils having good moisture and aeration. In humid tropical regions, it does not take more than 3-4 months for complete decomposition of detritus. However, under low temperature conditions (< 10°C) of soils, the rate of decomposition is very slow even if moisture and aeration are optimum.

(c) Moisture. An optimum moisture helps in quicker decomposition of detritus. Reduction in moisture reduces the rate of decomposition as in areas of prolonged dryness Tike tropical deserts where, otherwise, the temperature is quite high. Excessive moisture also impedes decomposition.

Temperature and soil moisture are the most important climatic factors that regulate decomposition through their effects on the activities of soil microbes.

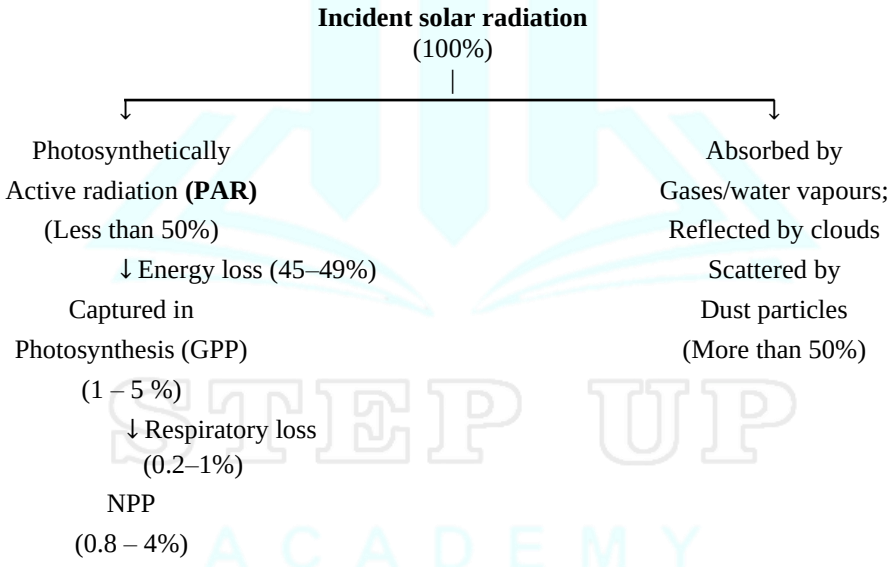
(d) Aeration. It is required for activity of decomposers and detrivores. A reduced aeration will slow down the process of decomposition.

So, from this discussion it can be enumerated that warm and moist environment favour decomposition whereas low temperature and anaerobiosis inhibit decomposition resulting in buildup of organic materials.

(iii) **Nutrient Immobilisation :** The phenomenon of incorporation, of nutrients in living microbes is called nutrient immobilization. After death of microbes, the immobilised nutrients become available again for solubilisation. Immobilisation protects the nutrients from being washed out and lost from ecosystem.

III. Energy Flow

Energy flow is sequential process of the movement of energy in an ecosystem through a series of organisms. Sun is the only source of energy for all ecosystems on earth (except for deep sea hydrothermal ecosystems).



Source of Energy :

Of the incident solar radiation less than 50% of it is photosynthetically active radiation (PAR) Plants capture only 2-10% of PAR or 1-5% of incident solar radiation synthesis of organic matter. Roughly 20% of it is consumed in respiration so that net primary productivity is 0.8-4% of incident radiation or 1.6-8% of PAR.

Energy does not remain trapped permanent in any organisms. It is either passed on to the higher trophic level or becomes available to detrivores or decomposers after the organism dies. Herbivores feed on producers Part of the energy is wasted in digestion and assimilation. Some of the assimilated food is broken down to release energy for performing body activities. A very small proportion becomes part of the body of herbivore Herbivores are eaten by primary carnivores, latter by secondary carnivores and so on. So, energy flow in an ecosystem is always **unidirectional** or one way i.e.,

Solar radiation → Producers → Herbivores→ Carnivores.

Thus, energy which passes from autotrophic plants to the herbivores does not pass back to the autotrophs.

Energy flow follows laws of thermodynamics:

This large expenditure of energy in the ecosystem is based on two basic laws of thermodynamics in accordance with the first law of thermodynamics (which states that energy is neither created nor destroyed, but can be transformed from one state to another), energy of sunlight can be transformed into energy of food and heat. According to

second law of thermodynamics, no transfer of energy occurs unless and until it is accompanied by degradation or dissipation of energy from concentrated to dispersed form. The transfer of energy from one organism to other is accompanied by degradation and loss of major part of food energy as heat. Energy of food is concentrated form while its highly dispersed form is heat.

IV. Food Chain

It is a sequence of living organisms which involves transfer of food energy from producers, through a series of organisms with repeated eating and being eaten is called food chain. Each level or step in a food chain where transfer of energy takes place is called **trophic level.**

Types of Food Chain :

(i) Grazing Food Chain (GFC) or Predator food chain

(ii) Detritus Food Chain (DFC) or Saprophytic food chain

(iii) Parasitic Food Chain (PFC) or Auxiliary food chain

(i) Grazing Food Chain (GFC) : Consists of producers, consumers and decomposers. Source of energy for such food chain is sun.

(a) Primary Producers (PP) : They are autotrophic organisms which fix up the solar energy and manufacture their own food from inorganic raw material. So, they form the base of food chain constituting first trophic level (T_1).

(b) Primary Consumers (PC) or Herbivores : These are animals which feed on green plants or plant products, so they constitute second trophic level (T_2).

(c) Secondary Consumers (SC) or Primary Carnivores : These are animals which feed on herbivores and form third trophic level (T_3).

(d) Tertiary Consumers (TC) or Secondary Carnivores: These are animals which feed on secondary consumers and constitute the fourth trophic level (T_4) and so on.

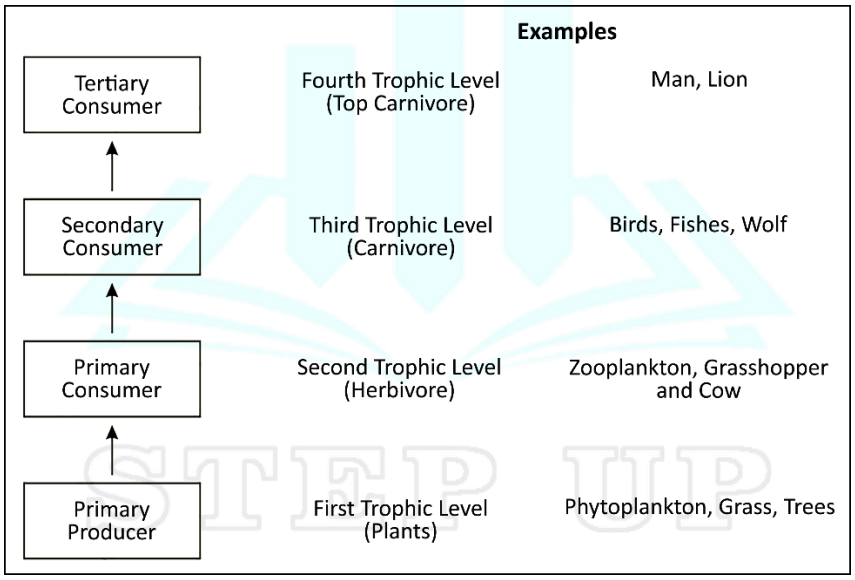


Fig. : Diagrammatic representation of trophic levels in an ecosystem

Terrestrial food chain :

Grass →	Grasshopper →	Frog →	Snake →	Eagle
PP	PC	SC	TC	Top consumer
T_1	T_2	T_3	T_4	T_5

Aquatic food chain :

Phytoplankton →	Zooplankton →	Small fish →	Large fish
PP	PC	SC	TC
T_1	T_2	T_3	T_4

GFC is major conduit of energy flow in aquatic ecosystem. Size of organisms commonly increase at higher trophic levels.

(ii) Detrius Food Chain (DFC) : Begins with detritus or dead organic matter. It is made up of decomposers which are heterotrophic organisms mainly fungi and bacteria. Detrivores act over it. Therefore, food energy present in detritus passed into them. Detrivores and decomposers are consumed by smaller carnivores which in turn become food for larger carnivores and so on. A common detritus food chain with earthworm as detrivore is given below.

Detritus → Earthworm → Sparrow → Falcon.

In terrestrial ecosystems, a much larger fraction of energy flows through DFC than through GFC.

(iii) Parasitic food chain/ Auxiliary food chain : Size of the organisms finally reduces at higher trophic level (parasite).

Tree → herbivore birds → lices and bugs.

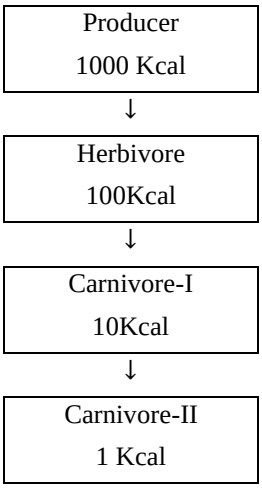
Food Web :

DFC may be connected with GFC chains at some levels. Some of the organisms of DFC are prey to the GFC animals. In ecosystem, linear food chains as shown above seldom exists, because every organism has alternate source of food. An animal may have preference for a particular prey, but if the latter has a small population, it may feed upon some other prey. Single animal may be eaten by different animals and thus, different food chains get interconnected and one animal may be a link in more than one food chain. The network of interconnected food chains at different trophic levels in a biotic community is termed food web.

Occurrence of food webs provides stability to ecosystem. Food webs operate because of taste preference for particular food and unavailability of food. One animal may feed upon organism of even different trophic level like, snakes may feed upon mice (herbivore) and frogs (carnivore), jackals are both carnivores and scavengers. Sparrow is a primary consumer when it eats seeds, fruits etc. and a secondary consumer when it eats insects and worms.

Ten Percent Law of Energy Transfer:

The law was proposed by **Lindeman** in 1942. The transfer of energy from one trophic level to another trophic level is accompanied by loss of energy at each level or step. When the plants are eaten by herbivore, about 10% of energy in the food is fixed into animal flesh while 90% is consumed in ingestion, respiration, maintenance of body heat and other activities. Similarly, when a carnivore consumes that herbivore, again about 10% of energy is fixed. Therefore, each transfer only 10% of the total energy is actually available to the next trophic level. It is called 10% law.



So, you can see residual energy decreases drastically within 2-3 trophic levels. **As a result, an ecosystem can support only a limited number of trophic levels hardly, 3-5. Respiratory loss gradually increases** in successive trophic levels. It is **20%, 30% and 60%** respectively at **producer, consumer and top carnivore level**.

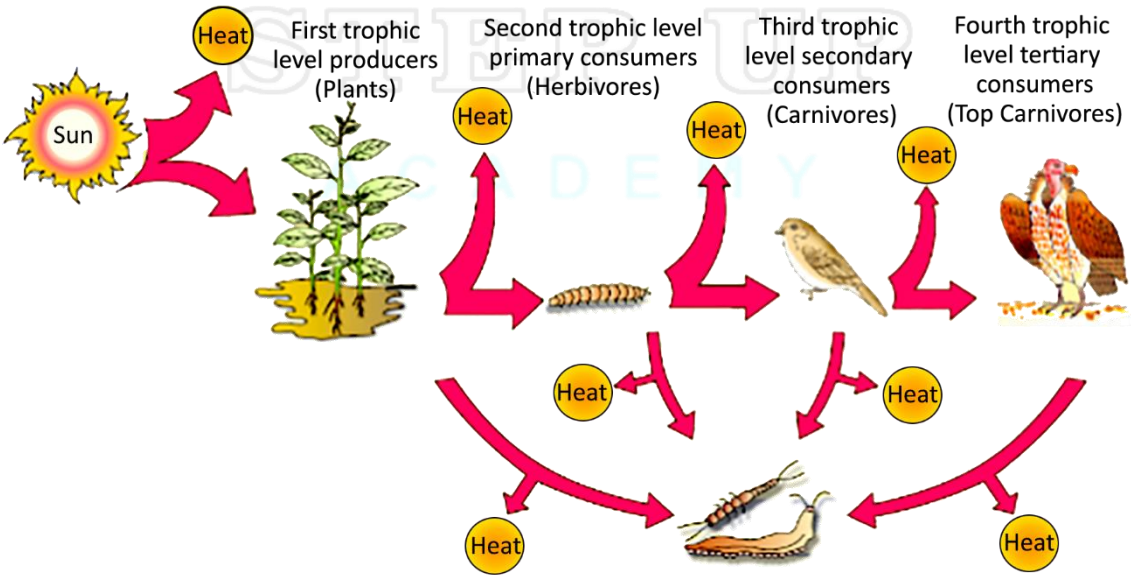


Fig. : Energy flow through different trophic levels

Standing state or quality : Amount of all the inorganic substances present in an ecosystem per unit area at a given time.

Standing crop : Amount of living material present in different trophic levels at a given time. It is expressed as the numbers or biomass of organisms per unit area. The

biomass of species is expressed in terms of either fresh or dry weight. Measurement of biomass in terms of either fresh or dry weight, Measurement of biomass in terms of dry weight is more preferred to avoid variations in weight due to season moisture differences in biomass.

ECOLOGICAL PYRAMIDS

They are graphical representations of various ecological parameters trophic levels of food chains with producers at the base, top cosuvosou at the apex and intermediate levels in between. Quantity at each level is indicated by length of bar in the graph. The common parameters used in preparing ecological pyramids are number of individuals, biomass and energy at different trophic levels. The three ecological pyramids that are usually studied are :

(i) Pyramid of Numbers

(a) Upright : In most ecosystem, the number of producers is maximum. During transfer of food at any trophic level, only 10 % of the food present in one trophic level becomes part of the next trophic level. 90 % of the food is either lost in wastage or broken down during cellular respiration for providing energy for various life activities. Producers, thus can support fewer herbivores is too small to support any other trophic level and do not act as prey to any other organism.

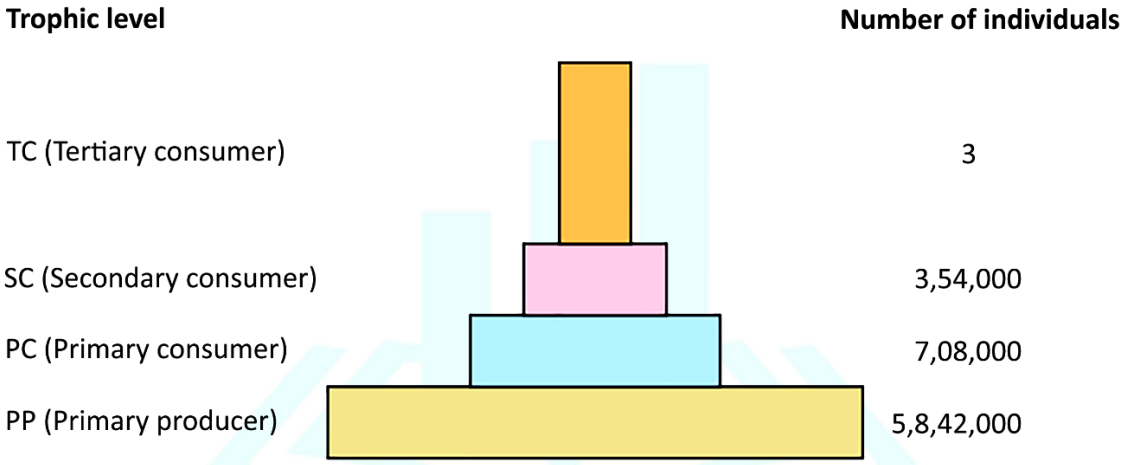


Fig.: Pyramid of number in a grassland ecosystem. For example, only three top carnivores are supported in an ecosystem based on production of nearly 6 million plants.

Thus, with each successive trophic level, numbers of individuals are decreasing. Therefore, the pyramid of number is upright e.g., Grassland ecosystem and Pond ecosystem.

(b) Inverted : in some cases at each successive trophic level, number of the organisms is higher than in preceding one and the size decreases gradually at successive levels. Thus, shape of pyramid may be inverted. For example, a large-sized tree (producers) may support and provide nourishment to several frugivorous birds. The number of ectoparasites like mites, ticks, lices, bugs etc. dependent upon birds for nourishment is much more than birds. The number thus, increases at each trophic level.

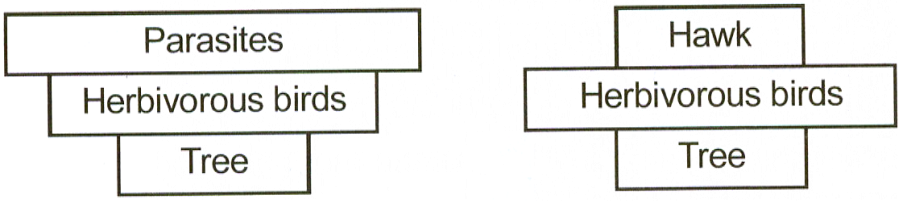


Fig.: Pyramid of number in three ecosystems (inverted and Spindle-shaped)

(c) Spindle : A tree supports a number of herbivore birds. The herbivore birds are eaten by one or two hawks of the area.

(ii) Pyramid of Biomass

Upright : Biomass is the amount of living matter (expressed as weight) at any particular trophic level at a given time. Pyramid of biomass in terrestrial ecosystems is usually upright.

For upright pyramid, total biomass of plants (producers) in a specific area is more than that of herbivores (primary consumers) and it gradually decreases at each successive trophic level. It is least in top carnivores e.g., tree and grassland ecosystems.

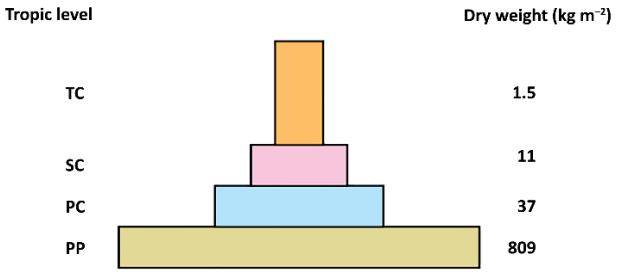


Fig.: Pyramid of biomass.

Showing a sharp decrease in biomass at higher trophic levels

Inverted : In aquatic ecosystem, the pyramid of biomass may be inverted. For example, biomass of zooplanktons is higher than that of phytoplankton as life span of former is longer and the latter multiply much faster through having shorter life span. A number of generations of phytoplankton's may thus be consumed by single generation of zooplanktons. Biomass of fish may still be larger as fishes are larger in size with longer life span and a number of generations of zooplanktons can be consumed by fishes. However, during transfer, only10% of the biomass of one generation is passed on to next trophic level. Sum total of biomass of benthic animals and brown algae exceeds the other producers and consumers in aquatic ecosystem.

Fig.: Inverted pyramid of biomass-small standing crop of phytoplankton supports large standing crop of zooplankton

(iii) Pyramid of Energy

The pyramid of energy is **always upright** because the flow of energy is **unidirectional** from producer to consumer level. The energy content is maximum in producers. The energy decreases at each trophic level of food chain, as part of the energy is lost as heat a major part of energy is liberated during respiration for use in various activities. Only 10% of the energy of previous trophic level is received by next trophic level as proposed by **10 percent law of Lindeman** (1942). Just to illustrate 10,00,000 J of solar energy is needed to produce 10,000 J of energy stored in a plant (if plants trap 1% solar energy). Herbivores, feeding upon plant, will retain 1,000 J of available stored energy and carnivores feeding upon them will gain only 100 J of usable energy giving an upright pyramid shape as given below:

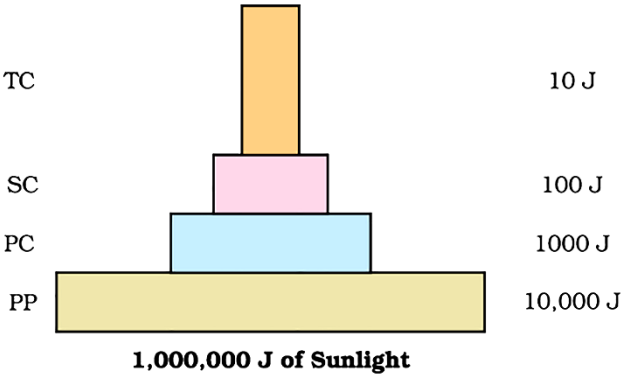


Fig.: An ideal pyramid of energy. Observe that primary producers convert only 1 % of the energy in the sunlight available to them into NPP

In most ecosystem, all the pyramids of numbers of energy and biomass are upright. Producers are more in number and biomass than the carnivores. Also, energy at a lower trophic level is always more than at a higher level.

Note : Any calculation of energy content, biomass or numbers has to include all organisms at that trophic level.

LIMITATIONS OF ECOLOGICAL PYRAMID

(i) It does not take into account the same species belonging to two or more trophic levels, e.g., insecrtivorous plants.

(ii) It assumes a simple food chain and does not accommodate a food web.

Saprophytes, decomposers, microbes and detrivores are not given any place in ecological pyramids.

ECOLOGICAL SUCCESSION (BY HULT)

Biotic community is seldom static. Its composition changes with time due to interactions between biotic and abiotic components. This change is orderly and sequential, parallel with the changes in the physical environment. These changes lead finally to a community that is **in near equilibrium** with the environment and is called **climax community.** Such gradual and fairly predictable changes in the species composition of a given area are collectively called **ecological succession.** During succession, some species colonies an area and their populations become more numerous, whereas populations of other species decline and even disappear.

Types Successional Communities

(i) Pioneer community : The first biotic community that develops in a bare area is termed as pioneer community. e.g., Lichens on rock, phytoplankton and zooplanktons in pond.

(ii) Transitional or seral community : The pioneer community is followed by a specific orderly sequence of series of plant communities know as seral communities . e.g., Bryophytes, herbs, shrubs in xerosere , submerged, floating plants in pond.

(iii) Climax community : The last community in biotic succession which is relatively stable and is in near equilibrium with the environment of that area is called climax community. e.g., Forests.

The entire series of communities occurring in biotic succession is called sere. The individual transitional communities are termed **seral stages or seral communities.**

Type of Succession

(i) Depending upon nature of habitat it starts, it is two types i.e.,

Xerosere or Xerarch succession	Hydrosere or Hydrarch succession
Takes place in dry areas like rock (lithosere), sand (psammosere) and saline conditions halosere.	Starts in aquatic habitat.

(ii) Depending upon the type of nudity of the area, it is two types :

Primary succession	Secondary succession
(a) It starts at barren area, never having vegetation of any type or where no living organism ever existed. (b) Cooled volcanic lava, sand dunes, igneous rocks, newly exposed sea or newly submerged terrestrial habitats in water, etc., are the areas where primary succession starts. (c) It is very difficult for the pioneer community to get established in these areas. (d) It takes natural processes several hundred to several thousand years to produce fertile soil on bare rock thus, it takes a very long time to reach climax.	(a) It starts in areas that somehow lost all the living organisms that existed there. (b) It occurs in areas such as in abandoned farm lands, burned or cut forests, lands that have been flooded. (c) Pioneer community establishes with comparatively more ease. (d) Since some soil or sediment is present, succession is faster. Here, climax is also reached more quickly.

Process of Succession

Major steps in a primary autotrophic succession are as follows :

1. **Nudation :** Exposure of an area.
2. **Migration :** The process of dispersal of seeds, spores and other structure of propagation of the species to bare area is known as migration.
3. **Germination :** It occurs when conditions are favourable.
4. **Ecesis :** Successful germination of propagules and its establishment in a bare area is known as ecesis.
5. **Colonisation and Aggregation :** After ecesis, the individuals of the species increase in number as a result of reproduction.
6. **Competition and Co-action :** Due to limited resources, species show both inter and intraspecific competition. This results in elimination of unsuitable and weaker plants.
7. **Invasion :** Various other types of plants try to establish in the spaces left by the elimination of previous plants due to competition.
8. **Reaction :** The newly arrived plants interrupt with the existing ones. As a result of reaction, environment is modified and becomes unsuitable for the existing community which sooner or later is replaced by another community.
9. **Stabilisation :** The process when the final climax community becomes more or less stabilised for a longer period of time and-it can maintain itself in equilibrium with the climate of the area. As compared to transitional communities, **the climax community has larger size of individuals, complex organization, complex food chains and food webs, more efficient energy use and more nutrient conservation.**

Ecosystem Characteristics that change during succession :

(i) Change in diversity of species i.e., some species colonise an area and their populations become more numerous, whereas populations of other species decline and even disappear.

(ii) Little diversity to high degree of diversity i.e., increase in the number of species.

(iii) Total biomass increases.

(iv) Increase in humus content of soil.

(v) Aquatic or dry conditions to mesic conditions i.e., both Hydrarch and Xerarch succession lead to medium water conditions (mesic), neither too dry (xeric) nor too wet (hydric).

(vi) Vegetational changes in turn affect food and shelter for various types of animals. Thus, as succession proceeds, the number and types of animals and decomposers also change.

The present-day communities in the world have come to be because of succession that has occurred over millions of years since life started on earth. Actually, succession and evolution would have been parallel processes at that time.

Succession of Plants

Xerarch Succession :

Primary succession on rocks is xerarch. The successional series progress from xeric to mesic conditions.

(i) Pioneer Community : (Lichen stage) The pioneer lichens on such habitats are usually crustose lichens, e.g., Graphis, Rhizocarpon. The propagules of these lichens settle and get established on wet rock surface soon after rainfall. They can tolerate desiccation and high temperature. The acidic substance produced by lichens corrodes the rock surface forming small depressions and release minerals needed for the growth of lichens. The dead and decaying organic matter of the lichens along with sand particles, brought by wind, get collected in depressions and forms a little bit of soil by mixing with weathered rock particles. The habitat becomes suitable for foliose lichens like Parmelia. Foliose lichens compete with crustose lichens and slowly replace the latter due to their larger size. They increase shading of rocks, more accumulation of organic matter and formation of larger depressions. This accelerates the process of soil formation and makes the habitat **more suitable for next seral stage,** the moss stage.

(ii) Transitional Communities :

(a) Moss stage : Due to interaction of foliose lichens, the habitat becomes suitable for hardy mosses (e.g., Torfula, Grimmia) to grow. Mosses, being larger in size and having gregarious habit, shade the lichens and replace them. Their rhizoids can penetrate deeper. Growth of mosses leads to accumulation of more soil and organic matter which can retain moisture for a longer span and soon the habitat is occupied by moisture loving mosses (e.g., Hypnum, Bryum).

(b) **Annual grass stage :** During rainy season, the compact mat formed by mosses on weathered rock retain sufficient moisture and the habitat thus, becomes suitable for germination of seeds of annual grasses and hardy herbs, e.g., Aristida, Poe, Eleusine, etc. Their roots penetrate deeper and cause more weathering of rocks. They replace mosses, grow for a couple of months and their death and decay results in increased organic matter. This mixes with weathered rock particles to form soil and thus process of soil formation continues.

(c) Perennial grass stage : Due to increasing moisture and soil in rock crevices, annual grasses are replaced by perennial grasses. These grasses like Heteropogon, Gymbopogon, etc. spread very fast due to the presence of runners and rhizomes.

(d) Shrub stage : The habitat occupied by perennial grasses soon become suitable for invasion of xerophytic shrubs like Zizyphus, Rubus. Rhus, Capparis etc. These shrubs soon get established in such habitats, replacing the perennial grasses. As shrubs are larger in size their roots penetrate deeper, causing more fragmentation of rock and hence more accumulation of soil.

(iii) Climax community : Shrubs are soon replaced by hardy trees forming stable climax forest community The nature of climax forest depends upon the climate of that area.

Sequence of various stages in a sere can be represented as

Lichens → Bryophytes → Herbs → Shrubs → Forest.

Hydrarch Succession :

The succession in aquatic habitat like freshly formed pond is hydrosere. The successional series progress from hydric to mesic conditions.

(i) Pioneer Community : It is formed by the **phytoplankton's** i.e., the minute microscopic autotrophic organisms like diatoms, unicellular, colonial or filamentous green algae and blue-green algae The spores of these organisms reach the newly formed pond through wind or animals. They multiply rapidly due to which it becomes suitable habitat for zooplanktons which feed upon them. The organic matter formed by death and decay of planktons, mixes with clay and silt at the bottom of pond to form soft mud The habitat becomes suitable for the growth of next stage.

(ii) Transitional Communities :

(a) Submerged plant stage : With their roots they are anchored in the mud at the bottom of water body. e.g., Mynophyllum, Hydrilla, Vallisneria, Potamogeton etc.

(b) Submerged free-floating plant stage : Due to the accumulation of dead and decaying remains of submerged plant bottom level is raised as well as ponds become rich in minerals (nutrients) which become suitable for free-floating plants, e.g., Azolla, Wolffia. Pistia etc.

(c) Reed-swamp stage : More shallowing of plants occur due to continued siltation which paves way for the growth of rooted emergent plants I.e., reeds (amphibious plants), e.g., Typha, Sagittaria, Phragmites etc.

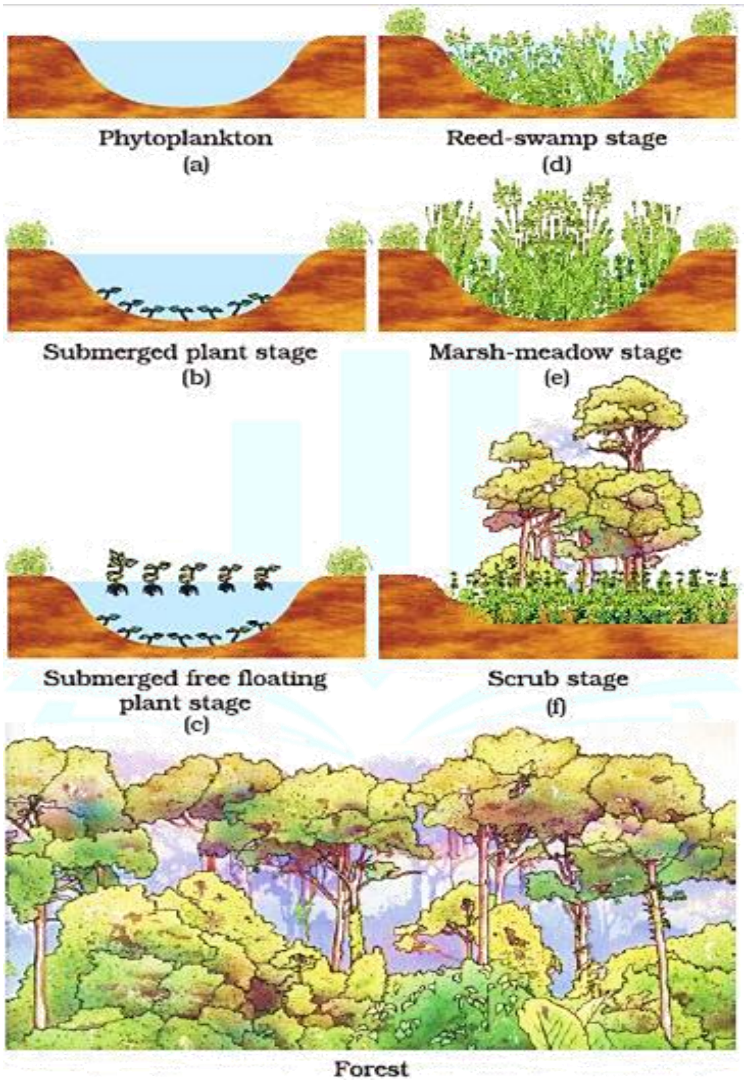


Fig. : Diagrammatic representation of primary succession in pond

(d) Marsh-meadow stage : Reed-swamp stage is invaded by marshy plants. With increased settling of silt and deposition of dead organic matter derived from floating and rooted species, the pond becomes shallower until it gets transformed into terrestrial habitat, e.g. Carex, Juncas, Cyperus.

(e) Shrub stage : Marsh-meadow stage is replaced by shrubs, e.g. Populus, AInus.

(iii) Climax community : The scrub stage is replaced by trees which grows to greater heights. The nature of climax community depends on the climate of that area. e.g. Forest.

Phytoplankton → Submerged plant stage → Submerged free-floating plant stage → Reed-swamp stage → Marsh-meadow stage → Scrub stage → Forest

NUTRIENT CYCLE

Biogenetic Nutrients : They are essential elements required by organisms for their body building and metabolism which are provided by earth. The amount of nutrients present in the soil at any given time is referred to as the standing state. It varies in different kinds of ecosystems and also on seasonal basis.

Circulation or exchange of biogenetic nutrients between the living and non-living c**omponents is called nutrient cycle or biogeochemical cycle** (at global scale).

Whole living matter is made up of nutrients, either as structural components, biochemicals as enzymes. An ecosystem has a limited supply of biogenetic nutrients in its abiotic environment. They are picked by producers and made part of organic matter. From producers the nutrients in the form of organic matter, pass to the higher trophic level. They are released back to abiotic environment by decomposers acting on organic wastes and deed bodies of organisms. In this way the same nutrients repeatedly move through living and non-living components of the ecosystem.

The whole of biogenetic nutrients is not always in circulation. The nutrients exist in two states:

Reservoir Pool	Cycling Pool
(a) Reservoir of biogenetic nutrients from which they are slowly transferred to cycling pool. (b) Nutrients are in relatively inactive form e.g. Nitrogen gas in atmosphere.	(a) In this pool the biogenetic nutrients are repeatedly exchanged between the abiotic and biotic components of biosphere. (b) Nutrients are present in active/ionic form.

Types of Biogeochemical Cycles

(i) Gaseous cycle

(a) Exchange of nutrients occurs in gaseous or vapour form.

(b) Biogeochemical is non-mineral.

(c) Reservoir pool is atmosphere or hydrosphere.

e.g., Nitrogen, Carbon, Oxygen, Hydrogen cycle.

(ii) Secondary cycle

(a) Biogeochemical is mineral.

(b) Reservoir pool is earth's crust or lithosphere.

e.g., Sulphur, Phosphorous cycle.

Function of reservoir is to meet the deficit which occurs due to imbalance in the rate of influx and efflux.

Carbon Cycle

Importance of carbon : Carbon is a component of all organic compounds of protoplasm like carbohydrates, lipids, proteins, nucleic acids, enzymes, hormones etc. It constitutes 49% of dry weight of organisms and is next only to water in abundance.

Source of carbon :

(a) Out of the total quantity of global carbon, 71% carbon is found dissolved in oceans.

(b) CO;, in the atmosphere (This oceanic reservior regulates the amount of CO_2 in the atmosphere).

(c) Carbonates and graphites in rocks. -

(d) Fossil fuels.

Circulation : Carbon cycling occurs through atmosphere, ocean and through living and dead organisms.

Utilisation : According to one estimate, 4 x 10^{13} kg of carbon is fixed in the biosphere through photosynthesis annually.

Some amount of fixed carbon is lost to sediments, shells, skeletons and removed from circulation.

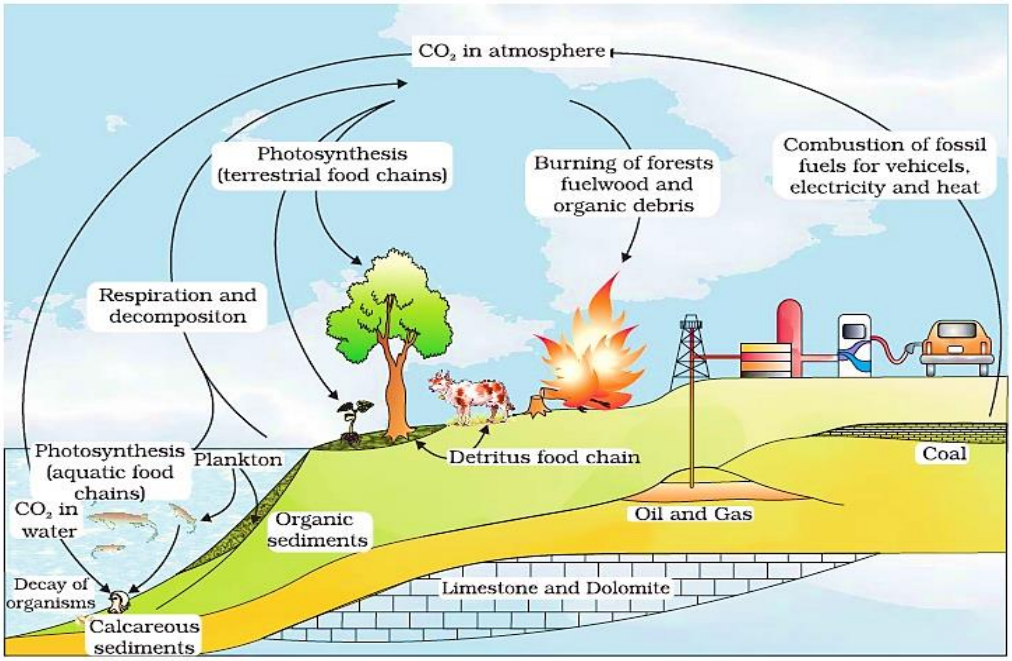


Fig. : Simplified model of carbon cycle in the biosphere

Addition :

(a) A considerable amount of carbon returns to the atmosphere as CO_2 through respiratory activities of the producers and consumers.

(b) Decomposers also contribute substantially to CO_2 pool by their processing of waste materials and dead organic matter of land or oceans.

(c) Burning of wood, forest fire and combustion of organic matter, fossil fuels, volcanic activities are additional sources for releasing CO_2 in the atmosphere.

(d) Human activities have significantly influenced the carbon cycle i.e., rapid deforestation, transport, massive burning of fossil fuels increases the rate of carbon dioxide into atmosphere.

Phosphorous Cycle

Importance of Phosphorous : Phosphorous is a major component of biological membranes, nucleic acids and cellular energy transfer system. Many animals also need large quantities of this element to make shells, bones and teeth. After nitrogen, phosphorous is the second most critical element.

Main Sources : The natural reservoir of phosphorous is rock which contains phosphorous in the form of phosphates.

Utilisation and Addition: Small amount of phosphate is always added to soil solution through weathering of rocks.

(i) It is absorbed by the roots of the plants.

(ii) Herbivores and other animals obtain this element from plants.

(iii) Animal excretion and dead bodies of organisms are acted upon by decomposers.

(iv) Phosphorous released in the process becomes available for reutilisation of plants. Difference from carbon cycle :

Difference from carbon cycle :

(i) No respiratory release of phosphorous into atmosphere.

(ii) Inputs of phosphorous through rainfall are much smaller.

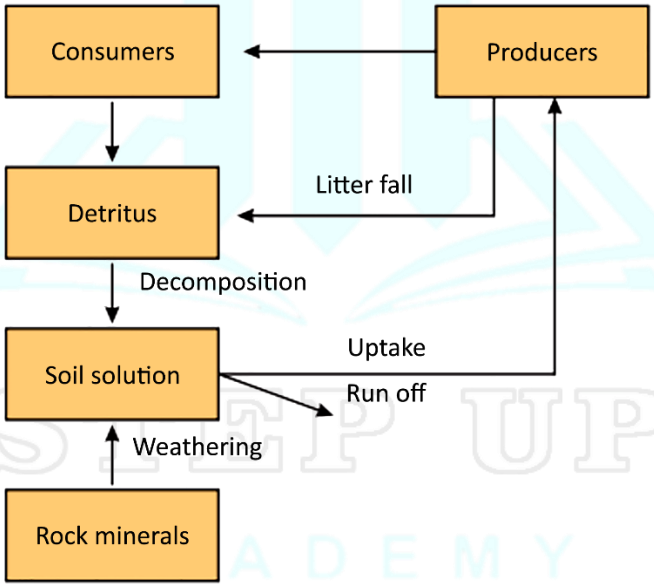


Fig. Simplified model of phosphorous cycle in the biosphere

Ecosystem Services →

The products of ecosystem processes which have environmental, aesthetic and indirect economic value are named as ecosystem services. For best services the ecosystems must be healthy. Following ecosystem services are

(i) Purification of air and water by healthy forest ecosystem

(ii) Mitigate droughts and floods

(iii) Nutrient cycles

(iv) Generating fertile soils

(v) Providing wild life habitat

(vi) Maintaining biodiversity

(vii) Pollinating crops

(viii) Provide storage site for carbon

(ix) Provide aesthetic, cultural and spiritual values

Robert Costanza and his colleagues have put an average price tag of US $33 trillion a year on these fundamental services i.e., nearly twice the value of a global GNP-US $18 trillion. The cost is distributed as such:

(i) Soil formation - 50 %

(ii) Recreation - < 10%

(iii) Nutrient cycling - < 10%

(iv) Climate regulation – 6%

(v) Habitat for wildlife – 6%

Chapter 13
Biodiversity and Conservation

Introduction:

If an alien from a distant galaxy were to visit our planet Earth, the first thing that would amaze and baffle him would most probably be the enormous diversity of life that he would encounter. Even for humans, the rich variety of living organisms with which they share this planet never ceases to astonish and fascinate us. Biodiversity is inherent in the occurrence of various types of environmental conditions in different parts of an area as well as earth and the presence of various forms of life adapted to these different environmental regimes. The common man would find it hard to believe that there are more than 20,000 species of ants, 3,00,000 species of beetles, 28,000 species of fishes and nearly 20,000 species of orchids. Biodiversity is not uniform. It is tremendous in some places, moderate in others and low in certain regions. Ecologists and evolutionary biologists are trying to understand the significance of such a tremendous diversity. After all, why are these so many species? Did such diversity exist all the time or is it only of recent development? How has this diversity come into existence? What is the importance of biodiversity to the biosphere? What is its importance to human beings? What would be the effect of lesser diversity on biosphere as well as on human interests?.

BIODIVERSITY

The term biodiversity was popularised by sociobiologist Edward Wilson to describe the combined diversity (or heterogeneity) at all the levels of biological organisation right from macromolecules within the cells, genes, species, ecosystems and biomes.

There are three most important hierarchical levels of biodiversity:

(i) Genetic diversity: A single species might show high diversity at the genetic level over its distributional range. It means genetic diversity is a measure of variety in genetic information contained in the organisms. Within a species genetic diversity occurs in the differences of alleles, entire genes and chromosomal structures. Genetic diversity enables a population to adapt to its environment and the changes occurring the environment. It results in variation in potency and concentration of the active chemical (reserpine) being present in medicinal plant *Rauwolfia vomitoria* growing in different Himalayan ranges. It helps in formation of **ecotype** and Play key role in process of speciation. More than 50,000 genetically different strains of rice, and 1,000 varieties of mango occur in India due to genetic variations.

(ii) Species diversity: It refers to the variety of species within a region. For example, Western Ghats have greater amphibian species diversity as compared to Eastern Ghats.

(a) Species diversity is product of **species richness** and **species evenness**.

(b) Species richness is the number of species present within a unit area.

(c) Species evenness or species equitability is the proportionate number of individuals of different species (taxonomic groups). Communities where species are represented by more or less equal number of individuals exhibit evenness. Others where one or more species have more individuals than others show dominance or unevenness.

(d) Maximum taxonomic diversity occurs where species of taxonomically different groups occur in almost equal abundance.

(iii) Ecological diversity (Community diversity) : It is the variety of ecosystems which indicate diversity in the number of niches, trophic levels, food webs, nutrient cycles and ecological processes sustaining energy flow. For example ecosystem diversity is high in India because of the occurrence of a large number of ecosystems like deserts, rain forests, mangroves, coral reefs, wetlands, estuaries, and alpine meadows. It is quite low in small countries e.g., Scandinavian country like Norway.

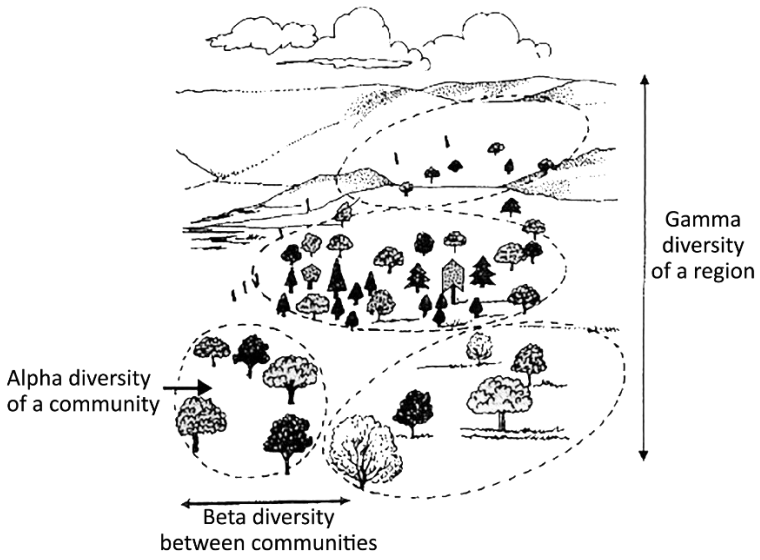


Fig.: Types of ecological diversity (community diversity)

It has taken millions of years of evolution, to accumulate this diversity in nature, but we could lose all that wealth in less than two centuries, if the present rates of species losses continue. Biodiversity and its conservation are now vital environmental issues of international concern as more and more people around the world begin to realise the critical importance of biodiversity for our survival and well-being on this planet.

How many Species are there on Earth and How many in India?

According to the **International Union of Conservation of Nature and Natural Resources (IUCN, 2004)**, the total number of plant and animal species described so far is slightly more than 1.5 million, but we have no clear idea of how many species are yet to be discovered and described. Estimates of species vary widely and many of them are only educated guesses. Scientists estimate the number of species present in tropics by comparing species richness between tropical and temperate areas. For many taxonomic groups, inventories are more complete in temperate than in tropical countries. Estimates are made of their number in tropics on the basis of temperate tropical species richness of some exhaustively studied groups like insects. On this basis, scientists have calculated that the total number of species in the world ranges from 20 to 50 million. **Robert May**, with his conservative and scientifically sound estimate, places the number of global species diversity at about 7 million.

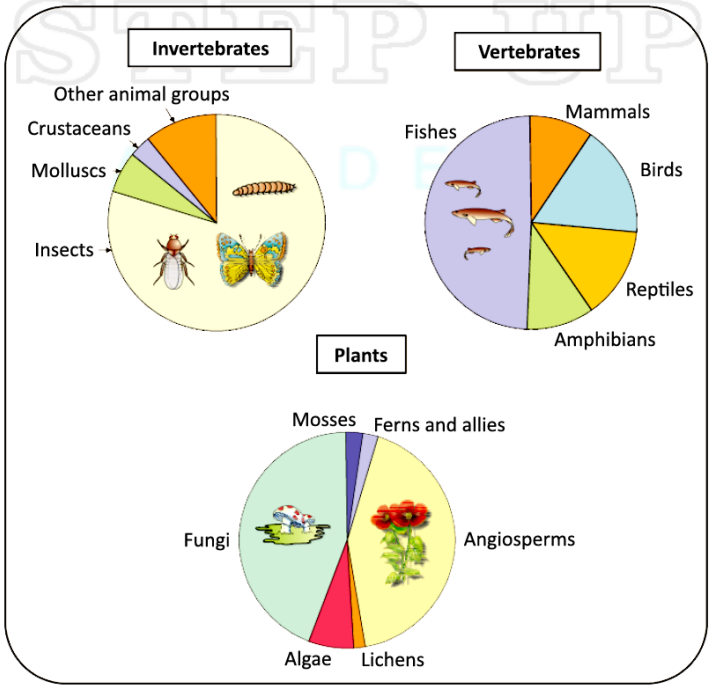


Fig. : Representing global biodiversity: Proportionate number of species of major taxa of invertebrates, vertebrates and plants

Some interesting aspects about earth's biodiversity based on the correctly available species inventories :

(1) Number of animal species is more than 70 percent Plants (including algae, fungi, bryophytes. Gymnosperms and angiosperms) account for nearly 22% of the total.

(2) Among animals, insects are the most species-rich taxonomic group, making more than 70 percent of the total. It means, out of energy 10 animals on this planet, 7 are insects.

(3) Number of fungi species (72,000) in.the world is more ten the combined total of the. species of fishes (28,000), amphibians (4780), reptiles (7150) and mammals (4650).

Estimates (1-3), mentioned above, do not give any figures for prokaryotes. Biologists are not sure about how many prokaryotic species there might be. The possible reasons are as follows:

(i) Conventional taxonomic methods are not suitable for identification and characterisation of microbial species.

(ii) Many species are simply not culturable under laboratory conditions.

If biochemical or molecular criteria are employed for distinguishing species for prokaryotes, then their diversity may run into millions.

Biodiversity in India : India is divided into 10 bio-geographical regions. India with only 2.4% of the world's land area possesses 8.1% species diversity of the world due to varying physical conditions and species grouping. It is because India is one of the 12 mega diversity countries of the world. There are nearly 45,000 species of plants and **twice as many** animal species. If we accept May's global estimates, only 22 of the total species have been recorded so far. Applying this proportion to India's diversity figures, we estimate that there are probably more than 1,00,000 plant species and more than 3,00,000 animal species yet to be discovered and described. It will require a large trained manpower of taxonomists and lot of time to complete the inventory of the biological wealth of our country However, a very large number of species that are yet to be discovered are facing the threat of becoming extinct even before we discover them.

Nature's biological library is burning even before we catalogued the titles of all the books stocked there.

Patterns of Biodiversity

The diversity of plants and animals is not uniform throughout the world but shows a rather uneven distribution. There are many interesting patterns in diversity like latitudinal, altitudinal, geographical, topographical humidity gradients but most well - known being the latitudinal gradient in diversity.

(i) Latitudinal gradients: As we move from low to high latitude i.e. from the equator to the poles the biodiversity decreases. In other words, the biodiversity is minimum in the arctic region, moderate in temperate area and maximum in tropical regions (latitudinal range of 23.5° N to 23.5°S).

Examples of high diversity in tropical regions:

(a) Colombia located near the equator (tropical region) has about 1,400 species of birds New York (41° N) in temperate area has 105 species and Greenland (71° N) in arctic area possess 56 species of birds.

(b) India has more than 1,200 species of birds because most of the land area of our country lies in tropics.

(c) A forest in a tropical region like Equator has 10 times more species of vascular plants as compared to a forest of equal area in a temperate region like the Midwest of the USA.

(d) **Tropical Amazonian rain forest in South America has the greatest biodiversity on earth.**

These rain forests might have at least 2 million insect species yet to be discovered and named.

Why biodiversity is rich in tropics?

Ecologists and evolutionary biologists have proposed various hypothesis to explain the cause of high diversity in tropics. Some important ones are as follows:

(a) Speciation is a function of time. Temperate areas have undergone frequent glaciations in the past. It killed most of the species. No such diversification occurred in tropics where species continued to flourish and evolve undisturbed for millions of years.

(b) Warm temperatures and high humidity in tropical areas provide favorable conditions throughout the year. Therefore, tropical environments unlike temperate ones, are less seasonal, relatively more constant and predictable. Such constant and favorable environment has helped tropical organisms to gain more niche specialization and lead to a greater species diversity.

(c) Tropical areas receive more solar energy over the year as they are near to equator. Thus tropical communities are more productive that can support a wider range of species.

(ii) Species-Area relationships: German naturalist and geographer **Alexander von Humboldt** while exploring the South American jungles, found that within a region, species richness increased with increasing explored area but only up to a limit. The relationship between species richness and area turned out to be a rectangular hyperbola for a wide variety of organisms like angiosperm plants, birds, bats and

freshwater fishes. The relationship is a straight line on a logarithmic scale.

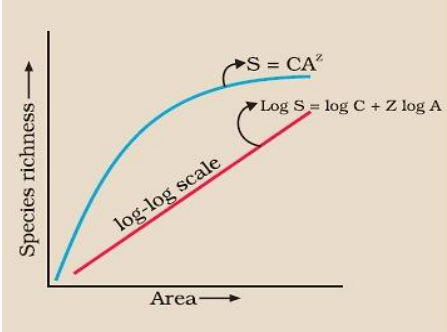


Fig.: Graph showing species area relationship.

Note that on log scale the relationship becomes linear

$S = CA^Z$

Or $\log S = C + Z \log A$

Where,

S = Species richness

A = Area

Z = Slope of the line (regression coefficient)

C = Y – intercept

Significance of slope of regression (Z) in a species-area relationship: Slope of regression or regression coefficient of species-area relationship indicates that species richness decreases with the decrease in area. The value of slope of regression (Z) of species-area relationships lies in the range of 0.1 to 0.2 when analysis is done among small areas like plants in Britain, birds in California or molluses in New York state. However, if the species-area relationship is done for very large areas like the entire continent, the slope of the line is much steep with value of Z in the range of 0.6 to 1.2. For example, it is 1.15for frugivorous (fruit-eating) birds and mammals in the tropical forests of different continents. Thus, larger the explored area, more is steepness of the slope of line.

The Importance of Species Diversity to the Ecosystem

Number of species in a community really matter to the functioning of the ecosystem because rich biodiversity is important for stability, productivity, resilience, alternative pathways and health of ecosystems.

(i) Stability: Ecologists, consider that communities with more species tend to be more stable than those with less species. What exactly is stability for a biological community? Let us discuss it.

(a) A stable community should not show too much variation in productivity from year to year.

(b) It must be resistant or resilient to occasional natural as well as man-made disturbances.

(c) It must be resistant to invasions by alien species.

David Tilman's long-term ecosystem experiments using outdoor plots confirmed that these attributes are linked to species richness in a community. He found that

(a) Plots with more species showed less year-to-year variation in total biomass.

(b) Increased diversity contributed to higher productivity.

Now it is clear that species diversity is important for productivity, stability and resilience.

(ii) Ecosystem health: It is often believed that little harm would occur to ecosystem if a few species become extinct. There should not be much difference if one of the tree frog species is lost forever from. Western Ghats ecosystems or number of ant species is reduced from 20,000 to 15,000. Apparently there might not appear any difference for some time. However, rich biodiversity is not only essential for ecosystem health but, also for survival of human race on earth. It is because larger number of species have higher number of niches, more interactions and more inter-relationships. The enslich effect of reduction in biodiversity has been explained by **Paul Ehrlich** through **Rivet popper hypothesis**. In an airplane, (ecosystem) all parts are joined together using thousands of rivets (species). If every passenger travelling in it starts popping a rivet to take home (causing a species to become extinct), it may not affect flight safety (proper functioning of the ecosystem) initially, but as more and more rivets are removed, the plane becomes dangerously weak over a period of time. Furthermore, which rivet is removed may also be critical. Loss of rivets on the wings (key species that drive major ecosystem functions) is obviously a more serious threat to flight safety than loss of a few rivets on the seats or windows inside the plane.

Airplane	Ecosystem
Riverts	Species
Rivets on the wings	Key species

Loss of Biodiversity

While it is doubtful if any new species are being added (through speciation) into the earth's treasury of species, there is no doubt about their continuing losses. The biological wealth of our planet has been declining rapidly. Complete disappearance or extinction of a species results in complete loss of genetic information contained in it.

Natural extinction: With the change in environmental conditions, some species disappear and others, which are more adapted to changed conditions, take their place. This loss of species which occurred in the geological past at a very slow rate, is called natural extinction.

Mass extinction: It is the dying off or extermination of a large number of species due to catastrophes. During the

long period (> 3 billion years) since the origin and diversification of life on earth there were five **episodes of mass extinction of species.**

Anthropogenic extinction: It is extermination of species caused directly or indirectly by human activities like habitat destruction, over-exploitation, hunting, pollution etc.

(a) Colonisation of tropical Pacific Islands by humans have resulted in extinction of more than 2,000 species of native birds.

(b) The IUCN Red List (2004) documents the extinction of 784 species in the last 500 years, it includes 338 vertebrates, 359 invertebrates and 87 plants. Some examples of important recent extinctions include the Dodo (Mauritius), Quagga (Africa), Thylacine (Australia), Steller's sea cow (Russia), and three subspecies (Bali, Javan, Caspian) of tiger. In the last 20 years, 27 species have become extinct.

(c) Extinction across taxa are not random. For example, some groups like amphibians appear to be more vulnerable to extinction.

Presently some 15,500 species world-wide are facing the threat of extinction. They include:

(1) 12 percent of all bird species

(2) 23 percent of all mammal species

(3) 31 percent of all gymnosperm species

(4) 32 percent of all amphibian species

(d) Anthropogenic extinction is causing a **sixth mass extinction** of species. It is 100 - 1,000 times more faster than the rate of natural extinctions. Ecologists warn that if the present trends continue, nearly half of all the species on earth might be wiped out within the next 100 years.

Results of loss of biodiversity: Loss of biodiversity in a region may lead to

(i) Decline in plant production or productivity,

(ii) Reduced resistance to environmental perturbations like drought,

(iii) Increased variability of ecosystem processes like productivity, water use, pest and disease cycles.

Causes of biodiversity losses: The world is facing accelerated rates of species extinctions, largely due to human activities. There are four major causes of biodiversity losses, called **The Evil Quartet'**(It is the sobriquet used to describe them).

(i) Habitat loss and fragmentation: It is the most important cause driving animals and plants to extinction.

(a) When people cut down trees, fill a wetland, plough a grassland or burn a forest, the natural habitat of a species is changed or destroyed. These changes can kill or force out many plants, animals, and microorganisms as well as disrupt complex interactions among the species. The most dramatic examples of habitat loss come from tropical rain forests. These forests once occupied 14% of the earth's land area. Today they occur on only 6% of land area. By the time you finish reading this chapter, 1000 more hectares of rain forest would have been lost. The **Amazon rain forest ('Lungs of the Planet')** harbouring probably millions of species is being cut and cleared for cultivating soya beans or for conversion to grasslands for raising beef cattle.

(b) Besides total loss, the degradation of many habitats by pollution also threatens the survival of many species. Pollution may reduce and eliminate populations of sensitive species. For example, pesticide linked decline of fish-eating birds and falcons. Lead poisoning is another major cause of mortality of many species, such as ducks, swans and cranes. Eutrophication (nutrient enrichment) of water bodies drastically reduces species diversity.

(c) Large habitats are broken into small fragments in habitat fragmentation. It is due to various human activities like human settlements, building of roads, digging of canals etc. Forest patches having croplands, orchards, plantations and urban settlements on their outskirts are examples of fragmented habitats. Animals requiring large territories (e.g. mammals, birds) and migrating animals are badly affected, leading to population declines.

(ii) Over-exploitation: Humans have always been dependent on nature for food and shelter, but when 'need' turns to 'greed', it leads to over-exploitation of natural resources. It means, biological systems should not be exploited beyond the degree of their renewability. Overexploitation of a particular species reduces size of its population to an extent so that it becomes vulnerable to extinction. Dodo, Steller's sea cow and Passenger pigeon have become extinct in the last 500 years due to Overexploitation by humans. Some commercially important species of marine fishes are likely to become endangered because marine fish populations are being overharvested all over the world.

(iii) Alien species invasions: New species entering a geographical region are called exotic or alien or non-native species. When alien species are introduced unintentionally or deliberately for whatever purpose, they may cause disappearance of native or indigenous species through changed biotic interactions.

A few examples of exotic species are as follows:

(a) **Nile perch**, a large predator fish was introduced into Lake Victoria of East Africa. Nile perch killed and eliminated ecologically unique assemblage of over 200 species of **cichlid fish** that were endemic to this freshwater aquatic system.

(b) Carrot grass (Parthenium) is a weed which came into India as a contaminant with imported wheat. It has occupied all open areas exterminating many herbs and shrubs.

(c) Lantana is a straggling shrub of tropical America which got introduced in India accidentally. Today it has become a serious weed which has replaced many species in forests.

(d) Water hyacinth (Eichhornia) was introduced by Europeans in India. It has clogged water bodies including wetlands at many places resulting in death of several aquatic plants and animals.

(e) The recent illegal introduction of the African **catfish Claries gariepinus** for aquaculture purposes is posing a threat to the indigenous **catfishes in our rivers**.

(iv) Co-extinctions: There are many obligate associations amongst different species in ecosystems. When one of them becomes extinct, the plant and animal species associated with it in an obligatory way also become extinct. For example, coevolved plant-pollinator mutualism will result in extinction of one partner if the other is eliminated in nature. If the host fish becomes extinct, all the parasites exclusively found on it will also become extinct.

Susceptibility to Extinction

Species more susceptible to extinction have following the population characteristics.

(i) Large body size (e.g., Rhinoceros, Lion)

(ii) Small population size and low reproductive rate (e.g., Giant Panda, Blue Whale).

(iii) High trophic level in food chain (e.g., Bald Eagle, Bengal Tiger).

(iv) Fixed habitat and migratory routes (e.g., Whooping Crane, Blue Whale).

Red Data Book and IUCN

IUCN is **International Union for Conservation of Nature and Natural Resources**, which is now called **World Conservation Union** [WCU]. It has H.Q. at Morges, Switzerland. It maintains a **Red Data Book** or **Red list** which is a catalogue of threatened plants and animals facing risk of extinction.

Concept of threatened species : Threatened species [T] is the one which is liable to become extinct if they are not provided with proper habitat, food and protection so that they can realise their full biotic potential.

Red list has eight categories of species : **Extinct, in the Wild, Critically Endangered, Vulnerable, Lower Risk, Data Deficient, and Not Evaluated.**

Examples of threatened species in India

S.No.	Category	Plant	Animal
1.	Critically endangered	Berberis nilghinensis	Sus salvanius (Pigmy hog)
2.	Endangered	Bentinckia nicobarica, Aconitum (Indian Aconite), Blue vanda (orchid), Podophyllum, (Medicinal plant) Nepenthes, (Pitcher plant) Rauwolfia serpentina, Santalum album (Sandal wood), Cycas beddomei	Asiatic Lion, Giant Panda, One horn Rhinoceros, Kashmiri stag (Hangul), Red Panda (Aiiurus), Indian Elephant, Blue Whale, Lion tailed Macaque, Musk Deer, Tiger, Indian Wild Ass, Great Indian Bustard, Siberian crane.
3.	Vulnerable	Cupressus cashmeriana	Antelope cervicapara (Black Buck)

Biodiversity Conservation : –

Ecosystems are undergoing change due to several factors like pollution, invasive species, over-exploitation by humans, climate change etc. Most people are beginning to recognise that diversity at all levels-genetic, species and ecological is important and needs to be conserved.

Why Should We Conserve Biodiversity?

There are many reasons (all equally important) why should we conserve biodiversity. They can be grouped into three categories: narrowly utilitarian, broadly utilitarian, and ethical.

(i) Narrowly utilitarian (Direct or economic uses): Humans derive countless direct economic benefits from nature.

(a) Food: All the food like we eat comes from plants and animals e.g., cereals, pulses, fruits, eggs, meats etc.

(b) Firewood: It is used as source of energy for cooking and heating. .

(c) Fibre: Jute, flax, hemp, cotton, coir are the source of natural fibres.

(d) Construction material: Wood is used as timber in construction work, furniture, sports goods, musical instruments etc.

(e) Industrial products: Tannins, lubricants, dyes, resins, perfumes, paper, rubber are some of the industrial products obtained from plants.

(f) Drugs: More than 25 of the drugs currently sold in the market worldwide are derived from a mere 120 species of plants. About 25,000 species of plants contribute to the traditional medicines used by native peoples around the world. Many more medicinally useful plants especially in tropical rain forests, waiting to be explored.

Drug	Source	Application
Morphine	Dried latex from unripe capsules of Papaver somniferum	Analgesic
Quinine	Bark of Cinchona ledgeriana and C.officinalis	Antimalarial

Bioprospecting : Exploring molecular, genetic and species-level diversity for products of economic importance is going on vigorously. Nations with rich biodiversity are expected to reap enormous benefits.

(ii) Broadly utilitarian (Ecosystem services): Biodiversity plays a major role in many ecosystem services that nature provides.

(a) Oxygen: Plants are replenishing 0_2 of the atmosphere due to their photosynthetic activity. Amazon rain forest is estimated to produce 20 of it.

(b) Pollination: A number of organisms like bees, bumblebees, birds, bats are involved in pollination of plants which is essential for formation of fruits and seeds. If humans perform this duty, the cost would be many billion dollars.

(c) Aesthetic pleasure: Biodiversity has a lot of aesthetic and attraction value. It provides a lot of pleasures of walking through thick woods, watching spring flowers in full bloom or waking upto a bulbul's song in the morning.

(d) Flood and Erosion control: Plant roots hold the soil particles against moving wind and water and thus prevent soil erosion. Plants also increase the porosity of soil and thereby allow water to percolate down into the soil. They help to retain water and prevent run off of rain water. Litter and humus of plants act as a sponge, retaining most of the rain water. As the soil is porous, the retained water percolates downwardly and is stored as underground water.

(iii) Ethical: There are millions of plant, animal and microbial species who evolved just as we have evolved and are sharing the planet with us. No organism is useless. Every species has an intrinsic value, though it may not be of direct use to us. It is therefore, our moral and ethical duty not to destroy them. Instead, we should take care of their well-being so as to pass the rich biological legacy to future generations.

How Do We Conserve Biodiversity?

There are two basic strategies of biodiversity conservation, in-situ (on site) and ex-situ (off site).

(i) In-situ conservation: The in-situ strategies emphasise protection of whole ecosystem, therefore its biodiversity at all levels is protected. It means we save the entire forest to save the tiger. However, many nations find it unrealistic and economically not feasible to conserve all their biological wealth. Invariably, the number of species waiting to be saved from extinction far exceeds the conservation resources available. On a global basis, this problem has been addressed by eminent conservationists.

In-situ conservation strategies are of two types: hot spots and protected areas.

(a) Hot spots: Concept of hot spots was developed to designate priority areas for in-situ conservation. The hot spots are the richest and the most threatened reservoirs of plant and animal life on earth. The key criteria for determining a hot spot are:

(1) Very high levels of species richness.

(2) High degree of endemism (species confined to that region and not found anywhere else).

(3) Degree of threat, which is measured in terms of habitat loss.

Initially, 25 hot spots were identified globally. But, now the number is raised to 34 occupying earth's land area of less than 2%. The number of species they collectively harbour is extremely high and strict protection of these hot spots could reduce the ongoing mass extinctions by almost 30%. India has three hotspots - **(1) Western Ghats** and **Sri Lanka (2) Indo-Burma** and **(3) Himalaya,** and these extend into the neighbouring countries also.

Biodiversity hot spots in India :

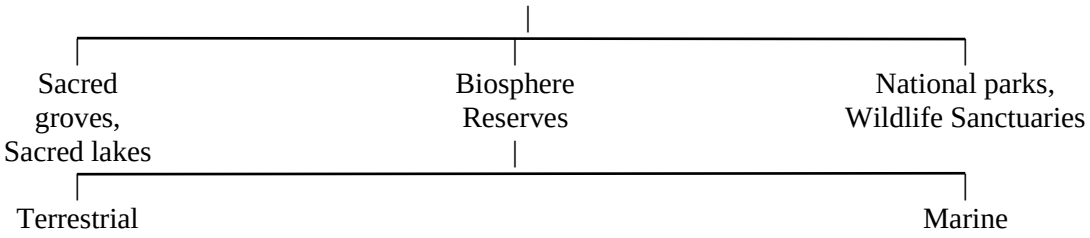
(1) Western Ghats and Sri Lanka: They occur along the western coast of India through Maharashtra, Karnataka, Tamil Nadu and Kerala extending over to Sri Lanka. Southern Western Ghats are known as Malabar. The Agasthyamalai hills, the Silent Valley and the new Amambalam Reserve, are the major centres of diversity.

(2) **Indo-Burma:** It extends from Bhutan to Myanmar covering most of the North-Eastern India.

(3) Himalaya: It is one of the richest hot spot of biodiversity.

(b) Protected areas: These are areas of land and/or sea, especially dedicated to the protection and maintenance of biological diversity, and of natural and associated cultural resources. These are managed through legal or other effective means.

Protected Area Network



India has 14 biosphere reserves, 90 national parks and 448 wildlife sanctuaries covering 4.7 of the land surface, as against 10 internationally suggested norm.

(1) National parks: These are areas maintained by government and reserved for of wildlife (flora and fauna both). Grazing, felling of trees, habitat manipulation and cultivation are not allowed. The earliest national parks, the **Yellowstone** in USA and **The Royal** near Sydney Australia were selected for their scenic beauty and recreational values. The Jim **Corbett National Park** was the first National Park established in India.

(2) Sanctuaries: These are tracts of land where animals (fauna) are protected from all types of exploitation and habitat disturbance. Collection of forest products, harvesting of timber, tilling of land, private ownership etc. are allowed.

(3) Biosphere reserves: These are a special category of protected areas of land and/or coastal environments, wherein **tribal people are an integral component of the system**. These are representative examples of natural biomes and contain unique biological communities. The concept of Biosphere Reserves was launched in 1975 as a part of UNESCO's Man and Biosphere Programme (MAB). There are **14 biosphere reserves in India.** A Biosphere Reserve consists of **core, buffer and transition zones.** The **natural** or **core zone** comprises an undisturbed and legally protected ecosystem. The **buffer zone** surrounds the core area, and is managed to accommodate a greater research and educational activities. The **transition zone**, the outermost part of the Biosphere Reserve, is an area of active, cooperation between reserve management and the local people, wherein, activities like settlements, cropping, forestry, recreation and other economic uses continue in harmony with conservation goals.

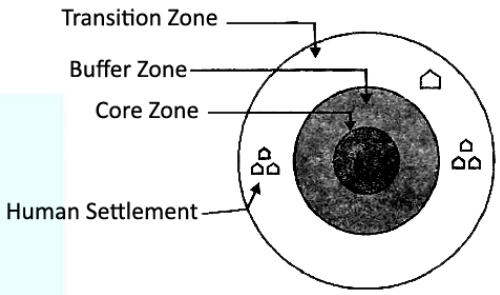


Fig.: The zonation in a terrestrial Biosphere Reserve

The main functions of biosphere reserves are :

(i) Conservation

(ii) Development

(iii) Scientific research, monitoring and education

(4) Sacred groves: In many cultures, tracts, of forest were set aside, and all the trees and wildlife within were venerated and given total protection. These are also known as **Islands of Pristine Forests.** These are found in several parts of India:

(1) Khasi and Jaintia Hills in

(2) Aravalli Hills of Rajasthan

(3) Western Ghat regions of Kamataka and Maharashtra

(4) Sarquia, Chanda and Bastar areas of Madhya Pradesh

In Meghalaya, the sacred groves (sacred forest) are the last refuges for a large nurftfei of rare and threatened plants.

Sacred lakes : Pushkar lake in Rajasthan; Khecheopairi lake in Sikkim.

Sacred plants : Odmum sanctum (Tuisi), F/cus religiosa, Elaeocarpus floribundus (Rudraksha) etc.

(ii) Ex-situ conservation: In this type of conservation strategies, threatened animals and plants are taken out from their natural habitat and placed in special setting where they can be protected and given special care. If an animal or plant is endangered or threatened and needs urgent measures to save it from extinction, ex-situ conservation is the desirable approach.

Ex-situ conservation

Sacred plants, Home gardens	Seed banks, Field gene bank, Cryopreservation	Botanical gardens, Arboreta, Zoological parks, Aquaria, Wildlife safari parks

There are many animals that have become extinct in the wild but continue to be maintained in zoological parks. In recent years, ex-situ conservation has advanced beyond keeping threatened species in enclosures. Now, gametes of threatened species can be preserved in viable and fertile condition for long periods using cryopreservation techniques, eggs can be fertilised in-vitro, and plants can be propagated using tissue culture methods. Seeds of different genetic strains of commercially important plants can be kept for long periods in seed banks.

CONVENTIONS ON BIODIVERSITY

The historic Convention on Biological Diversity (The Earth Summit') held in Rio de Janeiro in 1992,called upon all nations to take appropriate measures for conservation of biodiversity and sustainable utilization of its benefits.

In a follow-up**, The World Summit on Sustainable Development held in 2002 in Johannesburg, South Africa,** 190 countries pledged their commitment to achieve by 2010, a significant reduction in the current rate of biodiversity loss at global, regional and local levels.

	Animals	National Parks/Sanctuaries
1.	**Lion** (Panthera leopersica) protection started in 1972	• Gir National Park, Junagarh (Gujarat).
2.	Tiger (Pantheratighs) protection started in 1973	• Corbett National Park [Nainital; Uttarakhand] • Hazaribagh Sanctuary, [Hazaribagh; Bihar] • Sundarbans Tiger Reserve [24 Pargana; Bengal] • Ranthambore National Park [Savaii Madhupur; Rajasthan] • Kanha National Park [Madhya Pradesh] • Bandipora National Park [Mysore; Karnataka] • Nagarjuna Sagar Srisailam [Guntur; Andhra Pradesh] (Largest tiger reserve)
3.	**Snow Leopard** (Panthera uncial)	• Khangchendzonga National Park [Gangtok ; Sikkim]
4.	**Rhino** (Rhinoceros unicornis) started in 1987	• Kazi Ranga National Park [Sibs agar Jorhat] • Manas Sanctuary [Assam] • Jaladhar Sanctuary [West Bengal]
5.	**Elephants (**Elephas maximus started in 1991	• Pereyra Sanctuary [Kerala] • Simlipal Sanctuary [Orissa] • Manas Sanctuary [Assam] • Bandipora National Park [Mysore; Karnataka]

NEERI : National Environment Engineering Research Institute.

UNEP : United Nations Environment Programme.

CAZRI : Central Arid Zone Research Institute (Jodhpur).

IBWL : Indian Board of Wild Life.

CBD : Convention on Biological Diversity (Promoted by The Earth Summit) – 1992

Cryopreservation: In-vitro conservation in which tissue, organs, embryos, seed etc. are stored at very low temperature of-196ºC (temperature of liquid nitrogen).